

Biolubricants : How to choose the most appropriate ultimate biodegradability test method ?

1. Introduction

The use of bio-based materials is beneficial with reference to the fossil resources depletion, the climate change and the water and soil pollution. In some application the lubricant is directly widespread in the nature due to its normal use like in the loss lubrication ; in some other applications, leakages or accidents leads to contaminate the environment.

Moreover, it is recognised in the CEN 16227 that, in Europe, more than 1 million ton per year is not recovered and more than 7 millions tons of lubricants remains every year in the environment.

The biodegradability of a product is a key parameter which is needed for many directives like REACH, GHS but also for the Vessel General Permit (VGP) in marine applications, the European Ecolabel as well as many National ecologic label (Blue Angel, White Swan,...). This is also a major property in the evaluation of LCA, an exposure scenario or to fill in the data in a MSDS or technical sheet for transport, waste management,... so its evaluation has to be done with the most appropriate test method. In this paper, only the aerobic biodegradation will be reviewed.



2. Overview of the ultimate biodegradability test methods

2.1. Primary and Ultimate biodegradation

Many terms are used to define a test substance as biodegradable – primary, potentially, fully, readily, ultimate,... The first important difference that must be done

is between the primary and ultimate as well as readily or inherently biodegradation :

The primary biodegradation is 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. The laboratory tests which are carried out to evaluate this primary biodegradation are mainly based on a determination of the residual test substance content after a incubation period. In the eighties, the test CEC L-33 approved in 1993 (obsolete now) based on residual oil content determined by IR spectrophotometry after solvent extraction at the end of 21 days incubation period has been developed. This test was first dedicated to 2-strokes outboard engine oils. The french method NF T 60-198, based on the same principle, extended the scope to many types of lubricants like the hydraulic fluids. More recently, the primary biodegradability test CEC L-103 evaluates the residual oil content by chromatography.

Even if these tests give a good indication for the potential of biodegradation, they are based on the loss of parent substance rather than mineralisation. Moreover, it can lead to erroneous high value as it becomes difficult to extract completely short polar hydrocarbon chains.

The ultimate biodegradation (aerobic) is the level of degradation achieved when the test compound is totally utilised by micro-organisms resulting in the production of carbon dioxide, nitrate, sulfate, mineral salts, water and new microbial cellular constituents (biomass).

A chemical is defined as readily biodegradable if this chemical has passed certain specified screening tests for ultimate biodegradability; *these tests are so stringent that, according to OECD guidelines, it is assumed that compounds which reach the pass level will completely biodegrade in aquatic environments under aerobic conditions.* The test methods usually used are the OECD 301 A/B/C/D/E/F and OECD 310 guideline and their equivalent methods.

Inherent biodegradability tests possess a high capacity for degradation to take place. These test procedures allow prolonged exposure of the test substance to microorganisms and a low test substance to biomass ratio, which makes the tests powerful. Some of these tests may be conducted using microorganisms that have previously been exposed to the test substance, which frequently results in adaptation leading to a significantly more extensive degradation of the chemical.

A substance yielding a positive result in a test of this type may be classified as inherently biodegradable, which, preferably, should be qualified by one of the terms "with pre-adaptation" or "without pre-adaptation" as appropriate.

Because of the favourable conditions employed in these tests, a rapid biodegradation in the environment of inherently biodegradable chemicals cannot generally be assumed. However, if an inherent test is negative this could indicate the potential for environmental persistence of the organic compound.

2.2. Abiotic transformation

Chemicals in aquatic environments, soil and air may be transformed by abiotic processes such as transformation can be an important step in the pathway for degradation of chemicals in the environment. Although abiotic transformation in itself is only primary degradation, the products formed by such abiotic processes may be biodegraded further by microorganisms.

2.3. Ultimate biodegradability test methods

First, the aerobic biodegradability should be examined in a screening test for ready Biodegradability.

In case of a negative result in a test for ready biodegradability, the biodegradation of the chemical may be examined in a simulation test to obtain data describing the biodegradation rate in the environment.

Alternatively or supplementary a screening test for inherent biodegradability may be conducted for generation of data describing the potential biodegradability under optimised aerobic conditions, such as those which may potentially occur in biological sewage treatment plants.

Since inherent biodegradability can be considered to be a specific property of a chemical, it is not necessary to define limits on test duration or biodegradation rates.

