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INTERSPECIES COMPETITION IN COLONIZED POROUS PELLETS

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Abstract—Packed-bed bioreactors filled with diatomaceous earth (D.E.) pellets were used to evaluate the effects of competition between inoculated and invading microbial species on the spatial and temporal distribution of microorganisms within an individual pellet. The (D.E.) pellets were cylinders 6 mm in diameter and 5–10 mm long with a mean pore diameter of 20 μm. Bench-scale experiments evaluated competition between two distinct microbial species: *Pseudomonas aeruginosa*, a motile, obligate aerobe ($\mu_{max} = 0.4\ h^{-1}$) and *Klebsiella pneumoniae*, a non-motile, facultative organism ($\mu_{max} = 2.0\ h^{-1}$). Organism growth rate appeared to be more important than motility or order of introduction in determining organism spatial and temporal distribution within the pellets. Pilot-scale experiments used pellets colonized with a pseudomonad growing on chlorobenzene as the sole carbon and energy source. Organic-rich ground water containing benzene, chlorobenzene and a population of indigenous microorganisms was used as feed. Pellet concentrations of the inoculated pseudomonad dropped from 10^9 to 10^6 colony forming units (cfu) ml^{-1} pellet volume over 15 days. These experiments demonstrate that inoculated organisms within porous packing media may undergo significant loss in colonization numbers when faced with competition from faster growing organisms.

Key words—colonization, biodegradation, microbial competition, survival, diatomaceous earth pellets

INTRODUCTION

Above-ground biotreatment of contaminated groundwater necessitates the stabilization of a microbial population capable of utilizing the target contaminant. Such treatment normally involves pumping the contaminated water to the surface via recovery wells, passing the water through a medium colonized with microorganisms, then disposal via reinjection or surface application. Such above-ground bioreactors utilize a variety of media designed to increase surface area available for microbial colonization per unit reactor volume. Attempts to maximize surface area have led to the use of porous media for microbial cell retention. One such medium (R-635, Manville Inc., Lompoc, Calif.) is a diatomaceous earth (D.E.) pellet. An expected benefit of such porous pellets is a degree of protection from both surface fluid shear conditions and microbial competition for cells colonizing the pellet interior. A nominal pore size of 20 μm provides a high pellet surface area, and to a large degree prevents advective flow within the pellets, yet allows diffusive transport of cells, substrate, and electron acceptor and colonization of interior pellet surfaces (Fig. 1).

Efforts to enhance biodegradation have led to the isolation of bacterial strains capable of degrading one or several otherwise recalcitrant organic compounds, frequently as the sole carbon and energy source. Such strains are typically isolated from a contaminated soil site or from activated sludge (Lee *et al.*, 1988). The isolation/selection procedure involves exposure of the inoculum to increasing concentrations of the target contaminant followed by selection of the most vigorously growing colonies (Omenn, 1986). These efforts have been successful with a diverse range of compounds such as aromatics (naphthalene, styrene, benzene, toluene, xylene), chlorinated aromatics (chlorobenzene), halogenated aliphatics (chloroform, bromodichloromethane, trichloroethylene, tetrachloroethylene) and polychlorinated biphenyls (McCarty *et al.*, 1984; Focht and Brunner, 1985).

For *in situ* bioremediation, reintroduction of these isolates into soil systems has met with limited success. Inoculated organisms are often quickly displaced by indigenous soil organisms. Goldstein *et al.* (1985) cite four reasons for such failures: the concentration of the target compound is too low, the environment contains some substance or organisms that inhibit growth, the inoculated organism uses a substrate other than the target compound, or the substrate is not accessible to the organism. Protozoan predation may also significantly reduce bacterial populations (Madsen *et al.*, 1991). In cases where some success with inoculated organisms has been reported, controls often show contaminant removal commensurate with uninoculated controls (Focht and Brunner, 1985; Westlake *et al.*, 1978).

Above-ground bioreactors offer the advantage of a potentially sterile site for colonization by inoculated

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Fig. 1. Scanning electron micrograph of the pellet interior. The nominal pellet pore size is approx. 20 μm.

bacteria. Since groundwater may contain as many as 10^6 cfu/ml of naturally occurring microorganisms (Bitton and Gerba, 1984), the initial absence of competing microbes allows unhindered colonization by the inoculated organism. Despite these advantages, the abundance of bacteria in most groundwater insures that a competitive environment will exist once the system is exposed to groundwater. Furthermore, the use of inoculated organisms in bioreactors has met with limited success because inoculated consortia may not perform in field reactors as it did in the laboratory (Sojka *et al.*, 1989). Bartha (1986) suggests that repeated inoculations may be necessary to degrade many pollutants, particularly if sufficient, easily degradable organic carbon exists for survival of competing organisms.

