KBBPPS
Knowledge Based Bio-based Products' Pre-Standardization

Work package 6

Biodegradability

Deliverable N° 6.5: Biodegradability standards assessment report

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1 Introduction

The European project KBBPPS (Knowledge Based Bio-based Products' Pre-Standardization, see also www.kbbpps.eu) aims at increasing the uptake speed of standards and certification systems for bio-based products. The project concentrates on pre-standardisation research for bio-based products, including developing test methods for determining the bio-based carbon content, the biomass content, biodegradability and functionality issues. The objective is to integrate the results of the research project into further standardization work of CEN.

The objective of WP6 “Biodegradability” within the KBBPPS project is to develop and validate a test methodology for the evaluation of biodegradability in freshwater and soil for bio-based lubricants and bio-based solvents, starting from what exists at present.

Deliverable D6.5 summarises the executed work in WP6 and gives guidance towards the interlaboratory testing that are planned for the follow-up project Open-BIO in WP5 “In situ biodegradation”.

Advisory partner SCION contributed to the interlaboratory test and is gratefully acknowledged. Moreover, also the participants of CEN/TC 19/WG 33/TF “Biodegradation” are acknowledges for their input.

2 Design and validation of biodegradation test methodologies in freshwater and in soil

2.1 Literature study

The work in WP6 was initiated by an extensive literature research reviewing existing biodegradation and toxicity test methodologies in different environments. Moreover existing labelling systems, certification schemes and standard specifications in which biodegradability is included were discussed (deliverable report D6.1, *Report on current relevant biodegradation and ecotoxicity standards*).

Due to the fact that freshwater, seawater, soil, anaerobic digesters and composting installations are characterised by significant differences with regard to microbial and/or fungal activity, temperature, chemical parameters, oxygen concentration, nutrient content, etc., biodegradability of a material is linked to the environment in which it occurs. Consequently the biodegradability and the biodegradation rate of a material can significantly vary between these different environments. The existing methodologies in the different environments were reviewed to evaluate their applicability towards bio-lubricants and bio-solvents. In this deliverable (D6.5) the focus is especially on freshwater and soil environment as the further work in KBBPPS focusses on these environments. In the follow-up project Open-BIO marine biodegradability and several managed end of life options are investigated in detail.

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. An overview of the most important available techniques and an example of some of the existing test methods is given in Figure 1.

Not each measurement technique is suitable in order to determine the biodegradability of a broad range of products. For example: methods based on dissolved organic carbon (DOC) are not suitable to evaluate biodegradability of insoluble (e.g. insoluble polymers) or poorly water soluble products (e.g. lubricants), while actively aerated systems in which air is bubbled through the systems are not suitable to evaluated volatile substances (e.g. solvents with a high volatility). Therefore, the most appropriate measurement techniques were selected in order to further develop a test methodology: OECD 301 B (comparable to ISO 9439 or ISO 14852) and OECD 301 F (comparable to ISO 9408 or ISO 14851) for bio-lubricants and OECD 301 D or OECD 301 F with limited headspace for bio-solvents. Moreover in order to increase the reproducibility between the laboratories, special care should be given towards the description of the addition methods, a suitable reference material and the inoculum source.

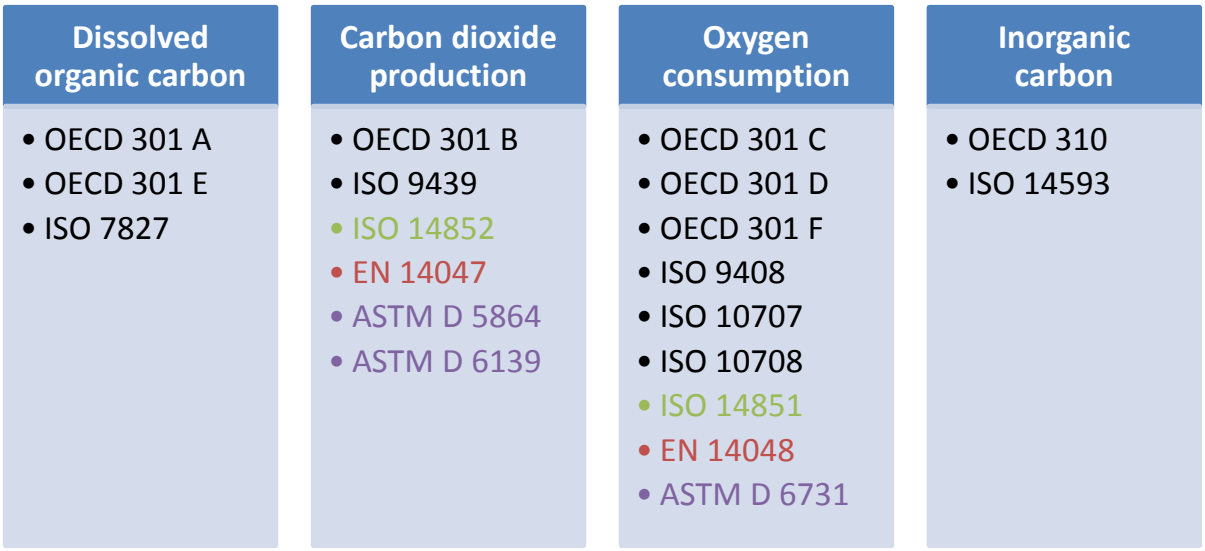

Figure 1. Overview with biodegradation measurement methods and some examples of existing test methods for Organic compounds – Plastics – Packaging – Lubricants.

With regard to environmental safety, it can also 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 poorly water soluble bio-lubricants and volatile bio-solvents to the testing systems as this can influence the test results. The American standard ASTM D 6081 provides adequate information towards the sample preparation and the interpretation of results of aquatic toxicity tests with lubricants.

Labelling systems (e.g. Ecolabel, Blue Angel label, etc.) and specifications (e.g. ISO 15380 and prEN 16807) for bio-lubricants have been developed, but a European or international labelling systems for bio-solvents (taking into account parameters like biodegradability, environmental safety, minimum bio-based content, etc.) is not available.

The literature review on methodologies in a soil environment revealed that some international standards are available about testing biodegradability of organic compounds and plastics in soil. Concerning bio-based lubricants and solvents an appropriate testing method is needed. This should be based on proper adaptation of testing methods for biodegradation of bio-based polymers in soil, combined with specifications and labelling analogous to those already available for biodegradable in soil plastics. The international test method ISO 17556 was selected as most appropriate starting point for the further developments/improvements. This methodology developed for plastics cannot be used as written. Some modifications towards sample addition and reference material are needed to make them suitable for bio-lubricants and to improve precision, repeatability and reproducibility.

2.2 Questionnaires

In a next phase questionnaires were sent to industry in order to investigate the needs and problems related to biodegradability, environmental safety and labelling of bio-based lubricants and bio-based solvents. The contact persons were provided by project partner NEN and were mainly participants from CEN/TC 19/WG 33 “Bio-lubricants” and CEN/TC 411/WG 2 “Bio-solvents”. The details regarding the questionnaires and corresponding results have been documented in deliverable report D6.2, *Draft biodegradability standard*.

The main conclusions related to lubricants were:

- According to lubricant industry the existing OECD methods are well established and there is no need to write completely new biodegradation testing methods or ecotoxicity testing methods for lubricants. Focus should be on improvement of biodegradation testing methods (addition methods for poorly water-soluble test items, reproducibility, variation in the inoculum, etc.).
- Currently biodegradation and ecotoxicity tests on lubricants are executed in an aquatic environment. These methods are well established. The participants were not in favour for an approach per environment as this increases complexity.
- All participants concluded that it would be easier to execute tests on the final product. When testing on the component level is considered, the use of the Lubricant Substance Classification list is very useful.
- 90 % absolute biodegradation in a freshwater biodegradation test within 28 days cannot be considered as an acceptance criteria as this is very difficult to obtain.
- The main market barriers for bio-lubricants are (1) high price, (2) high amount of tests (biodegradation, bioaccumulation and aquatic toxicity on different trophic levels) that are required to obtain a label, (3) fact that the use of bio-lubricants is not yet mandated in sensitive area’s (need for an European legislation), (4) differences between labelling systems (= high complexity) and (5) fast revision of the labelling systems.

The main conclusions related to solvents were:

- The existing OECD methods are well established and there is no need to write completely new biodegradation testing methods or ecotoxicity testing methods for solvents. No problems occur and no further improvement of the test methods is needed.
- The main market barriers are (1) the high price, (2) absence of clear specification system and reliable labelling system and (3) high amount of tests.

It was also checked with CEN/TC 411/WG 2 if additional work toward biodegradability and environmental safety of bio-solvents was required. According to CEN/TC 411/WG 2 no further research with regard to biodegradability and environmental safety of bio-based solvents is useful as this is already performed in the scope of REACH and CLP.

Based on the results of the questionnaires and the contacts with the CEN committees, it was decided that no further needs existed with regard to biodegradability and environmental safety of bio-based solvents, while additional research towards addition method and inoculum of

bio-based lubricants still deemed necessary in order to develop a methodology with a high repeatability and reproducibility.

2.3 Development test methodologies

In a next step some preliminary tests with respect to sample addition, reference material and inoculum were executed by OWS (freshwater and soil) and AUA (soil) in order to create some background information for the development of the test methodologies. The lubricant samples were provided by the contact persons that participated to the questionnaires. The details regarding these preliminary tests and corresponding results have been extensively reported in deliverable report D6.2, *Draft biodegradability standard*.

The most important conclusions from the preliminary tests in freshwater were:

- OECD 301F with some modifications towards reference material and addition method is a suitable method in order to evaluate biodegradability of lubricants in freshwater. (Note: During this phase no preliminary tests using OECD 301B were performed, but according to the other laboratories participating to the CEN/TC 19/WG 33 TF Biodegradation meetings, this methodology is also suitable and often used for lubricants.)
- Rapeseed oil, sunflower oil or HOSO (High Oleic Sunflower Oil) are suitable materials in order to be used as positive reference material for lubricants. The properties of these materials are more comparable with lubricants when compared to the standard reference materials (aniline, sodium benzoate, etc.) as suggested by OECD 301.
- Activated sludge should be specified as inoculum source. This inoculum is characterised by the highest biodegradation potential and in order to reduce the variability between laboratories, a final concentration of suspended solids in the mixture of 30 mg /l is recommended.
- A glass goblet and a filter paper are less suitable as addition method. Addition using an inert carrier (plastic carrier or stirrer) and addition in a solvent seem to be the most suitable methods among the investigated alternatives. Based on a first comparison between the addition in a solvent (hexane) and the addition on a stirrer, it was concluded that the addition method in hexane was characterised by less variation when compared to the addition method on the stirrer.

The most important conclusions from the preliminary tests in soil were:

- ISO 17556 with some modifications towards reference material and addition method is a suitable method in order to evaluate biodegradability of lubricants in soil.
- The inoculum source can significantly influence the biodegradation results. Based on the first preliminary tests, it seems that natural soil is somewhat more aggressive when compared to standard soil in order to biodegrade bio-based lubricants.
- A clay-loam type soil with a balanced presence of all clay, silt and sand soil components, coming from fertile agricultural areas, is a good natural matrix material for biodegradation in soil tests. Since the addition of the sample material perturbs the C:N

balance in the soil, nitrogen needs to be added. The current experiments with Clay Loam soil indicate that an amount of 0.1 g of N in nitric based fertilizer form has to be added per 1 g of added C. However, the ratio may depend on the soil type.

- Several testing methods were investigated and compared: the closed flask method with alkaline trap based on titration, the periodically aerated method with alkaline trap based on titration, the BOD manometric method based on oxygen demand and the continuously aerated method with IR-CO_2 sensor. It was shown that they are all compatible with comparable results and can be used alternatively. The BOD manometric method tends to slightly overestimate biodegradation due to oxygen consumption by nitrification.

Simultaneously with the first preliminary tests executed in freshwater and soil, biodegradation test methodologies in freshwater and in soil were developed for bio-lubricants (deliverable report D6.2, *Draft biodegradability standard*). Following freshwater biodegradation methodologies were developed in CEN/TC 19/WG 33 TF Biodegradation: (1) Method A (carbon conversion method): *Liquid petroleum products – Bio-lubricants - Determination of aerobic biological degradation of fully formulated lubricants in an aqueous solution – Test method using detection of CO_2 production* and (2) Method B (manometric respirometric method): *Liquid petroleum products – Bio-lubricants - Determination of aerobic biological degradation of fully formulated lubricants in an aqueous solution – Test method based on O_2 consumption*. Project partner OWS participated to the meetings and reviewed the methodologies. The test methodology in soil was developed within the project partners and was based on ISO 17556.

2.4 Interlaboratory testing

2.4.1 Phase 1

In the final phase of the project two interlaboratory tests were executed in order to evaluate the reproducibility of the developed test methodologies. The detailed results and biodegradation curves of the performed tests per partner can be retrieved in deliverable report D6.4, *Biodegradability method validation*. In the current deliverable report (Deliverable report 6.5) a comparison is made between the results of the different laboratories. The freshwater methodologies were evaluated by AUA, DLO-FBR, OWS and advisory partner SCION, while the soil methodology was evaluated by AUA, OWS and advisory partner SCION. The interlaboratory tests were executed on samples provided by CEN/TC 19/WG 33.

