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Project Title

Biodegradation of phase change products

ID **0611-AFG-01-- 1** Entry Date 6/14/2011

Project Summary

Overview: One homogenous test liquid was submitted for OECD 301B Biodegradation testing.

Results: The product formulation as defined by the manufacturer was used to calculate the theoretical carbon dioxide (ThCO₂) expected from the test sample and designated Sample 1 (PureTemp 4). Test sample is a homogeneous hydrophobic compound. The control material was selected from known readily biodegradable vegetable oils (rapeseed oil). The test sample demonstrated ready biodegradability as being met according to the conditions set forth in OECD 301B by day 4.7 and ultimately demonstrated 65% +/-5% biodegradation at the plateau of the biodegradation analysis by day 15 of the test timeframe (see figures). The control sample achieved 60% biodegradation on day 10 of the test with marginal increase up to day 18. Both 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.

Although demonstrating ready biodegradability the control sample biodegradability was below expected values (80 to 90%) at the plateau of the biodegradation analysis. As a naturally derived product some variability is expected and additional analysis is being conducted to determine batch to batch variability.

Method Name

<i>Sample #</i>	<i>Sample Name</i>	<i>Sample Notes</i>
OECD 301 B - Solution Biodegradation by CO₂ Evolution		
1	PureTemp 4	3 replicates
2	Reference Control (rapeseed oil)	3 replicates

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, 22C +/- 2C
- bacterial inoculum ~1E5 cfu/ml
- Graph and curve fit by 3 parameter sigmoid curve [f = a/(1+exp(-(x-x0)/b))]

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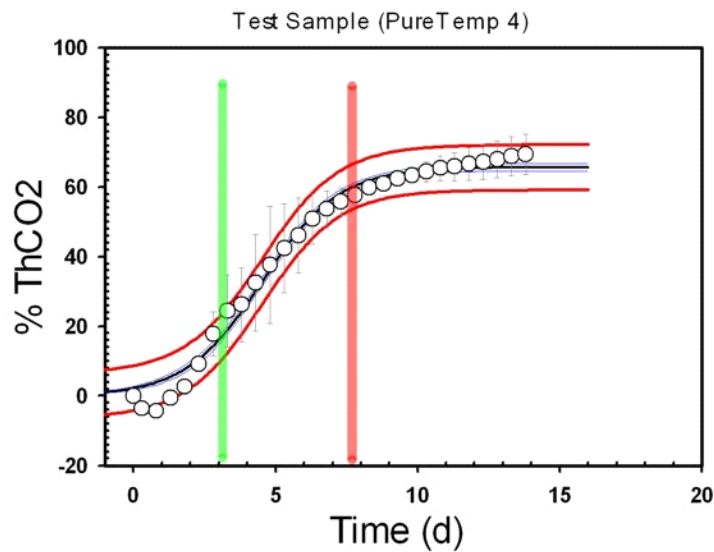
Image Table

Sample # **1** PureTemp 4

Test Method OECD 301 B - Solution Biodegradation by CO2 Evolution

Inoculum *Mixed Bacteria*

Biodegradation Time Course



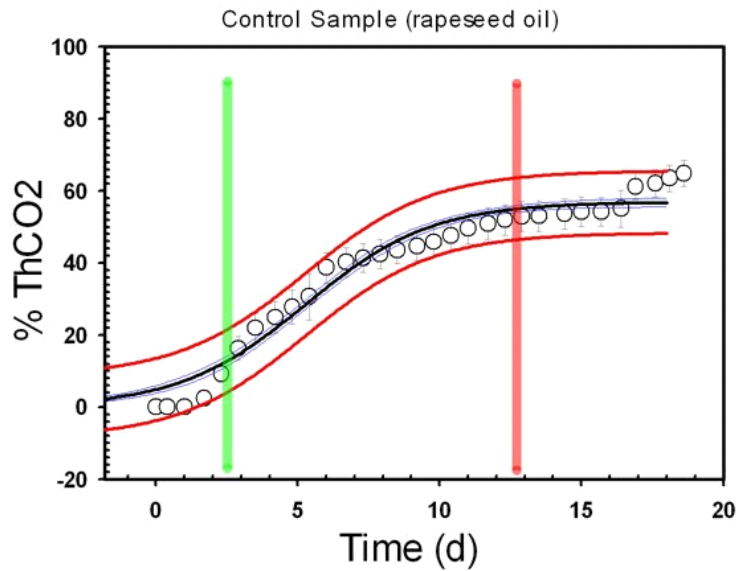
Sample achieved 60% ThCO₂ at 4.7 days from lag phase transition (green bar to red bar). No observed growth in abiotic sample or attenuation in test of (sample/control - 50:50) sample mix. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Sigmoid function, (S-shaped) curve fit is applied to calculate the statistical confidence for 99% (blue) and 95% (red) boundary lines.

Theoretical CO2 Calculation Table

PureTemp 4	component and total wt	Test Target DOC or TOC	Amount to add (g) based on formulation content			
Enter Target		0.020	0.0075			
Calculated Values	Test Sample Components	wt% contribution	Moles O2 required	Moles/g sample	ThOD (used) g	ThCO2 (produced) g
	PureTemp 4	0.020000	592.0	29600.0	0.0553	0.0200
					0.0000	0.0000
					0.0000	0.0000
					0.0000	0.0000
	100% Target			29600.0000	0.0553	0.0200
	90% Target				0.0498	0.0180
	60% Target				0.0332	0.0120

Sample #	2	Reference Control (rapeseed oil)
Test Method	OECD 301 B - Solution Biodegradation by CO2 Evolution	
Inoculum	Mixed Bacteria	

Biodegradation Time Course



Sample achieved 60% ThCO2 at 10 days from lag phase transition (green bar to red bar). Expected control sample biodegradation is below expected levels and is being reviewed with the material supplier. Average values are plotted with the standard deviation (+/- SD) for the time course of the test. Sigmoid function, (S-shaped) curve fit is applied to calculate the statistical confidence for 99% (blue) and 95% (red) boundary lines.

Image Table

Theoretical CO2 Calculation Table

Rapeseed (Canola)	Sample component and total wt	Test Target DOC or TOC	Amount to add (g) based on formulation content			
Enter Target		0.020	0.0071			
Calculated Values	Test Sample Components	wt% contribution	Moles O2 required	Moles/g sample	ThOD (used) g	ThCO2 (produced) g
	Palmitic - C16:0	0.00094	736.0	36800.0	0.0575	0.0009
	Stearic - C18:0	0.00036	832.0	41600.0	0.0586	0.0004
	Oleic - C18:1	0.01260	816.0	40800.0	0.0579	0.0126
	Linoleic - C18:2	0.00400	800.0	40000.0	0.0571	0.0040
	Linolenic - C18:3	0.00172	784.0	39200.0	0.0564	0.0017
	Eicosenoic - C20:1	0.00038	912.0	45600.0	0.0588	0.0004
	Erucic - C22:1	0.00000	992.0	49600.0	0.0590	0.0000
	100% Target	0.0200		293600.0	0.4054	0.0200
	90% Target				0.3649	0.0180
	60% Target				0.2432	0.0120