STATEMENT

KOPI

Biodegradability of oxodegradable LDPE film from Nordic Ecoflex Int. in line with ASTM D6954 (2013)

OWS nv evaluated the biodegradability of oxodegradable LDPE film (45 µm), produced by Nordic Ecoflex Int., Huginsvej 15, 4100 Ringsted, DENMARK, according to American standard ASTM D6954 *Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation* (2013). According to the manufacturer the film was produced from LDPE pellets to which PDQ-M Oxo additive (supplied by Willow Ridge) had been added.

Prior to biodegradation testing the film was weathered during 900 hours (= 37.5 days) in a weathering cycle of 20 hours UVA exposure (0.89 W/m² at 340 nm) at 50°C, followed by 4 hours condensation at 40°C according to ASTM D 5208 *Standard Practice for Fluorescent Ultraviolet (UV) Exposure of Photodegradable Plastics (Cycle A)* (2009), which is part of ASTM D6954 (2013). The actual biodegradation test was performed according to ASTM D5988 *Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residuals Plastic Materials after Composting* (2012).

The biodegradation of weathered plastic film, with a measured organic carbon content of 62.3%, started after a lag phase of about 200 days. After 400 days a biodegradation of 20.5% was measured. The biodegradation proceeded at a constant moderate rate and after 782 days a biodegradation of 64.0% ± 6.9% was reached. On a relative basis, with cellulose as the suitable reference substrate, a biodegradation of 61.1% was calculated. Moreover biodegradation of Weathered plastic film was still proceeding.

The American standard ASTM D6954 (2013) stipulates that for products consisting of a single polymer (homopolymers or random copolymers), 60% of the organic carbon must be converted to carbon dioxide before ending the test.

Gent, May 4th, 2016

NORDIC ECOFLEX - DENMARK
9/5-2016
OXO-BIODEGRADABLE PRODUCTS
Cell: +250(0) 787780456
EXECUTIVE DIRECTOR OFFICE

Bruno De Wilde
Lab Manager

KOPI

KOPI

FINAL REPORT
NordicEcoRW2-RMU-1/2

Natural soil biodegradation test on
Weathered plastic film

Author: Lynn Serbruyns
Sponsor: Nordic Ecoflex Int.
Huginsvej 15
4100 Ringsted
DENMARK

Through
Rwanda Bureau of Standards (RBS)
PO.Box 7099
Kicukiro-KK 15 Rd
Kigali
RWANDA

KOPI

Table of contents

1 Identification of the test

1.1 General information

Project number
NordicEcoRW2-RMU-1/2

Sponsor
Nordic Ecoflex Int.
Huginsvej 15
4100 Ringsted
DENMARK

Through
Rwanda Bureau of Standards (RBS)
PO.Box 7099
Kicukiro-KK 15 Rd
Kigali
RWANDA

Monitoring scientists
Mr. Antoine Mukunzi
antoine.mukunzi@rsb.gov.rw

Mr. Raymond Murenzi
raymond.murenzi@rbs.org.rw
Phone: +250 252 582 949

Testing facility
OWS nv
Dok Noord 5
9000 Gent
BELGIUM
Phone: +32 9 233 02 04
Fax: +32 9 233 28 25
bruno.dewilde@ows.be
lynn.serbruyns@ows.be

Test item
Weathered plastic film

Reference item
Cellulose

Test duration
782 days

1.2 Study personnel

Study Director:	Lynn Serbruyns
Replacement Study Director:	Bruno De Wilde
Study Director QA:	Steven Verstichel

1.3 Study schedule

Starting date study:	January 14th, 2014
Starting date experiments:	January 14th, 2014
Starting date of incubation:	January 15th, 2014
Completion date of incubation:	March 7th, 2016
Completion date of experiments:	March 24th, 2016
Completion date study:	April 7th, 2016
Total test duration:	782 days

1.4 Archiving

All raw data and records necessary to reconstruct the study and demonstrate adherence to the study plan will be maintained in the archives of OWS nv. These records include notebooks, study plan, study report, samples of test item and specimens. They will be stored in a file coded:

RMU-1/2

The training records of personnel are stored in the maps 'Organisation and Personnel'. These files are stored per person and administered by the Lab Quality Manager and the Assistant Lab Quality Manager.

