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Antimicrobials
Biodegradation
Discovery

Customer Report

Friday, August 31, 2012

CONTACT

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Elton Alderman
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Project Title

OECD 301B Biodegradation Test of Downhole Drilling Fluid Additive

ID **0712-AKV-01 -- 1** Entry Date 7/25/2012

Project Summary

Overview:
The OECD 301B method is designed to provide screening of chemicals for biodegradability in an aerobic aqueous medium. There are two biodegradable determinations that can be achieved for a biodegradable material by the OECD test protocol; Ready Biodegradability and Ultimate Biodegradability. Both requirements are based on the analytical determination of a materials' theoretical carbon content which is measured by the recovery of carbon dioxide (CO2) from the degrading test sample (% ThCO2).

To achieve a designation of Readily Biodegradable, samples are required to achieve a level and rate of biodegradation that results in a conversion from 10% to 60% ThCO2 within 10 days of the 28 test period. The level of degradation must be achieved within a strict timeframe of 10 days during the standard 28 day test period.

For Ultimate Biodegradability, the test sample must demonstrate 60% or greater biodegradation within a specified timeframe as determined by the outcome of the test procedure.

One test sample was submitted for OECD 301B biodegradation testing. Test sample (Pro One Downhole Drilling Fluid Additive) was provided as a dark tan hydrophobic material with 98.4% nonvolatile solids and a determined total organic carbon content of 72.6%.

Results: The control sample (sodium acetate) achieved 10% biodegradation by day 2 and 60% degradation by day 10, with a plateau of 69.6% biodegradation on day 19 of the test. The Test Sample achieved 60% degradation and Ultimate Biodegradability by day 16 with a plateau of 83% degradation by day 31 of the test period. The test sample demonstrated Ultimate Biodegradation according to the conditions set forth in OECD 301B requirements (see figures).

Test sample graphs were analyzed by curve fit to establish that a plateau of the rate of biodegradation was achieved prior to termination of the analysis (see figures).

Sample List

Method Name

Sample #	*Sample Name*	*Sample Notes*
OECD 301 B - Solution Biodegradation by CO2 Evolution		
1	Pro One Downhole Drilling Fluid Additive	
2	Control - Sodium Acetate	

Result Table

Contact	Pro One Lubricants	Elton Alderman	712-327-0262
Title	OECD 301B Biodegradation Test of Downhole Drilling Fluid Additive		
Project ID	0712-AKV-01 -- 1	Entry Date 7/25/2012	Test Start Date 7/25/2012

Sample Result Table *

Sample # 1 **Pro One Downhole Drilling Fluid Additive**

Test Method **OECD 301 B - Solution Biodegradation by CO2 Evolution**

		Interval	Result
Inoculum	***Mixed Environmental Organisms ()***		
Achieved 'Ultimate Biodegradation' requirement.		**16** day	**60** % ThCO2
Plateau level of degradation		**31** day	**83** % ThCO2

Image: **Test Sample**

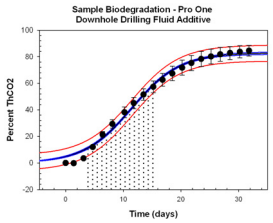

***Figure** - Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the statistical confidence for 95% (blue) and predicted 95% (red) boundary lines.*

Sample # 2 **Control - Sodium Acetate**

Test Method **OECD 301 B - Solution Biodegradation by CO2 Evolution**

		Interval	Result
Inoculum	***Mixed Environmental Organisms ()***		
Achieved 'Ready Biodegradation' Requirement		**10** day	**60** % ThCO2

Image: **Control Sample**

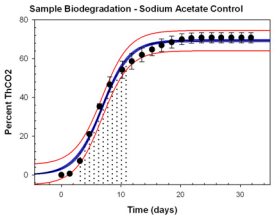

***Figure** - Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the statistical confidence for 95% (blue) and predicted 95% (red) boundary lines.*

plateau of biodegradation	**19** day	**69** % ThCO2

OECD 301 B - Solution Biodegradation

Test conditions:
- inoculum: Surface water from Skokie, IL water district.
- proportion and nature of industrial waste water in sewage: unknown, discharge from waste treatment facility within 1 mile.
- test duration and temperature: 28 days or as indicated, 22C +/- 2C
- bacterial inoculum ~1E5 cfu/ml

Legend
Sample Analysis
TC - Total Carbon determined by catalytic oxidation of the test sample.
IC - Inorganic Carbon
TOC - Total Organic Carbon - determined by the subtraction of TC from IC.
TN - Total Nitrogen determined by chemical luminescence.

