Presented by





bioremediation

GREASE AND ODOR CONTROL

the bioremediation solution

www.micro-solve.us

I. What is Micro-Solve[®]?

Micro-Solve® is a uniquely formulated blend of ingredients designed specifically to accelerate bacterial growth, and solve many of the problems associated with the handling and treatment of problems that arise when food waste, vegetable fat, animal fat and petroleum wastes are either intentionally or inadvertently dumped into wastewater systems. Micro-Solve® is a positive approach to control fat and grease build up in grease traps, lift stations, wet wells and collection systems.

In all living systems, all waste products are ultimately broken down by bacterial action. In order for the bacteria to work effectively, they must first survive in their environment. Secondly, these bacteria must be present in sufficient numbers to efficiently digest the "food" present. The "food" we are discussing are carbohydrates (both simple and complex), fatty acids, triglycerides and in some instances aliphatic hydrocarbons. Finally, the food must be in a form that is easily assimilated by the bacteria. Logically, the best way to digest this "food" is to use the bacteria already present in the waste stream. These bacteria have already shown an ability to survive in their own natural habitat. In order to digest the tough grease components of a waste stream Micro-Solve® utilizes dual action. First, it conditions the grease to make it readily available as "food" for the bacteria, and secondly, Micro-Solve® accelerates the growth of both aerobic and anaerobic bacteria that may be present. This dual action leads to a quicker biodegradation of the "food" present in the waste stream.

With the addition of Micro-Solve® to wastewater systems, the biological pathways are no longer inhibited due to hardened grease cakes and to the lack of facultative microorganisms and as a result, biological activity, growth, and metabolism are substantially increased. Tremendous benefits in wastewater systems can then be achieved including considerable increases in treatment efficiencies leading to substantial savings in operating costs.



Comments on the Nature and Virtues of



Micro-Solve® is a unique combination of ingredients designed to solve many of the problems associated with the handling and treatment of animal fat and petroleum wastes. It liquefies vegetable fats in grease interceptors, thus making them part of the wastewater flow that can be subjected to treatment by standard methods. Micro-Solve® liquefies the petroleum component of sludges that accumulate in pumps and tank bottoms where grit and tars may complicate handling, thus pumping the wastes for disposal or recovery is made possible.

Micro-Solve® solubilizes solid masses of oils and fats and, in a water medium, aids in the removal of these materials by phase separation; i.e., because oils are less dense than water. Micro-Solve® allows the oily contaminants to rise to the surface where they can be skimmed from the water for further treatment.

Micro-Solve® acts as a liquid-liquid extraction medium applied to solids such as grit or soil; it extracts the oily material from the solid material, where these oily contaminants occur as sediments in tanks or pits of water. Micro-Solve® and the greases that it holds, rise to the surface (phase separation) where removal by skimming is possible. Micro-Solve® is biodegradable under aerobic conditions and is completely compatible with aerobic activated sludge treatment of waste water. Laboratory tests suggest that Micro-Solve® may even enhance treatment in activated sludge systems by increasing availability of nutrients bound in insoluble fat and oil particles. Micro-Solve® brought this about by solubilizing soluble nutrients for use by the bacteria that effect treatment. A ten-fold increase in the number of bacteria found in domestic waste water was caused by the addition of Micro-Solve® to a laboratory model of an activated sludge treatment system. (Note: the oils and fats in domestic sewage, especially the solid particles, would normally; i.e., without Micro-Solve®, be absorbed onto the sludge particles and go to the anaerobic digester where it would either be unchanged or only partly digested. Most or all of it would remain with the sludge and go with it to a landfill or other sludge deposit.) Micro-Solve® should be used according to need. Enough should be applied to do the job at hand. In the case of waste water treatment for discharge of the effluent to natural waters, "enough" is the amount that dilution by the system under treatment keeps the concentration of Micro-Solve® in the discharged effluent to fifteen parts per million. This limit does not apply to sludge, soils, and pit treatment where further treatment of disposal does not involve natural waters.

We value your business and want to be sure you are kept abreast of current product information. For further information, please contact your Bio-Tech representative.

II. What are the Uses of Micro-Solve®?

Micro-Solve® is used in any wastewater application in which there is a problem of grease buildup, high Biochemical Oxygen Demand concentrations, high Total Suspended Solids, high Ammoniacal Nitrogen concentrations, high organic loading or a combination of the above. Micro-Solve® can be added to grease traps, lift stations, wet wells or just down the drain for pipe cleaning.

Micro-Solve® is initially dosed into the wastewater system at a ratio of 1:100 in grease traps, five to ten gallons for a lift station or wet well and a few ounces in drain systems. After one week, Micro-Solve® should be re-dosed at half of the initial dose rate. For optimum performance in grease traps and lift stations, a strong jet of water is used to agitate the mixture and improve aeration. At this point you will begin to see the grease peeling off the walls and solid grease cakes will appear softened. Every seven to ten days, a re-dosing of Micro-Solve® is suggested at 1:1000 in grease traps and 1-3 parts per million in other applications. After a time period of approximately three to six weeks, you should see that the grease problems are diminished.

Clients who are currently using Micro-Solve® are reporting dramatic reductions in grease (see reference letters, many clients), Hydrogen Sulfide (see reference letters, City of Ocala, FL), Ammoniacal Nitrogen (see reference letters, Engineering Science Inc.) and Biochemical Oxygen Demand (see reference letters, Keebler). Other reductions that have been seen are in Total Suspended Solids concentration, Sludge Wasting and Sludge Settling Rates. There also have been reports of the near elimination of the pumping out of grease traps (see reference letters, Keebler). Reports have also been received concerning the elimination of grease stoppages in wastewater collection lines (see reference letters, City of Jacksonville, Fl.).

All of these positive aspects are intensified by the fact that Micro-Solve® is 100% biodegradable, has little effluent toxicity and releases no hazardous organic compounds to the waste stream.



Before Micro-Solve®



After 8 weeks of Micro-Solve®

III. A Letter from the Inventor of Micro-Solve®



Dear Prospective Client,

We at Bio-Tech Industries hope that you will consider our product Micro-Solve® as a potential remedy for you wastewater treatment problems. Micro-Solve® is a product which increases the over all efficiency of natural bacterial actions to reduce levels of grease, Biochemical Oxygen Demand, Total Suspended Solids, Ammoniacal Nitrogen and other organic loading problems.

Additional testing has now been initiated to explore the use of Micro-Solve® as a bioremediation tool. Testing is also currently being undertaken to provide you, the client, with more detailed, up to date results for the reductions of parameters such as Biochemical Oxygen Demand, Total Suspended Solids, Ammoniacal Nitrogen, Total Kjeldahl Nitrogen, Oil & Grease and Total Phosphorus. Future projects will deal with the reduction of hydrogen sulfide in wastewater collection systems and the reduction of crude oil in the environment. The results for both sets of testing, now underway, should be available soon, so if you want a copy of them please call your sales representative at (512) 775-5358.

As a Micro-Solve® customer, should you observe reductions in parameters that we have not mentioned, please let us know so that we can explore these areas. Since we don't know the full potential of the products applications, we will appreciate any observations from our clients.

From grease build up to high concentrations of Biochemical Oxygen Demand, Micro-Solve will help to alleviate your wastewater treatment problems. Thank you for considering Micro-Solve®.

Sincerely,

David McGarva

IV. What is Bacterial Acceleration?

"Bacterial Acceleration" is defined as increasing the total number of bacteria in a system over the system naturally. That is to say that a product when added to a wastewater system increases the total bacterial count over what is seen with nothing added to the system.

Micro-Solve® works as a bacterial accelerant. When Micro-Solve® is added to wastewater, the bacteria grow much faster and multiply quicker than in a natural system. Since all bacteria must eat "food" to survive, these additional bacteria accelerate the biodegradation of human food waste, vegetable and animal fats and oils, and any other biodegradable organic substance in the system. In other words, these bacteria require a carbon source for survival. This carbon source, food, can be any organic substance in the wastewater, therefore, since Biochemical Oxygen Demand, Chemical Oxygen Demand and Oil and Grease are organic in nature, Micro-Solve® can help lower the concentrations of these parameters in the final effluent.

Micro-Solve® was tested by Drinking Water Research Center at Florida International University in Miami. The tests, shown on the next few pages, show that not only does Micro-Solve® accelerate the bacterial growth at use concentrations but, also, does not accelerate the harmful bacteria found in waste systems, such as Escherichia Coli (E. Coli), the bacteria associated with the intestinal commensal of warm blooded animals. This says that all of the "good" carbon eating bacteria are accelerated but the "bad" bacteria, E. Coli is not. As can be seen in the report, Micro-Solve® actually appears to inhibit E. coli in the test solutions.

So what does all of this mean in layman's terms? Micro-Solve® causes the bacteria to grow rapidly then the bacteria destroy human food particles, oils and greases. This reduces the concentrations of such parameters as Biochemical Oxygen Demand, Chemical Oxygen Demand, Total Suspended Solids and Oil and Grease. The bottom line is that with the use of Micro-Solve® as a maintenance additive, you will lower costs by pumping grease traps less, by not having to send maintenance crews out to unclog collection lines, and by lowering surcharges from the local treatment plant.



FLORIDA INTERNATIONAL UNIVERSITY TAMIAMI CAMPUS • MIAMI, FLORIDA 33199 • (308)884-2826

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.