The objective of the first part of the interlaboratory test in freshwater was the comparison between two different addition methods: (1) direct addition of sample on a stirrer and (2) solution of sample in a solvent (on request of CEN/TC 19/WG 33). The two different addition methods were compared using positive reference material HOSO (JA1405/06), two hydraulic oils, 1 gear oil and 2 greases (Table 1). KBBPPS project partner ECN executed the TOC analyses and the elemental analyses. These data were then redistributed to the participating laboratories as it was decided to test only the precision of the biodegradation test method and not the precision of the determination of the TOC analysis or the elemental analysis. This input was necessary for measuring biodegradation either by CO_2 production or by O_2 consumption.

Table 1. Overview of the samples for interlaboratory test (part 1).

Sample	Short description	MFA.-Nr.	Description
JA1405/01	01	111033888	ISO VG 10 hydraulic oil
JA1405/02	02	111033889	ISO VG 46 hydraulic oil
JA1405/03	03	111033890	ISO VG 320 gear oil
JA1405/04	04	111033891	Grease
JA1405/05	05	111033892	Grease
JA1405/06	06	111033896	High oleic sunflower oil (HOSO)

The test was performed by OWS with both addition methods (solvent and stirrer), and by AUA and SCION by means of the solvent addition method. Due to problems with available capacity DLO-FBR was not able to participate to the foreseen interlaboratory test. Consequently only the results of the laboratory tests executed at OWS could be used in order to compare the two addition methods.

A summary of the test set-up of the freshwater test is given in Table 2, while Table 3 shows an overview of the parameters of the inoculum.

Table 2. Overview of test set-up used in interlaboratory freshwater test (part 1).

Parameters	AUA	OWS	BMG	SCION
Type	Method B	Method B	Method B	Method A
Aeration	Stirred system (head-space provides the oxygen)	Stirred system (head-space provides the oxygen)	*	10 l/h air bubbles through the reac-tors
Reactor volume (L)	0.5	0.5	*	3
Temperature (°C)	25°C (± 1°C)	21°C (± 1°C)	*	25°C
Quantity of sample (mg)	10 (mg C)	10	*	60
Quantity of in-oculum (ml)	244 (mineral medium) + 0.76 (inoculum)	245 (mineral medium) + 5 (inoculum)	*	1200 (mineral medium) + 11.5 (inoculum)
Measurement method	O_2 consumption and CO_2 production (by means of titration)	O_2 consumption and CO_2 production (by means of titration)	O_2 consump-tion	O_2 and CO_2 level by IR detector

* No information was provided

Table 3. Characteristics of the inoculum and final medium of interlaboratory test (part 1).

Parameters	Characteristics inoculum (= as such)			
	AUA	OWS	BMG	SCION
Description inoculum	Activated sludge from municipal wastewater treatment plant	Activated sludge from mixture of 3 sources (2 municipal and 1 domestic wastewater treatment plant)	**	Activated sludge from municipal waste water treat-ment plant
pH	6.8	7.2	**	nd
TS (%)	nd	0.24	**	nd
VS (%, on TS)	nd	45.8	**	nd
TSS (g/l)	9.6	1.6*	**	3.16
VSS (g/l)	8.4	1.0*	**	1.5 g/l carbon
Total N (mg/l)	4.5	98	**	360
	Characteristics final medium (= mineral medium + inoculum)			
	AUA	OWS	BMG	SCION
Suspended solids (mg/l)	30	32	**	nd

* After centrifugation - ** No information was provided - nd = not determined

Method B was used in order to compare both addition methods at OWS. The biodegradation percentage was calculated based on the oxygen consumption and also as an additional check the biodegradation was calculated based on the titration of the absorbed CO_2 in the absorber. A summary of the results is shown in Figure 2.

The standard deviation in the series with the stirrer was higher when compared to the addition method in the solvent (= hexane). This was especially observed for ISO VG 46 hydraulic oil and for one grease (JA1405/04). For test item ISO VG 46 hydraulic oil, the biodegradation of one replicate remained increasing, while one replicate of grease JA1405/04 was significantly lagging behind in the test series with the addition on the stirrer. When these replicates are considered as outliers, the average biodegradation percentages of the remaining 2 replicates are comparable to the values obtained when using the addition method with the solvent (= hexane). The higher variation between the replicates is most probably caused by the fact that the magnetic field of the stirrer disturbed the analytical balance. This problem could be solved by using a plastic beaker to weight the stirrer and the sample (in order to avoid direct contact between the stirrer and the analytical balance).

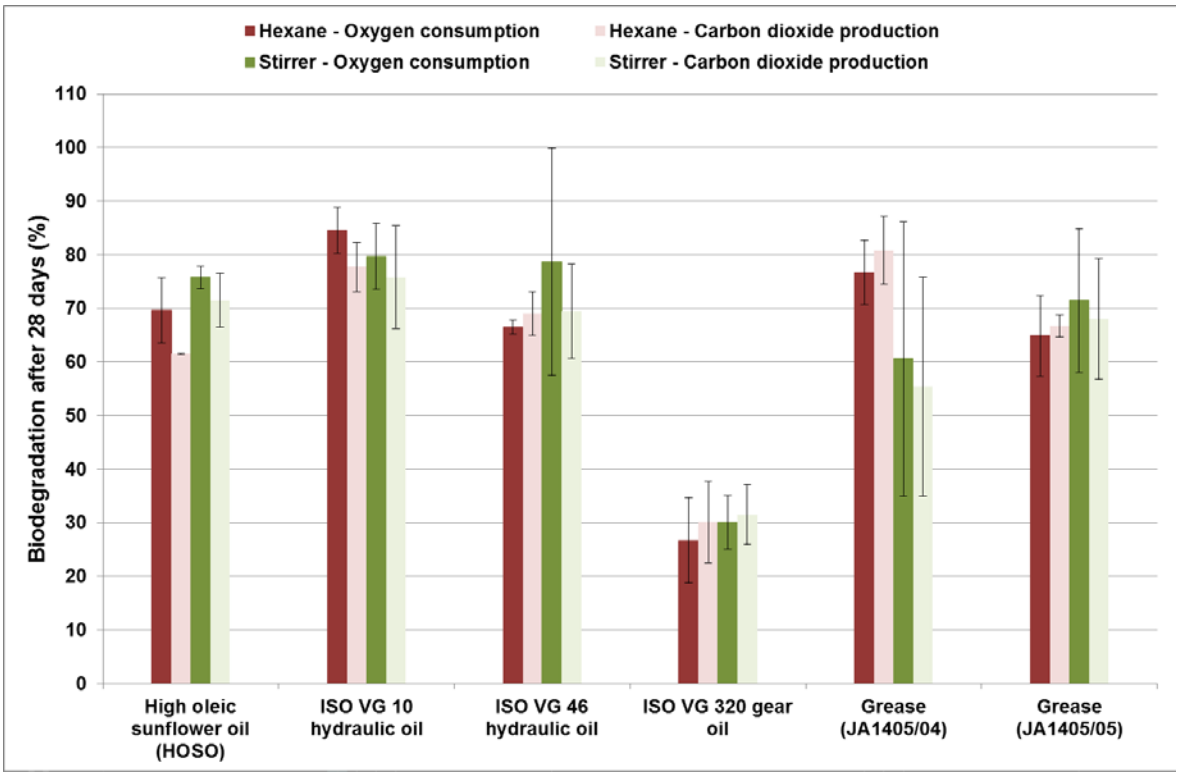

Figure 2. Summary of the biodegradation percentages of lubricants after 28 days (interlaboratory test – part 1) (OWS).

The above mentioned results were presented by OWS during the meeting of CEN/TC 19/WG 33 “TF Biodegradation” in Mannheim (August 2014). The results confirmed the expectations of the other participating laboratories (addition method in a solvent is the most optimal).

Table 4 shows the biodegradation percentages of the samples obtained after 28 days for all partners (OWS, BMG laboratory from CEN/TC 19/WG 33 and advisory partner SCION) using the addition method with the solvent. These results are also presented in a boxplot (Figure 3).

The background activity measured in the blank is also mentioned in Table 4. Following validation criteria are included in test methods based on the determination of the biodegradation based on oxygen consumption:

- OECD 301F: The oxygen uptake of the inoculum blank is normally 20-30 mg O_2/l and should not be greater than 60 mg/l in 28 days. Values higher than 60 mg/l require critical examination of the data and the experimental technique. [Remark: Normal duration OECD 301F is 28 days.]
- ISO 9408: The test is considered valid if the amount of BOD in the blank F_B at the end of the test, which is usually between 20 mg/l to 30 mg/l, does not exceed 60 mg/l after 28 days. [Remark: Usually the maximum test period shall not exceed 28 days.]

The background activities measured with method B (oxygen consumption) are for all partners < 60 mg/l after 28 days.

Following validation criteria are included in test methods based on the determination of the biodegradation based on carbon dioxide production:

- OECD 301B: The total CO_2 evolution in the inoculum blank at the end of the test should normally not exceed 40 ml/l medium. If values greater than 70 mg CO_2/l are obtained, the data and experimental technique should be examined critically. [Remark: Normal duration OECD 301B is 28 days.]
- ISO 9439: The test is considered valid if the concentration of CO_2 which has evolved from the blank F_B at the end of the test at a test volume of 3 l is about 40 mg/l and does not exceed 70 mg/l. [Remark: Usually the maximum test period shall not exceed 28 days.]

The background activities measured with method A or with method B (carbon dioxide production check) are below or around 70 mg CO_2/l for AUA and OWS, while the background activity of SCION was significantly higher 250 mg CO_2/l ± 37 mg CO_2/l and consequently reliability of the results becomes questionable. According to SCION the high background activity was caused by variability troubles from the CO_2 scrubbers.

When comparing the background activity measured by method B based on O_2 and CO_2, the difference should normally be approximately a factor 1.4 (MW CO_2 / MW O_2 = 44 / 32). Especially for the background activity measured at AUA, this factor is considerably higher. According to AUA the discrepancy may be related to the accuracy of the titration method. Consequently also the biodegradation percentages based on the CO_2 production should be interpreted with caution.

Samples JA1405/01 and JA1405/05 were only evaluated by one partner, which results in comparatively short boxplots. The other samples, which were evaluated by at least 2 part-

ners, were characterized by unrealistic high values (> 100%). From Table 4 and Figure 4 it can be deduced that these high maximum values were caused by the results of SCION (indicated in red). SCION had problems with their equipment (due to relatively low air pressures, bubbling air through 1 l of water caused large variations in flow resulting in large variations in CO_2 mass detected including the blanks). According to SCION the biodegradation percentages > 100% and the large variability between the replicates indicate that it is not possible to be confident in the results.

Table 4. Biodegradation percentages returned by participating laboratories (freshwater - after 28 days – addition method: solvent).

Lab	Method	Rep	Blank (mg O_2/l or mg CO_2/l)	Sample					
				06	01	02	03	04	05
OWS	Method B – O_2	1	37.9	73.9	86.1	65	18.1	74.3	65.2
		2	32.5	65.3	87.8	67	28	83.5	57.2
		3	36.6	nd	79.6	67.5	34.1	72.3	72.3
	Method B – CO_2	1	44.4	61.4	76.1	68.2	22.5	80.5	64.7
		2	40.8	61.5	82.8	65.3	30.1	87.3	66.4
		3	40.8	nd	74	73.5	37.8	74.6	69
	Method B – O_2	1	21.7	61	nd	nd	nd	nd	nd
		2	28.4	70.6	nd	nd	nd	nd	nd
		3	33.8	nd	nd	nd	nd	nd	nd
	Method B – CO_2	1	37.5	58.5	nd	nd	nd	nd	nd
		2	41.5	74.6	nd	nd	nd	nd	nd
		3	40.4	nd	nd	nd	nd	nd	nd
AUA	Method B – O_2	1	9.8	69.5	nd	72.5	50.5	nd	nd
		2	5.9	83.5	nd	72.2	48.8	nd	nd
		3	5.9	85.6	nd	65.3	52.5	nd	nd
	Method B – CO_2	1	49.6	73	nd	67.1	55.3	nd	nd
		2	70.8	58.8	nd	50.2	55.8	nd	nd
		3	67.2	81.8	nd	69.3	34.2	nd	nd
BMG	Method B – O_2	1	18.6	86.6	nd	nd	nd	nd	nd
		2	18.6	84.6	nd	nd	nd	nd	nd
		3	nd	nd	nd	nd	nd	nd	nd
SCION	Method A	1	292.7	128.7	nd	88.8	80.9	164.2	nd
		2	234.1	64.3	nd	130.1	80.4	95.8	nd
		3	225.1	132.9	nd	89.4	84.8	130.9	nd
Average				77.7	81.1	74.1	47.6	95.9	65.8
Stdev				21.0	5.5	18.1	21.4	31.3	5.1
Average (without SCION)				71.9		66.9	39.0	78.8	
Stdev (without SCION)				10.2		6.0	13.2	6.0	

