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DOE/ER/61887--T1

DOE Final Report

Transport of Subsurface Bacteria in Porous Media
Contract # DE-FG03-94ER61887

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The aims of our project have not changed significantly since the original proposal. Our primary goals were to support field experiments by screening strains of bacteria to find favorable transport characteristics among field isolates and to estimate collision efficiencies for those bacteria in typical Oyster site sediments. The data we obtained were disseminated to other members of the subprogram. For example, Tim Ginn of PNL incorporated our results into his field model; Aaron Mills used our work for comparison purposes; and John Wilson used our results to determine if there is a correlation between facies type and cell adhesion. Copies of all information were also sent to Mary DeFlaun of Envirogen for incorporation into the Sample Tables. In addition to the originally proposed work, we performed longer column studies, examining the effects of aluminum, iron, and water chemistry on bacterial transport, and beginning to understand the role of electrostatic interactions as determinants of biocolloid/collector affinity.

Listed below are the milestones achieved. Brief descriptions are provided for projects done in collaboration with members of the Subprogram. Papers, which have been submitted for peer review, are attached that describe our work in more detail.

1. Transport screening experiments involving bacterial isolates from the Oyster site were performed (Table 1). These experiments were conducted in MARK columns with artificial ground water and Oyster sediments. The organisms exhibited order of magnitude differences in their affinities for Oyster sediments, suggesting that results of the field experiment should be highly dependent on the selection of microorganisms. Note that only average affinity information is provided in Table 1. We continue to find that α, the collector affinity, is a function of depth within the MARK columns. Data for all isolates are not included in this summary, however, all data for each isolate were forwarded to Tim Ginn. These data include average α as a function of sediment depth, and C/C_0 vs. depth, in addition to velocity, pore volume, sediment size distribution, and column dimension data. We worked with sediments from the Oyster site and an artificial ground water that was designed on the basis of Oyster water quality measurements.

2. Using the most promising isolates, PL2W21 and PL2W31, MARK experiments were performed using borosilicate glass beads, quartz, and iron coated quartz for comparison. Artificial ground water was the mobile phase in all cases. As shown in Figure 1, the effective α was highly dependent on the collector material. PL2W31 exhibited less affinity for the sediment than for glass, quartz or iron-coated quartz.

3. We performed longer column experiments to verify the MARK column results and to more effectively compare our data to results obtained at the University of Virginia. These experiments were done in 30 cm with artificial groundwater, sediment, and bacterial strain PL2W31, the strain used for the field injects. Figure 2 shows concentration out of the column or breakthrough, and Figure 3 contains the bacterial

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adhesion information as a function of column depth. Our data was compared with similar studies performed at the University of Virginia. Together we determined the total amount of bacteria that was injected in the field study.

4. In addition, we performed various experiments to determine the transport properties and electrophoretic mobility of PL2W31 as a function of growth media. The experiments were done in MARK columns with Oyster sediment in artificial groundwater. Cells were grown in both acetate and glucose and we found that adhesion of PL2W31 was not a function of growth media. We determined that the electrophoretic mobility of this strain grown in acetate was -0.423± 0.007 μm cm/V s and for cells grown in glucose it was -0.419 ± 0.029 μm cm/V s. In conclusion we determined that growth media was not shown to affect either the affinity of the bacteria for the Oyster sediment or the electrophoretic mobility.

5. The final project we did in collaboration with other members of the Subprogram involved determining how bacterial transport varies with sediment type. We performed about 100 different MARK tests using PL2W31, artificial ground water and various facies samples obtained from the borrow pit. The results of the percent adhesion versus sample number are given in Table 2. These results were forwarded to members of the subprogram for correlation to particle size and iron content information.

