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COMPARATIVE ANALYSIS OF STANDARD TESTING METHODS FOR MEASURING FUELS & LUBRICANTS BIODEGRADATION

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Biodegradation in fresh water of materials such as plastics, lubricants, and other chemicals, which might end up in the environment after use, is of great importance. Therefore, certification of bio-based materials that may end up as waste in fresh water, with respect to their biodegradation behavior, is necessary. Standard testing methods for measuring biodegradation in fresh water are described in OECD guidelines, ASTM standards, ISO standards, etc. The most widely used testing methods for evaluating biodegradation of chemicals in aerobic aqueous medium are OECD 301 and OECD 310 for ready, and OECD 302 for inherent biodegradability, respectively. Similarly, the International standards ISO 7827, ISO 9408, ISO 9439, ISO 10707, ISO 10708, and ISO 14593 determine the biodegradability of organic compounds in an aerobic aqueous environment and are equivalent to OECD 301 and OECD 310. Test methods comparable to OECD 302 (inherent biodegradability) were also developed at ISO level (ISO 9887, ISO 9888). The American Standards ASTM D 5271 (plastics) and ASTM D 5864, ASTM D 6139 and ASTM D 6731 (lubricants) address the biodegradability of final products which are soluble, poorly soluble, or insoluble in water. Despite the availability of various testing methods for determining biodegradability in fresh water, further research is necessary for identifying the key factors affecting the accuracy and reproducibility of the results. Moreover, the corresponding specifications to characterize the biodegradability of bio-based complex materials in fresh water need to be further investigated.

Keywords: biodegradation, bio-based materials, standard testing methods, fresh water pollution.

Біорозщеплення в прісній воді таких матеріалів, як пластик, мастильних матеріалів та інших хімічних речовин, які можуть у навколишньому середовищі (після використання) мати велике значення. Таким чином, є необхідною сертифікація біоматеріалів. Стандартні методи випробувань для вимірювання біодеградації в прісній воді описані в рекомендації OECD, стандартах ASTM, стандартах ISO і т.д. Найбільш використовувані методи тестування для оцінювання біодеградації хімічних речовин в аеробному водному середовищі OECD 301 і OECD 310, і OECD 302, притаманному біологічному розкладанню. Крім того, міжнародні стандарти ISO 7827, ISO 9408, ISO 9439, ISO 10707, ISO 10708 та ISO 14593 визначають здатність до біологічного розкладання органічних сполук у аеробному водному середовищі і еквівалентні OECD 301 і OECD 310. Методи випробувань співставні з OECD 302 (внутрішньо властивого біологічного розкладання) також були розроблені на рівні ISO (ISO 9887, ISO 9888). Американські стандарти ASTM D 5271 (пластмаси) і ASTM D 5864, ASTM D 6139 і ASTM D 6731 (мастильні матеріали) адресовані здатністю до біологічного розкладання готової продукції, які розчинні, погано розчинні або не розчинні у воді. Незважаючи на наявність різних методів тестування для визначення здатності до біодеградації в прісній воді, необхідні подальші дослідження для виявлення основних факторів, що впливають на точність і відтворюваність результатів. Крім того, щоб провести подальше розслідування, необхідні відповідні специфікації для характеристики біодеградації біоскладних матеріалів у прісній воді.

Ключові слова: біорозщеплення, біоматеріали, стандарти методів випробувань, забруднення прісної води.

Introduction

With the increasing development of advanced aircraft for civil aviation fuel must meet a number of requirements related to efficiency, reliability and durability of its work and environmental safety fuel.

However, the vast majority of fuel and lubricants represents a significant environmental hazard because of its xenobiotic (alien to the biosphere) nature, often toxic and carcinogenic (the same fuel, and combustion products of their decomposition), low biodegradability and ability of bioaccumulation.

Considering these factors, actual was search and analysis of methods for biodegradability of aviation fuel to improve the environment [3–4]. Fuels and lubricants — oil, synthetic and vegetable oils, plastic and semi-fluid greases, fuel, lubricating — cooling technological means, on the one hand, are an integral and very important part of the technosphere, without which the latter, in fact, simply cannot function.

On the other hand, in the developing global environmental crisis, the growth of pollution of the biosphere, lubricants play a significant role: for example Putting waste lubricants only in aquatic ecosystems make up about 20 % of the total man-made pollution.

Some indicators — explosiveness, stability, composition and properties in terms of storage, transportation and use, volatility, biodegradability — represent a complex of characteristics associated with both environmental hazards and with the involvement of the product in the cycling of matter (biodegradable in the environment by microorganisms).