nd = not determined

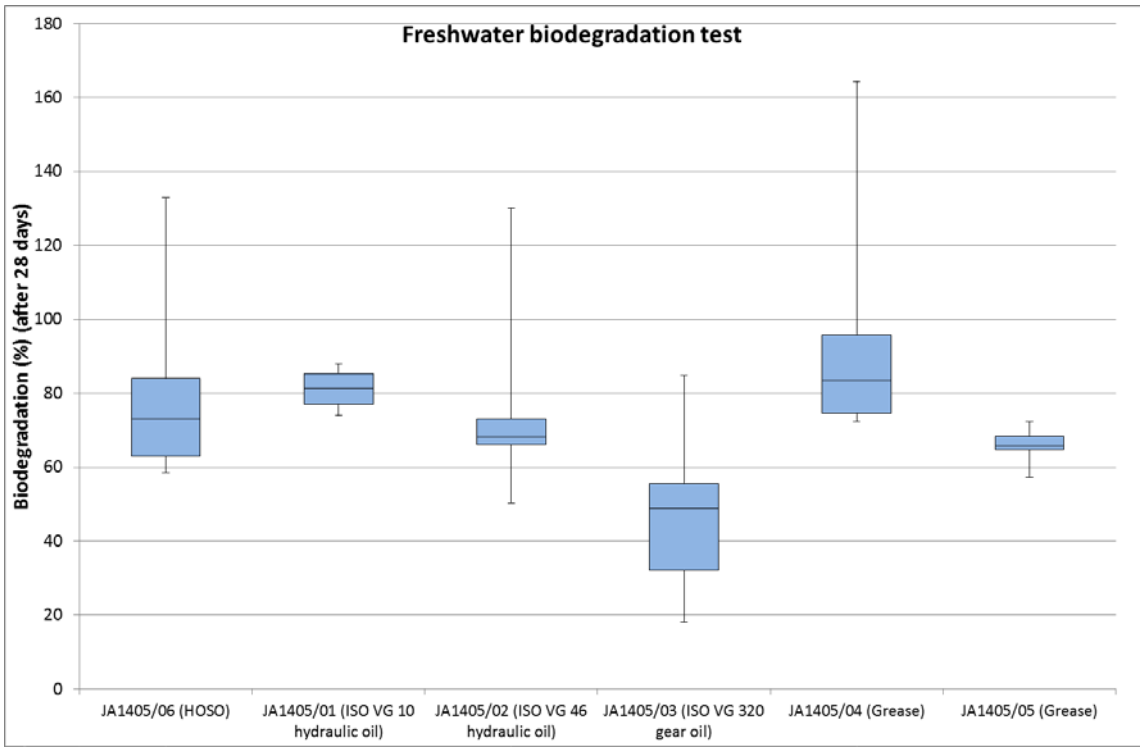

Figure 3. Boxplot with results of freshwater biodegradation test after 28 days (interlaboratory test – part 1) (all partners and 1 lab of CEN/TC 19/WG 33).

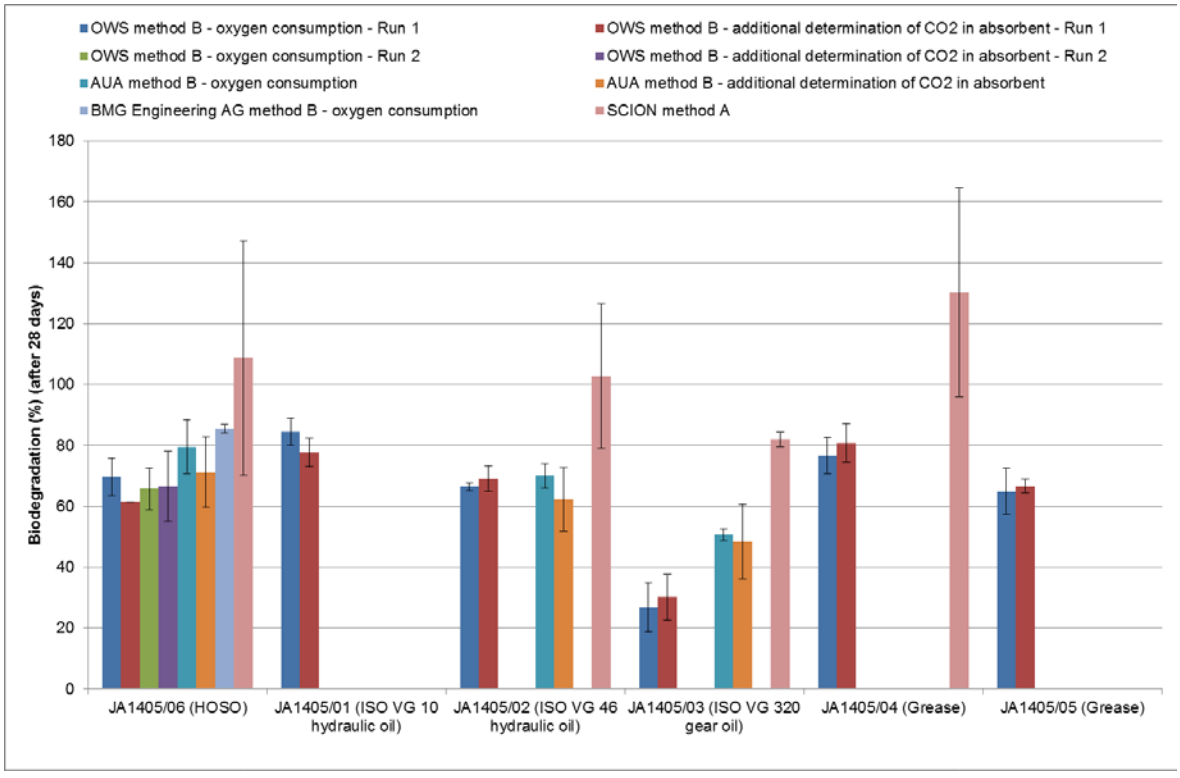

Figure 4. Summary of the biodegradation percentages of lubricants after 28 days (interlaboratory test – part 1) (all partners and 1 lab of CEN/TC 19/WG 33).

The soil methodology was evaluated by AUA, OWS and advisory partner SCION. A summary of the test set-up of the soil test is given in Table 5, while Table 6 shows an overview of the parameters of the inoculum. A high volatile solids content was measured for the soil of SCION. SCION confirmed that no large or obvious plant material was present in the soil. It could be possible that a significant amount of degraded plant material is present in the soil. The high value for the moisture content of the soil from New Zealand is questionable.

Table 5. Overview of test set-up used in interlaboratory soil test (part 1).

Parameters	OWS Natural soil	OWS Standard soil	AUA	SCION
Type	Closed CO_2 apparatus (ASTM D 5988)	Closed CO_2 apparatus (ASTM D 5988)	Closed CO_2 apparatus (ASTM D 5988)	Respirometer (ISO 14855)
Reactor volume (L)	4	4	4	3
Temperature (°C)	25°C±2°C	25°C±2°C	25°C±2°C	25°C±2°C
Quantity of sample (g)	1	3	1 g of C	1.5-2.9
Quantity of inoculum (g)	500	300	300 (dry weight)	400
Measurement method	CO_2 production (titration)	CO_2 production (titration)	CO_2 production (titration)	CO_2 production (IR analysis)
Addition of nutrients	No	Yes (see ISO 17556)	0.1 g N in the form of nitric salt	0.94 g KNO_3 = 0.13 g N

Table 6. Characteristics of the inoculum of interlaboratory test (part 1).

Parameters	Characteristics inoculum			
	OWS	OWS	AUA	SCION
Description inoculum	Mixture of 3 natural soils (sandy + 2 forest soils)	Standard soil (ISO 17556:2012)	Natural soil (clay-loam type)	Natural soil
Dry matter (DM, % on wet weight basis)	78.8	89.8	86	28.4
Moisture content (% on wet weight basis)	21.2	10.2	14	71.6
Volatile solids (VS, % on DM)	8.1	4.2	2	19.0
Ash content (% on DM)	91.9	95.8	98	81.0
pH	7.6	7.9	7.7	6.7
EC (µS/cm)	180	1300	3500	nd
WHC_{tot} (%)	52.5	25.2	nd	95
Moisture content (% on DM basis)	26.6	12.7	40	-
Moisture content (% on DM basis) on WHC_{tot} (%)	50.6	50.7	80.0 (after addition of water)	-
Total N (mg/kg DM)	4500	13900	1500	4410

The results of the biodegradation test in soil on these samples are given in Figure 5 (after 30 days), Figure 6 (after 60 days), Figure 7 (after 150 days) and Figure 8 (after 150 days – box-plot). At SCION only results up to 60 days were available.

Test item JA1405/03 (ISO VG 320 gear oil) is clearly characterised by a lower biodegradation in soil when compared to the other samples. From the boxplot after 150 days, it can be concluded that the boxplots are relatively short. Consequently, it can be concluded that the variation between the results of the different laboratories, using Belgian soil, standard soil (as defined in ISO 17556) and Greek soil, is relatively low.

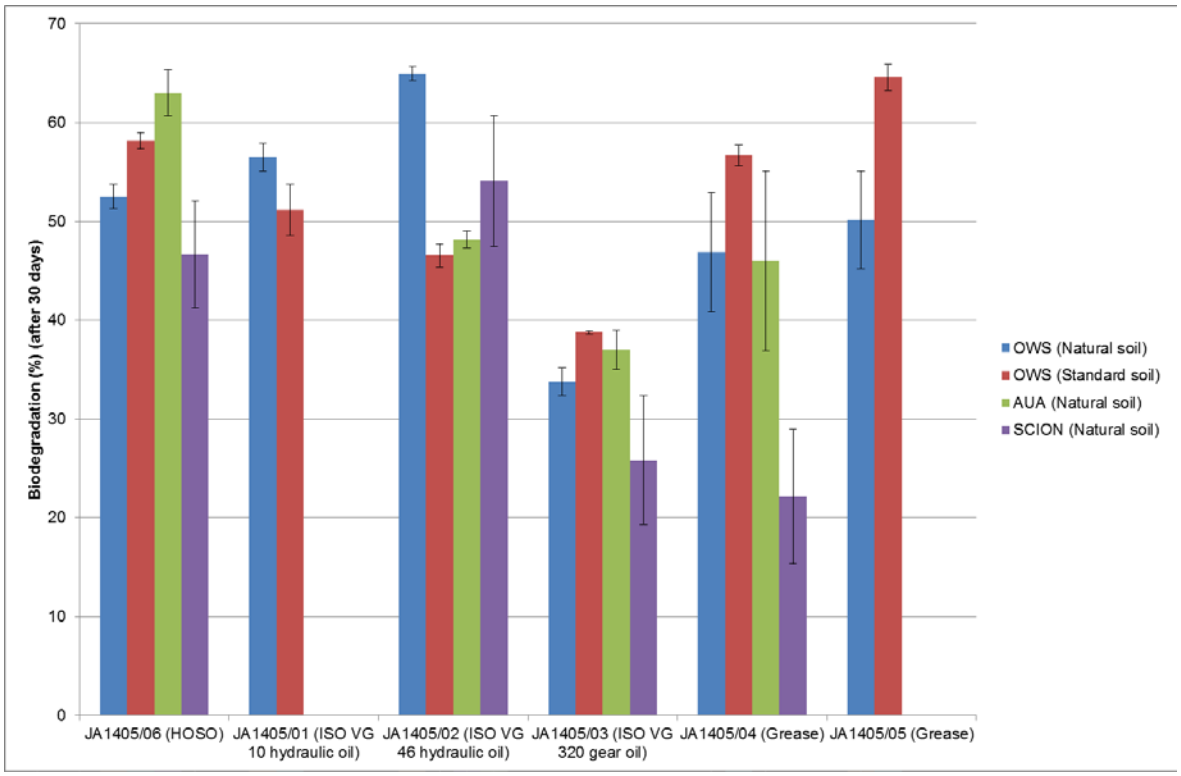

Figure 5. Results biodegradation test in soil after 30 days.

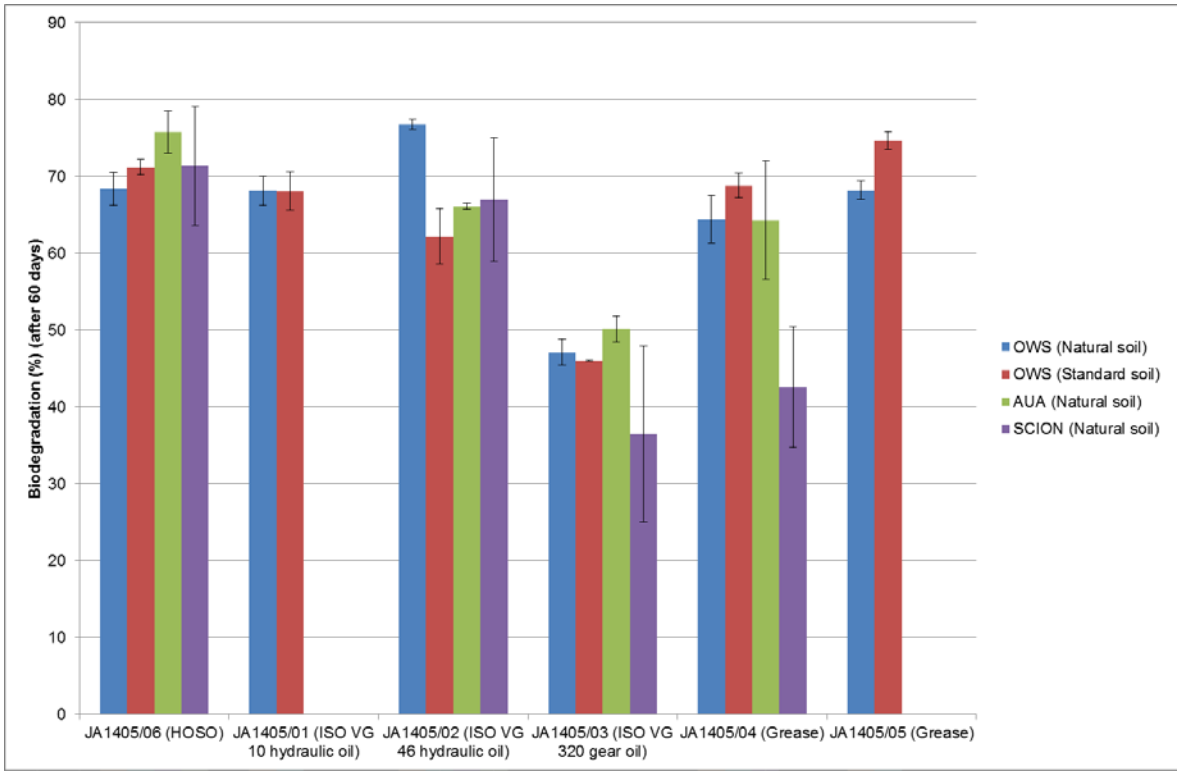

Figure 6. Results biodegradation test in soil after 60 days.

