

# The Effect of a Vegetable Oil Additive on Diesel Fuel Biodeterioration

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## Summary

A mixture of diesel fuel with 5% vegetable oil was tested for relative resistance to microbial growth. No difference was noted for plain diesel fuel with and without a proprietary additive. Two commercially available biocides used for fuel/bottom-water preservation were evaluated in the diesel/vegetable oil mixture and, under the conditions used, only methylenebisthiocyanate was successful while a boron derivative proved ineffective.

## I. Introduction

The global implications of applied microbiology have been informally and formally (Emejuaiwe *et al.* 1981) recognized. Frequently these implications involve recognition of economic and political considerations as well as microbiological ones. Thus, the addition of vegetable oil (VO) to diesel fuel oil was done for the former reason and without regard for any potential microbiological problems.

The use of vegetable and animal oils for heating and illumination predate the use of fossil fuels for those purposes. We must assume that domestic availability of the VO involved in this study not only made its use economically advantageous as an additive, but also did not result in any functional problems (e.g., equipment fouling, inefficient burning).

Microbial growth in stored fossil fuel has been recognized and addressed for at least 20 years (Knecht & Watkins 1963, Rogers & Kaplan 1982, Neihof & May 1982, Genner & Hill 1981). Thus, growth in bottom-water condensate does not impoverish the fuel even with minimal turnover and long-term storage but rather produces secondary effects such as corrosion and mechanical plugging. It is these latter problems that are of concern.

We received a contaminated sample of a bottom-water diesel fuel mixture. The fuel contained 5% by volume of a domestically produced VO and a proprietary fuel additive. The question posed related to the contribution of each additive to potential microbial problems and, in addition, the evaluation of two preservatives available in the local marketplace.

## II. Materials and Methods

### Inoculum

#### Biodeterioration Study

The following mixture of bacteria, yeasts, and mycelial fungi isolated from several sources was used as the definitive inoculum:

- Bacteria: *Pseudomonas aeruginosa*  
*Pseudomonas maltophila*  
*Bacillus* sp.  
Gram positive cocci  
Gram negative nonfermentative rod
- Yeasts: Unidentified budding yeast (Y1)  
Unidentified budding yeast (Y2)
- Fungi: *Cladosporium resinae*  
*Penicillium* sp.  
*Oidium* sp.  
*Fusarium* sp.  
*Cephalosporium* sp.

This was prepared by adding each isolate to Bushnell-Haas (Bushnell & Haas 1941) mineral salts medium, overlaying with untreated diesel fuel, and incubating three days at room temperature.

#### Preservative Study

The inocula contained the above mixture and the control bottom-water from the completed biodeterioration study in a 1:1 ratio.

In each case, the inocula were blended with a mechanical mixer to break up clumps and diluted 1:10 with fresh Bushnell-Haas medium. This suspension was added to the test systems at 1% of the fuel volume.

### Test Systems

#### Biodeterioration Study

We used a 250 ml separatory funnel containing 250 ml of fuel mixture plus 2.5 ml of the mixed microbial inoculum for each system (Fig. 1).

1. No. 2 diesel oil - control
2. No. 2 diesel oil + 5% vegetable oil
3. No. 2 diesel oil + 168 ppm Ethyl EDA-2 (Ethyl Distillate Additive 2, a combination of active materials providing fuel improvement performance in: detergency, dispersancy, stability, metal deactivation, anti-corrosion, conductivity, and control of emulsifying properties)
4. No. 2 diesel oil + 5% vegetable oil + 168 ppm Ethyl EDA-2

