



FINAL REPORT

Evaluation of Additives in Liquid Fuels

PROTOCOL
ASTM E1259

ORDER Number
371000995

PREPARED FOR:

Fuelade Technologies, LLC

Jason Dobranic, Ph.D.

EMSL Analytical, Inc.

107 Haddon Avenue, Westmont, NJ 08108

Phone: (856) 858-4800 Fax: (856) 858-0648 Web: <http://www.emsl.com>





Certificate of Analysis

Client: Fuelade Technologies, LLC

Contact: Peter Franz

Project: ASTM E1259

Product : Fuelade

EMSL NO: 371000995

Sample received: 2/1/2010

Start date: 2/16/2010

Report date: 3/5/2010

Challenge Bacteria: *Pseudomonas aeruginosa* ATCC No. 33988

Challenge Fungi: *Hormoconis resinae* ATCC No. 20495

Challenge Yeast: *Candida (Yarrowia) tropicalis* ATCC No. 18138

Experimental Summary: The testing procedure was designed after discussions between EMSL Analytical, the testing company, and the client, Fuelade Technologies, LLC. The protocol followed was ASTM E1259, Standard Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C. All testing was conducted in our Westmont Microbiology Laboratory.

Procedure:

In order to determine the effect the fuel additive has on microbial growth, three test organisms; *Pseudomonas aeruginosa*, *Hormoconis resinae*, and *Candida tropicalis*, were used to set up small scale, 1 liter, microcosms. Microcosms were first inoculated with bottom water (Bushnell-Haas broth) and the previously stated organisms. Each bottle was overlaid with a 10:1 ratio of diesel to bottom water. Three sample types were examined: 1) the test sample, an inoculated liter of diesel fuel plus the additive; 2) a positive control, an inoculated liter of diesel fuel without additive; and 3) a negative control, an un-inoculated liter of diesel fuel plus the additive. All samples were tested in replicate and an initial count of the microorganisms was obtained by standard plate counts on Tryptic Soy agar (TSA) for bacteria, and Malt Extract agar (MEA) for yeast and fungus. Agar plates were incubated for 3-5 days then examined using a light box. Samples were collected again 48 h later to observe the effect of the fuel additive on microbial growth in the diesel fuel.



Experimental Results:

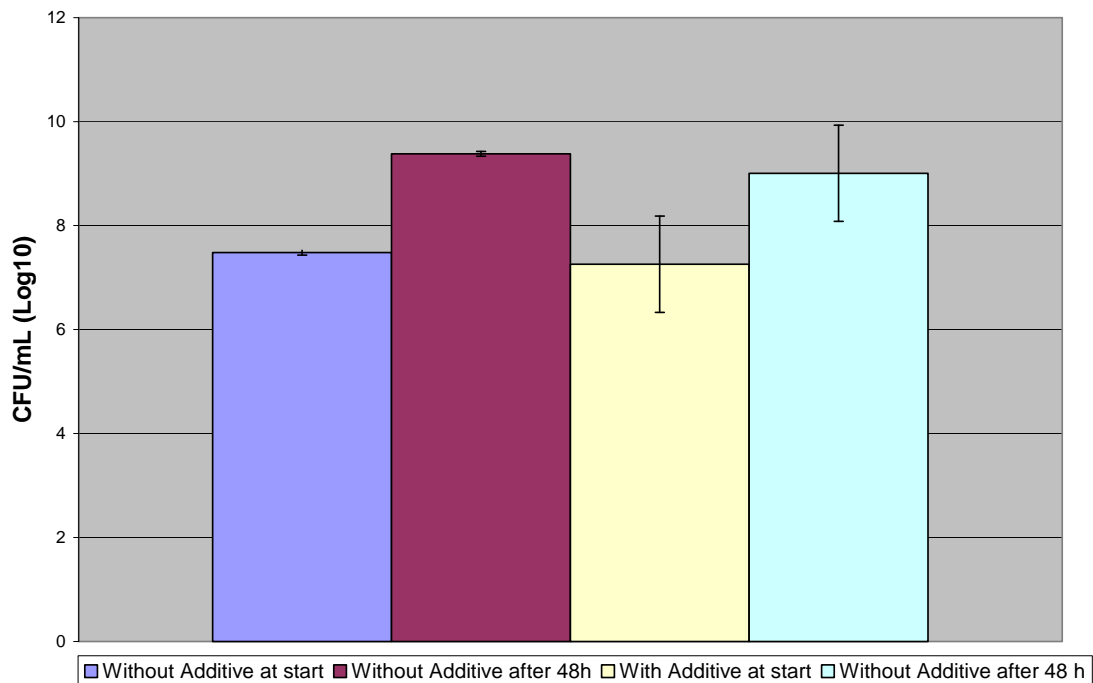
Table 1. Microcosm results at start and after 48 hr incubation.

Microorganism	Without Additive (CFU/mL)		With Additive (CFU/mL)	
	At Start	After 48 h	At Start	After 48 h
<i>P. aeruginosa</i>	3.02E+07	2.40E+09	1.81E+07	1.01E+09
<i>C. tropicalis</i>	1.28E+06	7.80E+05	8.30E+05	9.00E+05
<i>H. resinae</i>	8.00E+04	5.00E+04	1.13E+05	6.50E+04

Un-inoculated microcosms consisting of additive and fuel did not show any growth at start or after 48 hr (<10 CFU/mL).