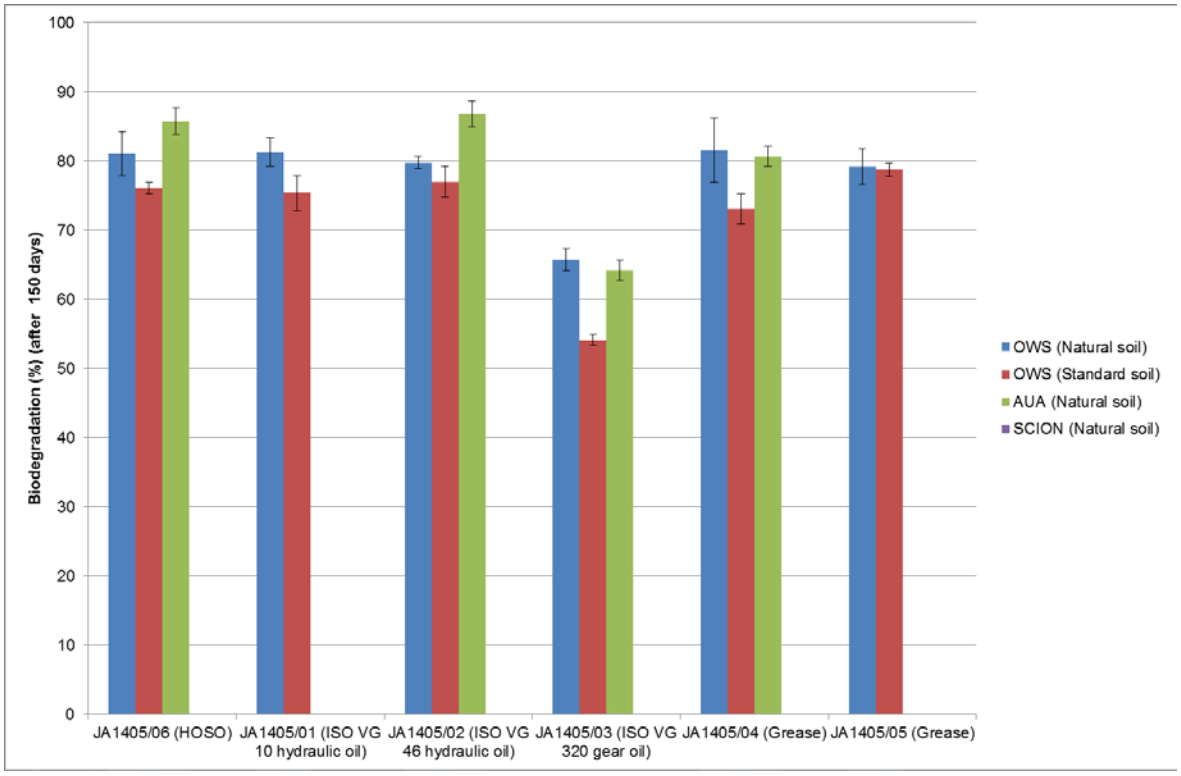

Figure 7. Results biodegradation test in soil after 150 days (AUA and OWS).

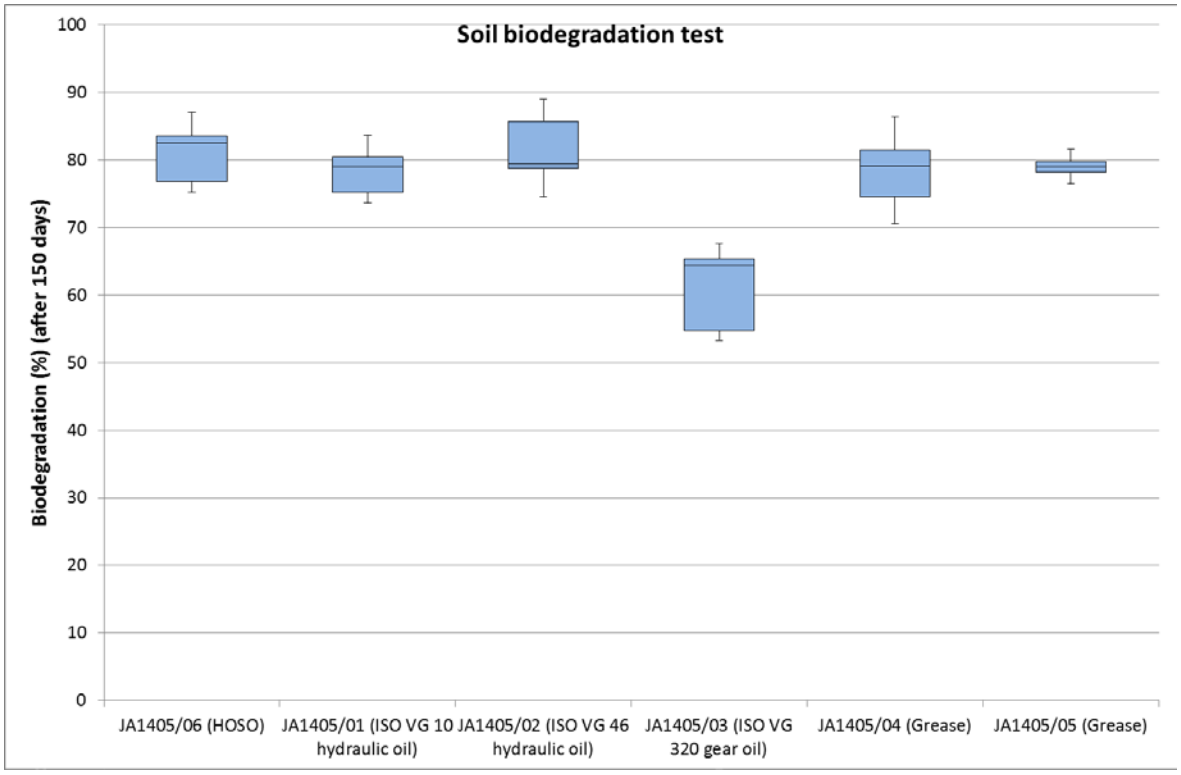

Figure 8. Boxplot with results biodegradation test in soil after 150 days (AUA and OWS).

In Table 7 the biodegradation percentages returned by the participating laboratories after 150 days are shown.

From Table 7, it is seen that the background activity (= CO_2 production in the blank series) in the natural soil is significantly higher when compared to the standard soil as prescribed by ISO 17556:2012. In order to be able to evaluate the signal to noise ratio is high enough, the cumulative carbon dioxide production in natural soil and standard soil is given in Figure 9. In the natural soil series, 1 g sample was added to 500 g natural soil, while 3 g sample was added to 300 g standard soil in the standard soil series[1]. From the data shown in Figure 9 it can be concluded that (1) a higher sample concentration and (2) the use of standard soil are both increasing the signal to noise ratio.

A rather comparable background activity is measured in the Belgian soil and the Greek soil after 150 days. In order to be able to compare the background activity also with the soil from New Zealand, the cumulative carbon dioxide production after approximately 60 days (results were provided by SCION till 60 days) in the natural soil from Belgium (OWS), the standard soil, the natural soil from Greece (AUA) and the natural soil from New Zealand (SCION) is given in Figure 10. The activity in the blank is clearly higher in the natural soil from New Zea-

[1] More sample has been added to the standard soil as this was from a practical point of view possible (due to the lower background activity of standard soil, less titrations are needed). Moreover, 1% sample was added in order to be able to perform toxicity tests after the biodegradation phase on the standard soil series.

land. This can be explained by the fact that volatile solids content in the soil of New Zealand was significantly higher when compared to the Belgian and Greek soil.

Table 7. Biodegradation percentages returned by participating laboratories (soil – after approximately 150 days).

Lab	Soil	Rep	Blank (mg CO_2/kg DM)	Sample					
				06	01	02	03	04	05
OWS	N	1	7080	83.4	80.8	80.8	65.3	81.3	76.4
		2	6880	82.5	83.6	79.4	64.4	86.4	81.6
		3	6930	77.4	79.6	79.1	67.6	77.0	79.6
	S	1	2800	76.2	78.4	78.8	53.3	74.1	78.4
		2	2800	75.2	74.2	74.5	54.4	74.6	79.9
		3	3410	76.8	73.6	77.7	54.7	70.6	78.1
AUA	N	1	7522	83.5	nd	85.7	64.4	81.5	nd
		2	7905	86.8	nd	85.9	65.6	81.5	nd
		3	8002	87.1	nd	89.0	62.6	79.1	nd
Average				81.0	78.3	81.2	61.4	78.5	79.0
Stdev				4.6	3.9	4.6	5.6	4.8	1.8

nd = not determined
N = Natural soil – S = Standard soil

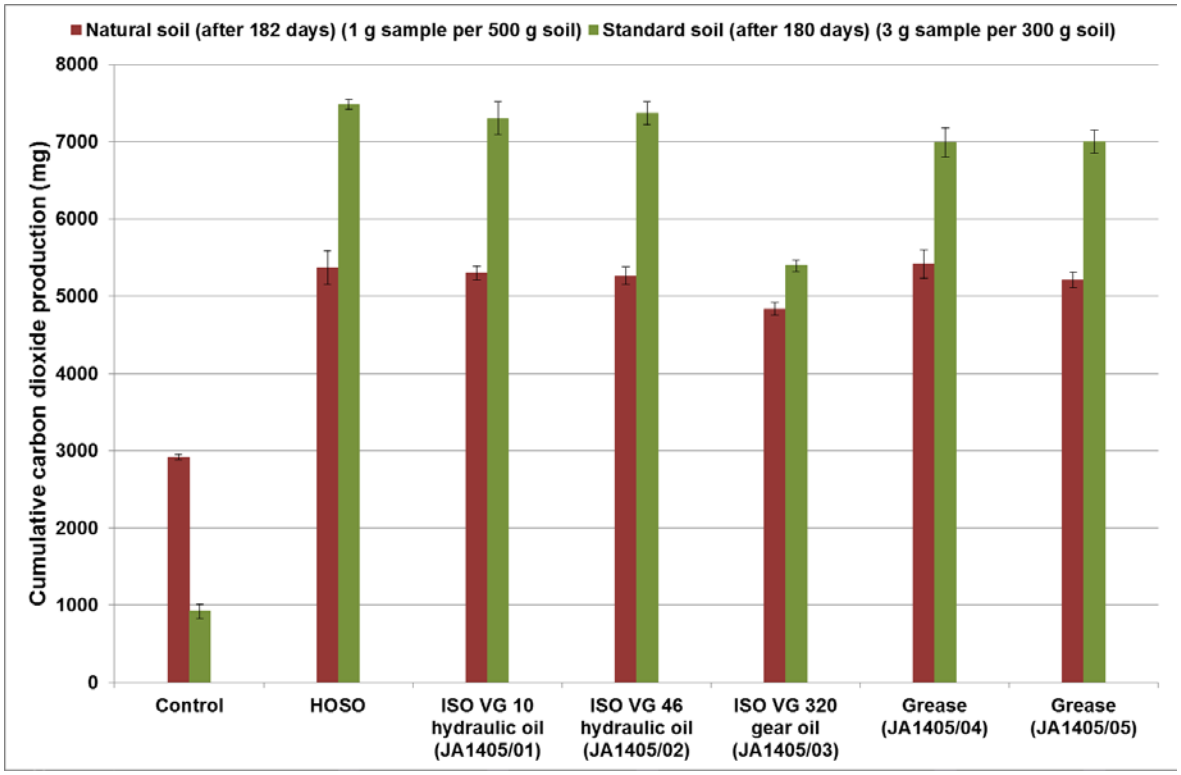

Figure 9. Cumulative carbon dioxde production in natural soil and standard soil after approximately 180 days.

