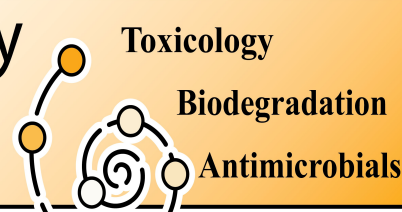




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Product Test Laboratory

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Customer Report

Thursday, June 27, 2019

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Engineered Fluids LLC
2202 Timberloch Place
Suite 118
The Woodlands TX

David Sundin
903-521-3775
David.Sundin@engineeredfluids.com

Project Title

Biodegradation Testing

ID

0419-CBH-01 -- 1

Entry Date 4/22/2019

Project Summary

ASTM D5864 Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components is designed to provide a measurement of the degree of aerobic aquatic biodegradation of fully formulated lubricants exposed to an environmental inoculum under laboratory conditions.

Product of degradation by microorganisms in this test method is carbon dioxide (CO₂). CO₂ evolution is recorded for a minimum of 28 days. Total organic carbon is determined analytically for each sample and is used as the reference for the determination of the theoretical percentage of carbon dioxide (%ThCO₂) that can be produced by microbiological degradation of the test sample. The value of %ThCO₂ is determined at the final reading or plateau for the test sample (see graphs) and reported as the biodegradation achieved.

Sample 1 (EC-100) achieved a degradation of 90% within the test parameters. The theoretical value for maximal degradation was determined for each sample following curve fit analysis of the degradation curve (see graphs), and would be expected to achieve a biodegradation of 99%. The test microbial inoculum was derived from soil and was pre-acclimated to the test sample for 14 days prior to initiation of testing.

Recommended Reading

Online Resource for Product Development, Testing, and Inquiry

<http://www.situbiosciences.com/lubricant-biodegradation-and-toxicity-testing/>

<http://www.situbiosciences.com/biodegradation-doc-co2-o2-measurements-and-method/>

<http://www.situbiosciences.com/biodegradation/astm-d5864-lubricant-biodegradation-test/>

Sample List

Method Name

Sample #

Sample Name

Sample Notes

ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components

1 EC-100

2 Sodium Acetate

Project - Images

1 EC-100

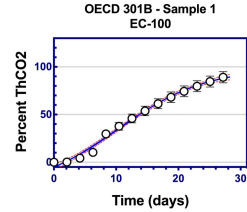
Result Table

Contact	Engineered Fluids LLC	David Sundin	903-521-3775
Title	Biodegradation Testing		
Project ID	0419-CBH-01 -- 1	Entry Date 4/22/2019	Test Start Date 4/25/2019

Result Table *

Test Method	ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components		
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Sample #	1	EC-100	
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Inoculum	Interval (units)	Result (units)
<i>Notes Section</i>		
Total Organic Carbon of the sample	0 day	81 %TOC
plateau not yet achieved	28 day	90 % ThCO ₂
Image: figure Figure – The sample graph shows the test chamber carbon dioxide (CO₂) measurement as the percent of theoretical maximum (% ThCO₂). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line).		
		
pH	0 day	7.6 pH
pH	28 day	7.2 pH
bacterial concentration	0 day	14000 CFU/mL
bacterial concentration	28 day	310000 CFU/mL

Result Table *

Test Method

ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components

Sample

2

Sodium Acetate

Inoculum

Notes Section

Total Organic Carbon of the sample

Interval (units)

0 day

Result (units)

26 %TOC

plateau achieved

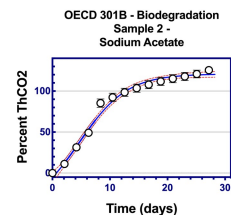
28 day

99 % ThCO₂

Image:

figure

Figure – The sample graph shows the test chamber carbon dioxide (CO₂) measurement as the percent of theoretical maximum (% ThCO₂). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line).



pH

0 day

7.6 pH

pH

28 day

7 pH

bacterial concentration

0 day

14000 CFU/mL

bacterial concentration

28 day

220000 CFU/mL

Test Method

Project - Images

Sample

1

EC-100

Inoculum

Notes Section

Interval (units)

time - 0

Result (units)

sample image

Image:

sample



ASTM D5864 - Solution Biodegradation**Test conditions:**

- inoculum: surface water from Skokie, IL water district acclimated to test media (4 day incubation and refiltered)
- test duration and temperature: 28 days or as indicated, 22C +/- 2C
- bacterial inoculum ~1E6 cfu/ml
- pH of the samples is pH 7.0 +/- 0.3 at the beginning of the test unless noted.

