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Determination of final biodegradability. OEDC 301F ¨manometric respirometry method¨.

Report

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2 Introduction

Elizabeth Dominguez from the Olipes company sent us a lubricant called Maxifluid BBO to determine its final biodegradability using the OECD 301F method. This report includes the study of the biodegradability in aqueous medium of the Olipes lubricant.

Figure 1. Maxifluid BBO

According to the commission's decision of June 24, 2011, which establishes the ecological criteria for granting the European Union Ecolabel to lubricants, regarding biodegradability and toxicity, lubricants must comply with the requirements specified in criterion 3 (additional aquatic toxicity requirements) and in criterion 4 (biodegradability and bioaccumulation potential).

Summarizing, the afore mentioned criteria, the lubricant and their main components, must meet the following requirements regarding biodegradation.

- A substance will be considered as final biodegradable, if in the test of 28 days based on the decrease of oxygen or the generation of carbon dioxide the biodegradation is ≥ 60%.

There are several internationally recognized methods for evaluating the biodegradability of lubricants and their components. The OECD standard tests are those recognized in Europe.

In this report biodegradation is determined with the OECD 301F test.

3 Ready Biodegradability

3.1 Abbreviations and definitions

Primary Biodegradable	The alteration in the chemical structure of a substance, brought about by biological action, resulting in the loss of a specific property of that substance.
Ultimate Biodegradation (aerobic)	The level of degradation achieved when the test compound is totally utilised by micro-organisms resulting in the production of carbon dioxide, water, mineral salts and new microbial cellular constituents.
Readily Biodegradable	An arbitrary classification on chemicals which have passed certain specified screening test for ultimate biodegradability; these tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environments under aerobic conditions.
TC	Total carbon is the sum of the organic and inorganic carbon present in a sample
TOC	Total organic carbon of a sample is the sum of the organic carbon in solution and in suspension
TIC	Total inorganic carbon
IC	Inorganic Carbon
BOD	Biochemical oxygen demand (mg) is the amount of oxygen consumed during oxidation of a test compound with hot, acidic dichromate; it provides a measure of the amount of oxidable matter present; also expressed as mg oxygen consumed per mg of test compound
DOC	Dissolved organic carbon is the organic carbon present in solution or that which passes through a 0,45 µm filter or remains in the supernatant after centrifuging at approx. 4000 g (about 40.000 m/s^2) for 15 min.
DO	Dissolved oxygen (mg/l) is the concentration of oxygen dissolved in an aqueous sample
ThOD	Theoretical oxygen demand (mg) is the total amount of oxygen required to oxidise a chemical compound completely; it is calculated from the molecular formula and is expressed as mg required per mg of the test compound
ThCO2	Theoretical carbon dioxide (mg) is the quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test compound when fully mineralized; also expressed as mg of carbon dioxide evolved per mg test compound

3.2 Elemental analysis of Maxifluid BBO called lubricant.

For the determination of %C, %H, N% and O% of the sample, the TruSpec CHNS equipment from LECO has been used. It is an analyzer of carbon, nitrogen and total hydrogen in macro samples, both solid and liquid. The operating principle on which the instrument is based is that of the total combustion of the sample - Dumas Method, and subsequent determination of each element by means of independent detectors that eliminate the matrix effect and the use of chromatographic columns for the prior separation of the gases. In the TruSpec Analyzer the analysis cycle consists of three phases: purge, burn and detection.

Figure 2 - LECO TruSpec Elemental Analysis Analyzer.

3.2.1 Results

Sample	Mass (gr.)	% Nitrogen	% Carbon	% Hydrogen	% Oxygen
Maxifluid BBO	0,11782	0,022	77,9	12,4	9,67