D.E. pellets have been widely used as a bioreactor packing material. Such pellets compare favorably with other common substratum materials such as sand, anthracite and charcoal in their ability to attach and retain organisms (Caunt and Chase, 1988). Several researchers have reported sustained degradation of BTEX compounds and chlorinated solvents using D.E. pellets inoculated with compound specific

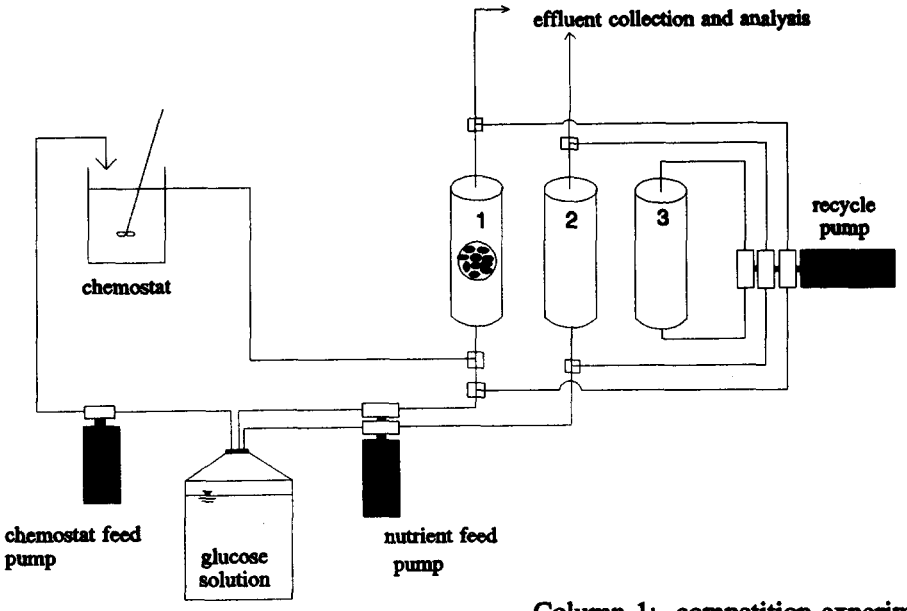

Fig. 2. Experimental system for competition experiments: chemostat, three column reactors and the necessary supporting equipment.

bacteria isolated from sewage sludge (Friday *et al.*, 1988). Such reports indicate that the porous pellets offer a suitable colonization surface, but since these studies were performed using tap water as column influent, no conclusions can be drawn about the longevity of the inoculated organisms with a microbially diverse influent feed. Other research (Pettigrew *et al.*, 1991; Nishino *et al.*, 1992) indicates that inoculated, compound-specific organisms may be outcompeted from pellet interior and exterior surfaces in under 2 weeks when exposed to naturally occurring organisms.

The goal of this research was to quantitatively analyze the processes influencing the survival of inoculated organisms within D.E. pellets. Experiments sought to determine the persistence of individual bacterial species when exposed to competition from other species, and elucidate the role of bacterial growth rate and motility in organism survival. To accomplish these objectives, two experimental programs were conducted: (1) examination of the effects of order of introduction and organism growth rate on species persistence in D.E. pellets using two well characterized bacteria; and (2) determination of the persistence of an inoculated pseudomonad in a pilot-scale bioreactor operated to degrade a benzene–chlorobenzene mixture from contaminated groundwater.

MATERIALS AND METHODS

Inoculation sequence experiments

A continuous flow, packed-bed reactor system with an attached chemostat (Fig. 2) was used to study the effects of order of introduction and organism growth rate on the survival of two bacterial species in D.E. pellets. Pellets (R-635) were obtained from Manville Inc. of Lompoc, Calif. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were chosen for these competition experiments for the following reasons: (1) their growth rates differ by a factor of five; (2) *Pseudomonas* is motile and *Klebsiella* is not; and (3) their kinetic coefficients have been well characterized (Table 1). Three experiments were performed to evaluate intrapellet competition between these two organisms. In experiments 1 and 2, pellets were colonized with *Pseudomonas aeruginosa* and challenged with *Klebsiella pneumoniae*. These experiments were run for 10 and 21 days, respectively. In experiment 3,

Table 1. Properties of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

Property	*P. aeruginosa*	*K. pneumoniae*
Motility	polar flagella	non-motile
Respiration	obligate aerobe	facultative anaerobe
Metabolism	chemoorganotroph	chemoorganotroph
Maximum growth rate	$0.40 \pm 0.01\ h^{-1}$	$2.00 \pm 0.3\ h^{-1}$*
Half-saturation coefficient	$2.5 \pm 0.5\ g\ m^{-3}$*	$1.4 \pm 0.5\ g\ m^{-3}$*

*Siebel and Characklis (1991).

which ran for 10 days, *Klebsiella* was the colonizing organism and *Pseudomonas* the challenger.