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

		Exposure T	ime, hours		
Tc	То	T ₂₄	T ₄₈	T ₇₂	T ₉₆
U²	U	U	U	U	U
U	U	U	U	U	U
0	28(8)	4(1)	20,000	>30,000	>30,000
0	44(10)	62(14)	>30,000	>30,000	>30,000
0	120(23)	>30,000	>30,000	>30,000	>30,000
U	U	U	U	U	U
0	1300(150)	1600(70)	1600(200)	760(100)	2300(460)
	U ² U 0 0 U	U ² U U U 0 28(8) 0 44(10) 0 120(23) U U	Tc ¹ To T ₂₄ U ² U U U U 0 28(8) 4(1) 0 44(10) 62(14) 0 120(23) >30,000 U U U	U ² U U U U U U U 0 28(8) 4(1) 20,000 0 44(10) 62(14) >30,000 0 120(23) >30,000 >30,000 U U U U	Tc¹ To T24 T48 T72 U² U U U U U U U U U 0 28(8) 4(1) 20,000 >30,000 0 44(10) 62(14) >30,000 >30,000 0 120(23) >30,000 >30,000 >30,000 U U U U U

¹ Uninoculated control (no microorganisms added to solutions)

²Unreadable; 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	Tc¹	То	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
			-				

¹Uninoculated control (no microorganisms added to solutions)

²Unreadable; emulsion formed, no microbial colonies discernible

Report of Studies of Micro-Solve

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 warmblooded 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 pourplated 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.

		-	Time Exposure		
	Test Organism	Тс	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

		Time Hour	's
Dilution	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

Table 3. Sewage Grease Remaining after 120 Hrs. Shaking in Micro-Solve Solutions (5g Wet Weight, Test Sample).

Dilution, in Water		t ining g. . Dry Wt.	Wt.Remaining % of Test	% Solids Of Wet	% Dry Weight Of Test
	wa wi	. Lify WL	Sample	Weight	Sample
			(A)		
	0.25	<0.05	5.0	<0.5	0*
1:100	1.0	0.5	20.0	50.0	17.5
1:160	1.0	0.5	20.0	50.0	17.5
1:200	2.5	1.5	50.0	60.0	52.6
1:1000	2.0	1.0	40.0	57.0	35.0
1:2000	3.5	2.0	70.0	57.0	57.0
0:1			70.0	•	51,15
(Water Control)					

Less than 0.05g remained; virtually the percentage was nil.



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DRINKING WATER RESEARCH CENTER

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

LAW & COMPANY

Consulting and Analytical Chemists

1763 MONTREAL CIRCLE TUCKER, GA.

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Chemical Report

Number: 815871 Received: 10/01/96

5750 Mr. Dave McGarva Bio-Tech 994 Blanding Blvd-Bldg. 118 Orange Park, Fl. 32065

Description: Microsolve

Project Overview

This project was constructed to evaluate Microsolve in its efficiency towards bioremediating animal grease, vegetable grease and hair in an environment similar to a clogged drain pipe.

The project was set up using one gallon glass jars with a mixture of greases and hair as the contamination. The jars were placed on a paddle stirrer and allowed to stir once per day. Microsolve was dosed at the manufactures recommended dosing rate. The test was run in full light.

The samples were allowed to bioremediate for four months. The samples were then sieved with 3/4" and a 1/4" stacked sieves. The grease and hair was weighed then extracted to remove the grease and the residual hair was weighed. The breakdown of the remaining grease and hair was determined.

Project Setup and Initiation

A synthetic grease clog was made from hair, vegetable grease and animal grease. The greases were mixed together and cooked at 360° F for two hours to remove any residual bactericides. The greases used were Crisco for the vegetable grease and lard for the animal grease. Two pounds of each grease was used. Hair was obtained from a local hair cutting establishment. To the grease, 125 grams of the hair was added. This was all mixed well and allowed to cool. Once cool, the mixture was semisolid and could be handled and sampled with ease.

Microsolve was set up to be analyzed in duplicate. A wide mouth clear glass jar was loaded with 50 grams of the hair/grease mixture. To the jar, three liters of dechlorinated water was added. Microsolve was then dosed according to the instructions on the product. A control was setup also in duplicate with 50 grams of the mixture and three liters of water. All jars were placed on a paddle stirred and were allowed to stir for thirty minutes.

The samples were stirred for fifteen minutes daily Monday through Friday. The jars were redosed as per manufacture's directions. When dosing occurred, the samples were allowed to stir for thirty minutes.

The samples were allowed to remediate at room temperature. The room which holds the stirring unit is not heated however, and the temperature of the samples dropped at times down to approximately 45° F.

Results

The hair and grease residue upon the completion of the test is contained in the following table.

ID	Hair/Grease Residue (g)
Control A	48.57
Control B	48.62
Microsolve A	13.98
Microsolve B	13.84

The averages for the duplicates are in the following table.

ID	Average Hair/Grease Residue (g)
Control	48.60
Microsolve	13.91

The percent removal of the hair/grease mixture is as follows.

ID	Percent Removal of Hair/Greas	
Control A	2.86	
Control B	2.76	
Microsolve A	72.04	
Microsolve B	72.32	

The average percent removal for the duplicates is as follows.

ID	Average Percent Removal of Hair/Grease	
Control	2.81	
Microsolve	72.18	

The following table contains the breakdown by sieve size, by hair and by grease:

ID	Residual Grease/Hair (g)	Residual Hair (g)
Control A 3/4"	47.20	3.46
Control A 1/4"	1.37	0.27
Control B 3/4"	46.43	3.59
Control B 1/4"	2.19	0.32
Microsolve A 3/4"	5.47	1.43
Microsolve A 1/4"	8.51	2.15
Microsolve B 3/4"	3.11	1.26
Microsolve B 1/4"	10.73	2.54

Discussion

The results of the bioremediation of vegetable grease, animal grease and hair show that Microsolve shows reduction. The control was reduced only by an average of 2.81% while Microsolve showed an average reduction of 72.18%.

Visual observations were also noted for the test. Both of the Microsolve samples became very turbid early in the testing and the color of the solution changed to a brownish yellow and remained that way through the duration of testing. The control showed little or no change in color or clarity. One would surmise that the turbidity and the color change that occurred in the samples, may be attributable to bacterial activity.

The testing was completed at room temperature. The room the test was run in however is not heated. This allowed the testing vessels to drop in temperature to approximately 40°F at times during the test period. The original length of the project was set at ninety days, but after some of these cooler temperatures were realized, the testing was allowed to continue for thirty days longer. The total length of biremediation of the grease and hair was 120 days.

Conclusions

The breakdown of grease and hair clogs in water drain systems should be accelerated by the use of Microsolve. By showing good reduction in the test, one would expect this information to be valid for a sanitary drain system.

Respectfully Submitted, LAW & COMPANY

By: Thomas & Lanty

June 12, 1997

Mr. Dave McGarva Bio Tech Industries 994 Blanding Boulevard, Suite 118 Orange Park, Fl. 32065

Dear Dave,

After careful review of the Law & Company report concerning the project to evaluate the efficiency of Microsolve towards grease and hair, I have a few further insights into the conclusions. Microsolve definitely appears to reduce levels of grease even at temperatures as low as 45°F. This is an amazing feat since bacterial action decreases as the temperatures decreases. One would surmise then that at higher temperatures, around 60-80°F, Microsolve would promote bacterial destruction of grease at even a faster rate.

The turbidity that was observed in the testing is a sign of bacterial activity. The change in color and turbidity, then, is attributed to the greater bacterial action in the Microsolve tests.

The reduction of grease and hair by an average of 72.18% is very promising. However, if one realizes that the hair did not bioremediate, the reduction calculated from the report results is actually closer to 88.4%. This reduction is the grease removed compared to the original grease added to the system. The hair weights were subtracted out of both the starting material and the final residue weight.

Overall, this report has very promising results. An 88.4% removal of grease is outstanding. You must realize that the major problem in grease traps, lift stations, and wet wells is mainly animal and vegetable oils and greases, the laboratory bioremediation results will be seen in the actual field results from the use of Microsolve.

If you need any further assistance in this matter, please feel free to contact me.

Sincerely,

Larry M. Gwinn, Jr.

V. The Biodegradability and Toxicity of Micro-Solve®

Micro-Solve® is totally and completely non-toxic to waste water systems, when used as directed, whether in grease straps, the collection system or in an activated sludge treatment system. Three sets of testing have been completed that illustrate this point. First, the Drinking Water Research Center of Florida International University performed two tests, biodegradability and chronic/acute toxicity. Law & Company, Consulting and Analytical Chemists, of Tucker, Georgia performed priority pollutant testing utilizing a bench scaled grease trap.

The biodegradability testing, contained on the next few pages, indicate that in an aerobic system, Micro-Solve® is biodegradable in concentrations of up to 10 mg/1. The chronic toxicity testing, contained on the next few pages, indicates that Micro-Solve® at 10 mg/1 and lower should not interfere with reproduction and growth of Fathead Minnows in natural waters and that a 5 mg/1 should not interfere with the growth of water flea, Daphnia Pulex. Of course, one must point out that the concentrations used will never occur in natural waters. The normal dosage rates of Micro-Solve® our applied to waste water stream, between dilution and biodegration, the final concentration in the waste water plant will be near zero, therefore, after treatment at the plant, no Micro-Solve® will be released into natural waters.

As can be seen, Micro-Solve®, when applied according to the manufacturers use instructions, is not toxic to the environment or the local wastewater treatment plant. We, at Bio-tech Distribution, Inc., pride ourselves in making sure that our product is safe and non-toxic.