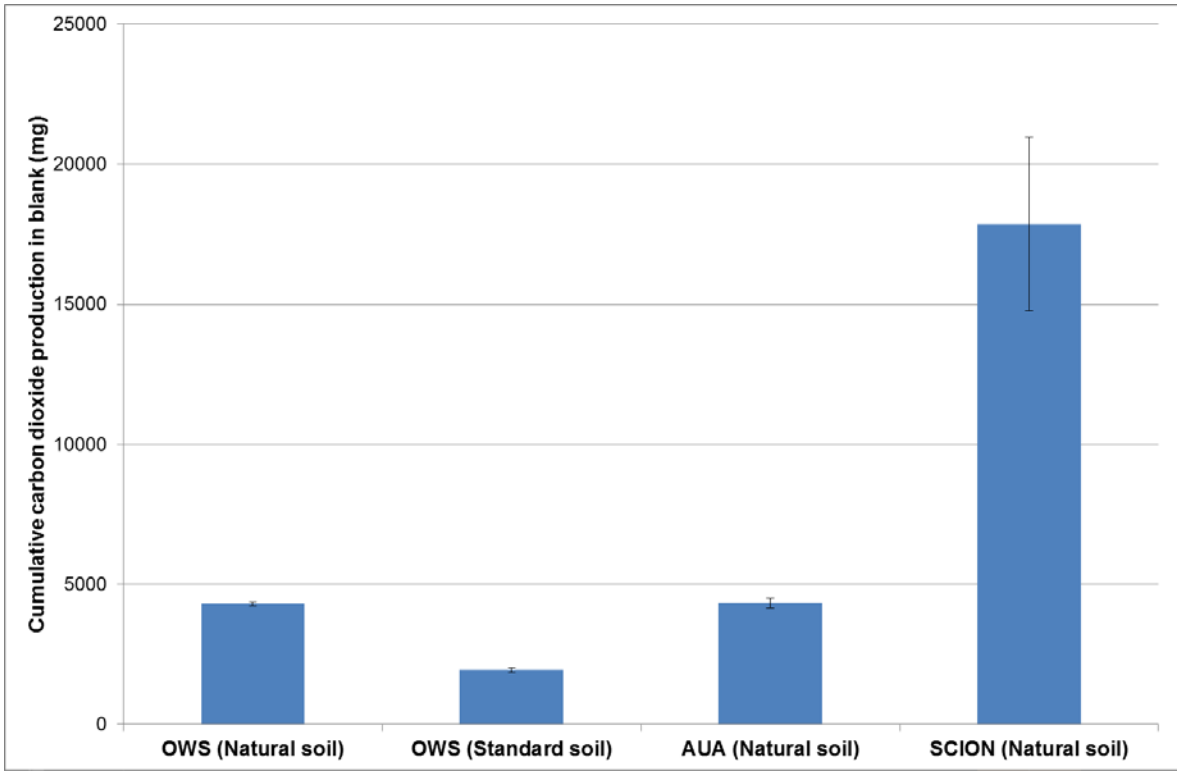

Figure 10. Cumulative carbon dioxide production in natural soil from Belgium (OWS), in standard soil, in natural soil from Greece (AUA) and in natural soil from New Zealand (SCION) after 60 days.

2.4.2 Phase 2

The objective of the second part of the interlaboratory test in freshwater was the evaluation of the reproducibility between the laboratories (as requested by CEN/TC 19/WG 33). The test was executed by 3 laboratories of CEN/TC 19/WG 33 (freshwater methodology), AUA (freshwater and soil), DLO-FBR (freshwater), OWS (freshwater and soil) and advisory partner SCION (freshwater and soil). The test was executed on three samples provided by CEN/TC 19/WG 33:

- Panolin hydraulic oil HEES ISO VG 46, ester-based (Sample A in tables)
- Round robin sample B 15/017-1 (Sample B in tables)
- ACR1411/08 - MFA 111037480 (Sample C in tables)

These samples were characterised by a varying degree of biodegradation: high, medium and low. KBBPPS project partner ECN executed the TOC analyses and the elemental analyses. These data were then redistributed to the participating laboratories. This was done to only test the precision of the test method and not the precision of the determination of the TOC analysis or the elemental analysis.

A summary of the test set-up of the freshwater test is given in Table 8, while Table 9 shows an overview of the parameters of the inoculum. It must be noted that OWS and AUA have used the sample amount as mentioned in the first version of the freshwater methodology (100 mg/l ThOD) (based on ISO 9408), while DLO used the sample amount as mentioned in the second version of the freshwater methodology (170 mg/l ThOD) (based on ISO 14851).

Table 8. Overview of test set-up used in interlaboratory freshwater test (part 2).

Parameters	OWS	OWS	AUA	SCION	DLO
Type	Method A	Method B	Method B	Method A	Method B
Active aeration	50 ml/min air bubbled through reactors	Stirred system (headspace provides O_2)	Stirred system (headspace provides O_2)	10 L/h air bubbled through reactors	Stirred system (headspace provides O_2)
Reactor volume (L)	2	0.5	0.5	3	1
Temperature (°C)	21°C (± 1°C)	21°C (± 1°C)	25°C	25°C (± 2°C)	20°C (± 1°C)
Quantity of sample (mg)	25	10	8-14 mg C	60	50-55
Quantity of inoculum (ml)	1176 (mineral medium) + 24 (inoculum)	245 (mineral medium) + 5 (inoculum)	244 (mineral medium) + 0.96 (inoculum)	1200 (mineral medium) + 11.5 (inoculum)	500 (mineral medium) + 5 (inoculum)
Pre-treatment inoculum	Sieving and aeration for 48 hours	Sieving and aeration for 48 hours			Sieving and aeration for 48 hours
Measurement method	CO_2 production (titration)	O_2 consumption and CO_2 production (titration)	O_2 consumption and CO_2 production (titration)	O_2 and CO_2 levels by IR detector	O_2 consumption and CO_2 production (titration)

Table 9. Characteristics of the inoculum and final medium of interlaboratory test (part 2).

Parameters	Characteristics inoculum (= as such)				
	OWS	AUA	SCION	DLO - Run 1	DLO - Run 2
Description inoculum	Activated sludge from 2 municipal wastewater treatment plants and 1 domestic wastewater treatment plant	Urban sewage	Activated sludge from Rotorua District Council Sewerage treatment plant	Activated sludge from the final aeration tank of the plant treating predominantly the domestic sewage of the village of Bennekom (NL)	Activated sludge from the final aeration tank of the plant treating predominantly the domestic sewage of the village of Bennekom (NL)
pH	6.9 / 6.9 / 6.9	6.8	nd	nd	6.62
TS (%)	0.25 / 0.24 / 0.22	nd	nd	nd	nd
VS (%, on TS)	52.7 / 51.1 / 47.9	87	nd	nd	nd
TSS (g/l)	1.9 / 1.8 / 1.5	7.6	3.16	2.98	3.10
VSS (g/l)	1.2 / 1.2 / 0.94	nd	1.5 g/l Carbon	nd	0.55 g/l
Total N (mg/l)	134 / 118 / 98	4.5	360	nd	170 mg/l
	Characteristics final medium (= mineral medium + inoculum)				
	OWS	AUA	SCION	DLO	DLO
Suspended solids (mg/l)	38 / 36 / 30	30	30	30	30

nd = not determined

The results of the freshwater tests after 28 days are summarized in Figure 11 and Table 10. The comparison between the laboratories was performed after 28 days as all laboratories provided results up to 28 days. DLO provided results up to 49 days (until a plateau in biodegradation was reached). These results are described in detail in deliverable report D6.4 *Biodegradability method validation*.

The background activity measured in the blank is also mentioned in Table 10. The background activities measured with method B (oxygen consumption) are for all partners < 60 mg/l after 28 days. A very low background activity was observed for the second run of DLO. The data of DLO showed that the sensors measured an initial pressure increase (= negative oxygen demand). The three blank vessels showed this phenomena and even though there was some further oxygen consumption visible, the end values sometimes did not climb above 0. Including a correction, a blank oxygen demand of approximately 26 mg/l is calculated, which is within the normal requirements. Theoretically, a pressure increase could be caused by an increasing temperature in the vessels (for example caused by the fact that the temperature in the laboratory is lower when compared to the temperature in the incubation room).

The background activities measured with method A or with method B (carbon dioxide production check) are below or around 70 mg CO_2/l for OWS, while the background activity of AUA and SCION was significantly higher (115 mg CO_2/l ± 10 mg CO_2/l and 250 mg CO_2/l ± 37 mg CO_2/l, respectively) and consequently reliability of the results becomes questionable (especially for SCION).

When comparing the background activity measured by method B based on O_2 and CO_2, the difference should normally be approximately a factor 1.4 (MW CO_2 / MW O_2 = 44 / 32). Especially for the background activity measured at AUA, this factor is considerably higher. According to AUA the discrepancy may be related to the accuracy of the titration method. Consequently also the biodegradation percentages based on the CO_2 production should be interpreted with caution.

Comparable as in the first part of the interlaboratory testing the results of SCION are characterised by a high variability and high (unrealistic) biodegradation percentages (indicated in red in Table 10). SCION had problems with their equipment (due to relatively low air pressures, bubbling air through 1 l of water caused large variations in flow resulting in large variations in CO_2 mass detected including the blanks). According to SCION the biodegradation percentages > 100% and the large variability between the replicates indicate that it is not possible to be confident in the results. Moreover, also the biodegradation of HOSO in run 1 method B (OWS) was characterised by a significant variation. This variation was caused by the presence of an outlier (the biodegradation of one of the three replicates was higher when compared to the other 2 replicates; outliers indicated in red in Table 10).

Figure 12 and Figure 13 represent the results of the freshwater biodegradation test after 28 days in a boxplot, including all data and excluding the data of SCION and the outlier, respectively. From Figure 13 the difference between the three samples can clearly be distinguished. Sample Panolin hydraulic soil HEES ISO VG 46, ester-based is clearly characterized by a high biodegradability, while Round robin sample B 15/017-1 and ACR1411/08 were characterized by a medium and low biodegradation, respectively.

When the outlier of OWS and the results of SCION are not taken into account, it can be concluded that the results of the freshwater interlaboratory test are rather comparable with the results of the interlaboratory test executed on 10 lubricant samples in order to evaluate the reproducibility of ASTM D 5864 which are reported in Research Report RR # D02-1584 (see Table 11). In the KBBPPS interlaboratory test the standard deviation varied between 6.7% and 10.6%, while the standard deviation in the ASTM interlaboratory test varied between 6.1% and 14.7%.

Results of the different partners were forwarded to CEN/TC 19/WG 33. These data were used by the CEN group in order to determine the reproducibility of the developed methodologies.

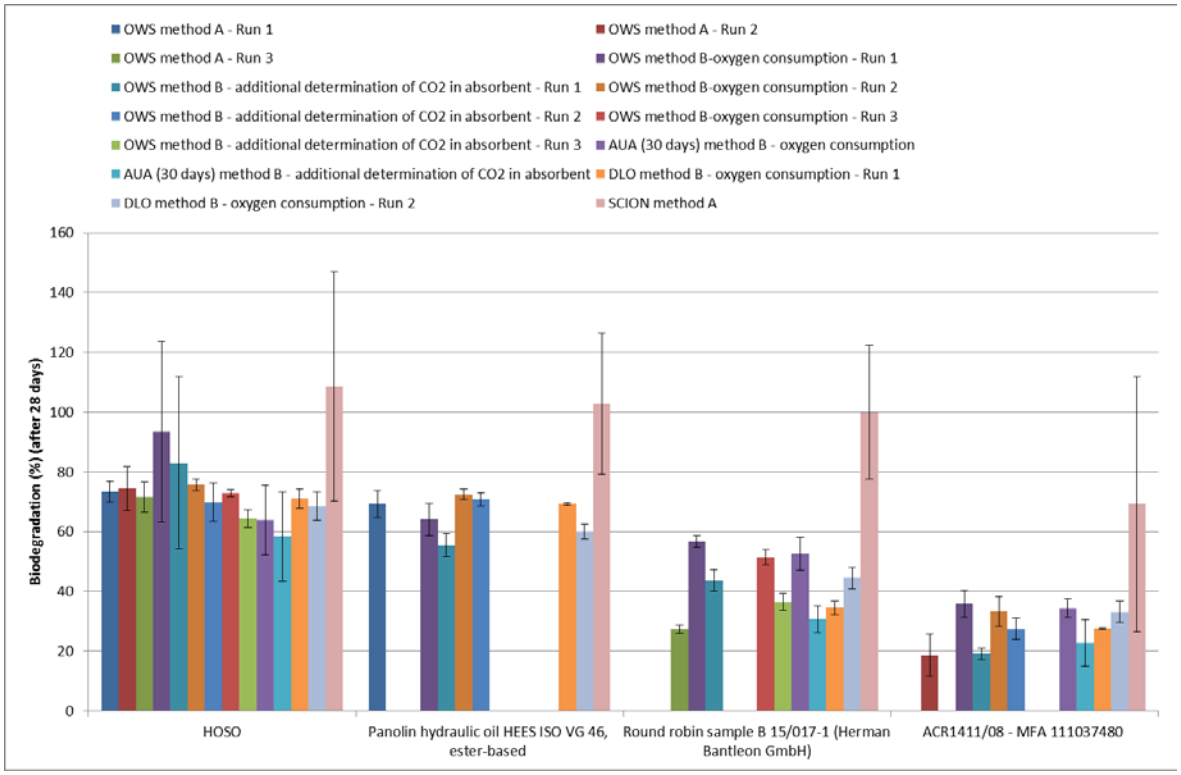

Figure 11. Summary of the biodegradation percentages of lubricants after 28 days (interlaboratory test – part 2).