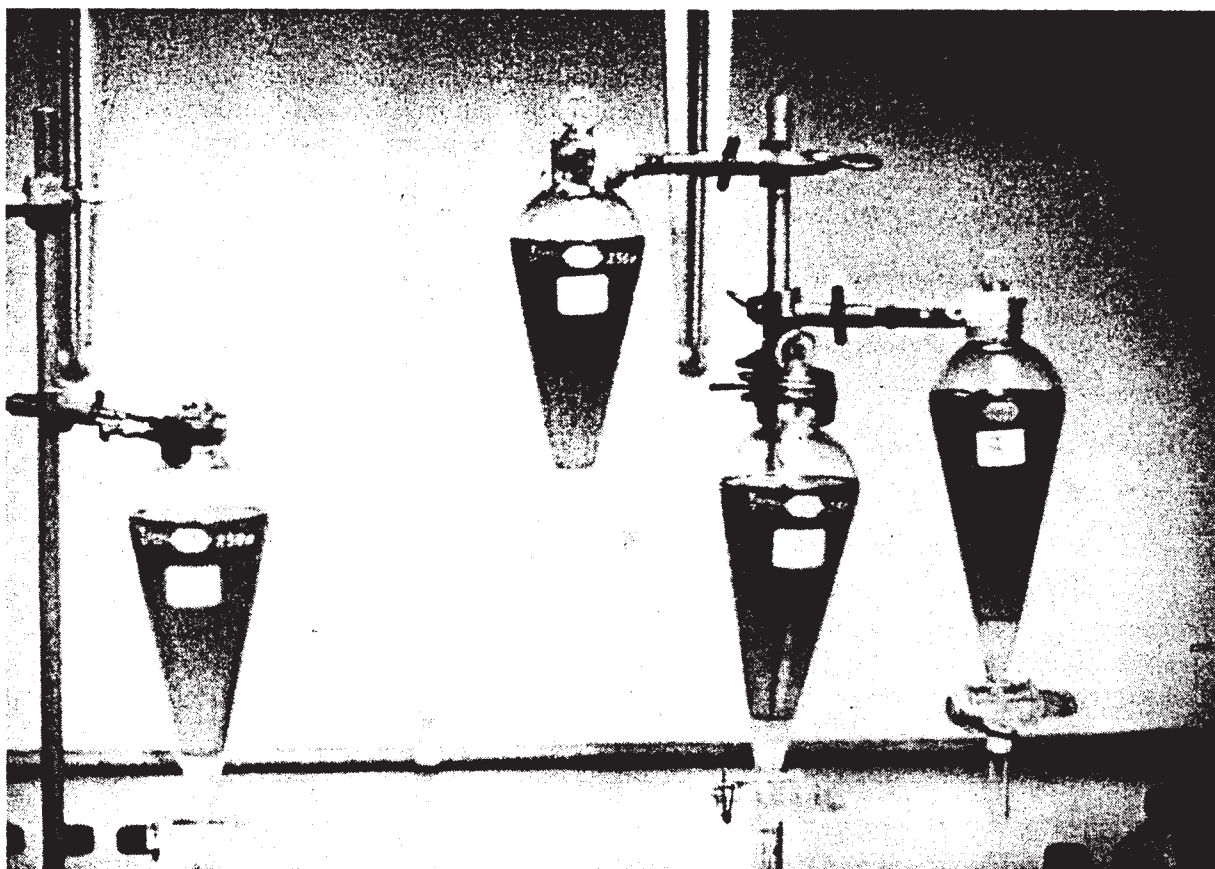


Fig. 1. Physical set-up for biodeterioration study

### *Sampling*

Each test system was analyzed quantitatively for microorganisms after five days of incubation, after seven days of incubation, and at weekly intervals for a total of eight weeks. For the first three weeks, the bottom 10 ml of liquid in each funnel were drawn off into a test tube. This 10 ml sample contained 2.5 ml of bottom-water, 7.5 ml of fuel, and the interface between them which contained the greatest number of microorganisms (especially fungal mycelial mats). The 10 ml sample was mixed on a vortex and then allowed to settle. The water portion was then analyzed. For the final five weeks, glass beads were added to the sampling test tube to aid in the disruption of fungal mycelial mats and slime that formed at the interface.