As a consequence of man-made multi-directional and biospheric processes, environmental and technical properties are also in conflict with each other. High stability of lubricants (thermal, oxidation, biological) is necessary to maintain a high level of technical properties and prolonged performance. On the contrary — its lowest level shows a good involves product in the cycling of matter [2].

Characteristic one aspect, generally, has its antipode differently: biodegradation/biostability, highly explosive/flammability.

In some cases, the technical properties as it can not “contradict” Environment; for example, modern lubricants (in particular — motor oil) inhibit the formation of deposits in engines and machinery, positive impact on car exhaust and do not cause deactivation of exhaust catalytic converters help to reduce oil consumption; good tribological properties help to reduce friction and wear, saving energy and reducing fuel consumption, that is, saving natural resources [4].

Literature overview

Our industry is responding because a vast quantity of industrial lubricants are finding their way into the environment. In fact, the National Oceanic and Atmospheric Administration estimates 700 million-plus gallons of petroleum enter the environment each year, more than half of which is through irresponsible and illegal disposal. Industry experts estimate that 70 to 80 % of hydraulic fl uids leave systems through leaks, spills, line breakage and fi tting failure. Petroleum is persistent and toxic. It damages living organisms including plants, animals and marine life for many years. In addition, the Coast Guard, EPA and local governments are increasing the range of responsibility of lubricant releases by adding significant fines and cleanup costs.

In addition to regulatory pressure, equipment operators are frequently faced with clients and stakeholders concerned with petroleum hydraulic fluids entering the environment.

Even a small amount of petroleum could contaminate an area and cause it to be classified as hazardous [1].

One of the solutions to the considered problem was use of biological or alternative fuels and lubricants. Biodegradation of organic material (natural or synthetic hydrocarbon compounds) is actually biochemical oxidation. It is initiated and performed by the enzymes of micro-organisms such as algae and microfungi. Although similar to combustion, this biochemical process is much longer. This process delivers energy to the micro-organisms. There is also another side reaction inside these “microbugs” that uses long-chain carboxylic acids for the formation of amino acids and proteins. This reaction makes the micro-organisms grow in size and number.

When oil or other organic material is spilled into natural water containing the usual micro-organisms, the initial speed of biodegradation is very slow, as not all “bugs” present will accept this material as “food.” Those that do will eat and grow in number and size, thus producing a faster biodegradation speed until this food (the added substrate) is fully consumed [2].

Customers have found the use of biodegradable and nontoxic lubricants to be suitable for environmentally sensitive applications in construction, mining, forestry, agriculture, hydroelectric dams, and various marine uses including dockside cargo handling, harbor dredging, off-shore drilling, stern tubes, azipods, and deck hydraulic equipment [1–2].

Aim of work

The main idea of this work is to analize the existing methods for fuel biodegrability determination.

This document will examine one of the two main properties of an environmentally aware lubricant, biodegradability.

A separate topic will address aquatic toxicity. Of course, a suitable environmentally aware lubricant will also provide required performance in the areas of viscosity, oxidation, wear, foaming, demulsibility, and other desired characteristics.

General information about the methods

OECD guidelines

OECD (Organization for economic co-operation and development) has developed several guidelines for testing of chemicals on biodegradation in an aerobic aqueous medium. As these guidelines are developed for chemicals (a form of matter that has a constant chemical composition and which cannot be separated into components by physical separation methods (without breaking chemical bonds)), these test methods are not always suitable in order to determine biodegradation of complex materials such as fuels & lubricants.

OECD guidelines do not provide explicit guidance with regard to biodegradability testing of poorly water-soluble substances (substances with a water solubility < 100 mg/l).

The only critical guidance provided is the applicability of a restricted range of analytical methods (OECD 301 B, C, D (±) and F and OECD 310) and the requirement of additional control vessels where emulsifiers, solvents and carriers are used. Whilst advocating the use of emulsifiers, solvents and carriers, none are specifically identified and no guidance is provided regarding the acceptable level.

Consequently, many approaches of introducing the test substance can be applied, which make it difficult to identify a set of core acceptable or workable solutions [ECHA (European Chemical Agency) (2012)]. For volatile substances analytical methods OECD 301 C (±), D and F (±) and OECD 310 are suitable [1–3].

OECD 301 and 310 are both designed in order to determine ready biodegradability, while OECD 302 determines inherent biodegradability. OECD 303 and 309 are both guidelines for simulation biodegradation tests in specific freshwater aquatic environments.