Table 10. Biodegradation percentages returned by participating laboratories (freshwater - after 28 days – addition method: solvent).

Lab	Method	Rep	Blank (mg O_2/l or mg CO_2/l)	Sample			
				HOSO	A	B	C
OWS	Method A	1	57.0	73.4	73.9	nd	nd
		2	53.3	76.8	68.6	nd	nd
		3	51.0	70.0	65.0	nd	nd
	Method A	1	60.0	76.8	nd	nd	26.9
		2	53.7	80.2	nd	nd	15.3
		3	45.6	66.2	nd	nd	13.8
	Method A	1	58.8	66.1	nd	25.9	nd
		2	53.3	72.6	nd	28.7	nd
		3	52.2	75.9	nd	27.5	nd
	Method B – O_2	1	14.9	127.7	61.9	54.5	31.5
		2	23.0	82.8	70.1	57.4	35.8
		3	16.3	70.1	60.1	58.1	40.4
	Method B – CO_2	1	37.4	114.8	54.7	45.2	16.8
		2	40.5	75.2	59.6	46.3	20.5
		3	46.4	59.1	52.1	39.5	20.0
	Method B – O_2	1	17.6	78.0	73.4	nd	32.6
		2	14.9	74.9	73.5	nd	28.5
		3	15.0	74.3	70.4	nd	38.6
	Method B – CO_2	1	36.2	63.0	69.2	nd	27.8
		2	32.6	70.8	73.2	nd	23.6
		3	33.6	75.9	70.2	nd	30.9
	Method B – O_2	1	17.7	72.1	nd	54.3	nd
		2	17.7	72.1	nd	49.6	nd
		3	23.1	74.2	nd	50.2	nd
	Method B – CO_2	1	36.7	66.3	nd	39.2	nd
		2	36.7	65.9	nd	36.6	nd
		3	40.6	61.0	nd	33.5	nd
AUA	Method B – O_2	1	13.0	74.5	nd	58.9	38.0
		2	34.0	51.4	nd	50.1	32.2
		3	16.0	66.0	nd	48.6	33.0
	Method B – CO_2	1	111.1	63.4	nd	34.4	18.7
		2	125.9	41.6	nd	25.7	31.9
		3	106.8	70.2	nd	31.8	18.0
DLO	Method B – O_2	1	28.2	73.5	69.0	32.9	27.7
		2	26.6	72.3	69.5	36.1	27.4
		3	28.4	67.5	nd	nd	nd
	Method B – O_2	1	-0.4	65.3	61.7	42.0	30.6
		2	2.5	66.3	58.4	47.0	35.7
		3	0.4	74.1	nd	nd	nd
SCION	Method A	1	292.7	128.7	88.8	87.6	23.1
		2	234.1	64.3	130.1	125.9	77.3
		3	225.1	132.9	89.4	86.5	107.2

Lab	Method	Rep	Blank (mg O_2/l or mg CO_2/l)	Sample			
				HOSO	A	B	C
Average (after 28d)				75.0	71.0	48.3	32.3
Stdev (after 28d)				18.5	16.1	21.6	18.9
Average (without outlier OWS + SCI-ON) (after 28 days)				69.7	66.0	42.2	27.8
Stdev (without outlier OWS + SCION) (after 28 days)				7.9	6.7	10.6	7.7

Table 11. Summary of results of research report RR# D02-1584.

Sample	Biodegradation	
	Average	Stdev
Vegetable oil hydraulic fluid	65.0	6.1
Vegetable oil-based grease	56.4	14.7
Low biodegradable synthetic PAO	21.1	8.8
Vegetable oil-Aluminum complex grease	19.3	11.9
High biodegradable synthetic hydraulic fluid	59.7	13.6
Mineral oil hydraulic fluid	26.0	6.4
High biodegradable synthetic PAO	58.8	12.2
High biodegradable synthetic ester	70.8	7.8
Moderately biodegradable synthetic PAO	29.5	8.9
Low erucic acid rapeseed	79.3	10.4

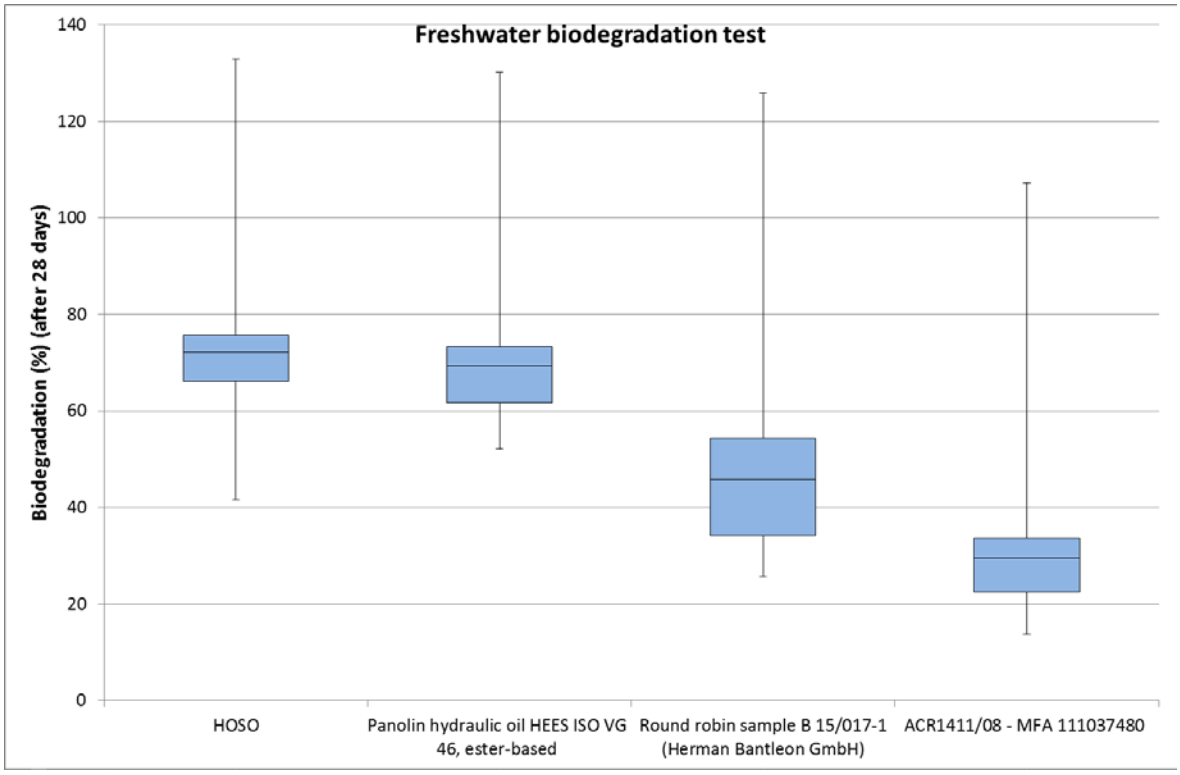

Figure 12. Boxplot with results biodegradation in freshwater after 28 days.

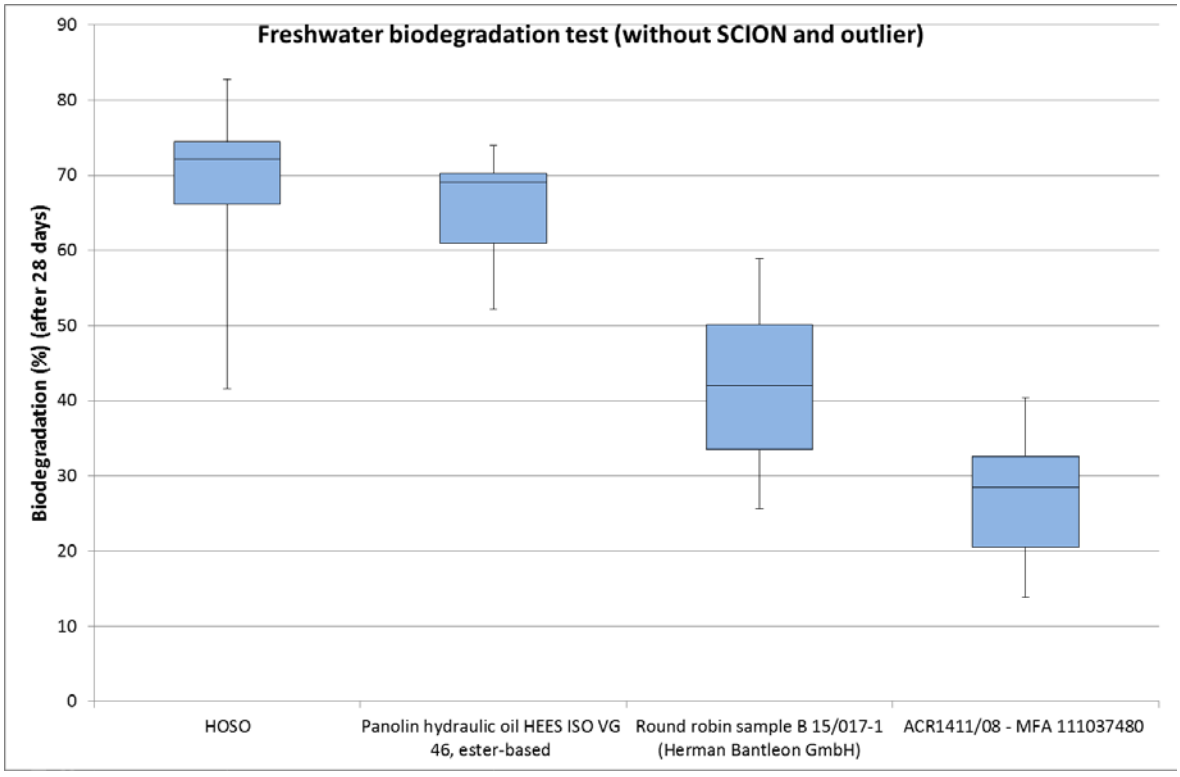

Figure 13. Boxplot with results biodegradation in freshwater after 28 days (without results SCION and outlier OWS).

In order to compare the biodegradation results per methodology (Method A versus Method B based on oxygen consumption versus Method B based on determination of CO_2 absorbed in absorbent), a boxplot was created comparing the methodologies per sample (see Figure 14 and Figure 15 (with and without results SCION and outlier OWS, respectively)).

Due to the fact that the results of SCION were characterised by unrealistic high biodegradation percentages, the results of Method A in Figure 15 were only obtained by 1 partner (OWS). Consequently, the comparison between the results of Method A and Method B is only a first indication, that needs to be evaluated later in the Open-BIO project. For the samples with the medium and low biodegradability, it was noticed that test Method A resulted in lower results when compared to Method B, while rather similar values were obtained for the samples with the high biodegradability.

When comparing the results of Method B based on oxygen consumption and based on carbon dioxide production, it was noticed for all samples that the biodegradation values based on the oxygen consumption were higher when compared to the values based on carbon dioxide production.

Possible reasons?

- Some of the produced CO_2 could be dissolved in the solution.
- Oxygen consumption by nitrification. However, at the end of the biodegradation test the nitrate content is normally evaluated by means of strips and no nitrate was detected. Possibly the strips are not sensitive enough to reveal small augmentations. Moreover, ATU was added to the reactors to inhibit the nitrification process.
- Necessary to describe titration method more in detail.

This will be further evaluated in the Open-BIO project (WP5 In-situ biodegradation).

The results of the freshwater tests executed per partner were forwarded to CEN/TC 19/WG 33 and they were used by the CEN group to calculate the reproducibility of the methodologies.

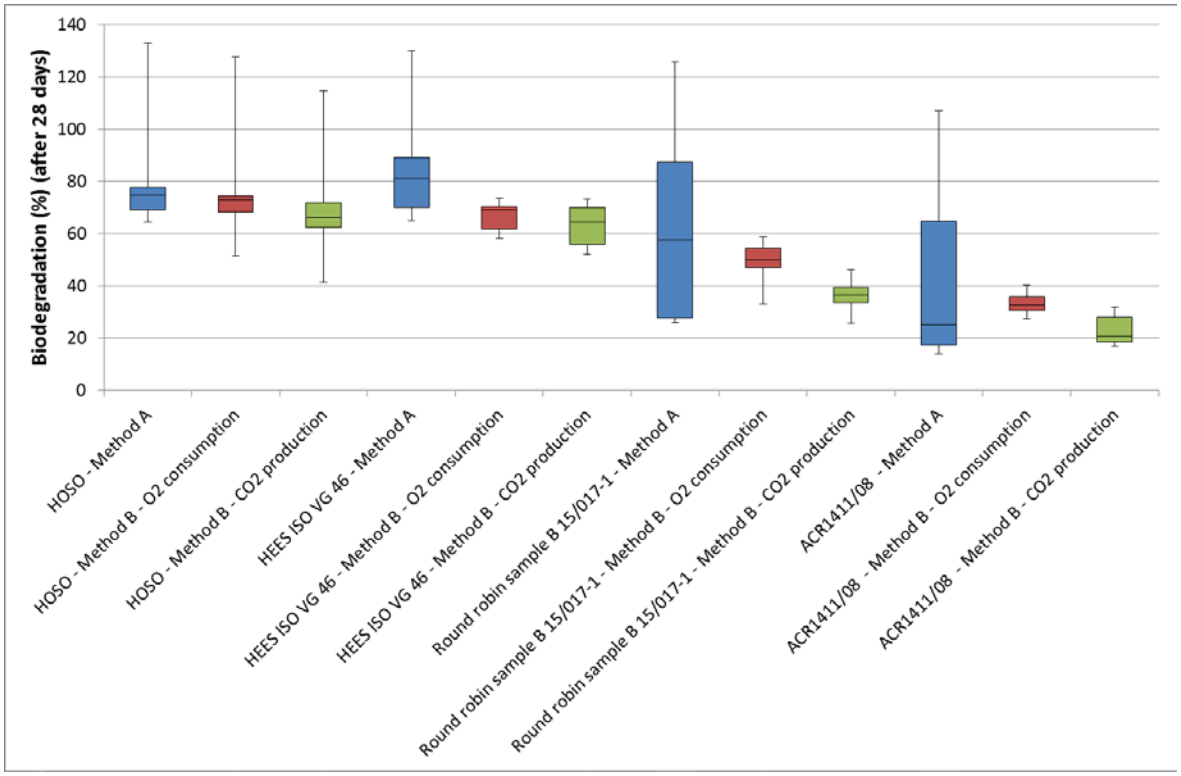

Figure 14. Boxplot with results freshwater biodegradation test after 28 days per method.