6. During the course of the project, we did a significant amount of laboratory experiments that were in direct support of the field work that lead to publications. A list of the projects is as follows, the papers for each project are attached.

a. We demonstrated the advantages of using capillary electrophoresis to determine electrophoretic mobilities of biological particles compared to other methods of determining mobilities. (Paper - Glynn, J.R. et al. "Capillary Electrophoresis Measurements of Electrophoretic Mobility for Colloidal Particles of Biological Interest", *Applied Environmental Microbiology*, under review.)

b. We determined that α is a function of depth within a saturated porous media for monodisperse, monoclonal suspensions of bacteria, and that a biomodal probability density function satisfactorily represents the α-distribution. Furthermore, the form of the distribution function was supported by capillary electrophoresis measurements. (Paper - Baygents, J.B. et al. "Variation of Surface Charge Density in Monoclonal Bacterial Populations: Implications for Transport Through Porous Media", *Environmental Science and Technology*, in press.)

c. We examined the effects of Fe-oxide on bacterial transport through porous media. (Martin et al., "Factors Affecting Bacterial Transport Through Aquifer Material for the Bioremediation of Hazardous Waste" *Proceedings of the 1995 Pacific Basin Conference on Hazardous Waste.* May 17-20 1995.)

d. We examined the effects of Al-oxide on bacterial transport. (Thombre et al., "the Effects of Al-oxide coatings on the retention of bacteria in porous media" In progress.)

7. Numerous oral presentations have been or will be made based on this research. A list follows.

a. J. Glynn, R. Arnold, K. Ogden, and J. Baygents "Electrokinetic Characterization of Monoclonal Bacterial Populations Via Capillary Electrophoresis" AIChE meeting, Miami Beach, FL 11/95.

b. B. Logan, K. Ogden, J. Baygents, Y. Sun, T. Martin, J. Glynn, and R. Arnold "Microbial and Chemical Determinants of Bacterial Mobility in Porous Media" ASM Conference, New Orleans, LA 5/96.
c. T. Martin "Effect of Fe-oxide on Bacterial Transport in Porous Media" Department of Chemical and Environmental Engineering, University of Arizona 3/95.
d. J. Glynn "Electrokinetic Characterization of Monoclonal Bacterial Populations Via Capillary Electrophoresis" Department of Chemical and Environmental Engineering, University of Arizona 3/95.
e. J. Glynn, R. Arnold, K. Ogden, and J. Baygents "Electrokinetic Characterization of Monoclonal Bacterial Populations Via Capillary Electrophoresis" World Congress, San Diego, CA 6/95.

8. This project has been used to fund 1 Ph. D. student, 4 MS students, and 3 undergraduate students.

Table 1. Effective α for Oyster Isolates

Bacterial Isolate	Average α
11A36	1.58
11A51	0.62
E1T2	0.29
E2W1	0.45
F3T3	1.22
PL2W21	0.69
PL2W31	0.05
PL2W32	0.05
PL2W33	*
PL2W34	*
11A-3-8	*
11A-4-3	*
SSB-5-3	*
SSB-5-9	*

Table 2: Descending Fraction Retained

Sample	Fraction Retained
05K104	0.2746
05K112	0.2205
05K102	0.1970
05Q094	0.1731
05K114	0.1713
07P034	0.1642
05K094	0.1626
05Q104	0.1467
07F034	0.1367
05K122	0.1358
04L084	0.1307
06F104	0.1307
05K082	0.1191
04F074	0.1113
05K084	0.1093
05K092	0.1063
05Q114	0.0970
07R014	0.0965
07R044	0.0955
03F044	0.0927
02A044	0.0905
07T074	0.0879
04F104	0.0844
07B034	0.0840
02S084	0.0822
03L044	0.0813
06F074	0.0802
05K034	0.0771
05K074	0.0744
07F104	0.0740
05K022	0.0731
05K062	0.0695
03L014	0.0682
01L044	0.0665
02Q084	0.0650
07F074	0.0637
01F074	0.0615
03L084	0.0605
05K072	0.0601
02K074	0.0594
05K032	0.0573
05i064	0.0554
05K014	0.0550
B5000060-90	0.0528
05O064	0.0524
05K024	0.0522
05K054	0.0520
05Q064	0.0518
06L084	0.0513
02K104	0.0488
05M064	0.0483
02Q044	0.0467
03T084	0.0467
01T084	0.0464
03B044	0.0461