In experiments 1–3, D.E. pellets were loosely packed into polycarbonate columns (packing dimensions: 9 cm long, 3.4 cm dia). The packed columns and feed solution (see below) were autoclaved at 121°C for 3–4 h to ensure sterile initial conditions. An experiment typically consisted of operating 3 columns in parallel; (1) an "initial colonization" control column; (2) a "single species" column which was operated in continuous flow but not subjected to a competing organism; and (3) a "competition column" which was colonized, run in continuous flow for 4–5 days to allow establishment of the inoculated organism, then challenged with a competing organism for 5–15 additional days. The single species column and the competition column were operated concurrently for the duration of each experiment, while the initial colonization column was operated only for 4–5 days to determine inoculated organism numbers before competition began. At the end of an experiment, pellets from each of these columns were dissected and the organisms within enumerated to determine the effects of microbial competition on both the inoculated organism and the challenging organism. All system pumps operated by peristaltic action. The chemostat feed pump and the plug-flow reactor feed pump were operated at a flow rate of 0.6 ml min^{-1} resulting in a hydraulic residence time of 45 min for the single species column and 23 min for the competition column (addition of chemostat effluent into the competition column created twice the flow in this column). Nutrients were added to both the chemostat and the packed bed column in the following concentrations: glucose, 15 mg l^{-1}; NH_4Cl, 7.2 mg l^{-1}; $MgSO_4 \cdot 7H_2O$, 2.0 mg l^{-1}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.001 mg l^{-1}; $ZnSO_4 \cdot 7H_2O$, 0.1 mg l^{-1}; $MnSO_4 \cdot H_2O$, 0.008 mg l^{-1}; $CuSO_4 \cdot 5H_2O$, 0.002 mg l^{-1}; $Na_2B_4O_7 \cdot 10H_2O$; 0.001 mg l^{-1}; $FeSO_4 \cdot 7H_2O$, 0.112 mg l^{-1}; $(HOOCH_2)_3N$, 0.4 mg l^{-1}; $CaCl_2 \cdot 2H_2O$, 11.0 mg l^{-1}; Na_2HPO_4, 213 mg l^{-1}; KH_2PO_4, 204 mg l^{-1}. The chemostat effluent cell concentrations for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, respectively, were 5×10^6 and 1×10^6 cfu ml^{-1} at a dilution rate of 0.6 h^{-1}. Initial colonization was accomplished by injecting a 2.5 ml cell suspension directly into the recycle stream of each reactor. Reactor fluid recycle was used for initial colonization only; no recycle flow occurred during column operation. The injected cell suspension was recycled for 3 days in a mineral salts buffer which was similar to the above nutrient feed minus the glucose. Reactor recycle flowrate during colonization was 0.6 ml min^{-1}.

Cell suspensions for inoculation were prepared by mixing 0.1 ml frozen stock culture, 1 ml of 100 ppm glucose solution and 100 ml sterile mineral salts buffer (pH 7) and incubating at 35°C for 48 h. The cell suspension was then centrifuged for 20 min at 17,000 *g* in a Sorvall refrigerated centrifuge. The supernatant was discarded and the cell pellet was resuspended in 20 ml sterile mineral salts buffer which resulted in a cell concentration of approx. 10^9 cfu ml^{-1}.

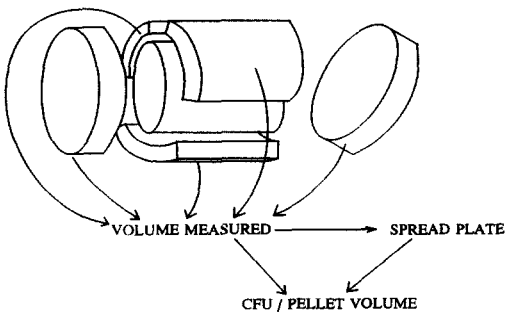

Fig. 3. Pellet sectioning technique: dissection of the pellet with a sterile razor blade such that cylindrical sections were removed and cell colonization determined.

During plug flow operation, reactors were sampled daily for effluent total organic carbon (TOC), effluent glucose and effluent viable cell numbers. At the end of each experiment, 5 pellets from the top, middle and bottom of each reactor were dissected. Concentric shells of each pellet were removed (Fig. 3) and suspended in calibrated test tubes for volume measurement. Homogenization and plating techniques were similar to those used for whole pellets. This technique allowed determination of the spatial distribution of interior cell colonization. Viable plate counts were done in triplicate on both R2A and *Pseudomonas* selective agars. Colony types were distinctive on R2A agar and *Klebsiella* will not grow on *Pseudomonas* selective agar. Plates were incubated at room temperature for 1–2 days.