After seven (7) years, all data and records will be destroyed or returned to the sponsor after agreement in writing by the involved Sponsor and the Study Director. In case no written agreement of the sponsor can be obtained after seven years, the data and records will be destroyed.

2 Confidentiality statement

The Testing Facility will treat strictly confidential all relevant information on the test item disclosed by the Sponsor as well as all results obtained in executing the test.

Bruno De Wilde
Lab Manager

3 GLP compliance statement

The test was performed in accordance with the OECD principles of Good Laboratory Practices (GLP).

Lynn Serbruyns
Study Director

4 Quality assurance audit statement

The results reported are in accordance with the study plan and raw data.

A quality control was executed on Apr-13-2016

This quality control ensures that the final report is complete and accurately reflects the conduct and raw data of the study.

Steven Verstichel
Study Director QA

5 Summary and conclusions

The aerobic biodegradation of test item Weathered plastic film was evaluated in a natural soil biodegradation test according to ASTM D5988 *Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residuals Plastic Materials after Composting* (2012), which is part of American standard ASTM D6954 *Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation* (2013). Prior to start-up test item Weathered plastic film was weathered during 900 hours (= 37.5 days) in a weathering cycle of 20 hours UVA exposure (0.89 W/m² at 340 nm) at 50°C, followed by 4 hours condensation at 40°C. The incubation temperature was continuously kept at 21°C ± 2°C. The total test duration was 782 days.

According to ASTM D5988 (2012) the test is considered valid when the standard deviation of the CO_2 production of the control reactors is less than 20% of the mean at the plateau phase or at the end of the test. After 782 days a value of 0.6% was reached, demonstrating the good precision of the test. Furthermore the degree of biodegradation of the reference material (cellulose) should be more than 70% at the plateau phase or at the end of the test. After 70 days cellulose was already degraded by 73.1%. At the end of the test (after 782 days) a plateau in biodegradation was reached at a level of 104.9% ± 5.0%. The biodegradation percentage above 100% can be explained by a synergistic effect, also called priming.

The biodegradation of Weathered plastic film started after a lag phase of about 200 days. After 400 days a biodegradation of 20.5% was measured. The biodegradation proceeded at a constant moderate rate and after 782 days a biodegradation of 64.0% ± 6.9% was reached. On a relative basis, with cellulose as the suitable reference substrate, a biodegradation of 61.1% was calculated. Moreover biodegradation of Weathered plastic film was still proceeding.

The American standard ASTM D6954 *Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation* (2013) stipulates that for products consisting of a single polymer (homopolymers or random copolymers), 60% of the organic carbon must be converted to carbon dioxide before ending the test. The 60% biodegradation requirement was reached for test item Weathered plastic film.

The results, obtained in this test, are valid for solid aerobic conditions only and cannot be directly used for aqueous or for anaerobic conditions. Other tests are more suited to simulate and examine the degradation under these circumstances.

6 Introduction

6.1 Principle of test method

With this test method, biodegradation of a test item in soil can be followed. The test item can either be a pure chemical or a compost sample with residual test product.

The test item is directly mixed with soil and incubated in the dark at ambient room temperature. Biodegradation is taking place through microbial activity. During the aerobic biodegradation in soil a mixture of gases, principally carbon dioxide and water, is produced. The CO_2 is captured in KOH and the CO_2 production is regularly determined by titration, which allows calculating the cumulative CO_2 production. The percentage of biodegradation can be calculated as the percentage of solid carbon of the test item, which has been converted to gaseous, mineral C under the form of CO_2. Also the kinetics of the biodegradation can be established.

The test is considered valid if:

- The degree of biodegradation of the reference material is more than 70% at the plateau phase or at the end of the test;
- The deviation of the CO_2 production in the different replicates of the control reactors (with standard soil only) is less than 20% of the mean at the plateau phase or at the end of the test.

6.2 Standard followed

- ASTM method D5988 *Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil* (2012)

7 Materials and methods

7.1 Test and reference item

Test item

Name:	Weathered plastic film
Description:	Film
Colour:	Transparent
Sample preparation:	Weathering during 900 hours (= 37.5 days) in a weathering cycle of 20 hours UVA exposure (0.89 W/m^2 at 340 nm) at 50°C, followed by 4 hours condensation at 40°C.

