

Customer Report

Biodegradability Testing

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prepared for:

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Antimicrobials ○ Biodegradation ○ Toxicology ○ Analytics ○ Product Development

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Table of Contents

Compliance.....	3
Abstract	3
Results and Discussion.....	3
Result Tables and Images.....	4
Introduction.....	8
Materials and Methods.....	8
Project (Item) List.....	10
Reference Substances.....	10
Reagents.....	10
Equipment List.....	10
Calculations.....	11
Statistical Methods.....	11
Report Compliance.....	12
Addendum.....	13

Compliance Statement

Testing is conducted according to the required criteria established for ISO 17025 Accredited laboratories. The laboratory is independently audited, verifying this compliance.

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. 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.

Abstract

The ASTM D5864 method is designed to determine the aerobic aquatic biodegradation of fully formulated lubricants or their components under laboratory conditions. A study was conducted to determine the biological degradation of the provided test item according to the ASTM D5864. Total carbon of the test substance is determined analytically for each sample and used as the reference for determining the percentage of carbon dioxide (% ThCO₂) produced by microbiological degradation.

Successful Biodegradability can be achieved when 60% (>=) of ThCO₂ is removed within 28 days of testing.

Results and Discussion

Results are provided in the Result Data Tables

The determined biodegradability of test **sample 1** was 87% ThCO₂ in a laboratory scale evaluation of the test sample under aerobic conditions.

The determined biodegradability of test **sample 2** was 82% ThCO₂ in a laboratory scale evaluation of the test sample under aerobic conditions.

Report Result Tables

Sample List

Sample #	Method Name	Sample Name	Sample Notes
ASTM D5864 - Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components			
1		ElectroCool EC-110 Coolant	
2		AmpCool AC-110 Coolant	
3		Positive Control	
Project - Images			
1		ElectroCool EC-110 Coolant	
2		AmpCool AC-110 Coolant	

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

Sample # 1 ElectroCool EC-110 Coolant

	<u>Interval</u>	<u>Result</u>
Inoculum: <i>Environmental Culture (activated sludge)</i> ()		
<i>Notes Section</i>		
Sample biodegradability	28 day	87 % ThCO ₂
sample as received	0 day	99 % Total Carbon
sample concentration at T0	0 day	20 ppm
initial pH	0 day	7.4 pH
final pH	28 day	7 pH
initial bacteria concentration	0 day	10300 CFU/mL
final bacteria concentration	28 day	13000 CFU/mL

Sample # 2 AmpCool AC-110 Coolant

	<u>Interval</u>	<u>Result</u>
Inoculum: <i>Environmental Culture (activated sludge)</i> ()		
<i>Notes Section</i>		
Sample biodegradability	28 day	82 % ThCO ₂
sample as received	0 day	99 % Total Carbon
sample concentration at T0	0 day	20 ppm
initial pH	0 day	7.4 pH
final pH	28 day	7 pH
initial bacteria concentration	0 day	10300 CFU/mL
final bacteria concentration	28 day	21000 CFU/mL

Sample # 3 Positive Control

	<u>Interval</u>	<u>Result</u>
Inoculum: <i>Environmental Culture (activated sludge)</i> ()		
<i>Notes Section</i>		
Control biodegradability	28 day	91 % ThCO ₂
sample as received	0 day	28 % Total Carbon
sample concentration at T0	0 day	20 ppm
initial pH	0 day	7.4 pH
final pH	28 day	7 pH
initial bacteria concentration	0 day	10300 CFU/mL
final bacteria concentration	28 day	11000 CFU/mL

Report Image

Sample # 1 ElectroCool EC-110 Coolant

Test Method Project - Images

Inoculum None

Image: sample

Timepoint: time - 0



Report Image

Sample # 2 AmpCool AC-110 Coolant**Test Method** Project - Images**Inoculum** None**Image:** sample**Timepoint:** time - 0

Introduction

Sample testing was conducted according to the list method following the specification of the laboratory ISO 17025 standards.

Additional information can be provided as needed to supplement the understanding of the ISO 17025 compliance requirements.

Additional testing and method information is provided for general reference.