Competition of Pseudomonas with native microflora

Pilot-scale bioreactors were constructed at Tyndall AFB, Fla, to degrade a mixture of aromatic compounds contaminating groundwater at Kelly Air Force Base, Tex. The packed-bed bioreactors used Manville R-635 D.E. pellets as the substratum for colonization. Columns were operated in a submerged manner and were fitted with sampling ports at the influent and effluent ends enabling removal of individual pellets. The flow system and reactor configuration were patterned after that used by Bouwer and McCarty (1982).

D.E. pellets were loosely packed into the reactors and initially colonized with *Pseudomonas* sp. JS 150, selected from sewage sludge for its capacity to grow on chlorobenzene as its sole carbon and energy source (Spain and Nishino, 1987). The reactor was first filled with a mineral salts medium diluted 1:1 with tap water. The reactor was drained and replaced at 24 h intervals (3 times) to select for attached cells. Chlorobenzene was supplied to the column in the vapor phase during colonization by the addition of chlorobenzene-saturated air to column. After colonization, columns were operated in a continuous manner (no recycle) using groundwater from the contaminated site at Kelly AFB, Tex. The groundwater was mixed with mineral salts buffer in a 1:3 ratio before injection into the column. A more detailed account of the operating conditions in these experiments is presented by Nishino *et al.* (1992)

During reactor operation, pellets were removed from the influent and effluent ends of the reactor at approx. 4 day intervals. Sampled pellets were suspended in mineral salts buffer and mailed overnight to the Center for Biofilm Engineering, Montana State University.

Scanning electron microscopy was performed on several pellets sent from Tyndall Air Force Base to gain qualitative evidence of cell penetration in the pellets (Fig. 1). Pellet samples were sectioned at the center with a sterilized razor blade to expose a radial face. Sections were dehydrated using successively more concentrated ethanol solutions as follows: 30, 50, 70, 90, 100% (each for 10 min). Samples were then critical point dried and gold sputter-coated. Observations were made with a JEOL 100CX (Peabody, Mass.) scanning electron microscope with an ASID-4D scanning attachment.

Cell colonization density within whole pellets was determined by suspending the pellet in 10 ml sterile water in a test tube of known diameter and measuring meniscus displacement with a micromanipulator. Pellet samples were then further diluted with an additional 10 ml sterile distilled water. Blending solution (Camper *et al.*, 1985) was added at 10 μl ml^{-1}, and the slurry was homogenized for 30 s at 20,000 rpm using a Tekmar tissuemizer. The homogenized mixture was then diluted and spread on plates in triplicate on both nutrient (R2A, Difco) and carbon-free, minimal salts (Noble, Difco) agars. The nutrient agar plates were incubated at room temperature for 2 days. The carbon-free agar plates were incubated in a chlorobenezene- and water-saturated atmosphere for 10–14 days. Colonies were counted

after the incubation period and the arithmetic mean of the three observations was used as the colony forming unit (cfu) count. The dilution counted contained between 30 and 300 cfu per plate.

RESULTS

Inoculation sequence experiments

In experiment 1, slower-growing *Pseudomonas* was inoculated into the pellets, and *Klebsiella* was continuously introduced via the addition of chemostat effluent to the reactor. The resulting effect was a decrease of one order of magnitude in *Pseudomonas* numbers in interior pellet sections and one-half order of magnitude in exterior sections. Invading *Klebsiella* colonized the outermost section only marginally less than the inoculated *Pseudomonas* in the 10 day experiment. Interior colonization by *Klebsiella* was significantly less than *Pseudomonas* (Fig. 4). Error bars in all figures represent the 95% confidence interval about the mean. The competitive effects of *Klebsiella* thus caused *Pseudomonas* numbers to drop relative to the unchallenged control.

Experiment 2 was identical to experiment 1, but ran for 21 days. The longer time period resulted in more dense *Klebsiella* colonization than either challenged or unchallenged *Pseudomonas* at all pellet depths. *Klebsiella* not only outcompeted *Pseudomonas* in the same reactor, but also showed more dense colonization than unchallenged *Pseudomonas* (Fig. 5). *Klebsiella* colonization ranged from 3×10^8 cfu ml^{-1} in exterior sections to 10^7 cfu ml^{-1} in the innermost section. *Pseudomonas* response to this competition was to decrease slightly in the 0–1 and 1–2 m sections, but remain stable at the pellet center (2–3 mm) (Fig. 5).