For ultimate Biodegradation, a rates above 20% may be regarded as evidence of potential, primary biodegradability, whereas biodegradation rates the pass levels of either 60% (ThOD or ThCO₂) or 70% DOC practically represent complete ultimate degradation of the test substance as the remaining fraction of 30-40% of the test substance is assumed to be assimilated by the biomass or present asproducts of biosynthesis.

Biodegradability test methods description

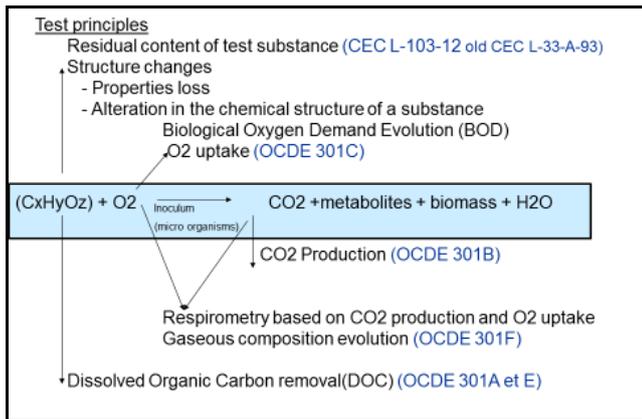
A solution, or suspension, of the test substance in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light.

The amount of DOC in the test solution due to the inoculum should be kept as low as possible compared with the amount of organic carbon due to the test substance. Allowance is made for the endogenous activity of the inoculum by running parallel blanks with inoculum but without test substance, although the endogenous activity of cells in the presence of a chemical will not exactly match that in the endogenous control.

A reference compound is run in parallel to check the operation of the procedures.

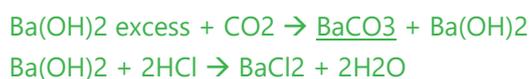
In general, degradation is followed by the determination of non-specific parameters such as DOC, CO₂ production and oxygen uptake and measurements are taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation. With automatic respirometers the measurement is continuous. DOC is sometimes measured in addition to another parameter but this is usually done only at the beginning and end of the test. Specific chemical analysis can also be used to assess primary degradation of the test substance and to determine the concentration of any intermediate substances formed. It is obligatory in the MITI method (301 C). Normally, the test lasts for 28 days. Tests however may be ended before 28 days, i.e. as soon as the biodegradation curve has reached a plateau for at least three determinations. Tests may also be prolonged beyond 28 days when the curve shows that biodegradation has started but that the plateau has not been reached by day 28, but in such cases the chemical would not be classed as readily biodegradable.

A summary of the test methods principle is included in the table hereafter :



The most requested tests for lubricants are carried out according to OECD 301B and OECD 301F; details of the test methods are given hereafter :

The modified Sturm test according to OECD 301B is based on the same principle that methods ISO 9439, NFT 90-306 and ASTM D 5864. The degradation is followed by the carbon dioxide production. The test substance is introduced by an appropriate means at a concentration between 10 and 20 mg of organic carbon per liter of test medium. The air used must be CO₂-free. Following biodegradation reaction, the organic carbon is finally transformed in CO₂; this CO₂ is entrained by the air and trapped in a Barium hydroxide solution which is in excess. Through a back titration, the remaining barium hydroxide is titrated by hydrochloric acid with phenolphthalein as the colorimetric indicator.



The CO₂ production is evaluated every 2-3 days at the beginning of the test, then every 4-5 days at the end of the test or when biodegradation reaches a plateau.

The biodegradation is expressed as the percentage of CO₂ produced corrected by the blank compared to the theoretical CO₂ which is calculated from the test substance concentration and the Total Organic Carbon determined by elementary analysis.

OECD 301F test is the manometric respiration. The biodegradation is followed by oxygen consumption registered in real respirometer. The CO₂ produced is absorbed by potassium hydroxide or another suitable absorbent. The Oxygen needed for the biodegradation is produced electronically to compensate the change in pressure or in volume.

The biological oxygen demand is calculated from the oxygen uptake, which is corrected by the blank, and the quantity of test substance.

The Biological Oxygen Demand (BOD) is expressed in mg O₂ per mg of test substance.

The biodegradability is expressed as the percentage of Theoretical Oxygen Demand (ThOD) which is the ratio between the Biological oxygen demand and the Theoretical oxygen demand.

