FLORIDA INTERNATIONAL UNIVERSITY
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DRINKING WATER RESEARCH CENTER

May 26, 1992

Mr. David McGarva, President
Bio-Tech Distribution Inc.
2774 Mesquite Drive
Orange Park, Florida 32065

Dear Mr. McGarva:

A report of microbial analysis of Micro-Solve is attached. Only aerobic culture results are given. Anaerobic cultures were also made, but require longer periods for analysis and these are not yet finished. Activated sludge treatment of sewage is an aerobic process, however, and the results given in this report should answer your initial questions.

As sludge digestion is an anaerobic process, the results of the anaerobic cultures will help you predict the effect of Micro-Solve on sludge treatment. You will receive these results early next week.

Yours very truly,

Frances Parsons, Ph.D.
Associate Professor

FP/mma

Enc.

Analysis of Micro-Solve®

Experiment #1

Determination of the effect of Micro-Solve® on microorganisms associated with sewage treatment.

Aerobic and anaerobic microorganisms obtained from activated sewage sludge were exposed to concentrations of Micro-Solve® in water that encompassed the concentrations found to be effective in field tests. The microorganisms were subjected to Micro-Solve® for a period equal to the longest exposure time expected in practical application of the product. Cultures were made of the water mixture at intervals during the exposure period to determine compatibility of the product and sludge microorganisms. Concentrations of Micro-Solve® in water were 1:5, 1:10, 1:100, 1:200 and 1:1000. Exposure time periods were 0, 24, 48, 72, 96 and 120 hours. Standard Method Agar. Standard Methods for the Examination of Water and Wastewater 1976. APHA Publishers, Washington, D.C. was the culture medium. All reagents, water, materials and methods used equaled the specifications of Standard Methods. Controls included cultures of the initial solutions, pure Micro-Solve®, water and culture medium. Control cultures were considered as unaffected on the basis of comparison with cultures of test solutions. All cultures were made in triplicate.

Results and Discussion:

Plate counts of aerobic cultures made at timed intervals during exposure of activated sludge microorganisms are shown in Table 1.

Undiluted Micro-Solve® and dilutions of Micro-Solve® in water and 1:5 and 1:10 produced a precipitate in the culture medium and prevented reliable interpretation of the observations. No growth was evident as colonies, but it was impossible to determine if microorganisms were present.

Cultures made immediately following addition of activated sludge organisms in all other dilutions of Micro-Solve® had no microbial growth. When compared to the water control cultures, inhibition of activated sludge microorganisms was 90% or greater.

After 24 hours exposure of activated sludge microorganisms to dilutions of Micro-Solve® of 1:100, 1:200, and 1:1000, cultures had growth that exceeded the counting limit of the technique used. These results are reported a “greater than 30,000/mL” (Table1). Tests are in progress to identify microorganisms in these cultures. Preliminary examination indicates that one organism or one group of similar organisms constitute most of the population in these cultures. Two ecological explanations of this phenomenon are possible: 1) the Micro-Solve® enhanced the growth of this organism, which then overtook other organisms present, and 2) Micro-Solve® inhibited other organisms present and allowed the non-susceptible fraction of the population to grow without competition.

The maximum concentration of Micro-Solve® in water that was produced when undiluted Micro-Solve® was added to solid grease cake was calculated to be approximately 1:160. This maximum concentration existed only temporarily as fluid entering the lift station diluted it. The concentration used in experiments reported here were greater and less than that use in the field. Other than a temporary inhibition of total microorganisms present in the activated sludge use of the test organisms, no inhibition of total microbial growth was observed.

The results of aerobic cultures are reported here. Sewage treatment is an aerobic process and should not be affected by the low concentration of Micro-Solve® that would be expected at activated sludge treatment plants. If undiluted, Micro-Solve® was used at individual points on the sewerage system and the dilution rate was sufficient, little effect on sewage treatment would be expected. In the test performed, Micro-Solve® enhanced the development of growth of sewage sludge organisms that dilutions from 1:100 to 1:1000 in water. Apparently Micro-Solve® had no deleterious effect on microorganisms associated with sewage treatment.

