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Microbial Product Testing
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Antimicrobials
Biodegradation
Discovery

Customer Report

Friday, June 14, 2013

CONTACT

Sutherland Products Inc.
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Morgan Sutherland
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Project Title

Biodegradation Test

ID **0413-ALK-01 -- 1** **r.** Entry Date 4/22/2013

Project Summary

The OECD 301B method is designed to provide the screening of chemicals for ready biodegradability in an aerobic aqueous medium. Samples are required to achieve a threshold of 60% degradation based on the maximum available carbon from a given sample formulation. Total carbon is determined analytically for each sample and used as the reference for the determination of the percentage of carbon dioxide (% ThCO2) produced by microbiological degradation.

For the purpose of determining biological degradation, two criteria can be achieved. Ready Biodegradability can be achieved by obtaining the 60% threshold within a 10 day window within a total of 28 days of testing. The second criteria, Ultimate Biodegradation, can be achieved if the amount of biodegradation meets or exceeds the 60% threshold at a time point determined in the test (e.g. when the rate of degradation reaches a plateau).

Four test samples were submitted for OECD 301B biodegradation testing (**CS13, CS13B, LS12, and PS03**). Test sample one achieved the required level of degradation for Ready Biodegradation according to the requirements of the OECD 301B standard by day 14 of the test. Test sample 2 to 4 achieved Ultimate Degradation by exceeding the 60% degradation threshold (see data table) at days 17, 24 and 32 respectively. Each test sample achieved the degradation plateau by the termination of the testing at day 41.

Each of the 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). The determination of the maximum degradation were derived from the curve fit and indicated on each graph. Sodium acetate is used as the positive control for the degradation testing.

Sample List

Method Name

Sample #	*Sample Name*	*Sample Notes*
OECD 301 B - Solution Biodegradation by CO2 Evolution		
1	CS13	
2	CS13-B	
3	LS12	
4	PS03	
5	Control - Sodium Acetate	

Result Table

Contact	Sutherland Products Inc.	Morgan Sutherland	336-949-4527
Title	Biodegradation Test		
Project ID	**0413-ALK-01 -- 1** **r.**	Entry Date 4/22/2013	Test Start Date 4/22/2013

Result Table *

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

Sample # **1** **CS13**

		Interval	Result
Inoculum	***Mixed Environmental Organisms ()***		
pH final 7.4; 10 day window - 4 to 14;		**14** day	**60** % ThCO2
ThCO2% plateau achieved; ThCO2 max = 95%		**38** day	**92** % ThCO2

Image: **Summary Graph**

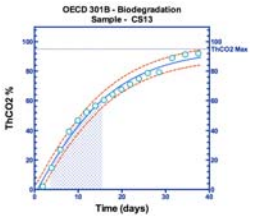


Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Sample # **2** **CS13-B**

		Interval	Result
Inoculum	***Mixed Environmental Organisms ()***		
pH final 7.4;		**17** day	**60** % ThCO2
ThCO2% plateau achieved. ThCO2% Max = 90%		**38** day	**82** % ThCO2

Image: **Summary Chart**

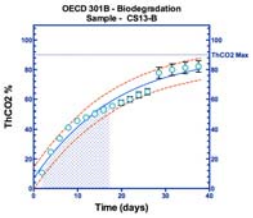


Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Result Table *

Sample # 3 **LS12**

	Interval	Result
Inoculum ***Mixed Environmental Organisms ()***		
pH final 7.4;	**24** **day**	**60** **% ThCO2**
ThCO2% plateau achieved. ThCO2% Max = 75%	**42** **day**	**72** **% ThCO2**

Image: **Summary Chart**

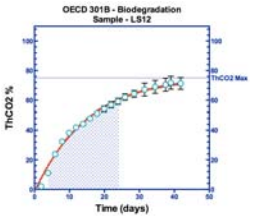


Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Sample # 4 **PS03**

	Interval	Result
Inoculum ***Mixed Environmental Organisms ()***		
pH final 7.6;	**32** **day**	**60** **% ThCO2**
ThCO2% plateau achieved. ThCO2% Max = 70%	**42** **day**	**72** **% ThCO2**