Recommended Reading Online Technical Resources

<http://www.situbiosciences.com/biodegradation/oecd-301b-biodegradation-test-co2-evolution/>

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

<http://www.situbiosciences.com/biodegradation-testing-overview/>

Methods

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

This method exposes the test material to a mixed microbial population derived from an activated sludge inoculum mixed with a nutrient medium to support microbial growth. The mixture is then incubated in a controlled environment with adequate aeration and temperature. During the incubation period, the biodegradation of the test material is monitored by measuring the production of carbon dioxide (CO₂) as an indicator of microbial activity. The evolved CO₂ is captured and quantified by infrared analysis. The extent of biodegradation is determined by comparing the amount of CO₂ produced by the test material to that produced by a reference substance, usually a known biodegradable compound. The biodegradation percentage is calculated based on the difference in CO₂ production between the test material and the reference substance.

Project - Images

Project images are provided for the submitted test samples. Images are taken for the samples as received to provide a reference for the materials submitted for testing. The provided images may or may not indicate other aspects of the sample condition but no analysis or inspection of the sample is conducted unless otherwise specifically noted in the project report.

Project List	(notes)
1	ElectroCool EC-110 Coolant
2	AmpCool AC-110 Coolant
3	Positive Control
4	Blank

Procedures

ASTM D5864

Inoculum

Test organisms were derived a municipal wastewater treatment facility. The total solids from the settled inoculum was less than 1%, and the bacteria concentration was determined by plating on TSA plates. A plate count was determined at the beginning and end of testing.

Total Carbon

The total carbon of the test sample was determined via catalytically aided combustion oxidation.

Biodegradation

Carbon dioxide concentration was measured at approximately 24-hour intervals. The test substance was added to 500 ml glass jars containing mineral medium. The test substance was directly added to the medium by weight on polystyrene sheets (approximately 20x20 mm). Three replicates of the test, control, and blank, were created. Samples were placed at 22 +/- 2 C in low-filtered light for the test duration. Following each measurement cycle, the test jars are purged with CO2 free.

The test system pH was determined at the beginning and the end of testing.

Reference Substance

Name: Sodium Benzoate

CAS: 532-32-1

Name: Sodium Acetate

CAS: 127-09-3

Name: Potassium phthalate crystals

CAS: 877-24-7

Name: Sodium Carbonate

CAS: 497-19-8

Name: Sodium Bicarbonate

CAS: 4144-55-8

Name: Dextrose

CAS: 50-99-07

Standards

pH 4
pH 10

Media Salts

Ammonium chloride
CAS: 12125-02-9

Sodium phosphate dibasic dihydrate
CAS: 10028-24-7

Potassium Phosphate Monobasic, anhydrous
CAS: 7778-77-0

Potassium phosphate dibasic, trihydrate
CAS: 16788-57-1

Equipment List

Laboratory and analytical balances
Laboratory Glassware
Laboratory Pipettes
Thermometers
Temperature data loggers
pH probe
Incubator
Spiral plater
Stir plate
Laboratory water
Carbon Analyzer

Test Organisms (by Method) *(Inventory ID / lot #)*

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

municipal sludge isolate; Dupage WW

AS053112

Calculations

Variable Slope Curve Fit:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogA} - X) * \text{HillSlope})})$$

A is the day 50% DOC is consumed measured at halfway between Bottom and Top. This is not the same as the response at Y=50.

HillSlope describes the steepness of the family of curves. It is derived from the curve fit analysis for the dataset and is a commonly used constant for first-order biological kinetics.

Top and **Bottom** are plateaus in the units of the Y-axis, (top = maximum %ThCO₂, bottom = net CO₂ production at time 0).

Statistical Methods

Replicate data were averaged by determination of the mean value (arithmetic average) for a selected measurement in the following manner:

$$A = S/N$$

A = average (or arithmetic mean)

S = the sum of the numbers in the set of interest (e.g., the sum of the numbers being averaged)

N = the number of terms (e.g., the number of items or numbers being averaged)

Standard deviation is a measurement of the variation around the Mean value. Built-in functions for Standard Deviation were performed within the analytic software used for calculating these values.

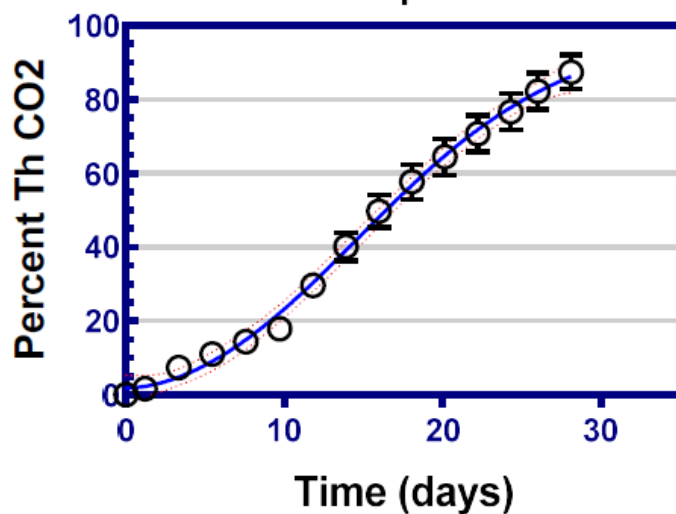
* This report is governed by and incorporates by reference the conditions of testing as posted on the date of issuance and is intended for the identified Project Owners exclusive use. Any copying or replication of this report to or for any other person or entity, or use of our company name or Service 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. The 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 the 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