After each sampling (except after the second week), the entire sample remaining following plating (about 9 ml) was returned to its separatory funnel. One milliliter of sterile Bushnell-Haas broth was added to each test system to maintain a level of 1% bottom-water. Following week two, the entire sample was discarded following plating. To bring the test systems back to their original volume, 7.5 ml of fresh fuel and 2.5 ml of sterile Bushnell-Haas broth were added to each system. This was done to simulate bottom-water drainage from a contaminated storage tank and the subsequent reintroduction of fresh fuel and bottom-water.

### *Preservative Study*

Two commercially available biocides - Biobor<sup>®</sup> JF (U.S. Borax Co.) and Amerstat WB<sup>®</sup> (Drew Chemical Corp., Subsidiary of U.S. Filter Corp.) - were added at indicated levels (Table 2) to No. 2 diesel oil containing 5% VO. We added 200 ml of each mixture plus 2 ml of inoculum (described above) to 250 ml screw-cap bottles pre-coated with siloxane (Glassclad<sup>™</sup> 18) to minimize water adherence. The systems were incubated at room temperature for one week and periodically shaken to maximize fuel/water/biocide mixing.

### Sampling

Because of the siloxane coating, the entire bottom-water portion of each test system remained on the bottom and was easy to remove. One milliliter of this bottom-water was withdrawn by pipet and plated by conventional pour plate techniques to quantitate the microbial population.

### Determination of Microbial Levels

All quantitation was done by standard methods using plate techniques. Bacterial populations were estimated in trypticase soy agar (BBL) and incubated at 30°C for 48 hours. Fungal and yeast populations were estimated in Sabouraud dextrose agar (DIFCO) with 50 ppm Gentamicin and incubated at 30°C for five days.

## III. Results and Discussion

The results of the nine samplings are shown in Table 1. The bacteria that were used in the inoculum adapted well in all four test systems. Approximately a four-log increase in bacteria occurred after five days of incubation. The levels of bacteria stayed elevated throughout the test period in all of the systems.

Table 1 Growth and survival of microbial populations in a diesel-vegetable oil mixture.