The duration of these tests is always 28 days, pre-exposure of the inoculum to the chemical is not allowed, the test substance is provided in a rather high concentration (2 to 100 mg/l) as the sole source of carbon for energy and growth while the amount of **DOC (dissolved organic compounds)** in the test solution due to the inoculum should be kept as low as possible compared to the amount of DOC due to the test substance. The endogenous activity of the inoculum is corrected by running parallel blank tests with inoculum but without test substance.

The pass levels for ready biodegradability for chemicals as prescribed by OECD 301 and OECD 310 are:

– 70 % removal of DOC

– Biodegradation > 60 % Theoretical Oxygen Demand (ThOD)

– Biodegradation > 60 % Theoretical carbon dioxide (ThCO2)

– Biodegradation > 60 % Theoretical inorganic carbon (ThIC)

The pass level is higher for methods based on the measurement of the residual sample (DOC), while the pass level is lower for methods, which are based on respirometric measurements.

This is caused by the fact that the biodegradation percentage based on CO_2 production and oxygen consumption is always less than the percentage determined by DOC removal due to the bacterial metabolism. Some of the organic carbon of the test substance is biochemically oxidized and converted to CO_2, while other fractions are synthesized into new cellular material or into organic metabolic products.

These fractions are not oxidised and do not contribute to the CO2 production. The biomass formation is related to different factors (nature of test substance, bacterial species, etc.)[3].

The pass levels need to be reached within a 10-d window, which starts when the degree of biodegradation has reached 10 % DOC, ThOD, ThCO2 or ThIC and must end before day 28 of the test. If chemicals are classified as readily biodegradable, the need for other ecological parameters (e.g. bioaccumulation and ecotoxicity) may be reduced. As no complete biodegradation needs to be demonstrated, these pass levels (60 or 70 %) for ready biodegradability are only reasonable for pure chemicals, but not necessarily for chemical mixtures or for chemicals containing significant proportions of impurities. OECD 302 A prescribes that test chemicals giving a result > 20 % loss of DOC may be regarded as inherently biodegradable, whereas a result > 70 % loss of DOC is evidence of ultimate biodeg-radability. No recommendations with regard to the interpretation of the results are given in guidelines OECD 302 B and OECD 302 C (Table 1).

Table 1

Overview of the OECD guidelines with regard to freshwater aquatic toxicity

Guideline	Adopted	Description
OECD 201	2006	Freshwater Alga and Cyanobacteria, Growth Inhibition Test
OECD 202	2004	Daphnia sp., Acute Immobilisation Test
OECD 203	1992	Fish, Acute Toxicity Test
OECD 204	1984	Fish, Prolonged Toxicity Test: 14-day Study
OECD 210	2010	Activated Sludge, Respiration Inhibition Test (Carbon & Ammonium Oxidation)
OECD 209	1992	Fish, Early-life Stage Toxicity Test
OECD 211	2012	Daphnia magna Reproduction Test
OECD 212	1998	Fish Short Term Reproduction Assay
OECD 215	2000	Fish, Juvenile Growth Test
OECD 221	2006	Lemna sp. Growth Inhibition Test
OECD 229	2012	Fish Short Term Reproduction Assay

The definitions mentioned in Annex B of ISO 7827 (2010) also mention that removal of 20 % DOC, consumption of 20 % ThOD or evolution of 20 % $ThCO_2$ in any degradation test within 28 days, with or without pre-exposure of the inoculum, indicates that the test substance in question is inherently biodegradable and non-persistent [1–3].

European standards

For testing the aerobic biodegradability of organic compounds and plastic materials in fresh water **CEN** (French: Comité Européen de Normalisation) refers to existing ISO standards.

International standards

ISO proposed a series of methods for evaluating the aerobic biodegradability in fresh water [3].

First the preparation method of the sample is selected based on the physico-chemical properties of the test substance (ECHA, 2012). These preparation methods are described in ISO 10634 (1995) "Water quality — Guidance for the preparation and treatment of poorly watersoluble organic compounds for the subsequent evaluation of their biodegradability in an aqueous medium". Four preparation techniques are described in this standard:

— Direct addition:

a. Test component is weighted and direct addition;

b. Using an inert support (for example: microscope slides);

c. Using a volatile solvent, which is removed prior to testing;

— Ultrasonic dispersion;

— Adsorption on an inert support (for example: silica gel or glass fibre filters);

— Dispersions or emulsions with an emulsifying agent.

After the pre-treatment, the biodegradation in an aerobic aqueous medium can be tested using the standard methods, but methods based on DOC measurements are normally not suitable. ISO has developed various test methods in order to determine the biodegradability of organic compounds and plastics in an aerobic aqueous environment.