TABLE 1

AEROBIC CULTURES OF ACTIVATED SLUDGE IN MICRO-SOLVE, PLATE COUNTS (s.d.) /mL

	Exposure Time, hours					
Dilution, Micro-Solve/Water	Tc[1]	To	T_{24}	T_{48}	T_{72}	T_{96}
1:5	U[2]	U	U	U	U	U
1:10	U	U	U	U	U	U
1:100	0	28(8)	4(1)	20,000	>30,000	>30,000
1:200	0	44(10)	62(14)	>30,000	>30,000	>30,000
1:1000	0	120(23)	>30,000	>30,000	>30,000	>30,000
1:0	U	U	U	U	U	U
0:1	0	1300(150)	1600(70)	1600(200)	760(100)	2300(460)

[1] Uninoculated control (no microorganisms added to solutions)

[2] Unreadable; emulsion formed, no microbial colonies seen

Analysis of Micro-Solve®

Experiment #1, Report No.2

Determination of the effect of Micro-Solve® on microorganisms associated with sewage treatment.

Aerobic cultures:
Plate counts of aerobic cultures made of solutions of Micro-Solve® and water that had been standing at room temperature for 120 hours are given on Table 1*. These results are compatible with those reported earlier. Counts greater than 30,000/mL of the test solutions were obtained for dilution of 1:100, 1:200, and 1:1000 of Micro-Solve® in water. The water control culture developed 1300 colonies/mL. As stated earlier report, microbial growth and solutions of Micro-Solve® was enhanced when compared with the water control.

Anaerobic cultures:
Plate counts of anaerobic cultures made of the same solutions described earlier, of Micro-Solve® in water to which activated sludge had been added, are shown in Table 2. These results are compatible with those obtained from aerobic cultures of the same solutions.

Plate counts that exceeded specifications of conventional methodology are reported in Table 2 as greater than 30,000 and (>30,000); counts that were less than specified as reliable for the plating technique used are reported as less than 30 (<30). Anaerobic cultures made immediately following addition of activated sludge microorganisms in Micro-Solve® dilutions of 1:100 and 1:1000 yielded fewer colonies than the water control. This indicates that some organisms were inhibited immediately. Recovery of microbial populations occurred rapidly as plate counts that exceeded those obtained from water controls were obtained within 24 hours exposure time in the 1:1000 dilution and 72 hours in the 1:100 dilution.

In dilutions of 1:100 and 1:200, anaerobic plate counts exceeded those obtained for the water control after 96 hours exposure. The counts obtained from the 1:1000 diluted to greater than those obtained from the water control after 24 hours exposure of activated sludge microorganisms to Micro-Solve®.

Other than a temporary, initial decrease in plate counts relative to water controls, there is no evidence that Micro-Solve® inhibited development of anaerobic microbial populations in activated sludge. Indeed, Micro-Solve® enhanced the growth of microorganisms in anaerobic culture, as compared with water controls.

TABLE 1*

AEROBIC CULTURES OF ACTIVATED SLUDGE IN MICRO-SOLVE, PLATE COUNTS (s.d.) /mL
(120 Hours Exposure Time)

Dilution, Micro-Solve/Water	Exposure Time (120 hours)
1:5	U**
1:10	U
1:100	>30,000
1:200	>30,000
1:1000	>30,000
1:0	U
0:1	1300(120)

*These values are the final result of the 5 day exposure of aerobic microorganisms to Micro-Solve, Table 1.

**Unreadable emulsion formed, no microbial colonies discernible.

TABLE 2

ANAEROBIC CULTURES OF ACTIVATED SLUDGE IN MICRO-SOLVE, PLATE COUNTS (s.d.) /mL

	Exposure Time, hours						
Dilution, Micro-Solve/Water	Tc[1]	To	T_{24}	T_{48}	T_{72}	T_{96}	T_{120}
1:5	U	U[2]	U	U	U	U	U
1:10	U	U	U	U	U	U	U
1:100	0	440(1)	135(35)	<30	12,000	>30,000	>30,000
1:200	0	<30	<30	<30	370(270)	>30,000	>30,000
1:1000	0	240(75)	>30,000	>30,000	>30,000	>30,000	>30,000
1:0	U	U	U	U	U	U	U
0:1	0	960(58)	1400(350)	1400(173)	1200(220)	930(14)	610(200)

[1] Uninoculated control (no microorganisms added to solutions)