Less satisfactorily the Chemical Oxygen Demand (COD) is used. Why using Chemical oxygen demand is less satisfactorily and can lead to false (overestimated) results?

The calculation of the theoretical oxygen demand is possible if you know the composition of the test substance and the molecular weight. You must at least know the content of Carbon, Hydrogen, Chlorine, Nitrogen, Sodium, Oxygen, Phosphorous and Sulfur. The distinction has to be done for the ThOD without nitrification or with nitrification.

For the compound:



The ThOD, without nitrification, would be:

$$ThOD_{NH_3} = \frac{16[2c + 1/2(h - cl - 3n) + 3s + 5/2p + 1/2na - o]mg/mg}{MW}$$

with nitrification:

$$ThOD_{NO_3} = \frac{16[2c + 1/2(h - cl) + 5/2n + 3s + 5/2p + 1/2na - o]mg/mg}{MW}$$

where MW = molecular weight

The COD is an oxidation by potassium dichromate and sulfuric acid which are very strong oxidant. Nevertheless, in some cases, the complete oxidation does not occur leading to a chemical oxygen demand lower than the Theoretical oxygen demand. *In these cases, the COD can lead to false results and the biodegradability can be overestimated.*



2.4. Factors which influence the aerobic biodegradation

Some factors have a positive effect on the aerobic biodegradation :

- ♦ Straight chain hydrocarbons
- ♦ Aliphatic short chain
- ♦ Presence of unsaturation
- ♦ Low molecular weight hydrocarbons
- ♦ No toxicity on microorganisms
- ♦ Oxygenates compounds
- ♦ Poor hydrolytic stability
- ♦ Conditions favorable substrate (binding sites)
- ♦ Favorable environmental conditions: High temperature , Low pressure
- ♦ Favorable aqueous medium - presence of nutrients
- ♦ Neutral pH
- ♦ Low interfacial tension with water
- ♦ Efficient microorganisms (enzyme for that substance to degrade, micro organisms adaptation)

2.5. Test methods applicability

Test substances which are soluble in water to at least 100 mg/l may be assessed by all methods, *provided they are non-volatile and non-adsorbing*. For those chemicals which are poorly soluble in water, volatile or adsorbing, suitable methods are indicated in the following table.

The MITI method neither solvents nor emulsifying agents are to be used.

Biodegradability : applicability of test methods

Analytical method	Norms				Suitability for compounds which are			
	OCDE	AFNOR	ISO	Others	Water soluble	Poorly water soluble	Volatile	Adsorbing
DOC disappearance	301A	T90-312	7827		YES	NO (> 100 mg/l)	NO	+/-
CO ₂ Production (essai Sturm modif.)	301B	T90-306	9439	ASTM 5864	YES	YES	NO	YES
O ₂ consumption Essai MITI (1)	301C	-	-	-	YES	YES	+/- C _{eau} /C _{air} ≥ 1	YES
Dissolved O ₂ Closed Bottle(1)	301D	-	10707	-	YES	+/- (> 2-10 mg/l)	YES	YES
DOC disappearance	301E	-	7827	-	YES	YES (> 40 mg/l)	NO	+/-
Manometric respirometry O ₂ consumption(1)	301F	T90-309	9408	-	YES	YES	+/-	YES

The test substance must not be toxic at test concentration
(1) Interference with nitrification

The main restrictions in the applicability of the biodegradability test methods are due to the substance properties and some specific test criteria.

The choice of a suitable test method is dependent of the test substance properties.

First the biodegradability is dedicated to *Organic compound*.

It is important to receive information on its composition, adsorption, water solubility, partition coefficient, dissociation constant in water and the presence of inorganic carbon or impurities.

2.6. Review of the specific test criteria

For all methods the substance must not be toxic at the test concentration.

The OECD 301A and 301E methods which are based on dissolved organic carbon removal are not suitable for volatile substance, neither for poorly water-soluble substance, less than 100mg/l.

The organic carbon content should be known and preferably, the proportions of major components should be known.

The OECD 301 B based on CO₂ production is not suitable for volatile substance. The Total organic carbon content should be known and preferably the proportions of major components. This method is not suitable for test substance with a high concentration of Inorganic carbon (for instance like carbonates) ; the inorganic carbon must be less the 5% of the Total carbon.