3.3 Description of the biodegradation test, method OECD 301F

The Final Biodegradability has been determined with the OECD 301F test, “Manometric Respirometric” Method.
A measured volume of inoculated mineral medium, containing a known concentration of test substance (100 mg test substance/l giving at least 50-100 mg ThOD/l) as the nominal sole source of organic carbon, is stirred in a closed flask at a constant temperature (± 1 ºC or closer) for up to 28 days. The consumption of oxygen is determined either by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in the respirometer flask, or from the change in volume or pressure (or a combination of the two) in the apparatus. Evolved carbon dioxide is absorbed in a solution of potassium hydroxide or another suitable absorbent. The amount of oxygen taken up by the microbial population during biodegradation of the test substance (corrected for uptake by blank inoculum, run in parallel) is expressed as a percentage of ThOD or, less satisfactorily, COD. Optionally, primary biodegradation may also be calculated from supplemental specific chemical analysis made at the beginning and end of incubation, and ultimate biodegradation by DOC analysis.
Degradation is monitored over a period of 28 days, or longer, if necessary, by automatic or manual determination of oxygen consumption.).

The incubation of the containers should be carried out in the dark or with diffused lighting, at a temperature that is in the range between 20- 25º C and that is varied by more than ± 1ºC during the test.

- The respirometer used is the following:

Figure 3- Lovibon OxiDirect BOD System

- The system of sensors for BOD, OxiDirect allows the exact and comfortable measurement of Biochemical Oxygen Demand (BOD). The consumed oxygen is determined through a pressure reduction in the closed BOD measurement system.

Figure 4: Lovibon ET Fridge Thermostat

3.4 Inoculum

The inoculum used is activated sludge from aeration tank of WWTP (wastewater treatment plant) from Mungia. Mungia is a Spanish municipality in the historical territory of province of Bizkaia. Microbiological activity of the inoculum used, from the Mungia WWTP on 27/11/2023. Slide cultures have been carried out with the inoculum of activated sludge from the Mungia WWTP. The microbial load of the sample is determined by comparing the colony density on the culture slide with the attached standards.

Figure 5: There is a bacterial growth of 10 7 CFU/ml (colony forming units per ml)

3.5 Results obtained in the OECD 301F test.

OECD 301F, MANOMETRIC RESPIROMETRY TEST OF MAXIFLUID BBO

1. LABORATORY: Tekniker
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2. DATE AT START OF TEST: 28/10/2023

3. TEST SUBSTANCE:

Name: **Maxifluid BBO**
The sample has been evaluated 4 times at different concentrations
Initial concentration in medium: Sample 1, 62,30 mg/l and Sample 2, 50,82 mg/l.
Volume in test flask: 244 ml
Elemental Analysis= %C=77,9, %H= 12,4, %O= 9,67 and %N=0,022
ThOD= 2,97 mg O_2/ mg substance, using Elemental Analyzer

4. INOCULUM
Source: The effluent of the second stage (biological) or sewage treatment plant localized in Mungia, a town with 10.000 habitants.
Pick up date: 27/10/2023
Treatment given: Filtrated by paper of filter paper
Suspended solid concentration in reaction mixture: **6,63 mg/l**
Microbiological activity: **10^7 CFU/ml**

5. REFERENCE SUSTANCE:

Sodium Benzoate Concentration = 136,3 mg/l and ThOD = 1,67mg O_2/ mg substance
$C_7H_5O_2Na$, Molecular Weight=144,10 g/mol;
Elemental Analysis= %C=58,33, %H= 3,47, %O= 22,22 y %Na=15,98

6. PRINCIPLE OF TEST
A measured volume of inoculated mineral medium, containing a known concentration of test substance (100 mg test substance/l giving at least 50-100 mg ThOD /l) as the nominal sole source of organic carbon, is stirred in a closed flask at a constant temperature of 21-22ºC. for up to 28days.
Note *, the rule that 100 mg test substance/l should give at least 50-100 mg ThOD/l is accomplished.

7. APPARATUS

Respirometer: DBO Oxidirect
Temperature control 20-25ºC, maintaining 1ºC: 21-22.0ºC

8. DATA AND REPORTING

Treatment of results:

The following tables first show the evolution of BOD and, in the second, the evolution of biodegradation over 28 days.

Table 1_ BOD evolution of the sample, the reference substance, and the blank (mg O_2/l), during the 28 days in the manometer.