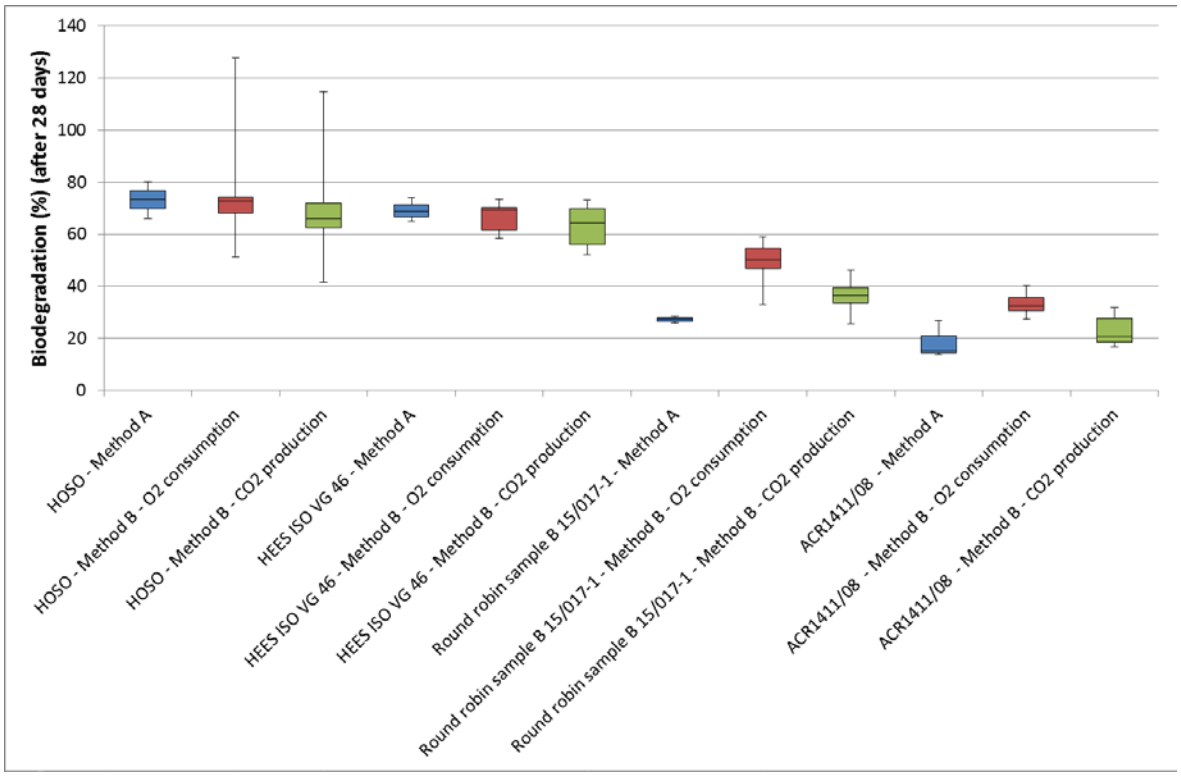

Figure 15. Boxplot with results biodegradation in freshwater after 28 days per method (without results SCION and outlier OWS).

The soil methodology was evaluated by AUA, OWS and advisory partner SCION. A summary of the test set-up of the soil test is given in Table 12, while Table 13 shows an overview of the parameters of the inoculum. A high volatile solids content was measured for the soil of SCION. SCION confirmed that no large or obvious plant material was present in the soil. It could be possible that a significant amount of degraded plant material is present in the soil. The high value for the moisture content of the soil from New Zealand is questionable.

Table 12. Overview of test set-up used in interlaboratory soil test (part 2).

Parameters	OWS Natural soil	OWS Standard soil	AUA	SCION
Type	Closed CO_2 apparatus (ASTM D 5988)	Closed CO_2 apparatus (ASTM D 5988)	Closed CO_2 apparatus (ASTM D 5988)	Respirometer (ISO 14855)
Reactor volume (L)	4	4	4	3
Temperature (°C)	25°C±2°C	25°C±2°C	25°C±2°C	25°C±2°C
Quantity of sample (g)	1	2	1g C	1.5-2.9
Quantity of inoculum (g)	500	500	300 g dry	400
Measurement method	CO_2 production (titration)	CO_2 production (titration)	CO_2 production (titration)	CO_2 production (IR gas analysis)
Addition of nutrients	No	Yes (as prescribed in ISO 17556)	0.1 g N in the form of nitric salt	Yes (0.94 g KNO_3 = 0.13 g N)

Table 13. Characteristics of the inoculum of interlaboratory test (part 2).

Parameters	Characteristics inoculum			
	OWS	OWS	AUA	SCION
Description inoculum	Natural soil	Standard soil	Natural soil - clay loam	Natural soil
Dry matter (DM, % on wet weight basis)	78.0	88.0	86.21	28.4
Moisture content (% on wet weight basis)	22.0	12.0	13.79	71.6
Volatile solids (VS, % on DM)	7.9	4.0	2.25	19.0
Ash content (% on DM)	92.1	96.0	97.75	81.0
pH	7.9	8.4	8	6.7
EC (µS/cm)	nd	1049	3500	nd
WHC_{tot} (%)	53.5	27.4	nd	95
Moisture content (% on DM basis)	28.2	13.6	40.00	-
Moisture content (% on DM basis) on WHC_{tot} (%)	52.7	49.8	80 (after addition of water)	-
Total N (mg/kg DM)	3800	1800	1500	4410

The results of the biodegradation test in soil on these samples are given in Figure 16 (after 30 days), Figure 17 (after 60 days), Figure 18 (after 120 days) and Figure 19 (after 120 days – boxplot) and in Table 14. At SCION only results up to 60 days were available.

Also in the soil biodegradability test the difference between the three samples can clearly be distinguished. Sample Panolin hydraulic soil HEES ISO VG 46, ester-based is clearly characterized by a high biodegradability comparable to the biodegradation of positive reference material HOSO, while Round robin sample B 15/017-1 and ACR1411/08 were characterized by a medium and low biodegradation, respectively. This is in line with the results of the freshwater biodegradation test.

From Figure 19 it can be concluded that the variation between the results is smaller for the easily biodegradable samples when compared to the samples characterised by a medium and low biodegradability. This was also observed in the Round Robin test that was executed in ISO 17556:2012 on microcrystalline cellulose reference material and starch/poly(butylene adipate-co-butylene terephthalate) blend test material. The standard deviations obtained in the Round Robin test on plastics was higher (Reference material: 69.19% ± 9.91% (natural soil) – 64.50% ± 10.91% (standard soil) // Test material: 39.39% ± 26.03% (natural soil) – 40.62% ± 25.96% (standard soil)) when compared to the standard deviations in this test. However, it must be noticed that significantly more laboratories (6) participated to the Round robin test on plastics and additionally also more test set-ups were evaluated per laboratory (up to 5).

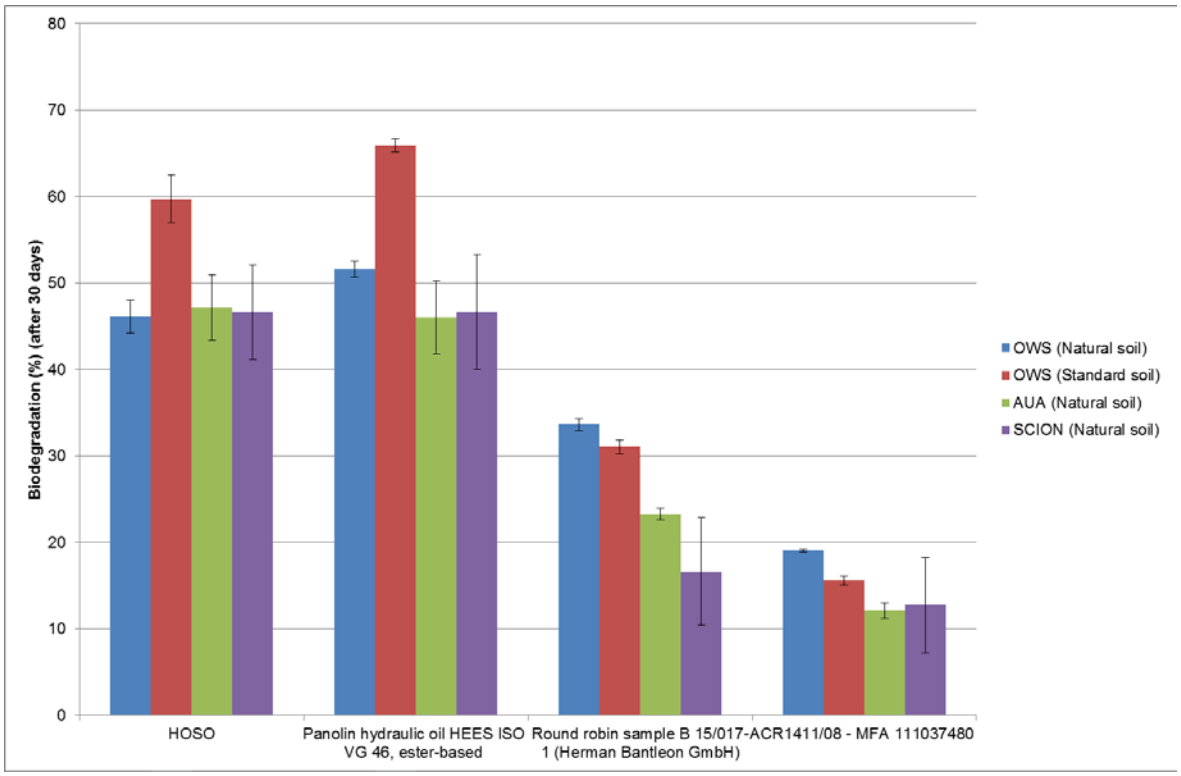

Figure 16. Results biodegradation test in soil after 30 days (AUA, OWS and SCION).

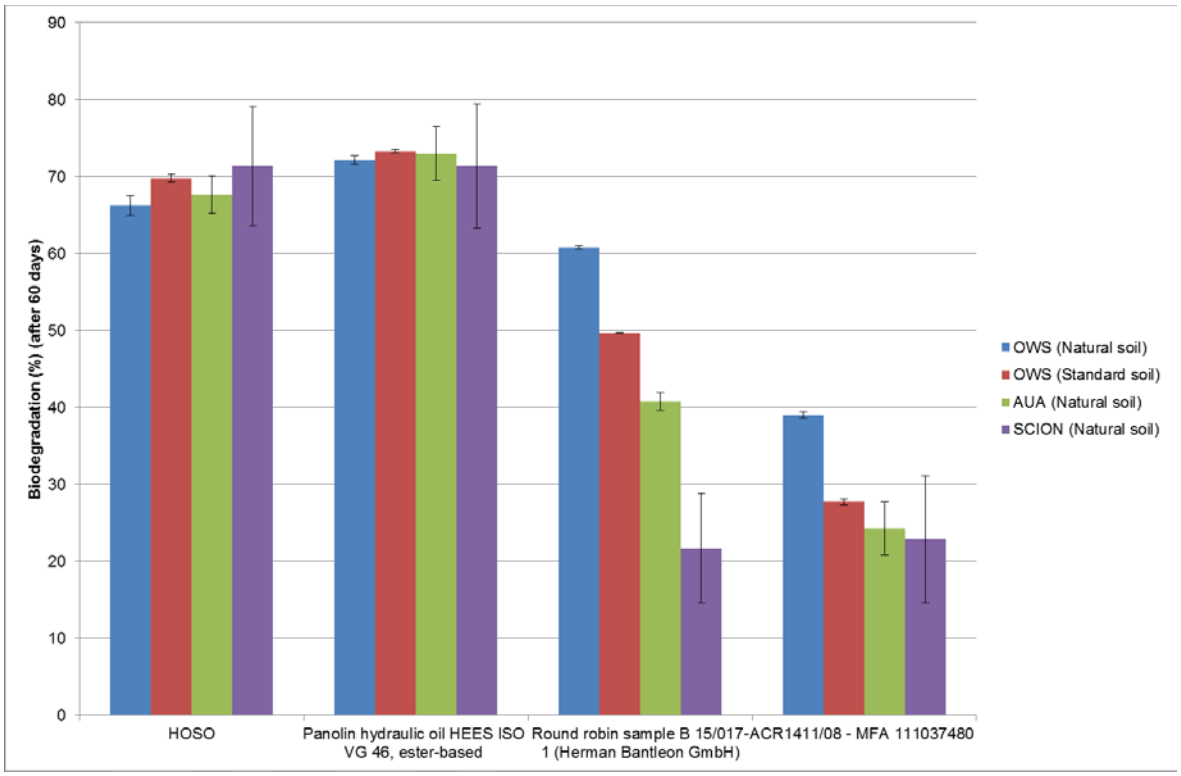

Figure 17. Results biodegradation test in soil after 60 days (AUA, OWS and SCION).