OECD 301C is based on oxygen uptake. In this method, precautions has to be taken for volatile substance.

The main restrictions (difficulties) of this test method is the preparation of the inoculum. The inoculum is prepared from micro-organisms coming from at least 10 sites mainly in areas were a variety of chemicals are used and discharged. Aerated inoculum is maintained using glucose, peptone and potassium orthophosphate. The inoculum can not be used before one month from its preparation and latest 4 months after its preparation so a new inoculum has to be prepared every 3 months.

This test method do not authorize the use of solvents or emulsifiers for poorly water-soluble test substance.

A dead gas space must be kept at a minimum.

The appropriate Theoretical oxygen demand has to be calculated with or without nitrification.

The result using the Chemical oxygen demand instead of ThOD is optional.

The OECD 301D guideline which is the close bottle test is based on dissolved oxygen depletion. The main restriction in this test is the limited amount of test substance which has to be used; indeed, the dissolved oxygen must be in excess and taken into account a concentration of 8-9 mg of dissolved oxygen per liter of test medium at test

temperature and the volume of the flask (about 300ml), only 2 to 5 mg of test substance per liter can be used. Precautions have also to be taken for volatile substance. Preferably the Theoretical oxygen demand should be known. If not and if the the Chemical oxygen demand is used, you must be sure that the oxidation of the test substance is complete otherwise, it will lead to false biodegradability test results.

The test OECD 301F based on oxygen consumption must be carried out in a real respirometer in which the oxygen is produced electronically; this test is carried out at a concentration around 100 mg of test substance per liter preventing the use of BOD flasks.

Precautions has to be taken for volatile substance.

As for tests OECD 301 C and OECD 301D, the theoretical oxygen demand should be known for biodegradation calculation but chemical oxygen demand can be used if it has been proven that the oxidation is complete ; if not, the result can be false.

3. Biodegradability on a poorly water-soluble organic compounds

Poorly water-soluble organic compounds are classified as difficult test substances and require appropriate preparation and introduction methods to achieve satisfactory and reliable test results. The ISO 10634 is a guidance for the preparation and treatment of poorly water-soluble organic compounds for the subsequent evaluation of their ultimate biodegradability in an aqueous medium.

Moreover the restrictions on the applicability and selection of the test methods dedicated to biolubricants. some precautions have to be taken.

It is a real competence and experience to conduct this test on water poorly soluble substances.

Various introduction methods exist :

- Direct addition after homogeneization
- Ultrasonic dispersion
- The use of emulsifier or solvents
- Adsorption on inert support
- Dispersions

Advantages and disadvantages from these various techniques depend on the physical and chemical properties of the substances to be tested. However, there remains many traps which must be avoided :

- Avoid reducing the Bio-availability of the organic compound.
- Avoid the organic compound has a harmful physical effect on micro-organisms.
- Avoid the adsorption of the test substance on the walls of the test vessel.
- Avoid an inoculum from an industrial sewage treatment plant or a pre-adapted inoculum.
- Avoid abiotic degradation such as hydrolysis, photolysis and abiotic oxidation.
- Avoid changing the chemical structure of the test substance.
- Maintain the test substance homogeneously in the test medium.
- Avoid interferences and contamination with any product (emulsifier, solvent) or material (support) used for the introduction of the poorly water-soluble organic compound.

One of the best way for the implementation of ultimate biodegradability tests on poorly water-soluble organic compounds, is an introduction technique which increase the bio-availability of the test substance and which avoids the mentioned above traps; the poorly water soluble test substance is introduced through the adsorption on a inert support.

4. Conclusion

Based on the applicability of the ultimate biodegradability test methods, the description of some techniques for introducing the poorly-soluble organic test compounds included direct addition, ultrasonic dispersion, adsorption on an inert support and dispersions or emulsions, the use of the theoretical oxygen demand (ThOC) which suppose to know the formula of the test substance and its purity, or relative proportions of major components, the restrictions for using the chemical oxygen demand in the biodegradation calculation, the difficulties to use a inoculum derived from a mixture of sources will result that **the most appropriate test fo the ultimate biodegradability evaluation on poorly water soluble organic compounds is the OECD 301B based on CO2 production using the adsorption on inert support as introduction technique.** This conclusion has been confirmed and reinforced by new EN 17181 test method dedicated to fully formulated lubricants which uses the same preparation mode.

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