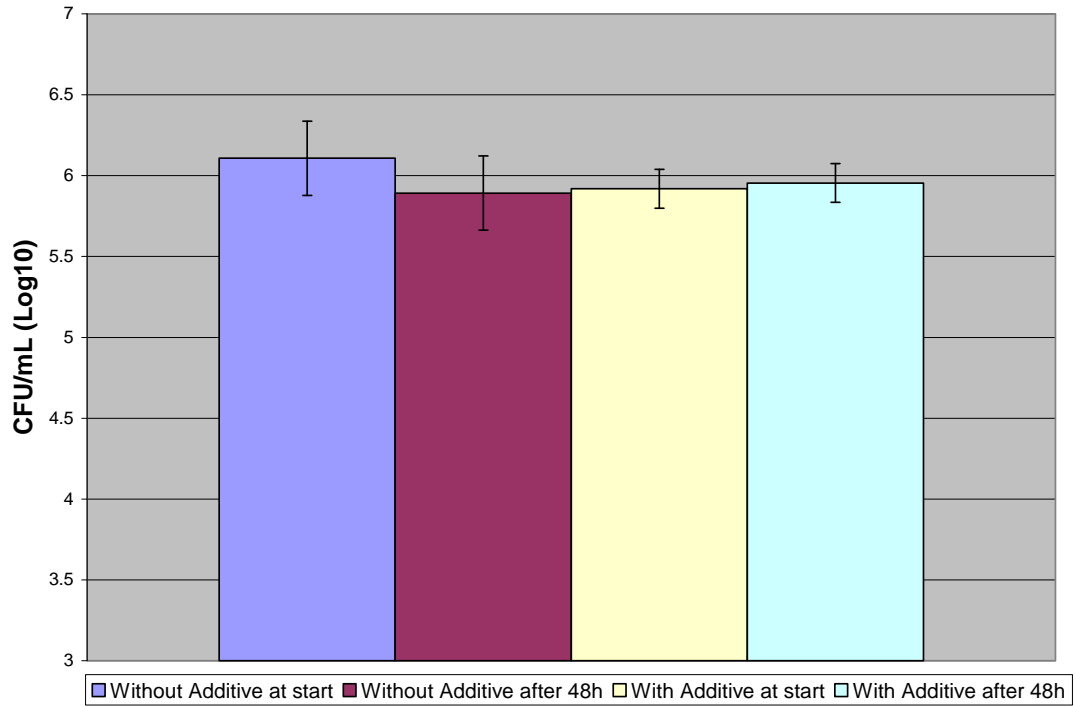
CFU = colony forming unit

***P. aeruginosa* Levels In Microcosms With and Without Additive**

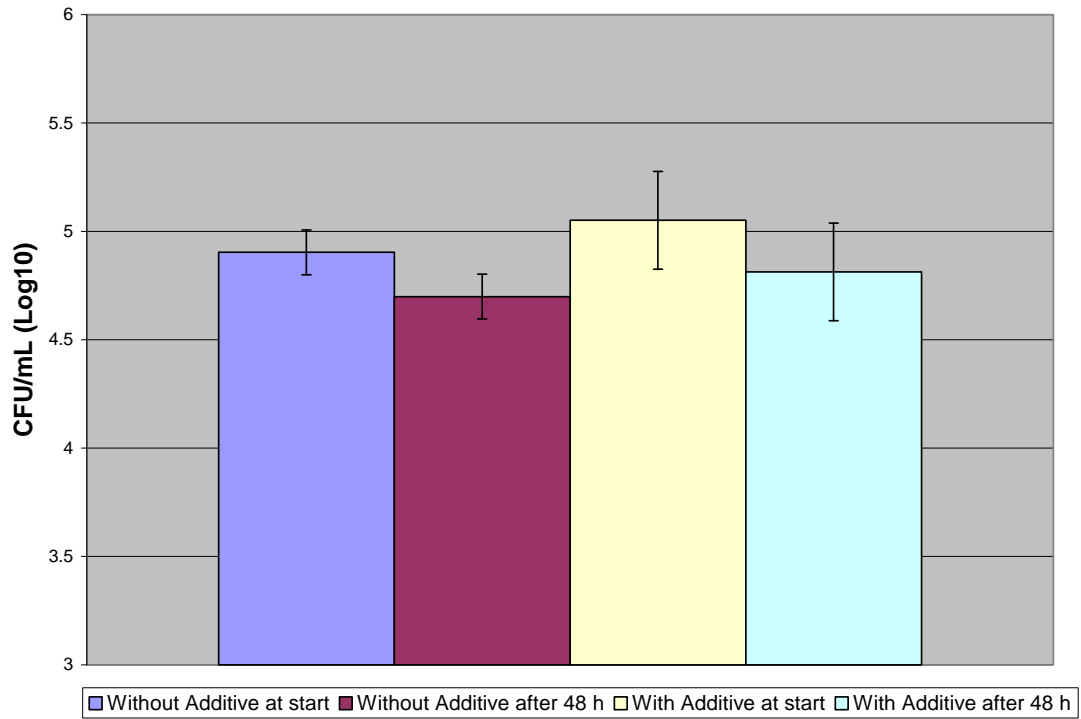




C. tropicalis Levels in Microcosms With and Without Additive



H. resinae Levels in Microcosms With and Without Additive





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Conclusions/Observations:

The Fuelade additive did not have any effect, either positive or negative, on the microbial growth in the diesel fuel microcosms tested.

Jason Dobranic, Ph.D.
National Director of Microbiology