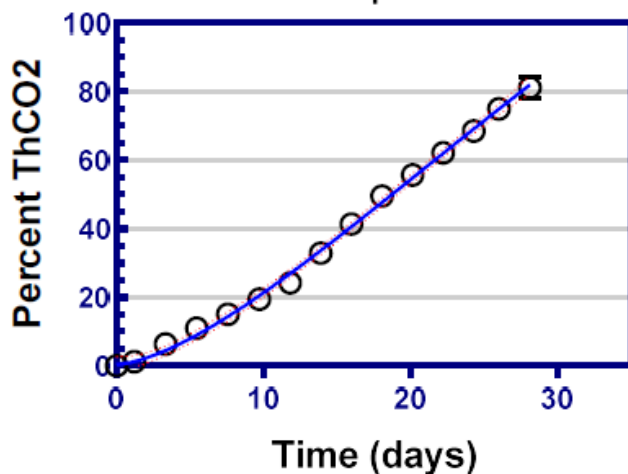
ASTM D5864 - Biodegradation Sample 1



Best-fit values	
S	0.4717
EC50	17.10
HillSlope	3.852
Top	103.8
Bottom	1.819
logEC50	1.233
Std. Error	
S	0.4372
EC50	1.263
HillSlope	2.763
Top	23.80
Bottom	1.744
95% CI (asymptotic)	
S	0.000 to 1.341
EC50	14.59 to 19.61
HillSlope	-1.644 to 9.347
Top	56.48 to 151.2
Bottom	-1.649 to 5.288
logEC50	1.164 to 1.293
Goodness of Fit	
Degrees of Freedom	82
R squared	0.9674
Sum of Squares	2523
Sy.x	5.546
Constraints	
S	S > 0
Number of points	
# of X values	87
# Y values analyzed	87

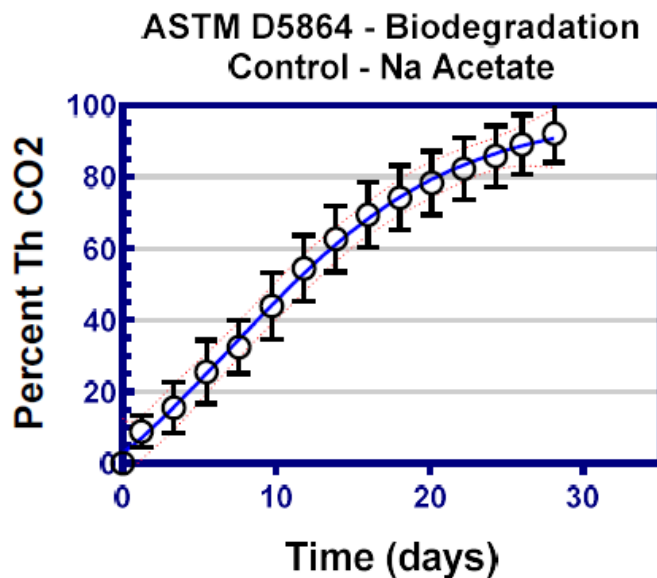
Time (days)	% ThCO2		
X	A:Y1	A:Y2	A:Y3
0.00	0.0	0.0	0.0
0.16	-2.1	0.1	-0.2
1.21	1.4	0.0	3.4
2.28	5.1	3.5	6.5
3.34	5.8	7.8	8.4
4.40	9.4	11.1	8.4
5.46	9.4	11.9	11.8
6.52	12.3	15.8	11.7
7.58	12.1	16.3	14.6
8.65	13.6	20.5	14.6
9.71	13.7	21.6	18.3
10.77	19.4	28.6	23.7
11.81	24.8	35.2	28.9
12.85	29.8	41.4	33.8
13.90	34.4	47.3	38.6
14.94	38.8	52.9	43.3
15.98	43.0	58.2	48.0
17.02	47.1	63.1	52.6
18.05	50.2	66.7	56.1
19.10	53.5	70.2	59.6
20.14	56.7	73.6	63.0
21.18	59.8	76.8	66.3
22.22	62.8	79.9	69.5
23.26	65.7	82.9	72.6
24.30	68.6	85.7	75.6
24.97	71.5	88.4	78.5
26.01	74.3	91.0	81.2
27.06	77.1	93.5	83.9
28.10	79.8	95.8	86.5