Reference item

Name:	Cellulose
Purity:	Native cellulose powder for thin layer chromatography (Avicel)
Physical form:	Powder
Colour:	White
Batch number:	K41853931105
Expiration date:	September 2020
Brand:	Merck Art. Nr. 2331

7.2 General procedure

The inoculum consists of a mixture of natural soils. Before use the soils are sieved on a screen of 2 mm. The fine fraction is the inoculum. It is recommended that the inoculum has a water content between 40% and 60% of the total water holding capacity and a pH between 6.0 and 8.0.

A defined amount of test and reference item is mixed with the inoculum (typically 1 g to 6.25 g of material with 500 g soil) and introduced into the reactors. The reactor also contains a beaker with KOH to absorb the carbon dioxide, released during the incubation and a beaker with water to prevent drying out of the soil. The reactors are closed airtight and placed in the dark at 21°C ± 2°C (see Figure 1). Also a technical control is taken along. These empty reactors only contain a beaker of water and a beaker of KOH and are used to correct for the CO_2 in the air in the headspace of the vessel.

The CO_2 production is determined by titration. The percentage of biodegradation is calculated as the percentage of solid carbon of the test item that has been converted to gaseous, mineral C under the form of CO_2. After each titration, a new beaker with KOH is put into the reactor. At the same time the soil is stirred and moistened if needed.

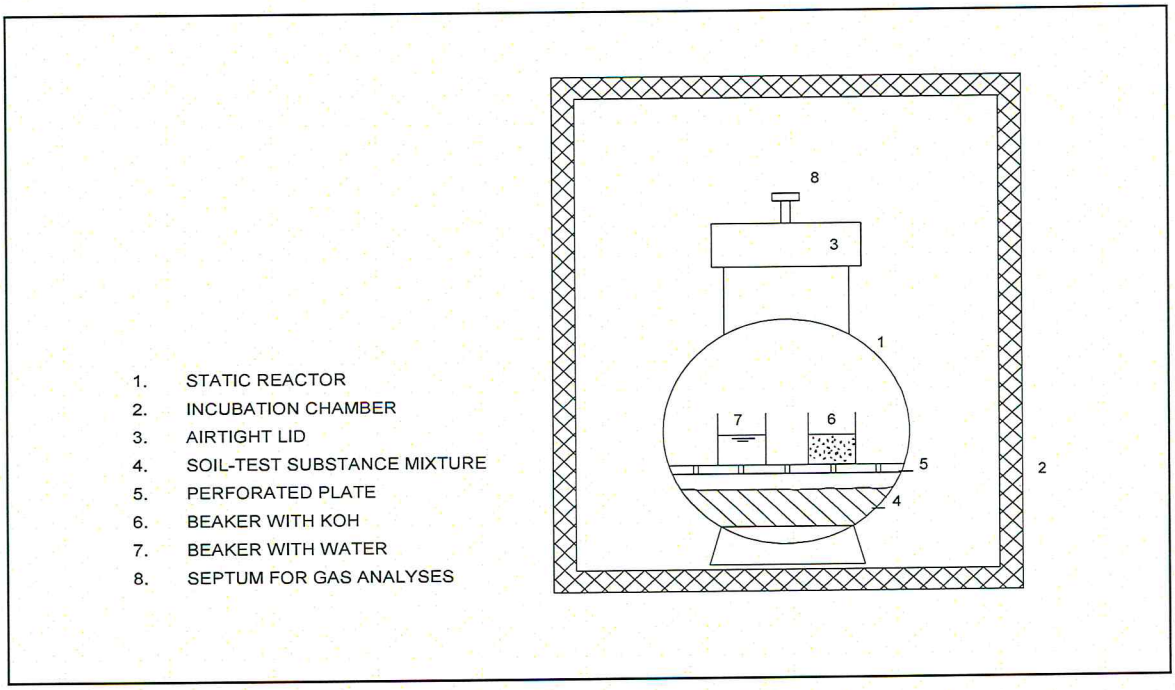

Figure 1. Set-up of soil biodegradability test.