Bio-Tech Industries

Manufacturers 994 Blanding Blvd., Suite 118 Orange Park, Fl.32065 Phone (904)272-6446 Fax (904)276-9662

Bio-Tech Distribution, Inc. 994 Blanding Blvd. Orange Park, Fl. 32065

1/2/98

Attn: All Sales and Technical Support Personnel

Subject: Toxicity Studies of Micro-Solve

Please find attached reports of the short term method used to determine the toxicity of Micro-Solve to fresh water fish.

Methods used for determination of toxicity were based on those published by the US-EPA, environmental monitoring and support laboratory, Cincinnati, Ohio (short-term methods for estimating the chronic toxicity of effluents and receiving waters to fresh water organisms, edited by W.B. Horning II and Cornelius I. Weber).

Attached reports were performed by Frances Parsons, Ph.D., Associate Professor and Pedro Lorenzo, Chemist, with the Drinking Water Research Center at Florida International University in Miami, Fl., 1-305-348-2826.

Sincerely,

David J. McGarva President LL/DM cc: Mr. J. Zanin

Mr. J. Devane

Short Term Method Used to Determine Toxicity

The method used to determine the toxicity of Micro-Solve® and freshwater fish was based on those published by the U.S. EPA, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio (Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, edited by Cap W. B. Horning, II and Cornelius I. Weber). Wall-eyed eggs were obtained, soon after fertilization from a commercial supply house and place in several concentrations of Micro-Solve® made with filtered, natural, upon water has outlined in Table 1.

Results of the tests are shown in Table 2. The larvae emerged during the second day of observation and were observed for period of six days following complete hatching.

No differences were seen between the test chambers the contained 10 mg/L Micro-Solve® and test chambers the contained clean, filtered, pond water. All eggs hatched and the larva appeared healthy and lively during the period of observation. Forty percent (total of 24 eggs) hatched in the test chambers the contained 100 mg/L of the test material in pond water. All 24 died within one hour of hatching. The remaining unhatched eggs kept their fresh appearance for two days, then became opaque and disintegrated over the following two days. Higher concentrations prevented hatching.

These results indicate the concentrations of 10 mg/L and lower should not interfere with reproduction and growth of fathead minnows in natural waters. In natural surface waters, volatilization, photolysis, and absorption can be expected to moderate the effect of low concentrations of such materials on the biota. Biodegradation can be expected to lower the concentration that would adversely affect higher life forms.

TABLE 1

SUMMARY OF TEST CONDITIONS FOR FATHEAD MINNOW EMBRYO-LARVAL SURVIVAL IN MICRO-SOLVE (WORKING TITLE)

Test Organism:

Fathead minnow (Pimephales promelas)

Test type:

Static

Duration:

Eight days (two days pre-hatch, six days post-hatch).

Temperature:

25c (+/- 2c)

Light:

Laboratory lighting; 16 h light, 8 h dark

Test Chamber Size:

250 mL

Test Solution Vol.:

100 mL

Test Concentrations:

10-, 100-, 1000-, 10,000-mg/L Micro-Solve and clean water

control.

Replicate Tests per

Concentration:

4

Organisms/Test Chamber:

15 eggs

Aeration:

Intermittent, to maintain DO at 50% saturation.

Dilution Water:

Filtered, natural, pond water. Ph, 7.2; total hardness,

250 (as carbonate); TOC, 16 mg/L.

Effects Observed:

Hatch and survival (for six days) of larvae.

TABLE 2

HATCH OF FATHEAD MINNOW EGGS AND SURVIVAL OF LARVAE EXPOSED FOR EIGHT DAYS TO MICRO-SOLVE (WORKING NAME)

Test Concentration mg/L	% Hatched	% Hatched		
10	100		100	
100	40		0*	
1,000	0		0	
10,000	0		0	
Control	100		100	

^{*} Died within one hour of hatching.

TABLE 3

RESULTS OF ACUTE TOXICITY TEST OF MICRO-SOLVE
ON
DAPHNIA PULEX AND PIMEPHALES PROMELAS

Concentration Micro-Solve, mg/L

Percent survived after 48 hours

Daphnia pulex Pimephales promelas 1.0 100 100 2.5 100 100			
			Pimephales promelas
2.5 100 100	1.0	100	100
	2.5	100	100
5.0 100 90	5.0	100	90
7.5 50 50	7.5	50	50
8.0 25 5	8.0	25	5
9.0 5	9.0	5	5
10.0 0	10.0	0	0

All individuals in control vessels remained viable for the test period and beyond.

Biodegradability Report

INTRODUCTION:

Tests were performed to determine the biodegradability of Micro-Solve®, a degreasing product designed 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 known concentrations of Micro-Solve®. 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 Micro-Solve® that remained unchanged.

METHODS AND MATERIALS:

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

Micro-Solve® was added to the microcosms to bring the final concentration to 10mg/L. Controls consisted of Micro-Solve® 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 Micro-Solve®. 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 Micro-Solve74 in the water plane.

Analysis for Micro-Solve® 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 form 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 Micro-Solve® in regent grade water made concurrently.

RESULTS AND DISCUSSION:

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

The aerobic column degraded 82% of the Micro-Solve® that was added daily at 10 mg/L. Eighteen percent of the daily dose could be recognized as unchanged Micro-Solve®. The anaerobic system degraded 21% daily of the added 10 mg/L Micro-Solve 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 Micro-Solve® and the columns received continual feed water containing a fixed concentration. The results indicate that Micro-Solve® is biodegradable, especially under aerobic conditions.

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

LAW & COMPANY

Consulting and Analytical Chemists

1763 MONTREAL CIRCLE TUCKER, GA. PHONE: 404-934-8200 FAX: 404-270-1700

Chemical Report

5750 Number 930532

Bio-Tech Industries 994 Blanding Blvd. Orange Park, Fl. 32065

Description: Effluent from Laboratory Scale Grease Trap

A laboratory scale grease trap was constructed. The product, Microsolve, was introduced into the system at its normal working dilution of 1 part Microsolve to 1000 parts water. Deionized water flowed throughout the grease trap at a rate equivalent to 3000 gallons per day in a 6 foot by 6 foot grease trap. The effluent from this lab scale grease trap was sampled every 15 minutes for 6 hours. This effluent was composited and analyzed.

pH (EPA 150.1)	5.8	mg/1
Biochemical Oxygen Demand (BOD) (5-day) (EPA 405.1)	<5	mg/1
Chemical Oxygen Demand (COD) (EPA 410.4)	<5	mg/1
Oil and Grease (EPA 413.1)	<5	mg/1
Total Suspended Solids (EPA 160.2)	<5	mg/1
Total Kjeldahl Nitrogen (N) (EPA 351.2)	0.86	mg/1
Total Cyanide (CN) (EPA 335.2)	<0.005	mg/1
Total Phenols (EPA 420.1)	<0.1	mg/1
	• • • • • • • • • • • • • • • • • • • •	
Antimony (Sb) (EPA 200.7)	< 0.05	mg/1
Arsenic (As))EPA 200.7)	<0.02	mg/1
Beryllium (be) (EPA 200.7)	< 0.005	mg/1
Barium (Ba) (EPA 200.7)	<0.05	mg/1
Cadmium (Cd) (EPA 200.7)	< 0.005	mg/1
Chromium (Cr) (EPA 200.7)	<0.02	mg/1
Hexavalent Chromium (Cr+6) (SM 3500D)	<0.04	mg/1
Copper (Cu) (EPA 200.7)	<0.02	mg/1
Lead (Pb) (EPA 200.7)	<0.02	•
	0.02	mg/1
Mercury (Hg) (EPA 245.1)	<0.002	mg/1
Nickel (Ni) (EPA 200.7)	<0.002	mg/1
Phosphorus (total as P) (EPA 200.7)	<0.02	-
Selenium (Se) (EPA 200.7)	<0.11	mg/1
Silver (Ag) (EPA 200.7)		mg/1
Thallium (TI) (EPA 200.7)	<0.01	mg/1
Zinc (Zn) (EPA 200.7)	<0.02	mg/1
210 (21) (C 7 200.1)	0.04	mg/1

Detection Limit Result

Volatiles (SpB) (EPA 601, 602)

Benzene	2	ND
Bromoform	2	ND
Carbon Tetrachloride	2	ND
Chlorobenzene.	2	ΝD
Chlorodibromomethane	2	ND
Chlorpethane	2	ND
2-Chloroethylvinyl ether	2	ND
Chioroform	2	ND
Dichlorobromomethane	2	ND
1, 1-dichloroetnane	2	ND
1, 2-dichloroethane	2	ND
1, 1-dichloroethylene	2	ND
1, 2-dichloropropane	2	ND
1, 2-dichioropropylene	2	ND
Ethylpenzene	2	ND
Methyl Bromide	2	ND
Methyl Chloride	2	ND
Methylene Chlonde	2	ND
1, 1, 2, 2-tetrachloroethane	2	ND
Tetrachloroethylene	2	ND
Toluene	2	5.3
1, 2-trans-dichioroethylene	2	ND
1, 1, 1-trichlorgethane	2	ND
1, 1, 2-trichioroetnane	2	ND
Trichioroethylene	2	ND
Vinyi Choride	2	ND
Xylenes	2	6.8
Votatiles (ppB) (EPA 624)		
Acrolein.	50	ND
Acrylonitrile	50	ND

Page 3 930532

Base/Neutral (ppB) (EPA 625)