[2] Unreadable; emulsion formed, no microbial colonies discernible

Results of a test to determine the effect of Micro-Solve on standard bacterial cultures are shown in Table 1. Pseudomonas aeruginosa, a common inhabitant of soil and sewage, was chosen to represent aerobic sewage treatment. Escherichia coli, the intestinal commensal of warm-blooded animals, was chosen to represent anaerobes (it is a facultative anaerobe) and indirectly anaerobic sludge digestion. The test was done primarily to determine differences that could be obtained in a standardized laboratory assay, which may be used to evaluate the toxicity of the product, and an assay designed to simulate actual application of the product. The standard bacterial cultures chosen for the test are commercial products commonly used in laboratory quality control procedures, and would logically be cultures of choice in any laboratory assay. Pseudomonas aeruginosa, American type Culture Collection (ATCC). Number 27853, and Escherichia coli, ATCC Number 25922, were originally obtained from a clinical specimen and a soil sample, respectively. Both were obtained from Difco Laboratories, Detroit, MI, as Bactrol disks, a lyopilized commercial product.

Dilution of Micro-Solve in water were made to encompass the concentrations encountered in application of the product. Dilutions of Micro-Solve were made in water alone to maximize contact with the bacteria.

After the specified contact periods, aliquots of the suspensions were removed and pour-plated in Standard Methods Agar to determine numbers of viable organisms. A control suspension was tested concurrently, which consisted of bacteria and water without Micro-Solve.

As shown in Table 1, Micro-Solve in water completely inhibited both bacteria as

compared with water alone. Their results were not surprising as it should be stressed that the experimental conditions were not similar to practice as were the conditions of the earlier experiment where sewage treatment organisms (mixed bacteria) were seen to increase in numbers in the presence of Micro-Solve. Thus we recommend that any evaluation of Micro-Solve, or any similar product, should be based on tests that simulate use rather than on artificially imposed laboratory conditions. Sewage treatment microorganisms are not expected to be exposed to Micro-Solve, or similar products, in simple water solutions. Sewage microorganisms are protected by sewage solids and solutes, which include complex lipids, carbohydrates, and nitrogenous compounds. The increase in numbers of sewage bacteria seen in the previous experiment, which was designed to simulate practice, indicated that conditions favorable for bacteria were enhanced by the presence of Micro-Solve. Increased numbers may have results from increased availability of nutrients.

Another test using the standard laboratory bacteria will be done to determine if sewage sludge solids and grease will protect these bacteria from the effects of Micro-Solve as was apparent when sewage sludge microorganisms were tested.

Results of a test (described in work proposed) to determine the dissolving capability of Micro-Solve for sewage grease are shown in Tables 2 and 3. In Table 2, it can be seen that a 1% solution of Micro-Solve dissolved 85% of the 5 g (wet weight) sample of sewage grease in 24 hours. Lower concentrations were not effective in 24 hrs. After four days contact, from 50% to 90% of the sewage grease was dissolved even at lowest the concentration of 0.05%. The increased dissolution seen over a 5-day period indicates that water was not limiting at 99% of the solution, and approximately 94% relative to the sewage grease on a wet weight basis. Twenty-four hours contact time probably is not practical in application of the product in the field, but then

85% dissolution of grease probably is not required to efficiently remove it from the sewage system. Micro-Solve also could be applied at concentrations greater than 1% directly to the grease cake to facilitate its removal more rapidly. The 1% concentration in this test was used only because that would result from applying the product full-strength to the fluid in a sewage lift station well. Direct application of Micro-Solve to the grease cake, of course, would result in faster, more thorough dissolution.

After five days of exposure to Micro-Solve, the remaining solid sewage grease was recovered from the test solution and measured. These results are shown in Table 3. In the 1% solution of Micro-Solve, the 5 g test sample of sewage grease had virtually disappeared. The grease that remained as solid material was more than 95.5% water (or volatile material) as compared with the original sample, which was 43% water or volatile material. Higher dilutions of Micro-Solve gave less dissolution of grease, but even at 0.1% concentration, 50% of the grease had dissolved. The apparent greater efficiency of the lower concentration, 0.05%, may only indicate that the sewage grease cake was not homogenous and that the individual 5 g sample originally contained more moisture, thus giving the appearance of dissolving to a greater extent.