Sample	Time of Sampling								
	5 Days	1 Week	2 Weeks	3 Weeks	4 Weeks	5 Weeks	6 Weeks	7 Weeks	8 Weeks
	BACTERIA/ml								
DO	$1.3 \times 10^8$	$1.4 \times 10^9$	$5.4 \times 10^8$	$8.8 \times 10^6$	$1.6 \times 10^8$	$3.5 \times 10^8$	$1.3 \times 10^8$	$7.6 \times 10^7$	$5.6 \times 10^7$
DO+VO	$1.3 \times 10^8$	$1.2 \times 10^9$	$8.5 \times 10^8$	$2.0 \times 10^8$	$1.0 \times 10^9$	$1.8 \times 10^8$	$3.9 \times 10^8$	$2.5 \times 10^8$	$3.0 \times 10^8$
DO+EDA	$1.1 \times 10^8$	$1.3 \times 10^9$	$1.2 \times 10^9$	$1.0 \times 10^7$	$7.3 \times 10^8$	$8.8 \times 10^8$	$4.0 \times 10^8$	$6.3 \times 10^8$	$7.3 \times 10^8$
DO+VO+EDA	$1.4 \times 10^8$	$1.5 \times 10^9$	$1.7 \times 10^9$	$3.6 \times 10^7$	$6.6 \times 10^8$	$7.5 \times 10^8$	$9.0 \times 10^8$	$3.5 \times 10^8$	$3.3 \times 10^8$
	FUNGI/ml								
DO	450	$6.0 \times 10^3$	$5.0 \times 10^4$	$1.5 \times 10^4$	$3.0 \times 10^4$	$7.9 \times 10^5$	$6.0 \times 10^5$	$1.0 \times 10^6$	$1.6 \times 10^5$
DO+VO	<10	$2.0 \times 10^3$	$7.0 \times 10^3$	$1.0 \times 10^5$	$1.1 \times 10^6$	$1.0 \times 10^6$	$1.4 \times 10^6$	$1.0 \times 10^6$	$1.0 \times 10^6$
DO+EDA	80	$1.7 \times 10^3$	$3.0 \times 10^4$	$1.0 \times 10^4$	350	$2.0 \times 10^3$	$1.0 \times 10^3$	$1.0 \times 10^3$	$2.0 \times 10^3$
DO+VO+EDA	30	150	$6.0 \times 10^4$	$9.4 \times 10^5$	$8.0 \times 10^4$	$8.2 \times 10^5$	$4.6 \times 10^5$	$5.2 \times 10^5$	$1.0 \times 10^5$
	YEAST/ml								
DO	$\sim 2.0 \times 10^5$	$5.0 \times 10^4$	$3.0 \times 10^4$	$2.0 \times 10^3$	$5.0 \times 10^4$	$5.0 \times 10^5$	$\sim 1.0 \times 10^6$	$\sim 8.0 \times 10^5$	$2.0 \times 10^6$
DO+VO	$3.0 \times 10^4$	$7.0 \times 10^3$	< $10^3$	$3.0 \times 10^4$	$3.0 \times 10^4$	$3.0 \times 10^5$	$\sim 1.0 \times 10^6$	$\sim 1.0 \times 10^6$	$1.0 \times 10^6$
DO+EDA	$\sim 5.0 \times 10^3$	$3.0 \times 10^4$	$4.0 \times 10^4$	$4.0 \times 10^5$	$1.0 \times 10^4$	$5.0 \times 10^3$	$\sim 1.0 \times 10^3$	$\sim 1.0 \times 10^4$	$1.0 \times 10^3$
DO+VO+EDA	$4.1 \times 10^4$	$6.0 \times 10^4$	$3.0 \times 10^4$	<10	$2.0 \times 10^5$	$6.5 \times 10^5$	$5.4 \times 10^4$	$\sim 3.0 \times 10^5$	$1.0 \times 10^5$

DO=No. 2 diesel oil-control. DO+VO=No.2 diesel oil + 5% vegetable oil.

DO+EDA=No. 2 diesel oil + 168 ppm Ethyl EDA-2. DO+VO+EDA = No. 2 diesel oil + 5% vegetable oil + 168 ppm Ethyl EDA-2.

Bottom-Water: Bushnell-Haas mineral salts medium at 1% of fuel level.

Inoculum (as zero time count after addition to Bushnell-Haas medium): Bacteria -  $6.7 \times 10^4$ /ml

Fungi - 85/ml

Yeast - <10/ml

Although no viable yeasts were detected in the inoculum (< 10/ml), the small numbers that were present were able to grow and survive in all of the test systems. The yeast counts are, where noted, approximated. In these approximated counts, the yeast colonies were obscured by overgrowth of bacteria. Major fluctuations in the yeast data may be due partially to this phenomenon and also due to clumping of organisms which made their enumeration difficult. The only significant factor in the yeast results is that EDA-2 reduced the counts significantly in straight diesel fuel and slightly in VO diesel fuel.

The fungi inoculated into the fuels also survived and grew. The most rapid growth appeared in the untreated straight diesel fuel. As the test proceeded, the results of the fungal counts mirrored those of the yeast counts in that the EDA-2 treated fuels showed reduction of fungi, particularly in the straight diesel fuel.

The visual appearance of the test systems containing EDA-2 was quite different from the two untreated systems. The untreated fuels showed a large amount of microbial growth adhering to the sides of the separatory funnels. The VO diesel fuel containing EDA-2 showed very little adherence, and the straight diesel fuel treated with EDA-2 showed virtually no adherence of microorganisms to the sides of the funnel. A comparison of the VO diesel and straight diesel fuel with no additives showed that more microbial growth occurred on the sides of the separatory funnel containing the VO diesel fuel.