Acenaphthylene 10 ND Anthracene 10 ND Benzidine 50 ND Benzo (a) anthracene 10 ND Benzo (a) pyrene 10 ND Benzo (a) pyrene 10 ND Benzo (ghi) perylene 10 ND Benzo (ghi) perylene 10 ND Benzo (k) fluoranthene 10 ND Bis (2-chloroscopropyl) ether 10 ND Butylisenzyl phthalate 10 ND 2-chloronaphthalene 10 ND 2-chlorophenyl phenyl ether 10 ND Chrysene 10 ND Dibenzo (a, h) anthracene 10 ND 1, 2 - dichlorobenzene 10 ND 1, 2 - dichlorobenzene 10 ND 1, 3 - dichlorobenzene 10 ND <th>Acenaphthene</th> <th>10</th> <th>ND</th>	Acenaphthene	10	ND
Benzicine 50 ND	Acenaphthylene	10	- 1-
Benzo (a) anthracene 10 ND Benzo (a) pyrene 10 ND 3, 4 -benzofiuoranthene 10 ND Benzo (ghi) peryiene 10 ND Benzo (k) fluoranthene 10 ND Bis (2-chloroetroxy) methane 10 ND Bis (2-chloroetroxy) ether 10 ND Bis (2-chloroetroxy) phthalate 10 ND Bis (2-chloroetroxy) phthalate 10 ND 4-bromophenyl phenyl ether 10 ND 4-bromophenyl phenyl ether 10 ND 2-chlorophenyl phenyl ether 10 ND Chrysene 10 ND Diberzo (a, h) anthracene 10 ND 1, 2, dichlorobenzene 10 ND 1, 3-dichlorobenzene 10 ND 3, 3-dichlorobenzene 10 ND Dietryl phthalate 10 ND Dietryl phthalate 10 ND Di-n-butyl phthalate 10 ND Di-n-butyl phthalate 10 ND Di-n-butyl phthalate 10 ND	Anthracene	10	-
Benzo (a) pyrene 10 ND 3, 4 -benzofiuoranthene 10 ND Benzo (ghi) perylene 10 ND Benzo (k) fluoranthene 10 ND Bis (2-chloroetroxy) methane 10 ND Bis (2-chloroetroxy) methane 10 ND Bis (2-chloroetroxy) ether 10 ND Bis (2-chloroexpropyr) ether 10 ND Bis (2-chloroexpropyr) ether 10 ND Bis (2-chloroexplexyl) phthalate 10 ND Bis (2-chloroexplexyl) phthalate 10 ND Bis (2-chloroexplexyl) phthalate 10 ND Chlorophenyl phenyl ether 10 ND Chlorophenyl phenyl ether 10 ND Chlorophenyl phenyl ether 10 ND Chrysene 10 ND Diberzo (a, h) anthracene 10 ND 1, 2, dichlorobenzene 10 ND 1, 3, 3-dichlorobenzene 10 ND 1, 3, 3-dichlorobenzene 10 ND	Benzidine	50	
3, 4 - benzofiuoranthene 10 ND	Benzo (a) anthracene	10	ND
Benzo (ghi) perylene	Benzo (a) pyrene	10	ND
Benzo (k) fluoranthene.	3, 4 -benzofiuoranthene.	10	ND
Benzo (k) fluoranthene.	Benzo (ghi) perylene	10	ND
Bis (2-chloroetryl) ether		10	ND
Bis (2-chiorosopropyf) ether 10	Bis (2-chloroethoxy) methane	10	ND
Bis (2-chioroscopropyl) ether 10 ND Bis (2-ethylhexyl) phthalate 10 ND 4-bromopnenyl phenyl ether 10 ND Butylbenzyl phthalate 10 ND 2-chlorophenyl phenyl ether 10 ND 4-chlorophenyl phenyl ether 10 ND Chrysene 10 ND Dibenzo (a, h) anthracene 10 ND 1, 2, dichlorobenzene 10 ND 1, 3 -dichlorobenzene 10 ND 1, 4 -denlorobenzene 10 ND 3, 3 -dichlorobenzidine 20 ND Dietryl phthalate 10 ND Dimethyl phthalate 10 ND Di -n-butyl phthalate 10 ND 2, 4 -dinitrotoluene 10 ND Di -n - octyl phthalate 10 ND Di -n - octyl phthalate 10 ND Di -n - octyl phthalate 10 ND	Bis (2-chloroethyl) ether	10	ND
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3, 3 - dichlorobenzidine 20 ND Dietryl phthalate 10 ND Di -n-butyl phthalate 10 ND 2, 4 - dinitrotoluene 10 ND 2, 6 - dinitrotoluene 10 ND Di - n - octyl phthalate 10 ND	1, 4 -dichlorobanzena	10	ND.
Dietr-yl phthalate 10 ND Dimethy: phthalate 10 ND Di -n-butyl phthalate 10 ND 2, 4 -dinitrotoluene 10 ND 2, 6 -dinitrotoluene 10 ND Di - n - octyl phthalate 10 ND		20	ND
Dimethyl pothalate		10	ND
Di -n-butyl phthalate 10 ND 2, 4 -dinitrotoluene 10 ND 2, 6 -dinitrotoluene 10 ND Di - n - octyl phthalate 10 ND	• •	10	ND
2, 6 -dinitrotoluene		10	
2, 6 -dinitrotoluene 10 ND Di - n - octyl phtnalate 10 ND	2, 4 -dinitrotoluene	10	ND
01-11-0c171 p.1210100	2, 6 -dinitrotoluene	10	ND
		10	ND
		50	ND

Base/Neutral (ppB) (EPA 625)

Fiurorambene	10	ND
Fluorene	10	ND
Hexachiorobenzene	10	ND
Hexachlorobutadiene	10	ND
Hexachlorocyclopentadiene	10	ND
Hexachioroethane	10	ND
Indeno (1, 2, 3 - cd) pyrene	10	ND
sophorone	10	ND
Naphthalene	10	ND
Nitrobenzene	10	ND
N - nrrosodimethylamine	10	ND
N - nitrosodi - n - propylamine	10	ND
N - nitrosodiphenylamine	10	ND
Phenanthrene	10	ND
Pyrene	10	ND
1, 2, 4 - trichlorobenzene	10	ND
Acid Compounds (ppB) (EPA 625)		
2 - chlorophenol	20	ND
2, 4 -dichlorophenol	10	ND
2, 4 -dimetnyiphenol	10	NE
4, 6 - dinitro - o - cresol	10	ND
2, 4 - dinitropnenot	50	NE
2 - nitropnenol	50	NE
4 - nitrophenol	10	ND
D - CNIOTO - m - Cresol.	50	ND
Pentachloropnenol	50	ND
Phenol	10	ND
2 A 6 - trichlorophenol	10	NC

	Pesticides	(ppB) (EPA	625) Detection Limit	Result
Aldrin			0.10	ND
Alpha-BHC			0.10	ИD
				ИD
			0.10	ИD
				ИD
Chlordane				ND
4,4'-DDT				ND
4,4'-DDE				ND
4,4'-DDD				ND
Dieldrin				ND
Alpha-endosulfan				ND
Beta-endosulfan .				ND
Endosulfan sulfat				ND
Endrin				ИD
Endrin aldehyde .				ND.
Heptachlor				ИÐ
Heptachlor epoxid				ИD
Toxaphene				ND
Methoxychlor				ир
PCB-1242	<i>.</i>		1.0	ИD
PCB-1254			1.0	ND
	<i></i>		1.0	ND
PCB-1232			1.0	ND
PCB-1248			1.0	1!D
PCB-1260			1.0	ND
PCB-1016		• • • • • • • • • •	1.0	ND
	Herbicides		<u>(ppB)</u> tection Limit	Result
2,4-D				ND ND
2,4,5-TP (silvex)		• • • • • • • • • • • • • • • • • • • •		ND
ND = None Detecte				
	***		11	
		LAW & CO	ully submitted, MPANY	,
		ву П	Comes E. J	anty
5 140000eh				

Samples are retained for a period of thirty to sixty days after completion of testing. After that time, samples are disposed of in an environmentally sound manner unless other arrangements are made by the client.

Bio-Tech Research and Product Development

Environmental Consulting Services State of Georgia Certified Analysts Licenses #012526

994 Blanding Blvd., Suite 118 Orange Park, FL 32065 Phone (904) 272-6446 Fax (904) 276-9662

Initial Forty Five Day Report

Remediation of Petroleum Contaminated Soil Utilizing Micro-Solve® Project Overview

On December 10, 1996, natural soil from Jacksonville was contaminated with 9.1% Aviation Jet Fuel in the laboratory. The soil was spit into five equal clear glass containers about 1.5 pounds of contaminate soil each. To all five containers, 13-13-13 fertilizer was added at commercial dosing rates. The soils were wet down with water. The first container was marked control. Nothing further was added to the control. The next four containers were marked samples 1 through 4. Sample 1 was dosed with Micro-Solve® at 1:500. Sample 2 was dosed with Micro-Solve® at 1:1000. No additional bacterial source was added to Samples 1 or 2. Sample 3 was dosed with Micro-Solve® at 1:500 Micro-Solve® to soil and dosed with a natural aerobic bacterial source to raise the initial bacterial count. Sample 4 was dosed with Micro-Solve® at 1:1000 Micro-Solve® to soil and dosed with the same amount of natural aerobic bacteria as Sample 3. All container were then mixed well making sure not to contaminate the contents. The containers were placed in a room where the ambient temperature remained approximately 70 degree F throughout the testing. Additional water was added everyday to keep the soils moist. The containers were stirred weekly. The containers were allowed to remediate for 45 days. Observations were made and Sample 2, the sample that looked to have the least remediation, was taken for analysis.