In experiment 3, when *Klebsiella* colonized the reactor and was challenged with *Pseudomonas*, there was very little change in *Klebsiella* cell numbers over the 10 days of the experiment. *Klebsiella* initially colonized all pellet surfaces at 10^7–10^8 cfu ml^{-1} and stayed at this level despite the *Pseudomonas* challenge. Despite continuous inoculation via chemostat effluent, *Pseudomonas* colonization was consistently 2 orders of magnitude below *Klebsiella* at all pellet depths (Fig. 6). In addition, *Pseudomonas* numbers were lower than in the previous two experiments.

Competition of Pseudomonas with native microflora

Two pilot-scale experiments were performed at Tyndall AFB, lasting 10 and 15 days, respectively. Scanning electron micrographs of pellets from the 10 day experiment qualitatively revealed that organisms were penetrating to pellet centers (Fig. 1). Over the 10 day duration of experiment the first pilot-scale experiment (PS1), chlorobenzene-degrading organisms in the pellets dropped from approx. 10^8 to 10^5 cfu ml^{-1} pellet, while numbers of total organisms in the pellets remained constant at 10^9 cfu ml^{-1} (Fig. 7).

In the second pilot-scale experiment (PS2), total viable cell counts within the pellet remained constant at 10^9 cfu ml^{-1} at the effluent end of the reactor. At the reactor influent, total viable cells increased from

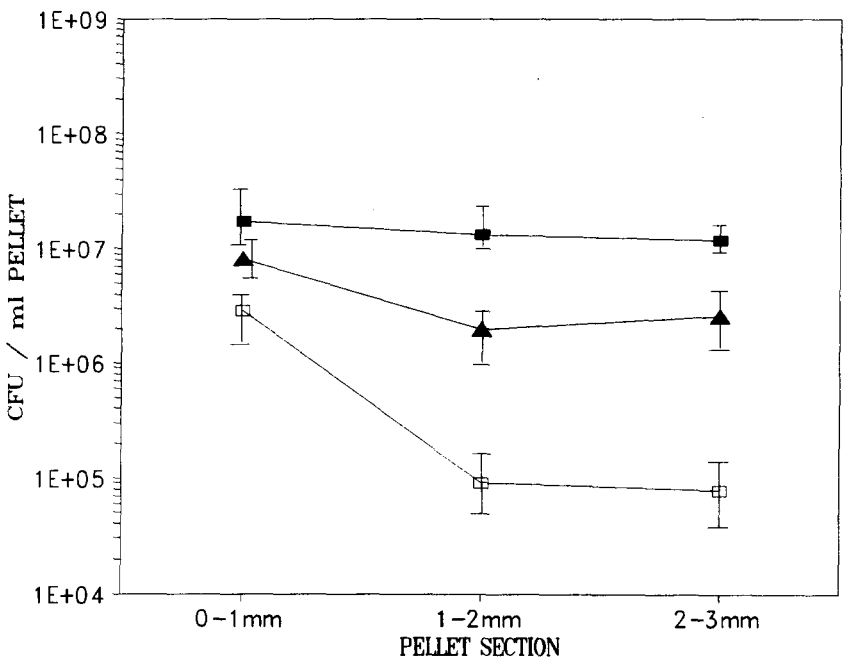

Fig. 4. Spatial distribution of unchallenged *Pseudomonas* (■), challenged *Pseudomonas* (▲) and *Klebsiella* (□) in pellets at day 10 of experiment 1. *Pseudomonas* was inoculated on day 0, then challenged with *Klebsiella* on day 4.

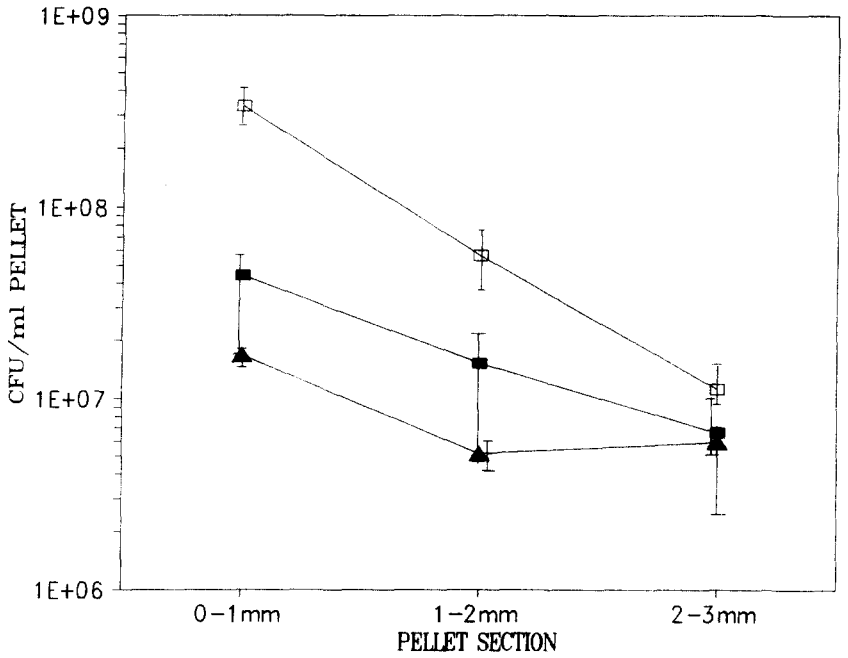

Fig. 5. Spatial distribution of unchallenged *Pseudomonas* (■), challenged *Pseudomonas* (▲) and *Klebsiella* (□) in pellets at day 21 of experiment 2. *Pseudomonas* was inoculated on day 0, then challenged with *Klebsiella* on day 4.