These standards are mainly based on the principles of the OECD guidelines, but the international standards are often more precise and clearer. Most of these international standards concern the determination of the biodegradability of organic compounds in aqueous media (ISO 7827, ISO 9408, ISO 9439, ISO 9887, ISO 9888, ISO 10707, ISO 10708, ISO 11733, ISO 14592, ISO 14593).

Only two standards are related to the biodegradability of plastic materials (ISO 14851 and ISO 14852), based on the same principles as ISO 9408 and ISO 9439, respectively [2–3].

The various ISO biodegradability standards differ with respect to microbial density, test item concentration, and test duration. Consequently these standards may exhibit different biodegradation potential for the same material.

Test method ISO 14592 is characterised by low microbial densities and very low test item concentrations (< 200 μg/l) as it is developed in order to evaluate the biodegradation of substances at low environmentally realistic concentrations in the aquatic environment. ISO 14592-1 is a batch test simulating standing water bodies (lakes or ponds), while ISO 14592-2 is a dynamic test simulating flowing waters (rivers).

ISO 10707 is performed in closed bottle without stirring or aeration and is also characterised by a rather low microbial density and a low test item concentration (2 mg/l). This method of low biodegradation potential is especially developed for volatile and inhibitory test compounds [1].

Test methods ISO 7827 (DOC), ISO 9408 (oxygen consumption), ISO 9439 (CO2 production), ISO 10708 (two-phase closed bottle) and ISO 14593 (CO2 headspace test) are all characterised by a higher biodegradation potential when compared to ISO 14592 and ISO 10707 as the microbial density and the test item concentration are considerably higher.

These standards are comparable to the OECD guidelines for ready biodegradability (OECD 301 and OECD 310). In spite of the similar biodegradation potential of these methods different results may be obtained due to differences in measurement techniques.

Test methods ISO 9887 (SCAS test) and ISO 9888 (Zahn-Wellens test) have a high inoculum concentration and may be extended beyond the usual duration of 28 days. The conditions as prescribed in these methods are optimal in order to allow the maximum biodegradation value of a test item. These methods can be used to determine the intrinsic biodegradation of chemicals, which corresponds to the OECD guidelines for inherent biodegradability.

ISO 11733 (activated sludge simulation test) is characterised by the highest inoculum concentration, as this test simulates the conditions of a wastewater treatment plant [2–3].

Different inoculum sources are prescribed by the various international standards for testing aerobic aqueous biodegradation of organic compounds. ISO prescribes that normally no preexposed inoculum should be used, especially in the case of standard tests simulating biodegradation behaviour in natural environments.

Depending on the purpose of a test, a preexposed inoculum may be used, provided this is clearly stated in the test report and the method of pre-exposure needs to be clarified.

The standards ISO 14851 and ISO 14852 are testing methods concerning final products (plastics). The main difference with the testing methods for organic compounds (ISO 9408 and ISO 9439) is:

1) the higher concentration of the sample;

2) the longer maximum duration; and

3) the possibility to use compost extract as inoculum. Both standards recommend that the test mixture should preferably contain about 103–106 CFU/ml. The tests are normally executed at a constant temperature between 20 and 25°C, but it is mentioned that higher temperatures might be appropriate when compost is used as inoculum.

Higher test item concentrations (up to 2000 mg/l of organic carbon) can also be tested in both methods on condition that an optimised test medium is used. An optimized medium is highly buffered and contains more inorganic nutrients.

ISO 14851 and ISO 14852 acknowledge that both the determination of the BOD (after taking into account nitrification) and the determination of the evolved CO2 are in some cases not enough to characterise the biodegradability of test materials with complex compositions. During a biodegradation test with a long duration the carbon in the test material is partly transformed to biomass but not biochemically oxidized. In this case, the measurements will not reach 100 % of the theoretical values even in case of complete biodegradation of the test material [2].

In order to confirm complete biodegradability, the carbon balance can be determined. The total carbon sum is based on five measurements:

1) carbon evolved as CO2;

2) carbon produced as biomass;

3) carbon transformed into watersoluble organic metabolites;

4) carbon determined as DOC; and

5) carbon remaining in the non-degraded polymer material.

American standards

ASTM (American Society for Testing and Materials) has developed three standard test methods for the determination of the aerobic aquatic biodegradation of lubricants (ASTM D 5864, ASTM D 6139, and ASTM D 6731) and one standard test method for predicting the biodegradability of liquid-based lubricants using a bio-kinetic model (ASTM D 7373).