Observations at 45 Days

At 45 days into the testing period, the sample containers were observed.

- **Control** the control showed no visible change in the sample container. There was the same marbling of water and petroleum that was visible at test initiation. The jar still smelled of Aviation Jet Fuel.
- **Sample 1** Sample 1 showed a much cleaner soil with the marbling effect almost gone. The water was a dark brown with a black petroleum layer on top. The smell of the jar was slight petroleum with a mild odor of anaerobic activity.

- Sample 2: Sample 2 showed a little less change than Sample 1. The marbling
 was somewhat less than Sample 1. The water was blackish with a large layer of
 black petroleum material on top. The smell was of a Jet Fuel type. Of the four
 samples, Sample 2 showed the least change.
- Sample 3: Sample 3 showed the marbling effect in the soil to be gone. The
 water was a light brown with almost no black petroleum on the top. The odor of
 the container was that of anaerobic activity. Of the four samples, Sample 3 and 4
 showed the most change.
- Sample 4: Sample 4 showed the marbling effect in the soil to be gone. The water was a light brown with almost no black petroleum on the top. The odor of the container was that of anaerobic activity. Of the four samples 3 and 4 showed the most change.

It was decided to analyze Sample 2 since that container showed the least change. The other containers were allowed to continue.

Results for Sample 2

Sample 2 was analyzed 45 days (January 25, 1997) from the initiation of the test period. All free liquids were poured off and the soil was to be analyzed for Total Petroleum Hydrocarbons by EPA Method 418.1 from the EPA Manual "Analysis of Waters and Wastewaters" EPA 600/4/79-020. The soil was determined to contain 4.34% TPH. The black layer on top of the water was analyzed by Infrared Spectroscopy and found to be chiefly aliphatic hydrocarbons. Sample 2, with Micro-Solve® at 1:1000 Micro-Solve® to water, showed 52.3% reduction of petroleum.

Sample 2, even though it showed the least visual change, was chosen to be analyzed. The theory behind this choice was that if Sample 2 showed significant change the other samples would be of much greater reduction and could continue in the testing. While Sample 2 shows 52.3% reduction, one must realize that the other samples have most likely shown greater reduction. Sampling will again be done in early March, and again, the sample with the least visual remediation will be chosen. The test should be complete 30-45 days after that when the best two samples and the control will be analyzed and the final results will be available.

Day 45 Conclusions

Micro-Solve® is apparently working to reduce the Total Petroleum Hydrocarbon contamination in soil. To this point in the test, one is very excited about the full potential of the remediation technology. Even though Micro-Solve® contains hydrocarbons, the reduction in the soil is evident. If the test continues with similar reduction rates, one could theorize a total cleanup in the 90 to 120 days.

Respectfully Submitted,

Larry M. Gwinn, Jr.

December 14, 1996

Larry M. Gwinn, Jr. 1706 Cumberland Court Smyrna, Ga. 30080

Mr. David McGarva Bio- Tech Distribution, Inc. 994 Blanding, Blvd. Suite 118 Orange Park, Fl. 32065

Dear Dave,

The first thirty days of the petroleum bioremediation testing with Micro-Solve is complete. I do, however, have concerns with the experimental conditions that occurred. As you know, the test condition in which we had trouble was temperature. The testing was attempted outdoors in order to show the most real world conditions possible. At the end of the third week, however, the samples froze due to the plummeting temperatures experienced in Georgia during that time period. Micro-solve showed what I believe to be encouraging results even though the conditions for the test deteriorated at the end of the third week. These results have to be listed as preliminary due to the freezing of the samples.

The contaminated soil samples were made by taking regular Georgia top soil and spiking it with approximately ten percent aviation jet fuel. Aviation jet fuel is also known as Kl kerosene. Fertilizer (13,13,13) was added to the soils at normal agricultural dosing rates. Micro-Solve® was added to the samples at a 1:500 ratio Micro-Solve® to soil. The samples were then wetted down with a spray mist of water. The resultant mixture was stirred and allowed to set outside under cover for the testing period. Lids were set on top of the containers so as to allow the test material to breath, but to avoid moisture loss.

The drop in temperature to approximately 20 degrees F occurred at the end of the third week. The containers of test material remained in the cold temperatures for two more days in a freeze thaw cycle. The last 4 days of testing had temperatures of above freezing.

Samples of the test mixture and control were taken at the end of the fourth week. They were analyzed for Total Petroleum Hydrocarbons by EPA Method 418.1 Modified for Soils. This is a quick and fairly accurate determination of petroleum contamination. The control showed a final concentration 9.6% on a wet basis. The Micro-Solve® treated soil showed a final concentration of 7.0% on a wet basis. Moisture levels were not determined due to the fact that the control and sample were kept at the same moisture level.

The above results indicate a 27.1 % decrease in the hydrocarbon contamination over the control. This appears to be quite impressive since the fourth week of bioremediation was

effectively wiped put. When the samples froze, one would expect a major kill of the bacteria in the system. Also, one would realize that the fourth week would have the highest rate of biodegradation due to the multiplication of bacteria. You would expect the bacteria to increase in total number week by week, and hence the fourth week the best. Since we lost the fourth week due to the major kill of bacteria, you could expect that the resultant bioremediation for a full four week test to be much higher in reduction.

I hope this letter addresses all of your concerns with the testing. If you have any questions, or comments, please feel free to call.

Sincerely,

Larry M. Gwinn, Jr.

Environmental Chemist



CM Services of O.P., Inc.

P.O. BOX 65756 3035 BRAVO COURT Orange Park, FL 32065-5756 (V) 904-276-9012 (F) 904-272-7751

E-mail: CMT@cmtchemmaster.com WWW: www.cmtchemmaster.com

Mr. David J. McGarva Bio-Tech Distribution Inc. 994 Blanding Blvd. Orange Park, FL 32065

Dear Mr.McGarva:

The following analytical information is provided as to the effects of soils remediation using Micro-Solve product for the reduction and or elimination of hydrocarbon contamination in earth soil.

A sample of soil was taken from a know site that had been contaminated with diesel fuel.

Sample container #002248 ,#002622 will be tested for the presence of Hydrocarbon (Aliphatic Petroleum)

GC method in accordance EPA 418.1

Testing Instrument: SRI 8610 Gas Chromatograph with a Flame lonization Detector and a small bore J&W SCIENTIFIC pack column .05 CFM hydrogen carrier gas. Isothermal 130c temperature program.

Report Generation: The Gas Chromatograph produce a printable graph. The graph is illustrated in retention time and peak height to total area. See attach report

Sample preparation: Ten grams of soil was placed in a 40 ml glass container. The sample was washed with 1cc HPLC grade Acetone. A 1ml sample of the liquefied slurry was injected into the GC.

Test Reaction: The solution enters the column where it is heated to 130c. The components in the solution separate by vaporization. As the components separate they will be carried out the column by the hydrogen gas The components are passed over the FID detector. A electrical signal is sent to the instrument (GC) and displayed as a peak on the graph.

Results: In graph one Sample #002248 peak area 1,2,3 represent the Acetone wash solution and peak 4,5 are hydrocarbons contamination found in the wash soil. (See attachment)

In graph two Sample #002622 peak area 1,2,3 represents the Acetone wash solution. As you can see peak 4,5 are absent. (See attachment)

It was noted that sample two # 002622 had been treated with Micro-Solve product in a attempt to remove the petroleum contamination and placed in sample container #002622. The test demonstrates that the hydrocarbons present in sample #002248 were in fact absent of in sample #002622.

Soils sample were received for Test conducted by Bio-tech Distribution on contaminated soils found in Remediation site in Jacksonville Florida.

CM Services Analytical Service,

CMT Chemical Management Technology, Inc

Orange Park Florida Since 1986



3035 Bravo Court Orange Park, FL. 32065 Phone 800-749-3248 www.cmtChemmaster.com

Dear Customer:

Friday, December 06, 2002

The following information is in response to the soil samples tested for rumination results.

Test Method: Gas Chromagratophy using a Flame Ionization Detector small bore pack column.

Testing sample: I gram of soil was diluted in 1 cc of HPLC grade acetone. The sample was Centrifuged to separate the soil solids for hydrocarbon particulate. The sample was then drawn into a 1ml syringe and injected using a 130C isothermal program in accordance with EPA methods.

One CMF of ultra pure Hydrogen was feed through the packed column. As the 1ml of sample is vaporized in the column compounds will be displayed on the screen as peaks. The retention time and area indicate Liquid components. As the sample vaporize each chemical will be illustrated as a peak on the graph.

The first large peak is the control dilution representing the HPLCc Acetone. 1cc to 1 gram of soil.

As you Can see the acetone contains three visible peaks at 1.0 to1.7 minutes the hydrocarbon is illustrated a 4.6 and 4.9 minutes on the graph.

Sample 2 Illustrated hydrocarbons contamination of 600ppm.

Sample 3 Illustrated hydrocarbon contamination was undetectable. Note: Small peak at 8.366 is a monomer and is not considered a Contamination.

See attached Graph.

If you have any Questions please feel free to call me .