10^9 to 10^{10} cfu ml^{-1} at about day 10. Entire pellet chlorobenzene-degrader counts remained constant at 10^6–10^7 cfu m^{-1} over the duration of the experiment at the reactor effluent, but dropped from approx. 10^6 to 10^5 at the reactor influent on day 3 then recovered again by day 10 (Fig. 8).

DISCUSSION

Inoculation sequence experiments

Experiments 1 and 2 indicate that *Klebsiella*, when continuously fed into a reactor, has the ability to overtake and outcompete the slower-growing, inoculated

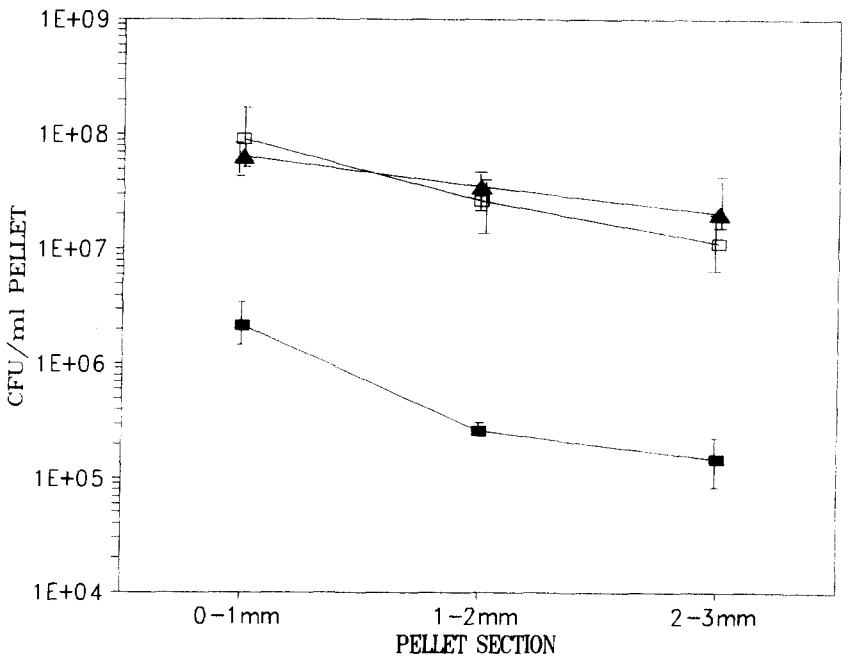

Fig. 6. Spatial distribution of unchallenged *Klebsiella* (▲), challenged *Klebsiella* (□) and *Pseudomonas* (■) in pellets at day 10 of experiment 3. *Klebsiella* was inoculated on day 0, then challenged with *Pseudomonas* on day 4.

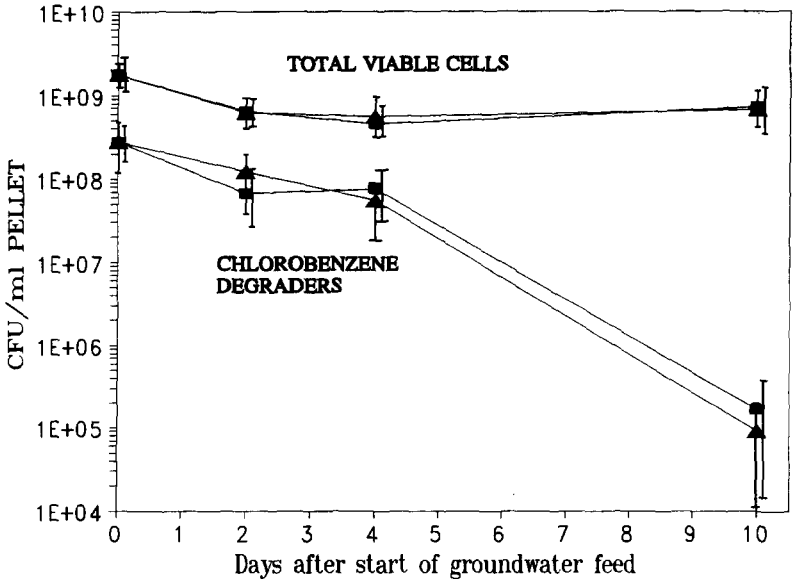

Fig. 7. The progression of pellet colonization in the first pilot-scale experiment for all pellet sections. Total viable cells and chlorobenzene-degraders at the reactor influent (■) and effluent (▲) are shown.