Image: **Summary Chart**

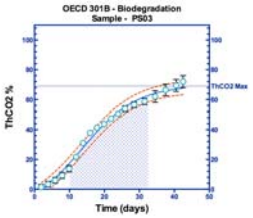


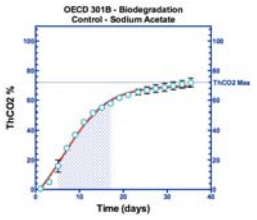
Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Result Table *

Sample # 5 **Control - Sodium Acetate**

	Interval	Result
Inoculum ***Mixed Environmental Organisms ()***		
pH final 7.8; 10d window 5 to 15	**16** day	**60** % ThCO2
ThCO2% plateau achieved. ThCO2% Max = 72%	**35** day	**72** % ThCO2

Image: **Summary Chart**



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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.

d p satchell

Don Satchell Ph.D., Technical Manager

Report Addendum

Friday, June 14, 2013

Project ID	0413-ALK-01 -- 1	Entry Date	4/22/2013	Test Start Date	4/22/2013

Image Table

Sample #	1	CS13

Test Method	OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Mixed Environmental Organisms*

Image: **Summary Graph**

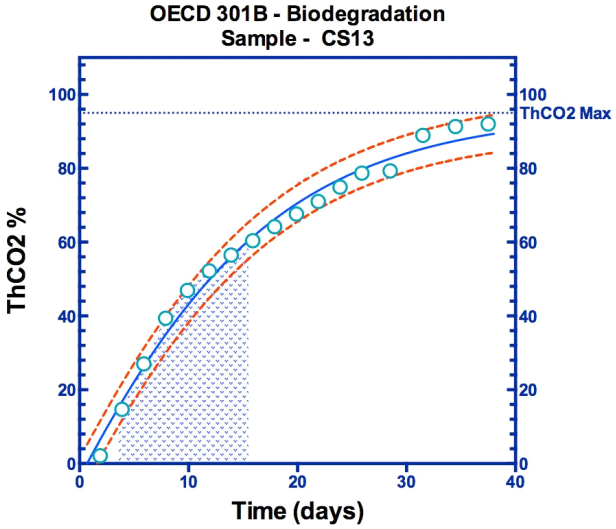

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Image Table

Sample #	2	CS13-B
Test Method		OECD 301 B - Solution Biodegradation by CO2 Evolution
Inoculum		*Mixed Environmental Organisms*

Image: **Summary Chart**

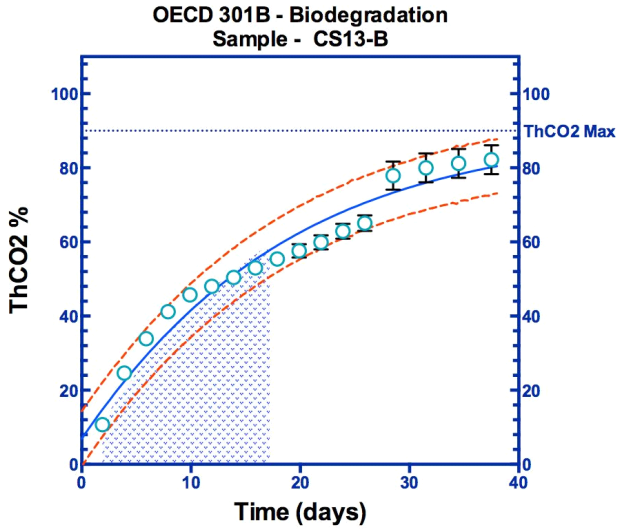

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Image Table

Sample #	3	LS12
Test Method		OECD 301 B - Solution Biodegradation by CO2 Evolution
Inoculum		*Mixed Environmental Organisms*