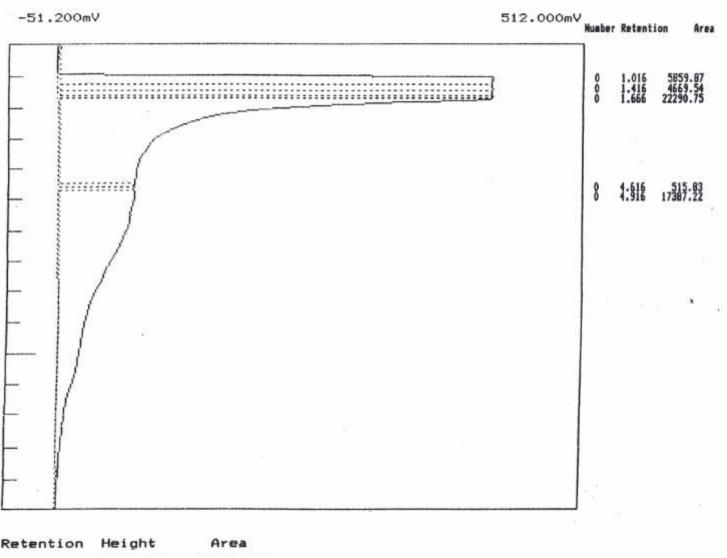
Sincerely Yours

Chuck Freeman PE CMT Engineering

JALL THEMOGRAG

CM Services GC 8610 Operator: Chuck Freeman Description: Soil Remediation test Conditions: 130C isothermal

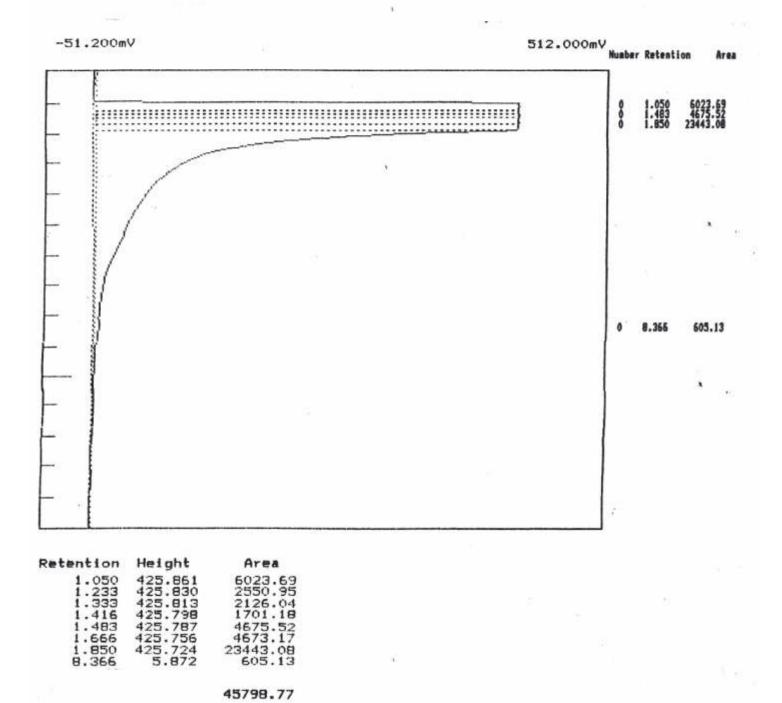
Detector: FID Date: 12/06/2002 Time: 13:18:21 Sample: 002248 (1)



etention	Height	Area		
1.016 1.233 1.416 1.600 1.666 4.466 4.616 4.916	425.350 425.295 425.249 425.203 425.186 74.682 74.445 74.670	5859.87 4670.05 4669.54 1698.80 22290.75 518.94 515.83 17387.22		
		57610.99		

CM Services GC 8610 Operator: Chuck Freeman Description: Soil Remediation test Conditions: 130C isothermal

Detector: FID Date: 12/06/2002 Time: 12:19:39 Sample: 002622 (2)



October 4, 1996

Mr; David Hicks Gainesville Public Utilities Environmental Services P.O. Box 2496 Gainesville, GA 30503

Dear Mr. Hicks,

First let me thank you for the opportunity to test Micro-Solve® in the grease trap of the Beef Corral Restaurant in Gainesville. It was a pleasure working with you on the project. I do, however, have some reservations concerning the methods used in spreadsheet analysis of the results. I also have some concerns about the sampling 'procedures used.

In order to get valuable results from a project such as the one we completed, one must obtain a sufficient amount of background results, a base line study. The baseline results used for calculating removal of the parameters tested in our project was totally inappropriate. The dates of samples collected and analyzed previous to the addition of Micro-Solve® were 5/13/93, 8117/93,4/11/94, 8/15/94 and 4/25/95. Some of these results were approximately two years old. Any waste stream in any environmental situation is going to change over such a period of time. Things can change such as Menu Items the restaurant offers, different cleaning agents being used and different pumping rates of the grease trap. One should realize the best background results would be obtained by taking samples irrunediately prior to the addition of Micro-Solve®. The best scenario for such a background would be to sample the effluent every week on the same day of the week for at least 90 days prior to the use of Micro-Solve®. Then sample the effluent in precisely the same manner following the addition. The reason one would pick the same day of the week to sample is that restaurants load the grease trap at different rates according to the number of persons that are served each day. One would expect much higher results on days such as Friday and Saturday when the restaurant is much more crowded than Monday or Tuesday when the crowds are apt to be much lighter.

The way the Micro-Solve® project was completed leads one to the conclusion that there are were too many variables involved such as the time elapse from the first samples being , taken to the start of the project and not taking the samples. on the same day of the week. This becomes very evident when you look at the Biochemical Oxygen Demand results prior to the addition of Micro-Solve, The high was 5450 mgll on 4/}1194 and the low was 753 mgll on 8/15/94. The results were averaged even with differences as great as these shown for Biochemical Oxygen Demand. The results for Total Suspended Solids, On and Grease, Phosphorus and Total Kieldahl Nitrogen all follow this same pattern.

Mr. David Hicks City of Gainesville, page 2

Another concern I would like to voice is the use of grab samples for the analysis of Biochemical Oxygen Demand, Total Suspended Solids, Phosphorus and Total Kjeldahl Nitrogen. Standard Methods and the Environmental Protection Agency's Manual Chemical Analysis of Water and Wastewater (600/4-79-020) both state that samples to be analyzed for the above parameters should be at a minimum a 24 hour timed composite but suggested 24 hour flow weighted composite. In addition to restaurants having different loading on different days of the week, restaurants also have different waste concentrations at different times of the day. For example, at lunch time one would expect higher results for tested parameters than at three in the afternoon. The reason for the difference is again the number of persons being served at the restaurant at any given time. Since restaurant grease traps have very short retention times, you would need to sample using the 24 hour composite method, whether it is timed or flow proportional.

I am currently in contact with a restaurant in Florida in order to conduct a very similar project. This time the concerns that I stated above will be addressed in such a manner as to show the best results possible. The work will be supervised by an environmental consultant and the analyses will be performed at a State of Florida Certified Laboratory. I will be happy to send you a copy of the final report when it is available.

I would like to propose to you that if the results for Micro-Solve® use in grease traps are encouraging, the City of Gainesville reconsider Micro-Solve® for use in grease traps in Gainesville restaurants. I believe that with better background results, sampling on the same day of the week and composite sampling Micro-Solve® will rise to the occasion. I hope if this is the case that you will indeed reconsider. Again, I would like to thank you for all your help with the Micro-Solve® Test Project. I hope to hear from you soon.

Sincerely,

Dave McGarva

cc: Tim Merritt, Mark Wyzalek



City of Gainesville PUBLIC UTILITIES DEPARTMENT

ENVIRONMENTAL SERVICES

P. O. BOX 2496 GAINESVILLE, GEORGIA 30503 (770) 532-7462 FAX (770) 534-1955

Director's Office - Construction - Management - Engineering - Meter Service Sanitary Sewer - Water Distribution - Water Pollution Control - Water Works



· To:

Tim Merritt, Environmental Services Chief

Copy to:

Tommy Furlow, Public Utilities Director

David Hicks, Industrial Pretreatment Inspector

From:

Mark Wyzalek, Chemist MW

Date:

August 10, 1995

Subject:

Environmental Services Laboratory Results; The Use of *Microsolve* for the Reduction of Oil and Grease in Grease Trap Effluent at Beef Corral Restaurant

Attached is a spreadsheet with the lab results of sampling of Beef Corral prior to and during the use of Microsolve in the grease trap at that location.

The results indicate that *Microsolve* was not effective in reducing oil and grease. The results show an average increase of oil and grease during the three months *Microsolve* was used in comparison with the average oil and grease prior to its use.

file: mw c:\esdnet\memos\pretreat\microslv.sam

Microsolve test at the Beef Corral downtown.

