SiTU Biosciences LLC
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Microbial Product Testing
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Customer Report

Thursday, April 10, 2014

CONTACT

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Project Title

Biodegradation Testing

ID **0214-AWF-01 -- 1** **r.** Entry Date 2/25/2014

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).

One test sample was submitted for OECD 301B biodegradation testing.
The respective summary result is listed here (see data tables and charts):

Sample 1 Blue Ocean WashBatch #4402MS14 – achieved the requirements for Ready Biodegradability by the OECD 301B standard.

Sample List

Method Name

Sample #	*Sample Name*	*Sample Notes*
OECD 301 B - Solution Biodegradation by CO2 Evolution		
1	Blue Ocean Wash Batch No: 4402MS14	
2	NaAc Control	

Result Table

Contact	Blue Ocean Oil Supply	Hannes Hattingh	
Title	Biodegradation Testing		
Project ID	0214-AWF-01 -- 1 r.	Entry Date 2/25/2014	Test Start Date 2/25/2014

Result Table *

Test Method OECD 301 B - Solution Biodegradation by CO2 Evolution

Sample # 1 **Blue Ocean Wash Batch No: 4402MS14**

	Interval	Result
Inoculum *Mixed Environmental Organisms ()*		
final ultimate degradation	28 day	98.5 % ThCO2
final sample pH	28 day	7.5 pH
Biodegradation; window = day 3.5 to day 13	8.5 day	Achieved
Image: Sample		

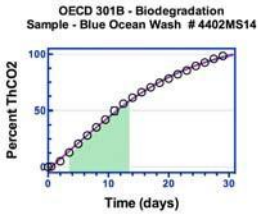


Figure *– The sample graph shows the test chamber carbon dioxide (CO2) measurement as the percent of theoretical maximum (% ThCO2). Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Curve fit is applied to calculate the predicted fit (blue line). Shading below the curve fit applies to the biodegradation requirement (10 to 60% TOC) for the determination of Ready Biodegradability and shows the required degradation within the 10 day-window to achieve Ready Biodegradation of the test sample.*

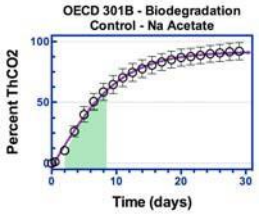
Sample TC = 3.8%	0 TOC TEST	20 mg/L TOC

Result Table *

Sample # 2 **NaAc Control**

	Interval	Result
Inoculum ***Mixed Environmental Organisms ()***		
final ultimate degradation	**28** day	**91.2** % ThCO2
final sample pH	**28** day	**7.5** pH
Biodegradation; window = day 2 to day 8.5	**8.5** day	**Achieved**

Image: **Control**



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	0 TOC TEST	**20** mg/L TOC

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.

Report Addendum

Thursday, April 10, 2014

Project ID	0214-AWF-01 -- 1	Entry Date 2/25/2014	Test Start Date 2/25/2014

Image Table

Sample #	1	Blue Ocean Wash Batch No: 4402MS14

Test Method	OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Mixed Environmental Organisms*

Image: Sample

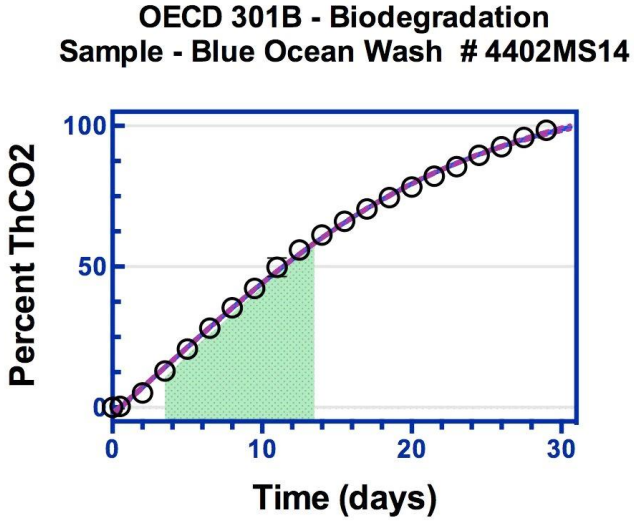

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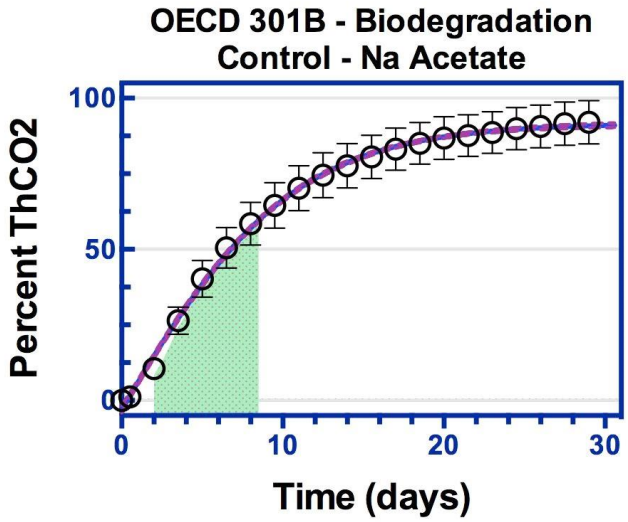
Image Table

Sample #	2	NaAc Control

Test Method	OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Mixed Environmental Organisms*

Image: Control



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