Legend*Sample Analysis*

TOC - Total Organic Carbon; by analytic combustion

Method Notes:

- no preadaptation to test sample unless noted
- no dispersion or WAF applied for sample unless noted
- Reference substance (control) is sodium acetate (NaAc) unless noted

* This report is governed by and incorporates by reference, the conditions of testing as posted on the date of issuance and is intended for the identified Project Owners exclusive use. Any copying or replication of this report to or for any other person or entity, or use of our company name or Service Mark is permitted only with our prior written consent. All images supplied as part of the report are provided as test result edification only and are the sole property of Situ Biosciences LLC and are copyright protected. Any exemption to the copyright of the report or images provided will be explicitly noted in this report.

This report sets forth our findings solely with respect to test samples identified herein. The results set forth in this report are not indicative or representative of the quality or characteristics of the lot from which a test sample was taken or any similar identical product unless specifically and expressly noted. Our report includes all tests requested and the results thereof based upon the information provided. Written notification within 60 days from the date of issuance of this report is required to address any material error or omission caused by the handling of the samples. Any such notification shall specifically address the issues related to the test samples supplied and testing conducted as provided in this report. A failure to raise such an issue within the prescribed time shall constitute your unqualified acceptance of the completeness of this report, the testing conducted, and the correctness of the report contents.

d p satchell Ph.D., Technical Manager

Report Addendum

Thursday, June 27, 2019

Project ID **0419-CBH-01 -- 1**

Entry Date 4/22/2019

Test Start Date 4/25/2019

Image Table

Sample # **1** EC-100

Test Method ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components

Inoculum

Image: figure

Timepoint: 28 day

OECD 301B - Sample 1 EC-100

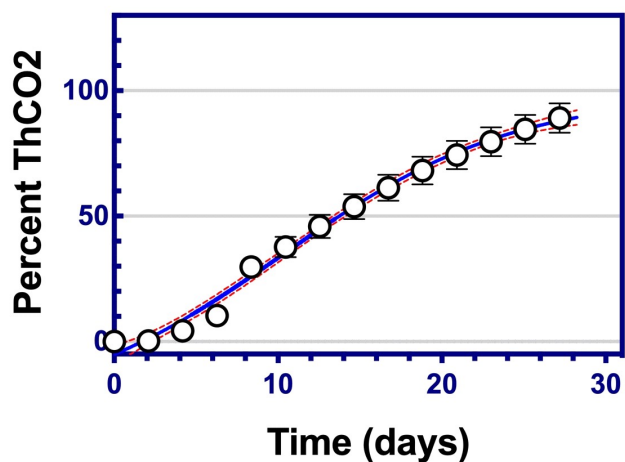


Figure – The sample graph shows the test chamber carbon dioxide (CO₂) measurement as the percent of theoretical maximum (% ThCO₂). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line).

Image Table

Sample #	2	Sodium Acetate
Test Method	ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components	
Inoculum		

Image: figure

Timepoint: 28 day

OECD 301B - Biodegradation Sample 2 - Sodium Acetate

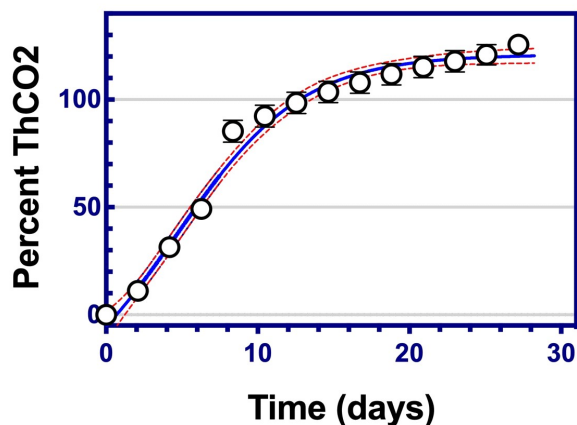


Figure – The sample graph shows the test chamber carbon dioxide (CO₂) measurement as the percent of theoretical maximum (% ThCO₂). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line).