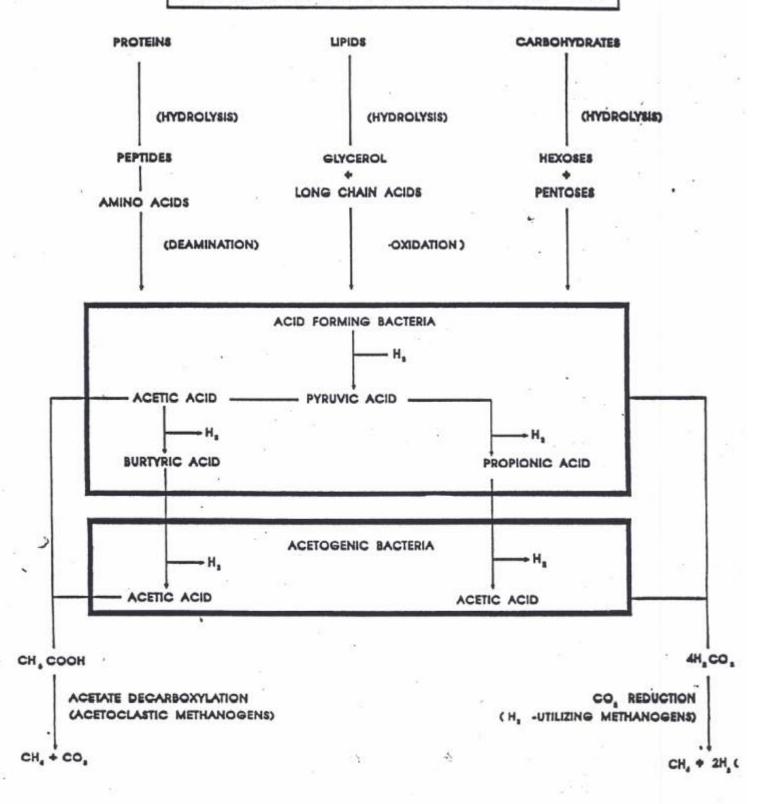
Three days of sampling were conducted by Dave McGarva May 25,26, & 27. This followed the grease trap being pumped approximately three weeks before, thus giving the grease trap time to stabilize before testing began.

Following the sampling five gallons of Microsolve was added to the grease trap on the morning of the 28th.

Since then approximately one quart has been added weekly.

SAMPLE	DATE	BOD	TSS	P	TKN	pH
		mg/L	mg/l	mg/l	mg/l	
BEEF CORRAL	05/13/93	2252	835	23.4	33.2	4.66
BEEF CORRAL	08/17/93	2145	540	22.8	32.6	4.32
BEEF CORRAL	04/11/94	5450	1315	41.46	67.9	4.85
BEEF CORRAL	08/15/94	753	208	21	15	6.51
average		2650	725	27	37	5.1
BEEF CORRAL-SYC.	04/25/95	909	722	10.36	24.25	4.67
BEEF CORRAL-SYC.	04/25/95	na	na .	10.03	29.25	na
Microsolve study begin		1				
BEEF CORRAL-SYC.	04/27/95	1250	638	12.52	43.2	4.38
BEEF CORRAL-SYC.	05/05/95	2025	848	23.84	40.3	5.23
BEEF CORRAL-SYC.	05/18/95	2210	557	18.24	28.7	4.93
BEEF CORRAL-SYC.	05/24/95	4540	536	25.41	29.1	6.44
BEEF CORRAL-SYC.	06/01/95	2030	620	24.56		
BEEF CORRAL-SYC.	06/01/95				35.2	4.68
BEEF CORRAL-SYC.	06/12/95	1280	390	13.3	22.2	4.77
BEEF CORRAL-SYC.	06/29/95	1530	612	14.43		
BEEF CORRAL-SYC.	06/29/95				20.4	4.76
BEEF CORRAL-SYC.	07/13/95	1370	394	14.81		
BEEF CORRAL-SYC.	07/13/95				26.3	4.62
BEEF CORRAL-SYC.	07/27/95	1320	416	12.58	19.65	4.55
BEEF CORRAL-SYC.	07/27/95					4.63
average		1679	521	16	29	4.5

ANAEROBIC DIGESTION AT THE MOLECULAR LEVEL



VI. Safety Data Sheet for Micro-Solve®

The following two pages contain the Material Safety Data Sheet (MSDS) for Micro-Solve®. This information is provided to aid you in safe materials handling procedures and to comply with applicable regulations.

page 1

Safety Data Sheet for Micro-Solve®

IDENTITY: Micro-Solve®

Manufacturer: Bio-Tech Industries Emergency Number

994 Blanding Blvd. 1-800-424-9300

Orange Park, FL 32065 Outside U.S.A. 1-706-527-3887

Information Number 1-904-272-6446

DATE PREPARED: 05/17/99

SECTION I HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

Hazardous Components: The identities of ingredients which are trade secrets are excluded from this section. Ingredients not identified in this section are not found on the IARC, NTP, or OSHA list of carcinogens. All components of this product are registered with the USEPA-TSCA, and this product is Authorized by The United States Department of Agriculture and is acceptable for use in sewage and/or drain lines of official establishments operating under the Federal meat, poultry, shell egg grading and egg products inspection programs.

SECTION II – PHYSICAL/CHEMICAL CHARACTERISTIC

Boiling point: 300-500F Specific Gravity (Water=1): 39.20F = 0.92

Vapor Pressure (mm Hg): <20 Melting Point: N/A

Vapor Density (Air = 1): <6 Evaporation Rate (Butyl Acetate =1): <1 Solubility in Water: NEGLIGIBLE (<.1% in distilled water at 500F) pH 5.8 – 6.2

Appearance and Odor: Clear green liquid with a distinct organic odor.

SECTION III – FIRE AND EXPLOSION HAZARD

Flash Point: 165 – 174F (PCC) Flammable Limits: None LEL: 3.4 UEL: 11.1

Extinguishing Media: Foam, (CO2), or dry chemical extinguisher.

Special Fire and Fighting Procedure: Apply above extinguisher media to keep exposed

containers cool. DO NOT enter fire without self-contained

OSHA approved, breathing Apparatus.

Full bunker gear.

SECTION IV – HEALTH HAZARD DATA

Acute Eyes: Can cause severe irritation, redness and blurry vision.

Skin: Prolonged or repeated contact, can cause moderate irritation, defatting & dermatitis.

Inhalation: Can cause Gastro-intestinal irritation, nausea, vomiting, diarrhea and/or headache.

Chronic Inhalation: May increase susceptibility to respiratory illness.

EMERGENCY FIRST AID:

Skin: Immediately flush skin with running water for 10 minutes. Remove

contaminated clothing immediately. Wash clothing before reuse.

Eyes: Flush immediately with running water for 15 minutes, occasionally lifting upper and lower lids, consult physician.

Inhalation: If affected, remove individual to fresh air.

Ingestion: DO NOT induce vomiting. Call a physician immediately. If conscious, give a

lot of water or milk DO NOT give anything by mouth to an unconscious or

convulsing person.

page 2

Safety Data Sheet for Micro-Solve®

EMERGENCY FIRST AID

Skin: Immediately flush skin with running water for 10 minutes. Remove contaminated clothing

immediately.

Wash clothing before reuse.

Eyes: Flush immediately with running water for 15 minutes, occasionally lifting upper and

Consult physician.

Inhalation: If affected, remove individual to fresh air. If breathing is difficult, administer oxygen and

get prompt Medical attention.

Ingestion: Do not induce vomiting. Call a physician immediately. If conscious, give a lot of water or

milk. Do not give anything by mouth to an unconscious or convulsing person.

SECTION V – REACTIVITY DATA

Stability: Stable

Incompatibility: Avoid contact with oxidizing materials

Hazardous Decomposition Products: Presence of air may yield minor amounts of nitrogen oxide.

Hazardous Polymerization: Will not occur.

SECTION VI – SPILL OR LEAK PROCEDURES

Isolate hazard and restrict entry. Eliminate all ignition In case of leak or spill:

> sources. Absorb with sand or any Inert non combustible, absorbent material, (DO NOT USE SAW DUST) and shovel

into approved containers.

Waste Disposal Method: Contaminated absorbent may be disposed of according to

Local, State & Federal Regulations for the products

containing, Distillates & Solvents.

SECTION VII - SPECIAL PROTECTION INFORMATION

Respiratory Protection: If ventilation is inadequate, wear a properly fitted MSA or OSHA

approved respirator

Protective Gloves: Neoprene gloves (strongly recommended) **Eve Protection:** Chemical splash goggles are recommended

Other Protection Equipment: If contact lenses are worn, the use of tight fitting safety goggles is

recommended

Wash hand thoroughly after handling & before eating, drinking, or **Work/Hygienic Practices:**

smoking.

SECTION VIII - SPECIAL PRECAUTIONS

Pre-cautions in Handling and Storage: Store in a cool well ventilated area away from heat, sparks,

and open flame. Keep containers tightly closed when not using. Keep out of reach of children and away from food. Open, pour and use only in WELL VENTILATED AREAS. Other Precautions: Containers, even emptied, will retain product residue and vapors. Do not cut, weld, or grind these drums.

ALWAYS OBEY HAZARD WARNINGS.

Container Disposal: Do no reuse container. Triple rinse and dispose of contents

and container in accordance with all FEDERAL, STATE, and

LOCAL LAWS AND REGULATIONS.

All statements information and data provided in this MSDS are believed to be accurate and reliable, but are presented without guarantee, warranty, or responsibility of any kind, expressed or implied on our part. Users should make their own investigations to determine the suitability of the information or products for their particular purpose. Nothing contained herein is intended as permission, inducement, or recommendations to violate any laws or to practice any invention by existing patents.



Food Safety and Inspection Service Regulatory Programs Building 306, BARC-East Beltsville, MD 20705

July 06, 1994

Ms. Sandra J. McGarva Bio-Tech Distribution Inc. 994 Blanding Blvd., Suite 118 Orange Park, FL 32065

Dear Ms. McGarva:

This is in reply to your request for compound authorization received on May 26, 1994 for your product Micro-Solve.