Sample	Fraction Retained
05S064	0.0454
05Q034	0.0451
01L014	0.0443
01F104	0.0439
05K044	0.0438
06B034	0.0438
05U064	0.0437
06F034	0.0431
02K034	0.0422
01F044	0.0417
03F074	0.0414
05Q074	0.0413
07J074	0.0412
04F034	0.0401
05K013	0.0399
06L044	0.0399
05M034	0.0396
06T064	0.0393
06L014	0.0390
05Q084	0.0379
05K052	0.0377
B5000130+05	0.0377
05K042	0.0374
03F104	0.0369
05Q054	0.0365
05G064	0.0362
05C064	0.0357
05K064	0.0351
05Q014	0.0343
01L084	0.0337
05C034	0.0324
05i034	0.0320
05Q044	0.0319
04B034	0.0316
05S034	0.0314
05Q024	0.0308
B5000060-40	0.0306
02Q014	0.0304
04L044	0.0304
05U034	0.0302
B5000010+05	0.0294
05A034	0.0280
04T064	0.0263
05K023	0.0261
07D074	0.0217
01B044	0.0206
05O034	0.0201
B5000060+20	0.0176
05E064	0.0169
05E034	0.0167
04L014	0.0163
05K012	0.0153
05A064	0.0150
05G034	0.0141
07R084	0.0123

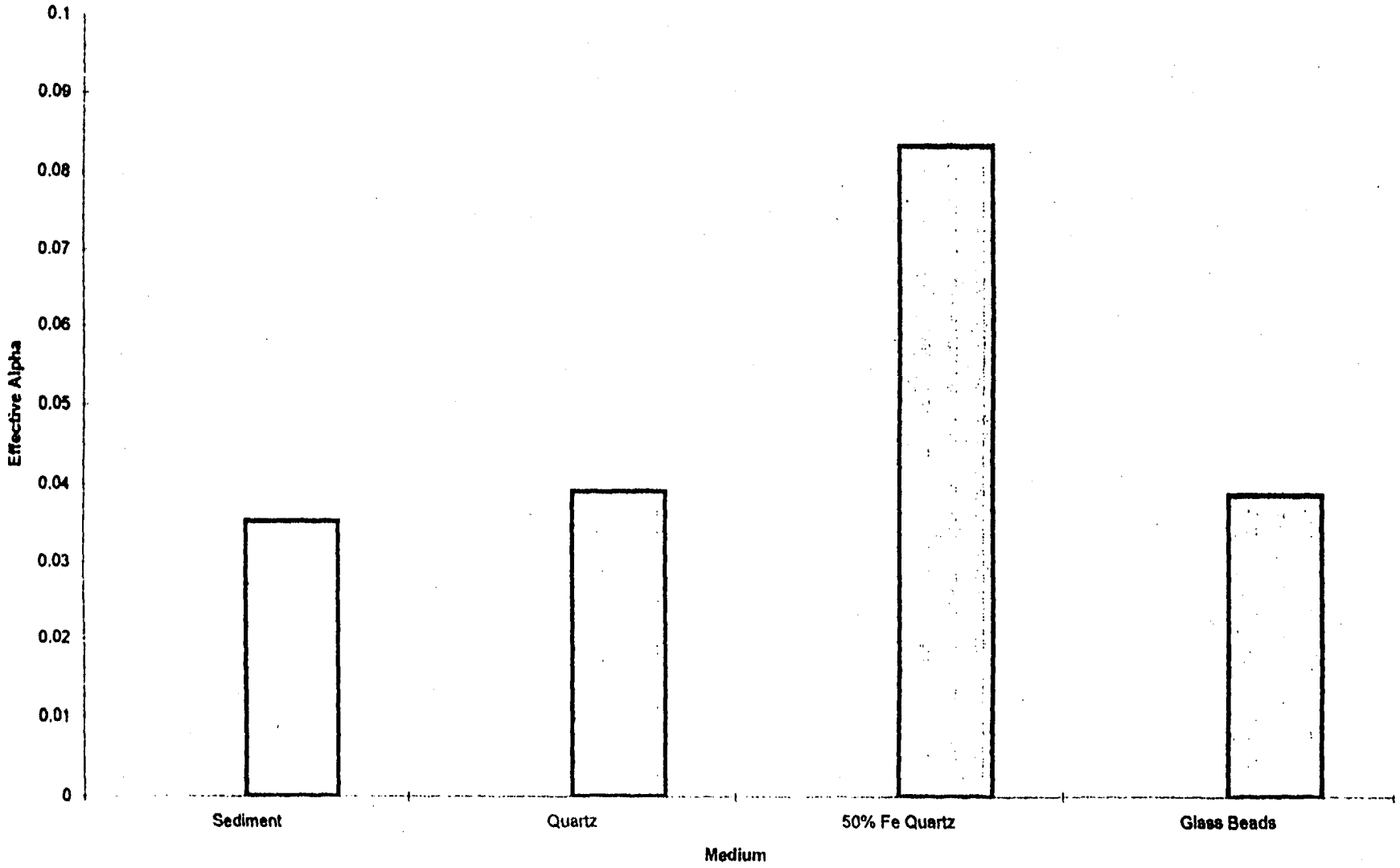

Figure 1. Alpha as a function of collector surface for bacterial strain PL2W31.