Standard test methods ASTM D 5864 and ASTM D 6139 are not designed for volatile lubricants or lubricants components, while standard test method ASTM D 6731 is suitable for evaluating the biodegradation of non-volatile as well as volatile lubricants [4–5].

Two other ASTM testing methods can be used to determine biodegradation of plastics in aqueous environment (ASTM D 5271 and ASTM D 6340).

Different inoculum options are prescribed such as activated sludge from wastewatertreatment plant, secondary effluent, surface water, soil extract, or mixture of sources. A criterion with regard to the quantity of micro-organisms in the inoculum is given.

Conclusions

Based on the literature review of the different biodegradation test methods in an aqueous aerobic freshwater environment it can be concluded that a sufficiently broad range of measurement techniques already exists.

The biodegradation rate can be determined based on the measurement of dissolved organic carbon, dissolved oxygen, CO_2 production, oxygen consumption or inorganic carbon. However, several improvements are required in order to enhance the reproducibility of the results and the credibility of the testing methods [4].

When testing poorly water soluble test items the addition of the test item to the test system needs to be adapted when compared to the addition of water soluble test items. Therefore the addition method as prescribed in the previous mentioned OECD, ISO and EN methods need to be modified. Such modifications are already described in ISO 10634, which was especially developed towards test items that are poorly water soluble.

When testing bio-lubricants and bio-solvents, more appropriate reference materials should be chosen when compared to the currently proposed water soluble and very easily degradable reference materials which are mentioned in the guidelines. Such a modification has already been introduced in biodegradation standards for plastics (ISO 14851 and ISO 14852).

No specific criteria are given in ASTM D 5864, ASTM D 6139 and ASTM D 6731. These test methods state that if a test material achieves a high degree of biodegradation, it may be assumed that the test material easily biodegrades in many aerobic aquatic environments. However, no specific pass level ("high" degree: > 60 %, > 70 %, > 90 %?) nor a maximum duration are given. This should be clearly defined in order to avoid miscommunications.

Based on the literature review of the available standards with regard to *environmental safety*, it can be concluded that a sufficiently broad range of testing methods towards freshwater organisms on different trophic levels (bacteria, algae, freshwater aquatic plants, crustacean and fish) already exists. For bio-lubricants and bio-solvents, additional attention is especially needed towards the addition of biolubricants that are poorly water soluble and volatile biosolvents to the testing systems as this can influence the test results [4–5].

There exist already documents with specific guidance toward the sample preparation and the interpretation of the results for difficult substances (OECD), poorly water soluble substances (ISO 14442) and lubricants (ASTM D 6081). These guidelines should be taken into account when testing biolubricants and bio-solvents (Table 2).

Table 2

General short overview of main methods

Methods	Substants	Duration of the test, days	Sensibility, %
OECD	Chemicals in an aerobic aqueous systems	28 days	~ 60–70 %
ISO	Aerobic biodegradation in fresh water	28 days	80 %
ASTM	Aquatic biodegradation of lubricants	28 days	> 60 %
CECL-33-A-93	Biodegradation of lubricants, mineral oils	21 days	> 80 %

The literature review on the *specifications* revealed that currently no clear specifications for biodegradation in fresh water exist on European and American level.

Therefore, there is an urgent need as standardisation can play an important role in the uptake of products such as bio-based lubricants and bio-based solvents and consequently can help to increase market transparency by providing reference methods and criteria [5].

REFERENCES

1. *De Wilde B*. (2005). International and national norms on biodegradability and certification procedures. — P. 145–181. Handbook of biodegradable polymers. Editor: Catia Bastioli. Rapra Technology Ltd., Shewsbury, UK. ISBN 1-85957-389-4.

2. *De Wilde B*. (2013). Degradable Polymers and Materials : Principles and Practice (2nd Edition), ACS Publications (in press), Chapter 3 “Biodegradation Testing Protocols”, 33–43. — Electronically published (http://pubs.acs.org/isbn/9780841228221).

3. *Painter H. A*. (1995). Detailed Review Paper on Biodegradability Testing. OECD, Environment Directorate.

4. *Евдокимов А. Ю*. Смазочные материалы и проблемы экологии / А. Ю. Евдокимов, И. Г. Фукс, Т. Н. Шабалина, Л. Н. Багдасаров. — М. : Нефть и газ, 2000. — 423 с.

5. *Папок К. К*. Химмотология топлив и смазочных масел / К. К. Папок. — М. : Воениздат, 1980. — 192 с.

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