Biodegradability Summary
ASTM D 5864-00 Report

Test Organization: Renewable Lubricants, Inc.
Test Operator: Aaron R. Harnar, Biochemist
Test Samples: RLI Bio-Hydraulic Formulations / Chevron Phillips Chemical PAO
Test Start Date: March 13, 2003
Test End Date: April 10, 2003

Inoculum Background

A sewage sludge sample for use in this test was obtained from the Hiram Wastewater Treatment Dept., Hiram OH 44234 (reference: Ken Young, Treatment Plant Manager, (330) 569-7860) March 11, 2003. The crude sludge sample was aerated overnight for inoculation the following day. After the sample had been allowed to settle for at least three hours, the 3L biodegradation flasks were inoculated with the recommended 30 ml of sludge supernatant on March 12. No inoculum pre-adaption techniques were utilized to enhance biodegradability results, with the exception two weeks prior to officially starting the test, sludge microorganisms were pre-exposed to the Bio-Ultimax ISO 68 test sample in order to enhance results as part of an optional inoculum pre-adaptation technique listed in ASTM 5864 Sec 8.3.1.

APHA Standard 9215 C. Heterotrophic Plate Count, Spread Plate Method, was followed to verify presence of live microorganism in sludge supernatant. Three days into the test, live inoculum subpopulations could be measured and were found to be around 250000 cfu/ml. This is significantly less than the ASTM recommended population of 10^6 cfu/ml and is believed to be due to decreased flow in the Hiram Wastewater Treatment Dept. during the Hiram College spring break.

Reference Substance

High oleic canola oil was listed as the biodegradable control for use with the water insoluble test formulations studied in this test. For additional references go to www.renewablelube.com Lab Information, Biobased and Biodegradable Testing, view Understanding Biobased/Biodegradable and the Industry's Standard Tests and Definitions.
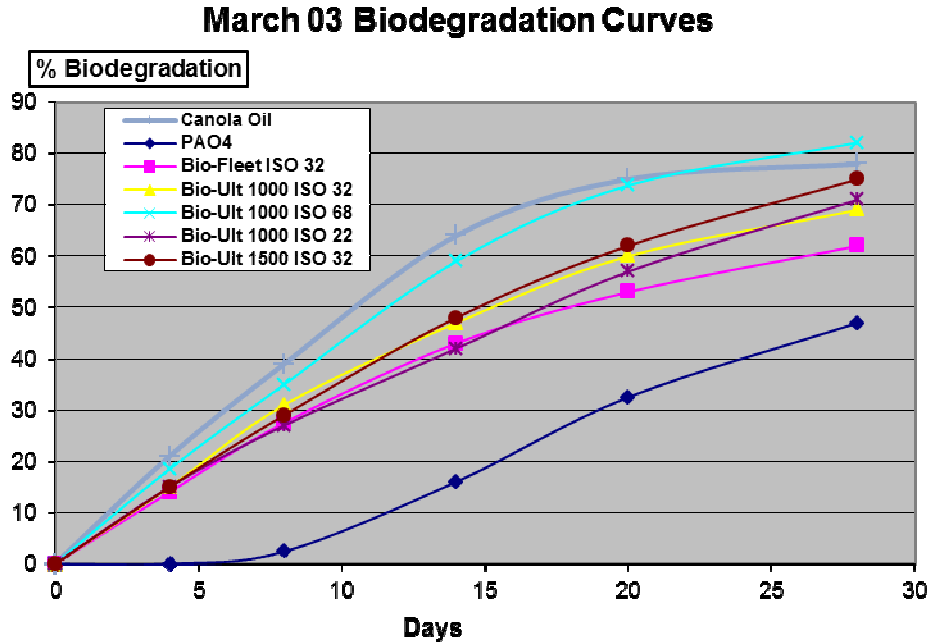http://www.renewablelube.com/lab.html

Reference and Test Substance Carbon Content

Robertson Microlit Laboratories, Inc., (29 Samson Ave., PO Box 927, Madison, NJ 07940, (973) 966-6668), measured the carbon content of the canola oil biodegradable control, five biobased hydraulic formulations and a PAO4 synthetic base oil tested in this study according to the procedure set forth in ASTM D 5291-02.

Substance ID	Carbon Content (%)
Canola oil	77.42
Bio-Ultimax ISO 32	81.59
Bio-Ultimax ISO 68	82.13
Bio-Fleet ISO 32	81.59
Bio-Ultimax ISO 22	81.97
Bio-Ultimax 1500 ISO 32	80.83
PAO4	85.62

Biodegradability Results

   By day 28 of the test, all test formulations except PAO4 exceeded the 60% biodegradation requirement of this test and could be deemed ASTM Pw1. A visual summary of each formulation showing the percent of biodegradation and demonstrating the biodegradation trends over the 28-day test period is provided below.

March 03 Biodegradation Curves

% Biodegradation

Legend:
- Canola Oil
- PAO4
- Bio-Fleet ISO 32
- Bio-Ult 1000 ISO 32
- Bio-Ult 1000 ISO 68
- Bio-Ult 1000 ISO 22
- Bio-Ult 1500 ISO 32

X-axis: Days
Y-axis: 0 to 90

Biodegradability Ratings

   ASTM D-5864 requires that the sample fluid should have at least a 60% yield of carbon dioxide (of the total measured carbon content) within 28 days for the "Ultimate Biodegradability Pw1" rating. Biodegradability ratings are identified in ASTM D-6046 (Standard Classification of Hydraulic Fluids for Environmental Impact). However, the Degradation/Accumulation Expert Group of the OECD Environment Committee classifies samples as" Inherently Biodegradable" if they demonstrate biodegradability greater than 20% in 28 days.

   In the tests, all five biobased hydraulic formulations had exceeded 60% biodegradation before 28 days. These materials can therefore be classified as Pw1, Ultimately Biodegradable or Readily Biodegradable according to ASTM D-6046. The OECD Environment Committee would classify PAO 4 as **inherently biodegradable** because it demonstrated biodegradability greater than 20% in 28 days. The Bio-Ultimax 1000 68 Hydraulic Fluid provided the best Biodegradability comparative to the canola oil control and this improvement over previous studies could be credited to the optional inoculum pre-adaptation technique used in this test.