Pseudomonas. The results (Figs 4 and 5) indicate that despite its lack of motility, *Klebsiella* surpassed *Pseudomonas* in numbers between the second and third weeks of exposure in the pellets. Although dominated by *Klebsiella*, *Pseudomonas* numbers did not drop significantly in experiment 2 compared to experiment 1. *Klebsiella* numbers increased steadily, but *Pseudomonas* numbers remained stable at 10^6–10^7 cfu ml^{-1} pellet.

Pellet section (distance from edge) did not seem to have a dramatic effect on *Pseudomonas* survival. In both experiments, there was less than 1 order of magnitude difference in interior section colonization compared to exterior. It appears that *Pseudomonas* cells remain viable in the pellet interior sections, but do not actively reproduce. Motility may facilitate recolonization of pellet interiors by *Pseudomonas* even if the death rate was relatively high. *Klebsiella* apparently grows in the pellet because of its ability to replicate under lower substrate concentrations as evidenced by its lower K_s and higher μ_{max} (Table 1).

Unchallenged *Pseudomonas* numbers increased slightly from the 10 day to the 21 day experiment. Virtually all of this growth occurred in the exterior sections (0–1 mm) while the 1–2 and 2–3 mm sections were almost identical for unchallenged *Pseudomonas*

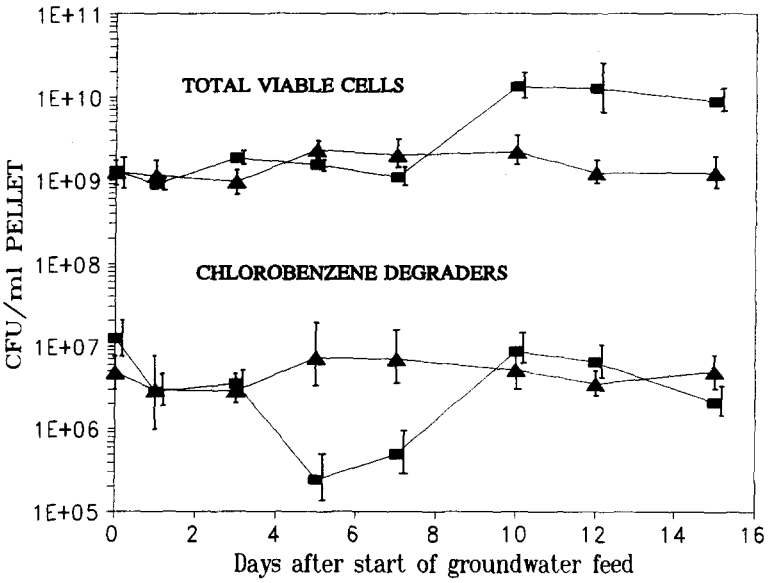

Fig. 8. The progression of pellet colonization in the second pilot-scale experiment. Total viable cells and chlorobenzene-degraders at the reactor influent (■) and effluent (▲) are shown.

in the two experiments (Figs 4 and 5). In pellet interior sections, unchallenged *Pseudomonas* did not grow to a cell density greater than $\sim 10^7$ cfu ml^{-1}. When subject to *Klebsiella* competition, challenged *Pseudomonas* does not drop much below this level either. In the innermost pellet section, invading *Klebsiella* outgrows *Pseudomonas*, but does not displace it.

Experiment 3 (Fig. 6) indicates that *Pseudomonas* is unable to significantly influence colonized *Klebsiella* in a 10 day experiment even though *Pseudomonas* was continuously inoculated. Two comparisons can be made with experiment 1 (*Pseudomonas* vs *Klebsiella*, 10 days). During experiment 1, challenged *Pseudomonas* numbers dropped as compared to unchallenged *Pseudomonas* (Fig. 4). There was virtually no difference between challenged and unchallenged *Klebsiella* during experiment 3 (Fig. 6). Therefore, the *Pseudomonas* challenge did not affect colonized *Klebsiella* compared to the reverse situation. Second, the progression for the *Klebsiella* 10 day invader (Fig. 4) and the *Pseudomonas* 10 day invader (Fig. 6) are very similar. Exterior section colonization is the same at 2×10^6 cfu ml^{-1}; and interior section colonization is slightly greater for *Pseudomonas*, as may be expected due to its motility.