TABLE 1. Survival of Aerobic and Facultative Anaerobic Bacteria in Solutions of Micro-Solve in Water, Plate Counts/mL recovered.

Dilution MS: Water	Test Organism	Tc	Time Exposure To	T24	T48
1 : 160	P.aeruginosa	0	0	0	0
	E. coli	0	0	0	0
1 : 1000	P.aeruginosa	0	0	0	0
	E. coli	0	0	0	0
	P.aeruginosa				
0 : 1	E. coli	0	800(120)	680(170)	>30,000
		0	570(140)	7700(2700)	>30,000

TABLE 2. Dissolution of Sewage Grease by Micro-Solve.

Estimated % dissolved

Dilution	Time Hours 24	96	120
1 : 100	85%	90	95
1 : 160	ND*	90	90
1 : 200	ND	90	90
1 : 1000	ND	75	75
1 : 2000	ND	50	50
0 : 1(Water Control)	ND	ND	ND

* Not Dissolved

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Introduction:

Tests were performed to determine the biodegradability of Microsolve, a degreasing product design for use where dilute solutions can enter the soil or bodies of water. Static microcosms and dynamic columns of environmental materials (i.e., water and soil) were constructed and injected with know concentrations of Microsolve. After a suitable period, samples of the water phase were removed from the test systems and analyzed by gas chromatography to determine the amounts of Microsolve that remained unchanged.

Methods and Materials:

Static microcosms of a typical ground water environment were constructed in 50ml septum bottles using pond water filtered through Whatman 100 to remove large suspended particles, and glacial till consisting of 35% sand, 34% clay and 31% silt. The soil-water ratio was 15-35 on a volume/volume basis.

Microsolve was added to the microcosms to bring the final concentration to 10mg/L. Controls consisted of Microsolve in sterile distilled water in identical septum bottles.

Dynamic test systems consisted of one-inch internal diameter glass columns eight inches long filled with glacial till and glass beads, 50-50 on a volume/volume basis. Three columns were arranged in parallel, two of which were test columns and one a control column. All were fed by a syringe pump at the rate of 50ml per day. The control column was sterilized and fed sterilized water containing Microsolve. One of the two test columns was kept under ambient atmosphere with the solubility of oxygen in the feed water at approximately 8.4mg/L. The other test column was maintained in an anaerobic state (i.e., no detectable dissolved oxygen) under a nitrogen atmosphere.

Microcosms and columns were foil wrapped to exclude light and prevent photo oxidation. All systems were incubated at 25C for a period of four weeks following analysis to determine time-zero concentrations of Microsolve in the water plane.

Analysis for Microsolve was done using a Hewlett-Packard gas chromatograph equipped with a flame ionization detector and a megabore column. Samples were taken from the water phase of the static microcosms and extracted with pentane prior to injection into the gas chromatograph. The fifty-ml daily effluent volumes from the columns were analyzed in the same way at time-zero and after four weeks operation. All chromatograms were compared with chromatograms of solutions of Microsolve in regent grade water made concurrently.

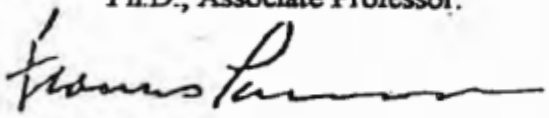
Results and discussion:

After four weeks incubation, 60% of the Microsolve remained in the water phase of the static microcosms; 40% had degraded to compounds not identified with the original added Microsolve.

The aerobic column degraded 82% of the Microsolve that was added daily at 10 mg/L. Eighteen percent of the daily dose could be recognized as unchanged Microsolve. The anaerobic system degraded 21% daily of the added 10 mg/L Microsolve and 79% remained unchanged.

The columns and microcosms represent environmental systems with active microbiota. The columns are dynamic; the microcosms static. Hence, real world situations bear a resemblance to both systems. The microcosms were dosed once with Microsolve and the columns received continual feed water containing a fixed concentration. The results indicate that Microsolve is biodegradable, especially under aerobic conditions.

These tests were performed by Pedro Lorenzo, Chemist, under the supervision of Frances Parsons, Ph.D., Associate Professor.



, Frances Parsons