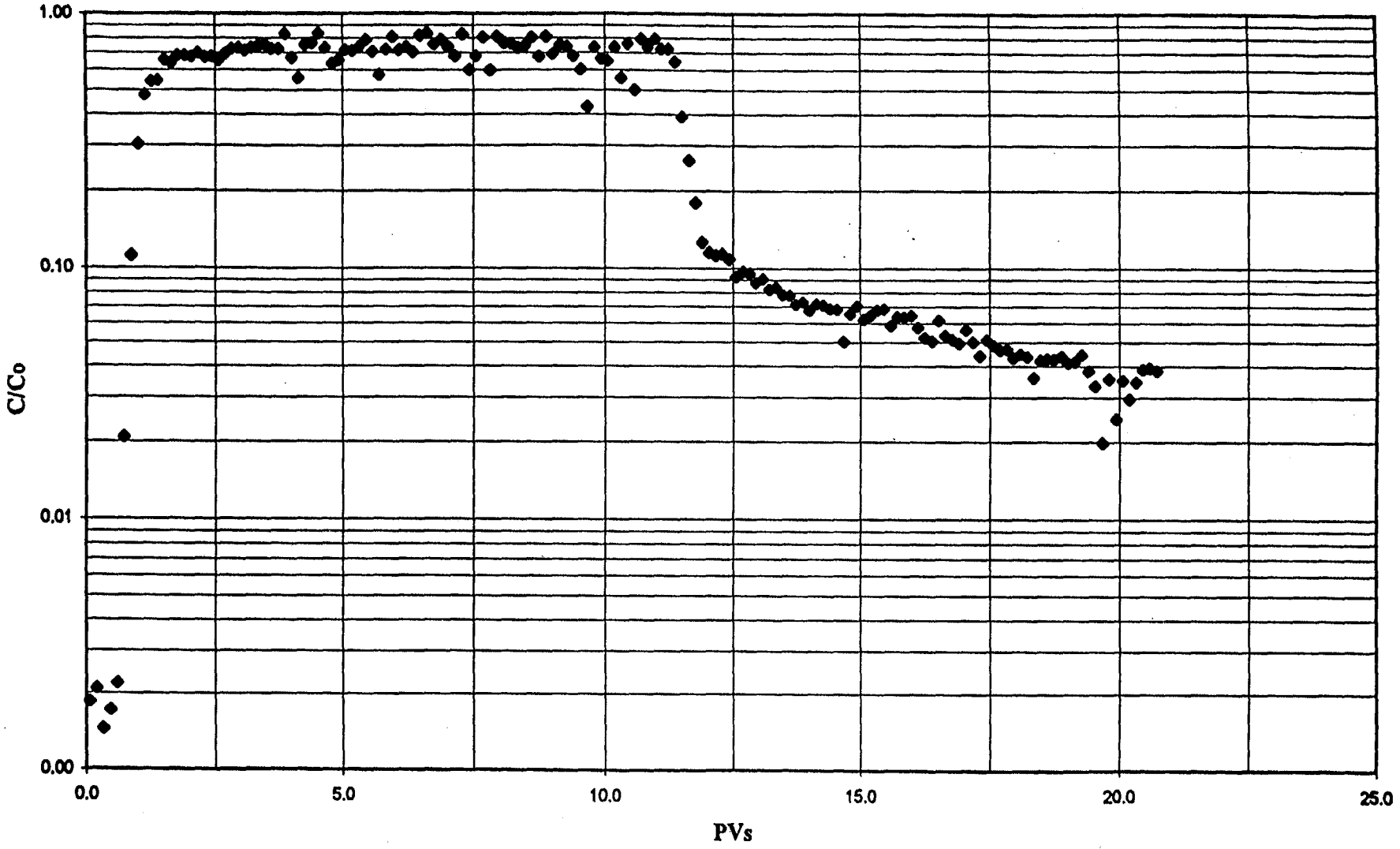

Figure '. Breakthrough curve for PL2W31 in AGW-2. The 30-cm column reactor was packed with washed, repacked Oyster sediment.