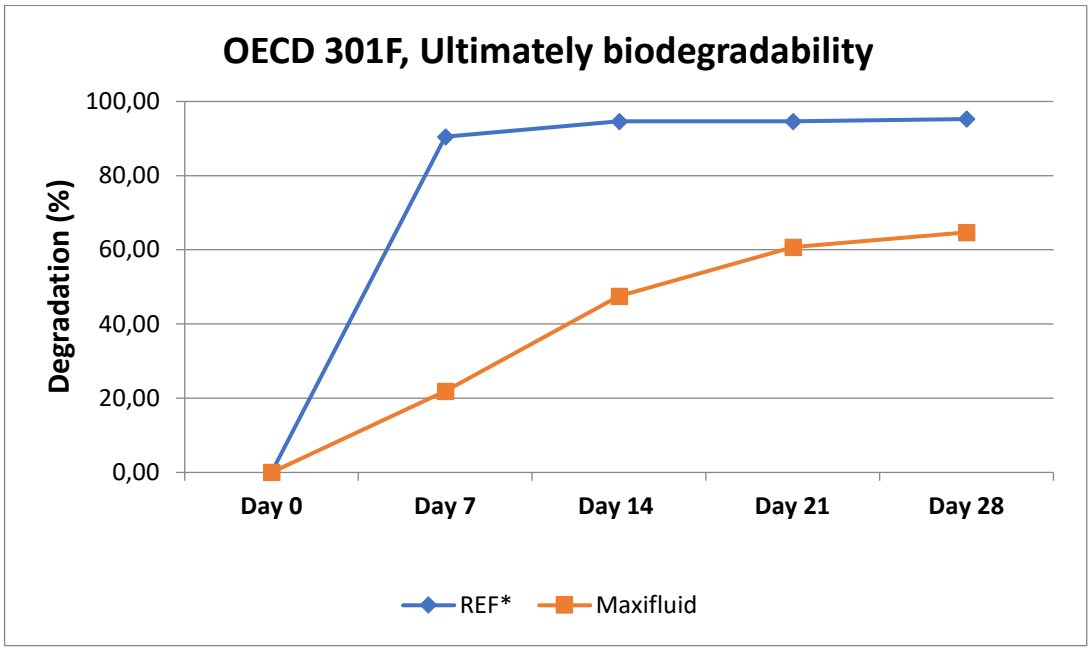
Time	Blank 1	Blank 2	Ref. 1	Ref. 2	Sample 1	Sample 2
[ ] mg/l	x	x	136,3	136,3	60,32	50,82
ThOD *	x	x	1,67	1,67	2,97	2,97
Day 0	0	0	0	0	0	0
Day 7	9	7	213	214	40	48
Day 14	10	8	225	223	77	97
day 21	11	14	230	225	113	114
Day 28	11	14	231	227	124	117

**(based on elemental analysis) mg O_2 /mg sample*
*** In the reference substance, the ThOD is calculated based on the theoretical formula*
[ ] Sample concentration

Table 2_Evaluation of biodegradability over 28 days of the sample and reference substance. Based on ThOD calculated by elemental analysis

Sample	ThOD (based on elemental analysis)	% Degradation				
		Day 0	Day 7	Day 14	day 21	**Day 28**
Ref. 1 Sodium Benzoate	1,67	0,00	90,24	**95,05**	95,75	96,19
Ref. 2. Sodium Benzoate		0,00	90,68	**94,2**	93,54	94,4
MAXIFLUID BBO	2,97	0,00	17,2	36,7	54,2	**60,2**
		0,00	26,4	58,2	67,2	**69,1**

9.BIODEGRADATION GRAPH:



3.6 Test validity criteria.

The test is considered valid if:

a) The reference substance be greater than 60% after 14 days. √

b) The oxygen uptake of inoculum blank is normally 20-30 mg O_2/l and should not be greater than 60 mg/l after 28 days. √

c) At the end of the test the pH should be between 6-8.5 √

The test performed meets the validity criteria.

4 Conclusions

Based on the results obtained with the OECD 301F test (Final Biodegradability Test) and in accordance with the biodegradability criteria of the European Ecolabel, the sample hipped from Olipes called **Maxifluid BBO can be considered as final biodegradable".**

A substance is ultimately biodegradable if, in the 28-day test based on oxygen depletion or carbon dioxide generation, the biodegradability is ≥ 60% of the theoretical maximum.