No apparent antibacterial activity by EDA-2 was observed; however, a two-log reduction in fungi and a three-log reduction in yeasts occurred in the test systems containing this additive. These reductions are not large but they are significant. Vegetable oil diesel fuel treated with EDA-2 showed a one-log reduction in fungi and yeasts. This reduction is rather small and is not considered significant.

A comparison of the two untreated fuels in all cases showed no significant differences in growth levels. These results lead to the conclusion that VO added to diesel fuel at this level (5%) would neither inhibit nor promote microbial growth in a long-term storage system.

The configuration of the test system (i.e., the separatory funnel) permitted optimal observation of the two phases and the interface and permitted the use of very small water levels while maintaining aqueous phase integrity. In addition, it facilitated bottom-water sampling and/or drainage. However, the sampling regimen is a form of destructive testing in which most of the bottom-water is consumed in the microbiological evaluation. This may be an artificial reduction in bioload. In the field, sample size is only a very small fraction of the bottom-water and will have no impact on the contamination level remaining. We are currently evaluating a test system that attempts to overcome this problem.

The results of the preservative test are shown in Table 2. Biobor did not work effectively in this system; perhaps the relatively high bottom-water level (1%) diluted and hydrolyzed the Biobor. The Amerstat bacterial survivors at the higher doses were all aerobic spore formers, not normally problems in these systems.

Table 2 Evaluation of two commercially available biocides in diesel oil with 5% vegetable oil.

Sample No.	Biocide/ppm	Bacteria/ml	Fungi/ml	Yeast/ml
1	None - control	$\sim 10^9$	$2.2 \times 10^4$	$\sim 1.3 \times 10^4$
2	<sup>1</sup> Biobor <sup>R</sup> JF/70	$\sim 10^9$	$1.2 \times 10^4$	$\sim 1.5 \times 10^4$
3	Biobor JF/140	$9.8 \times 10^7$	$6.0 \times 10^3$	$\sim 9.0 \times 10^3$
4	Biobor JF/210	$6.1 \times 10^7$	$8.0 \times 10^3$	$3.0 \times 10^4$
5	Biobor JF/280	$1.0 \times 10^8$	$2.1 \times 10^4$	$\sim 1.0 \times 10^5$
6	<sup>2</sup> Amerstat WB <sup>R</sup> /150	$\sim 10^9$	$5.5 \times 10^4$	$1.5 \times 10^4$
7	Amerstat WB/300	Fungal overgrowth	$2.5 \times 10^6$	$1.8 \times 10^6$
8	Amerstat WB/500	$7.0 \times 10^5$	<10	<10
9	Amerstat WB/700	$5.3 \times 10^4$	<10	<10
10	Amerstat WB/850	$1.0 \times 10^4$	<10	<10

<sup>1</sup>2,2'-oxybis(4,4,6-trimethyl-1,3,2-dioxaborinane/2,2'-(1-methyltrimethylene-dioxy)/bis-(4-methyl-1,3,2-/dioxaborinane)-95% active. Concentrations based on oil levels as per manufacturer's recommendation.

<sup>2</sup>methylene bithiocyanate - 10% active. Concentrations based on bottom-water level as per manufacturer's recommendation.

Biocide selection for diesel fuel preservation is limited by a number of constraints, including: efficacy, lack of mammalian toxicity, fuel and water solubility, low ash content, no effect on burners, and stability in storage. Efficacy, although of primary importance, can be overshadowed by cost factors. In this study, any added costs to control microbial activity, if deemed harmful, cannot exceed the savings produced by adding VO. Perhaps in the country of use, good housekeeping with better attention to bottom-water drainage and fuel turnover will mitigate the need for biocides.

This is not the first report of fossil fuel extension with biogenically derived oil (Fishinger *et al.* 1981), and certainly there are many unreported cases. However, this is the first study involving a potential microbiological problem. The results indicate that no difficulties would develop due to the biogenic additive.

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