Sample #	1	EC-100
Test Method	Project - Images	

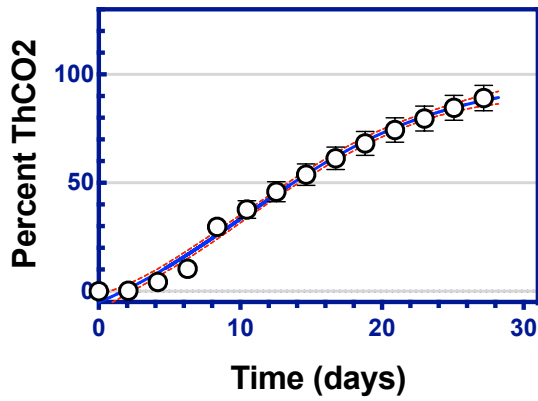
Inoculum

Image: sample

Timepoint: time - 0

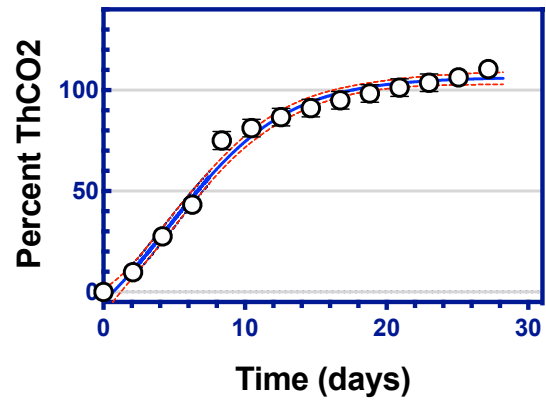


OECD 301B - Sample 1
EC-100



Time (days)	% ThCO2		
X	A:Y1	A:Y2	A:Y3
0.00	0.0	0.0	0.0
1.05	-0.1	0.0	-0.1
2.09	-0.2	0.5	0.3
3.14	1.4	2.2	1.8
4.18	3.7	5.1	4.1
5.23	7.0	9.0	7.3
6.27	9.6	11.9	9.5
7.32	25.9	29.8	23.8
8.36	28.7	33.5	26.9
9.41	32.6	37.6	30.2
10.46	37.0	42.1	34.0
11.50	40.9	46.5	37.9
12.55	45.0	50.9	41.7
13.59	49.0	55.1	45.6
14.64	52.9	59.1	49.3
15.68	56.7	63.0	53.0
16.73	60.7	66.8	56.5
17.77	64.4	70.5	59.9
18.82	67.3	74.1	63.2
19.87	70.2	77.4	66.3
20.91	73.2	80.5	69.4
21.96	75.8	83.2	72.1
23.00	78.3	86.0	74.8
24.05	80.4	88.6	77.5
25.09	83.0	91.0	79.8
26.14	85.4	93.4	82.1
27.18	87.6	95.6	84.2
28.23	90.1	97.7	86.0

OECD 301B - Biodegradation
Sample 2 -
Sodium Acetate



Time (days)	% ThCO2		
X	A:Y1	A:Y2	A:Y3
0.00	0.0	0.0	0.0
1.05	0.0	0.0	0.0
2.09	12.4	8.0	9.1
3.14	23.1	15.6	18.1
4.18	32.7	23.2	26.9
5.23	41.4	30.7	35.4
6.27	49.3	37.4	43.1
7.32	54.1	41.6	47.7
8.36	82.8	67.2	75.3
9.41	85.9	70.3	78.7
10.46	88.6	73.2	82.0
11.50	91.2	76.0	85.1
12.55	93.5	78.6	87.9
13.59	95.6	80.8	90.6
14.64	97.5	82.8	93.1
15.68	99.2	84.7	95.4
16.73	100.8	86.5	97.7
17.77	102.3	88.2	99.7
18.82	103.8	89.7	101.6
19.87	105.2	91.2	103.3
20.91	106.5	92.5	104.9
21.96	107.7	93.8	106.3
23.00	108.7	94.9	107.5
24.05	109.9	96.3	108.8
25.09	110.9	98.0	110.2
26.14	111.9	100.5	111.8
27.18	112.8	104.7	113.8
28.23	113.7	111.6	116.9