ASTM D5864 - Biodegradation Sample 2



Best-fit values	
Bottom	0.4066
Top	318.2
IC50	56.12
HillSlope	1.538
logIC50	1.749
Span	317.8
Std. Error	
Bottom	1.134
Top	174.5
IC50	30.52
HillSlope	0.1777
Span	175.2
95% CI (asymptotic)	
Bottom	-1.848 to 2.661
Top	-29.00 to 665.3
IC50	-4.572 to 116.8
HillSlope	1.184 to 1.891
logIC50	-infinity to 2.068
Span	-30.62 to 666.1
Goodness of Fit	
Degrees of Freedom	83
R squared	0.9830
Sum of Squares	1038
Sy.x	3.536
Number of points	
# of X values	87
# Y values analyzed	87

Time (days)	% ThCO2		
X	A:Y1	A:Y2	A:Y3
0.00	0.0	0.0	0.0
0.16	-2.1	-2.1	-2.1
1.21	1.0	1.0	1.6
2.28	4.3	1.8	1.6
3.34	8.2	5.8	5.1
4.40	11.7	9.0	5.6
5.46	14.6	9.2	9.1
6.52	15.2	12.4	12.6
7.58	17.8	15.0	12.3
8.65	18.4	18.3	15.7
9.71	22.1	20.6	15.7
10.77	22.1	20.6	15.7
11.81	27.4	24.6	20.5
12.85	32.4	28.3	25.0
13.90	37.2	31.9	29.6
14.94	41.8	35.3	34.3
15.98	46.2	38.6	39.3
17.02	50.3	41.9	44.2
18.05	54.3	45.3	49.0
19.10	57.3	47.9	52.5
20.14	60.4	50.7	56.1
21.18	63.4	53.6	59.7
22.22	66.5	56.6	63.2
23.26	69.6	59.8	66.6
24.30	72.8	62.9	70.0
24.97	75.9	66.1	73.3
26.01	79.1	69.3	76.4
27.06	82.5	72.4	79.3
28.10	86.0	75.5	82.2



Best-fit values	
S	0.3355
EC50	11.40
HillSlope	3.343
Top	99.83
Bottom	2.955
logEC50	1.057
Std. Error	
S	0.5266
EC50	1.282
HillSlope	4.087
Top	24.95
Bottom	4.831
95% CI (asymptotic)	
S	0.000 to 1.383
EC50	8.848 to 13.95
HillSlope	-4.787 to 11.47
Top	50.20 to 149.5
Bottom	-6.656 to 12.57
logEC50	0.9468 to 1.145
Goodness of Fit	
Degrees of Freedom	82
R squared	0.8624
Sum of Squares	11877
Sy.x	12.04
Constraints	
S	$S > 0$
Number of points	
# of X values	87
# Y values analyzed	87

Time (days)	% ThCO ₂		
X	A:Y1	A:Y2	A:Y3
0.00	0.0	0.0	0.0
0.16	5.9	4.0	4.2
1.21	17.8	4.7	4.1
2.28	17.8	4.7	11.7
3.34	28.9	5.3	12.4
4.40	38.4	14.0	19.4
5.46	43.1	14.0	19.4
6.52	46.5	13.9	24.8
7.58	46.0	20.6	30.7
8.65	51.7	20.9	30.7
9.71	62.4	33.0	36.5
10.77	68.1	38.8	41.7
11.81	73.0	43.9	46.5
12.85	77.3	48.4	50.7
13.90	81.1	52.5	54.6
14.94	84.6	56.1	58.1
15.98	87.7	59.3	61.3
17.02	89.9	61.7	63.7
18.05	92.2	64.1	66.2
19.10	94.2	66.3	68.5
20.14	96.1	68.4	70.7
21.18	98.0	70.4	72.8
22.22	99.7	72.3	74.9
23.26	101.3	74.1	76.9
24.30	102.8	75.9	78.8
24.97	104.3	77.6	80.7
26.01	105.6	79.3	82.5
27.06	106.9	80.9	84.3
28.10	108.1	82.4	85.9