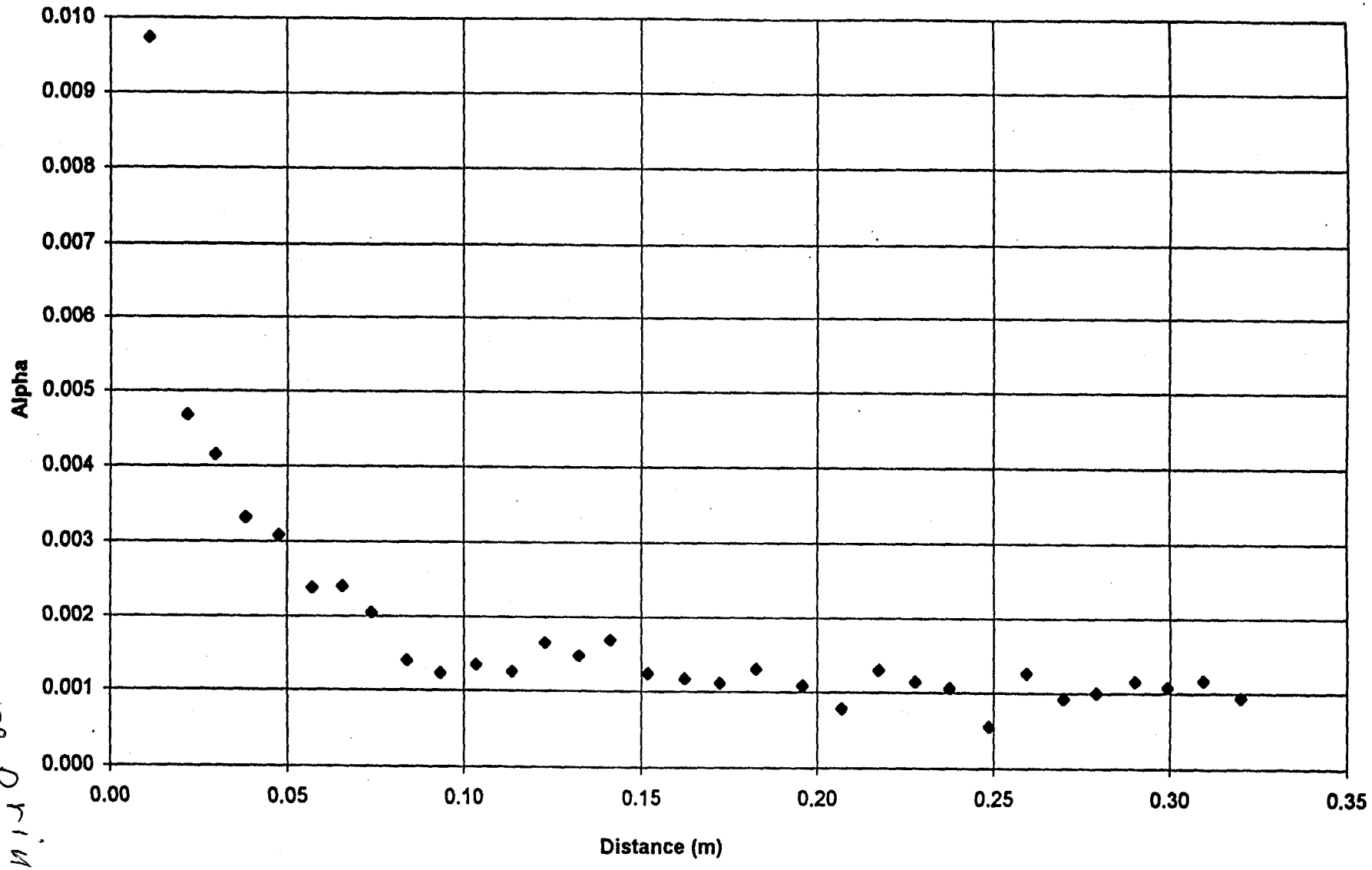

Figure **Depth-dependent calculation of α (collision efficiency) for PL2W31 in a 32-cm column reactor that was packed with washed, homogenized Oyster sediments. The mobile phase was AGW-2.**

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