%S - Percent Solids- is the dry (non-volatile) percent of the test sample.

For the sample analysis, percent solids is determined when estimating the weight of material to test. For biodegradable materials, the best degradability will be obtained with sample compositions that are linear organic (carbon containing) molecules lacking carbon to carbon double bonds. The total carbon (TC) provides an indication of the material composition, but does not provide information on chemical structure or function. Inorganic carbon is typically low in most biodegradable materials, and increases over the course of the test due to the action of the microorganisms in creating waste, or biological compounds that are generated from the consumption of the carbon based test sample. Total nitrogen can be an indication of nutrient abundance, but is not typically used as part of the test sample assessment.

Sample Result Table *

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d p satchell

Don Satchell Ph.D., Technical Manager

Report Addendum

Friday, August 31, 2012

Project ID	0712-AKV-01 -- 1	Entry Date	7/25/2012	Test Start Date	7/25/2012

Image Table

Sample #	1	Pro One Downhole Drilling Fluid Additive
Test Method		OECD 301 B - Solution Biodegradation by CO2 Evolution
Inoculum		*Mixed Environmental Organisms*

Image: **Test Sample**

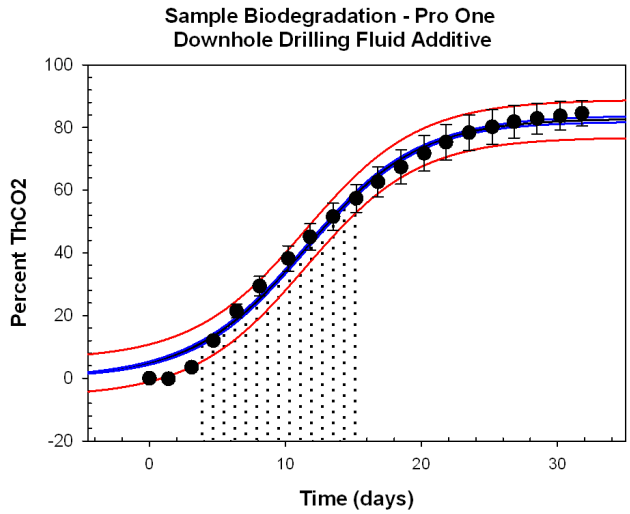

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the statistical confidence for 95% (blue) and predicted 95% (red) boundary lines.*

Image Table

Sample #	2	Control - Sodium Acetate

Test Method	OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Mixed Environmental Organisms*

Image: **Control Sample**

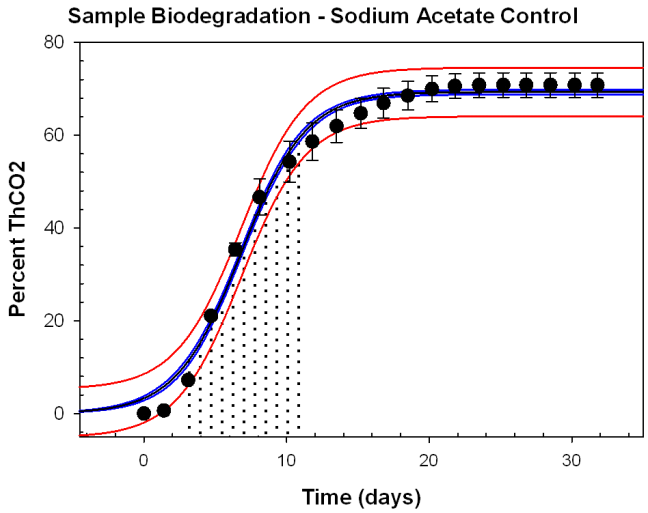

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the statistical confidence for 95% (blue) and predicted 95% (red) boundary lines.*