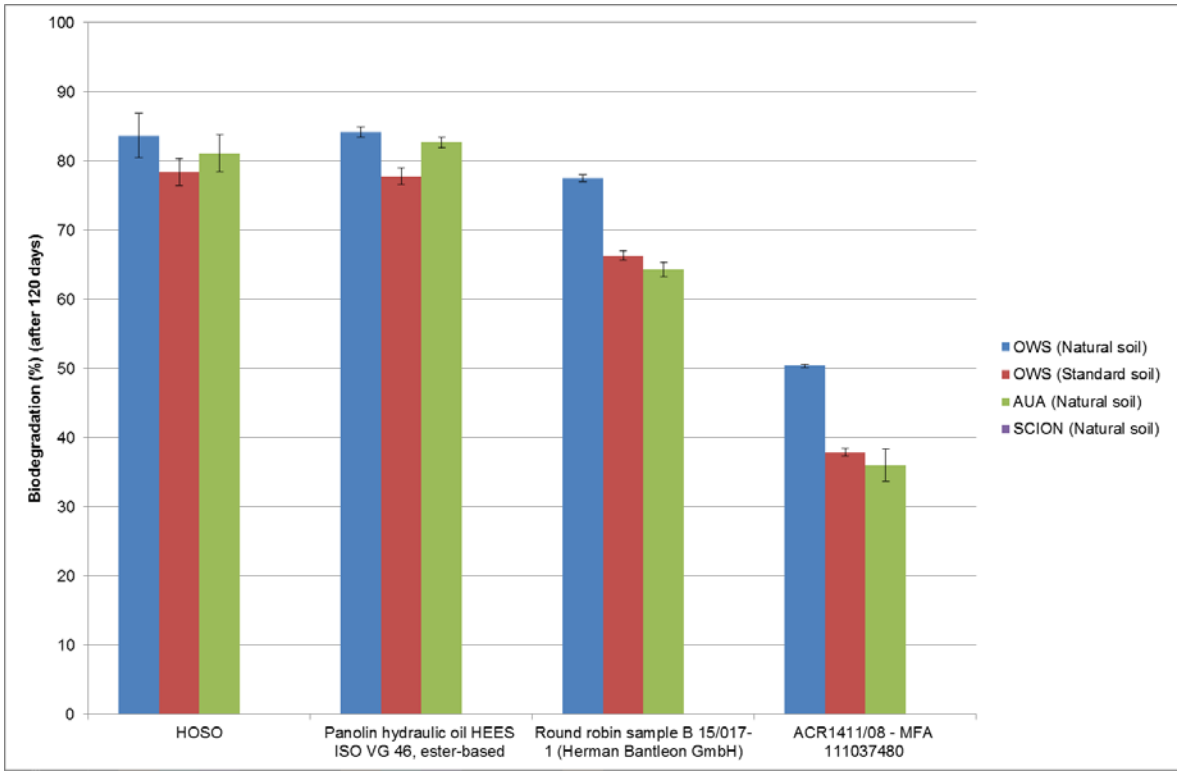

Figure 18. Results biodegradation test in soil after 120 days (AUA and OWS).

Table 14. Biodegradation percentages returned by participating laboratories (soil – after approximately 120 days).

Lab	Soil	Rep	Blank ($mg\ CO_2/kg\ DM$)	Sample			
				HOSO	A	B	C
OWS	Natural	1	5400	80.1	85.0	78.1	50.6
		2	5400	85.1	83.9	77.6	50.2
		3	5400	86.0	83.7	77.1	50.4
	Standard	1	3110	77.7	77.8	67.1	37.6
		2	3150	80.6	76.5	66.0	37.6
		3	3070	76.9	79.0	66.0	38.5
AUA	Natural	1	4733	84.2	82.3	64.6	33.5
		2	4785	79.9	83.6	65.1	38.2
		3	4444	79.3	82.3	63.2	36.4
Average				81.1	81.6	69.4	41.4
Stdev				3.2	3.0	6.2	6.9

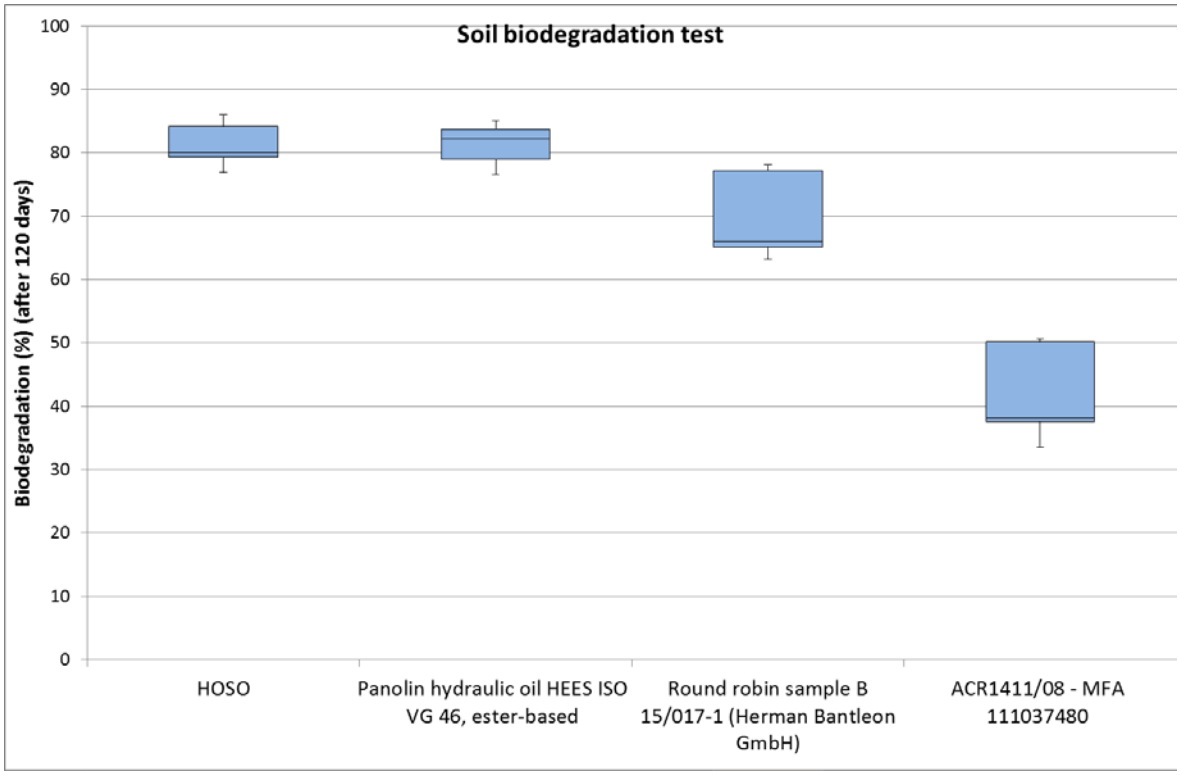

Figure 19. Boxplot with results biodegradation test in soil after 120 days (AUA and OWS).

The results of the soil biodegradation test of OWS were reported in the CEN/TC 19/WG 33/TF Biodegradation meeting in order to introduce the executed work with regard to soil biodegradability at the CEN group.

3 Follow-up work in Open-BIO

In the follow-up project Open-BIO it is foreseen that the developed methodologies for testing biodegradation of lubricants in freshwater and soil will be adjusted to be applicable to a broad range of bio-based products. The adjusted horizontal methodologies will then be tested and verified for bio-based plastics through Round robin tests and refined as needed.

Based on the experiences from the current interlaboratory tests, the test methodologies will first be refined and improved based on the experiences of the partners that participated to the interlaboratory testing in KBBPPS. An overview of the remarks of the partners is given below:

- Freshwater methodology - Method A
  - The methodology prescribes that incubation shall take place in dark or diffuse light. In order to exclude growth of algae, it would be better to remove diffuse light.
- Freshwater methodology - Method B
  - Titration methodology in order to double-check the biodegradation values obtained based on oxygen consumption should be described in detail. This include the amount and type of CO_2-absorbent, the molarity of the acid used for titration, whether the absorbent shall be titrated directly, of sampled so replicates are possible. (In case of sampling, how is prevented that CO_2 from the air interferes with the measurement, etc.)
  - In order to examine the reason of the difference between the values based on oxygen consumption and carbon dioxide production, an additional acidification step could be included in the methodology. The same approach as described in Method A could be used: *Determine the pH of all test flasks on the last day of test, acidify all the bottles with 1 to 10 ml of concentrated hydrochloric acid to decompose the carbonates and bicarbonates and close them immediately. Continue aeration for another 24 h, followed by determination of carbon dioxide for each flask.*
  - The methodology prescribes that incubation shall take place in dark or diffuse light. In order to exclude growth of algae, it would be better to remove diffuse light.
  - In order to avoid that an initial pressure increase in observed in the vessels, the temperature in the laboratory and the incubation room should be identical.
- Soil biodegradation methodology
  - In order to increase the signal to noise ratio a higher test item concentration could be suggested in combination with a low organic matter content in the natural soil. Also the use of standard soil could help to increase the signal to noise ratio.

4 Conclusion

The objective of WP6 “Biodegradability” was the development and validation of biodegradation methodologies in freshwater and soil for bio-lubricants. The methodologies in freshwater were developed in CEN/TC 19/WG 33/TF “Biodegradation”, while the methodology in soil was developed amongst the project partners. The developed methodologies are based on existing biodegradation methodologies for organic compounds and/or plastics. Modifications towards reference material, inoculum and sample addition were included in order to make the methods suitable/reproducible for bio-lubricants.

As reference material HOSO (High Oleic Sunflower Oil) was selected. The characteristics of this material lay closer to the characteristics of lubricants when compared to the positive reference materials as mentioned in the biodegradation methodologies for organic compounds (aniline, sodium acetate, etc.) and plastics (cellulose, etc.).

In order to decrease the variability between the laboratories, only 1 inoculum source (activated sludge) was prescribed in the freshwater biodegradation methodology.

Two interlaboratory tests were executed on request of CEN/TC 19/WG 33. The samples for these tests were provided by the CEN group.

The research question of the first interlaboratory test was “Which addition method is the most appropriate method to add lubricants to the test reactors for a freshwater biodegradation test?”. In order to investigate this, two addition methods ((1) addition by means of a solvent and (2) addition directly on the stirrer) were compared by means of 5 different samples. From the executed tests it could be concluded that the addition by means of a solvent resulted in results characterised by a lower variability between the replicates when compared to the addition directly on the stirrer.

The results of the first interlaboratory test were presented by OWS during the meeting of CEN/TC 19/WG 33 “TF Biodegradation” in Mannheim (August 2014). The results confirmed the expectations of the other participating laboratories (addition method in a solvent is the most optimal).

The research question of the second interlaboratory test was “Are the methodologies reproducible enough?”. In order to investigate this, the developed methodologies were used in order to determine the biodegradation of 3 samples with varying biodegradability (high – medium – low). The test was executed by 3 laboratories of CEN/TC 19/WG 33 (freshwater methodology), AUA (freshwater and soil), DLO-FBR (freshwater), OWS (freshwater and soil) and advisory partner SCION (freshwater and soil).

The results of the freshwater biodegradation test after 28 days were characterised by a maximum standard deviation around 10% (not taken into account the unrealistic high biodegradation percentages of SCION), while for the soil biodegradation test the standard deviation of the biodegradation percentages after 120 days was < 7%. Such standard deviations are comparable to or even lower than standard deviations reported in RR# D02-1584 (biodegradation in freshwater of lubricants) and ISO 17556:2012 (biodegradation in soil of plastics). The difference between the samples with high, medium and low biodegradation was clearly observed. Nevertheless, based on the experiences with the interlaboratory tests, research partners posed suggestions for further modification of the standard text (such as the light regime, and more detailed description of the titration methodology) to improve clarity and reproducibility.

The biodegradation results (freshwater) of the KBBPPS partners were forwarded to CEN/TC 19/WG 33 “TF Biodegradation”. The freshwater biodegradation data obtained in the KBBPPS project were used together with the data of the other laboratories that participated to the interlaboratory test by the CEN group in order to determine the reproducibility of the developed freshwater methodologies. The results of the biodegradation tests in freshwater and soil were also presented by OWS during the meeting of CEN/TC 19/WG 33 “TF Biodegradation” in Berlin (May 2015).

5 References

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