This product is acceptable for use in sewage and/or drain lines of official establishments operating under the Federal meat, poultry, shell egg grading, and egg products inspection programs.

Acceptance of compounds by this Department is in no way to be construed as an endorsement of the compounds or of any claims made for them.

If any change is made in the labeling information or formulation, the authorization for use in official plants becomes void immediately.

Sincerely,

John M. Damaré, Chief

Compounds and Packaging Branch

Product Assessment Division



Michelle M. Moore

Jacksonville, Florida 32207 904 308 3911 Main

1301 Riverplace Boulevard · Suite 1500

904 . 346 . 5559 MMoore@rtlaw.com 904 . 398 . 3911 Main 904 . 396 . 0663 Fax www.rtlaw.com

July 14, 2005

Mr. David McGarva BioTech Distribution, Inc. P.O. Box 65276 Orange Park, FL 32065

Re: Affidavit

Dear Mr. McGarva,

Enclosed you will find an affidavit regarding your Microsolve™ product. Please review it to ensure that it is accurate. If you find that it is factually incorrect in any way please contact me immediately so that it can be corrected before you have it notarized.

Regards,

Michelle M. Moore

Michelle M. Moore

Enclosures



AFFIDAVIT OF DAVID MCGARVA

BEFORE ME, the undersigned authority, this day personally appeared David McGarva, who, being first duly sworn, does hereby depose and say as follows:

- My name is David McGarva. I am more than eighteen (18) years of age and am competent to make this affidavit. The facts herein are based upon my personal knowledge and such facts are true and accurate.
- I have been working with the formulation and blending of chemicals for approximately 30 years.
- Currently, I am President of BioTech Industries, Inc., located in Orange Park, FL. The primary purpose of my company is to manufacture and distribute MicrosolveTM.
- MicrosolveTM is used for the break down and reduction of greases and oils in sewage and/or drain lines, septic tanks, pumping stations, and in wastewater treatment operations.
- MicrosolveTM, in addition to working as a solubilzer/demulsifier, also acts as a bacterial
 accelerant.
- 6. I began working on the chemical formulation for the original Microsolve™ in 1975. By the beginning of 1991 a working formula was in place and I, with the assistance of my wife, Sandra McGarva, began distributing this formulation later that year.
- 7. I recently formulated an improved formula for Microsolve™ and feel that when it is used as directed it is superior to the original working formula and produces better results than other products making similar claims. Additionally, Microsolve™ is environmentally friendly. Testing has shown that it has minimal effects on the environment and that it is not harmful to indigenous bacteria when used as directed.

 Presently Microsolve's™ formula is a trade secret, however I am in the process of procuring patent protection for it.

David McGarva, Affiant

SWORN TO AND SUBSCRIBED BEFORE ME, this ___ day of July, 2005, by David

McGarva, who is personally known to me or who has produced _____

identification.

SHANNON MCKEAN

Notary Public, State of Florida My comm. expires June 9, 2008 No. DD327791

Notary Public, State of Florida

Name:

My Commission expires: June 9, 2008 My Commission Number is: 032779

BIDDING SPECIFICATION

Micro-Solve®

Scope:

It is the internet of this specification to procure a product designed to solve many of the problems associated with handling and treatment of animal fat, grease, and oil. Product acts on grease by liquefying the solids into a condition that prevents the grease from caking and building up on the walls, thus improving the overall efficiency. It also accelerates the growth of naturally occurring digestive bacteria in both aerobic and anaerobic condition.

Description:

- 1. A non-water-soluble-based (that will float on water) solution containing a proven combination of de-mulsifiers, corrosion inhibitors, de-foamers, and organic compounds designed to accelerate growth of facultative sewage organisms.
- 2. Product to be completely bio-degradable.
- 3. Product must be proven to break down and bio-remediate at least (70) percent of grease and oil build-up.
- 4. Product must be acceptable for use in sewage and/or drain lines of official establishments operating under the Federal Meat, Poultry, Shell Egg Grading and Egg Products Inspection Programs by the United States of Agriculture.
- 5. Not an enzyme product
- 6. Not a bacteria product
- 7. Not an emulsifier
- 8. Aids in oil-water separation.
- o. Breaks Ionic bond of grease.
- 10. Has ionic bond and can effectively be used on collection lines.
- 11. Proven to bioremediate petroleum build-up in lift stations
- 12. NSF Registered
- 13. pH (5.8 6.2)

Application:

1. Designed for reducing and treating grease and oil accumulations and buildup in lift stations, force mains, gravity lines, grease traps and treatment facilities; anaerobic and aerobic.

Bio-Tech Industries, Inc.

Orange Park, Florida 32065 Phone 904-2726466 Fax 904-276-9662

Microbial populations contained in wastewater are ultimately responsible for the breakdown or treatment of waste. Frequently, insufficient amounts of these microorganisms are a limiting factor in the treatment of wastewater.

Micro-Solve® is a unique combination of traditional chemical ingredients designed to solve many of the problems associated with the handling and treatment of animal fat, vegetable grease and petroleum wastes.

Micro-Solve® is biodegradable under aerobic conditions and is completely compatible withe aerobic activated sludge treatment of wastewater. Laboratory tests show that Micro-Solve® enhances treatment in activated sludge systems by solubilizing nutrients, providing a more activated sludge treatment system. Micro-Solve® caused a ten fold increase in the number of bacteria found in wastewater.

With the addition of Micro-Solve® to a wastewater treatment facility, the biological pathways are no longer inhibited due to the lack of facultative microorganisms and as a result or biological activity, growth and metabolism are substantially increased. By stimulating these specific microorganisms, tremendous benefits in wastewater can be achieved including a considerable increase in treatment efficiency along with substantial operational savings.

Each treatment system is different, with a personality if it's own. Consequently there are variations as to how quickly treatment plants will respond to treatment with Micro-Solve®. Mich is dependent upon the extent of the accumulations and solids withing the plant, as well as the variability of loading. The following pattern can be considered typical.

FIRST WEEK: Very little change, perhaps a slight improvement in effluent quality and a decrease in odor levels.

SECOND WEEK: The system begins to clean itself. Grease and accumulated solids break away from walls. Undigested solids rise from their hiding places. Odors at this point should not be significant. This purging activity should continue for several days or weeks, depending on the amount of solids and the type of treatment system.

**NOTE: Until the system equalizes with organic matter completely digested, there could be a temporary increase in BOD being discharged. This due to additional metabolites being produced, which may be soluble. Until they are broken down to gases and water, the BOD level may be temporarily elevated.

THEREAFTER: Effluent quality will gradually improve. Settle ability increases. Systems typically stabilize within 30 to 90 days. For some activated sludge systems, it may be necessary to adjust the sludge pumping rate in order to maintain an appropriate level of MLSS. Many systems have been able to significantly reduce their sludge wasting.

Micro-Solve® should be used according to need. Enough should be used to do the job at hand. In case of wastewater treatment or discharge of the effluent to natural waters, "enough" is the amount that when diluted by the system maintains the concentration of Micro-Solve® in the treatment where further treatment or disposal does not involve natural waters.

DEGREE OF IMPROVEMENT

The improvement in effluent quality depends primarily on how the system was performing originally. I fa plant is operating at or near the theoretical optimum for that type of system, the expected improvement will generally be less than for a plant operating poorly. A primary plant may show significant improvements in the removal of suspended solids. Secondary plants likewise show improved suspended solids removal efficiency. Any BOD, nitrogen or phosphorus in the effluent associated with the suspended solids will also decrease significantly.

APPLICATION OF MICRO-SOLVE®

Each treatment facility using Micro-Solve® has to be individually engineered to determine the required dosage. Ideally, in continuous flow systems, Micro-Solve® should by applied as far upstream as practical. Introductions within the wastewater collection system has proven to be an effective means of maintaining lift stations while pre-treating the wastewater, thereby maximizing the effectiveness. Many plants apply Micro-Solve® at the head works of the plant itself. Treatment before any primary clarifiers is desirable, so BOD removals across the primary system can be maximized.

Reference Numbers

David McGarva: Chemist/Inventor of Micro-Solve® 904-272-6446 / 904-237-7627 Cell 1988 to present

Pump and Power Equipment

2001 to present Andy Anderson: works with cities and water companies in the Valley 210-781-9067

North Alamo Water Supply Corp

2001 to present Jesse Aguirre Cell 956-532-9600 Office 956-383-1618

Travis County Water Control Improvement District #17

2002 to present Isacc, Supervisor of WW Operations 512-844-9717

City of San Marcos 2002 to present Tony Salinas, W/WW Manager San Marcos, TX 512-738-7680

City of San Marcos 2002 to present Lloyd Juarez, WW Supervisor San Marcos, TX 78666 512-393-8010

City of Primera TX 2003 to present Merle Kenmerling, Operator cell 956-536-1066

City of Weslaco, TX
Marcello Cosme, Director of Utilities
956-5717481
2006 to present

City of Mission Omar Cantu, Plant Operator 2801 N. Holland Mission, TX 78572 956-584-4327 2006 to present

Laguna Madre Water District

(Port Isabel and South Padre Island) 2008 to present Mark Garza, WWTP Chief Operator (956)572-0395

City of Edinburg - 2012 to present Jose Anguiano, Jr., WWTP Chief Operator/WW Director (956)292-2045

City of Laredo - 2016 to present Angel Leon, WW Superintendent (956)721-2022

City of Pearsall - Jan 2016 to present Hector Gandara, UV Tech Operator (830)317-7073