Image: **Summary Chart**

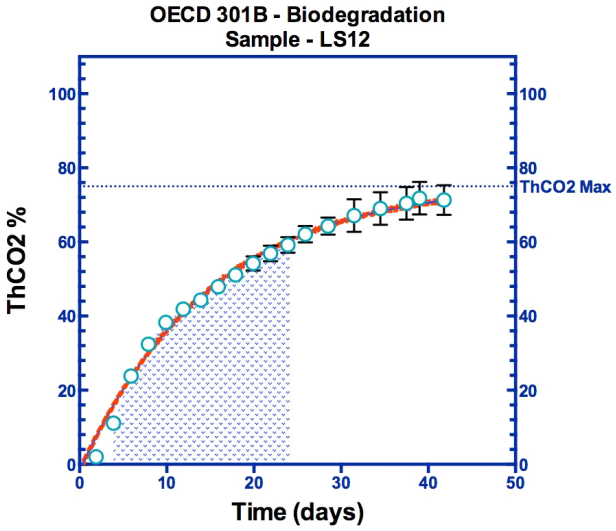

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Image Table

Sample #	4	PS03
Test Method		OECD 301 B - Solution Biodegradation by CO2 Evolution
Inoculum		*Mixed Environmental Organisms*

Image: **Summary Chart**

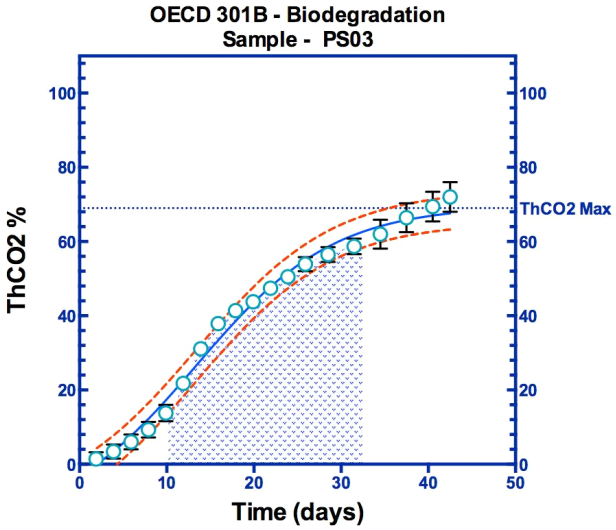

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*

Image Table

Sample #	5	Control - Sodium Acetate
Test Method		OECD 301 B - Solution Biodegradation by CO2 Evolution
Inoculum		*Mixed Environmental Organisms*

Image: **Summary Chart**

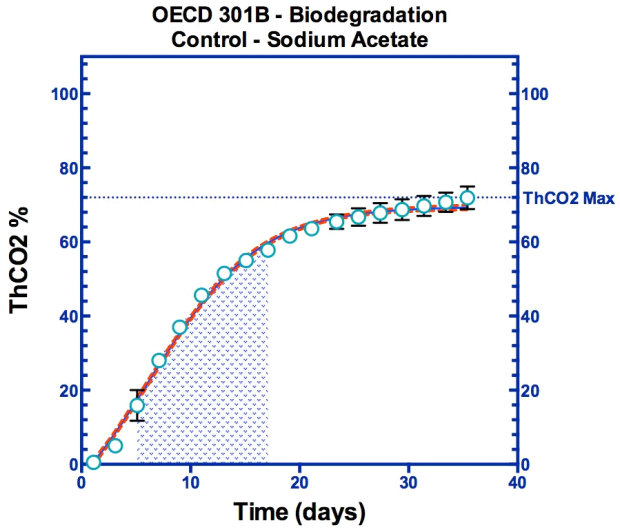

Figure *- Test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2) derived from the test sample. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line) and 95% confidence (red) boundary lines. Shading below the curve fit applies to the biodegradation window (10 to 60%) for the determination of Ready or Ultimate Biodegradability.*