Competition of Pseudomonas with native microflora

In both pilot-scale studies, native organisms were seen to maintain colonization densities near 10^9 cfu ml^{-1}. In the PS2, the increase from 10^9 to 10^{10} cfu ml^{-1} at day 10 could have been caused by an influx of cells from the influent groundwater or by a period of prolific growth brought on by an increase in easily assimilable organic material in the reactor influent. Neither cells nor non-contaminant organics were measured in column influent during these experiments.

Chlorobenzene degrading organisms in the two pilot studies showed different trends; a 1000 fold decrease in PS1 compared to nearly constant numbers in PS2. The transient decrease in chlorobenzene degrading organisms in PS2 observed over days 5–7 at the influent end of the reactor could have resulted from variations in influent concentrations of chlorobenzene or an influx of competing microorganisms. Since neither influent cells nor non-contaminant organics were measured during these experiments, possible reasons for the behavior of the chlorobenzene degraders are conjectural, though greater than 1 mg l^{-1} chlorobenzene was continuously present (Nishino *et al.*, 1992). Data reported in Spain and Nishino (1987) indicate this concentration is adequate for maintenance of *Pseudomonas* sp. JS 150, while chlorobenzene concentrations did not approach the range of inhibition.

Initial colonization was not as effective during PS2, but chlorobenzene degrader numbers did not drop in response to competition from native microbes. A possible reason is the maximum sustainable level of chlorobenzene degraders could be approx. 10^6 cfu ml^{-1} because of substrate or electron acceptor limitations in reactor influent. This would account for the drop in the PS1, and the maintenance of chlorobenzene degraders at this level in PS2. It should be noted that there is some risk of low dissolved oxygen levels due to the carbon-rich nature of the groundwater feed.

It is possible that the inoculated organism (*Pseudomonas* sp. JS150) may account for few or none of the chlorobenzene degraders measured in the latter stages of these experiments. Other research (Nishino *et al.*, 1992) reported similar chlorobenzene removals from both inoculated and uninoculated reactors indicating that there were indigenous chlorobenzene degraders present in this groundwater. The rapid population decrease in PS1 could have been due to the near total loss of the inoculum. The maintenance of chlorobenzene-degraders at a level of 10^6 cfu ml^{-1} after day 4 in PS1 and throughout PS2 would therefore have been due almost entirely to indigenous organisms. In this case, the inoculation in PS2 would have been ineffective; otherwise a higher initial chlorobenzene-degrader population would have been observed.

The results from both the inoculation sequence and pilot-scale experiments suggest two possible scenarios for organism behavior over longer periods: (1) when a faster growing organism continuously challenges a slower growing inoculated organism, the challenger may overtake and outcompete the inoculated organism. While not completely displaced, the inoculated species may persist, possibly in a quiescent state as suggested by Lewis and Gattie (1991), at cell densities several orders of magnitude below the faster growing challenger; (2) when a slower growing organism continuously challenges a faster growing inoculated organism, the disparities in growth rate determine the extent to which the challenger can compete. Where the growth rate differs by a factor of 5 (as in the case of the inoculation sequence experiments), the inoculum will maintain a dominant position.

CONCLUSIONS

Slower growing, inoculated *Pseudomonas aeruginosa* was overtaken, though not displaced, by faster growing *Klebsiella pneumoniae* in bench-scale studies under controlled conditions. Prior colonization within these pellets, while certainly offering some advantage in sustaining inoculated cultures over simple planktonic cell systems, does not indefinitely protect the inoculated species from the competitive effects of faster growing organisms in the influent stream. Motility effects were not as important as organism growth rate despite intra-pellet transport being primarily diffusive in nature. Non-motile *Klebsiella* was not prevented from colonizing pellet interior sections. *Klebsiella* was shown to outcompete slower-growing *Pseudomonas* at all pellet depths, indicating that overall growth rate is more important than either motility or order of introduction in determining organism spatial distribution in the reactor system studied. Likewise, experiments utilizing non-sterile contaminated

groundwater indicated that *Pseudomonas* sp. JS150 was not able to consistently maintain high population numbers when inoculated onto porous diatomaceous earth pellets and challenged with native microflora.

This work demonstrates under highly controlled laboratory conditions and under much less controlled field conditions the difficulty in maintaining a population of slow growing inoculated organisms at their level of inoculation in a competitive environment of faster growing, or continuously inoculated organisms. The implication of this research for the operation of above-ground biotreatment of contaminated groundwater is that if non-native contaminant degraders are utilized, continuous inoculation of these bacteria may be necessary to maintain a population at a level which facilitates contaminant degradation.

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This manuscript is dedicated to the memory of William G. Characklis, founder of the Center for Biofilm Engineering at Montana State University. Dr Characklis imbued all those he contacted with both his technical insight and love of life. He will be missed.

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