7.3 Analytical methods

Dry matter or total solids (TS)

The dry matter is determined by drying at 105°C for at least 14 hours and weighing, as described in 'METH L.009. Determination of moisture content'. The dry matter is given in percent on wet weight.

pH

The pH is measured with a pH meter after calibration with standard buffer solutions (pH = 4.00, pH = 7.00 and pH = 10.00), as described in 'METH L.006. Determination of pH and electrical conductivity'. Before inserting the electrode the sample is diluted with distilled water at a ratio of 5 to 1 (5 parts of demineralised water versus 1 part of sample) and thoroughly mixed, as described in 'METH L.012. Preparation of extracts and solutions'.

Salt content or electrical conductivity (EC)

The salt content is measured with a conductivity meter after calibration in a 0.01 M KCl and 0.1 M KCl solution, as described in 'METH L.006. Determination of pH and electrical conductivity'. Before inserting the electrode the sample is diluted with distilled water at a ratio of 5 to 1 (5 parts of distilled water versus 1 part of sample) and thoroughly mixed, as described in 'METH L.012. Preparation of extracts and analysis solutions'. The results are given in µS/cm.

Titration

The amount of CO_2 captured in the KOH solution (with the formation of K_2CO_3), is determined titrimetrically with 1N HCl. The titre of HCl is determined with a 1.0 N NaOH solution. The titration is done in two steps with an automatic titrator (Metrohm 888 Titrando). The first step involves the conversion of the excess of KOH to KCl and of K_2CO_3 to $KHCO_3$ (pH = 8.0). The second step involves the conversion of $KHCO_3$ to KCl and CO_2 (pH = 3.8). The amount of HCl used during the second titration step is a direct measure for the amount

of CO_2 which is captured (1 meq HCl titrated = 1 meq CO_2 captured). The results are given in ml.

Total nitrogen (N)

This analysis is done as described in 'METH L.005. Determination of total nitrogen'. In the presence of a catalysing agent (K_2SO_4-mixture) and under boiling conditions (380°C – 395°C) with a mixture of sulphuric acid-salicylic acid bound nitrogen is converted into the salt $(NH_4)_2SO_4$. Afterwards the ammonia is liberated using strong alkali and distilled for subsequent determination by titration. The ammonia is captured in a boric acid/indicator solution. Determination of ammonium ion in the distillate is done by titration with standard acid. The results are given in g per kg total solids.

Total organic carbon (TOC)

The TOC, total organic carbon, is determined in an external laboratory. In case the test item does not contain inorganic carbon an elemental CHN analysis is conducted according to DIN 51732 (2007-08). If this is not the case, the total organic carbon was determined by subtracting the total inorganic carbon content from the total carbon content in accordance with EN 15104 (2011). The results are given in per cent.

Total water holding capacity (WHC)

The water holding capacity (WHC) is determined by measuring the mass of water evaporating from the soil saturated with water when dried to constant mass at 105°C. This amount divided by the dry mass of the soil gives the total water holding capacity. This analysis is performed in triplicate. The analysis is described in 'METH L.310. Determination of the water holding capacity of a soil'.

Volatile solids (VS) - ash

The volatile solids and ash content is determined by heating the dried sample at 550°C for at least 4 hours and weighing, as described in 'METH L.010. Determination of organic matter and carbon content'. The results are given in percent on dry matter.

Weight determination

During the test 2 types of balances are used. A Sartorius AC 210 S with internal calibration (max. 200 g, d = 0.1 mg) for the determination of dry and volatile matter and for the weight of test and reference item. A Sartorius CP 12001 S (max. 12100 g, d = 0.1 g), Sartorius CPA 12001 S (max. 12100 g, d = 0.1 g), Sartorius AX6202 (max. 6200 g, d = 0.01 g), Acculab ATL-224 (max. 220 g; d = 0.1 mg) or Sartorius AX224 (max. 220 g; d = 0.1 mg) is used for weighing of the soil inoculum.

8 Results

8.1 Test conditions and set-up

A set of 12 equal vessels with a total volume of 4 l each was used, incubated in the dark at a constant temperature of 21°C ± 2°C. The test set-up is given in Table 1. Reference item cellulose was added as powder, while test item Weathered plastic film was added as pieces of film after weathering during 900 hours (= 37.5 days) in a weathering cycle of 20 hours UVA exposure at 50°C, followed by 4 hours condensation at 40°C. The weathering resulted in a reduction in average molecular weight from a value of 190000 g/mol at start to 5100 g/mol. Reactors 0, 00 and 000 were technical controls. These empty reactors only contained a beaker of water and a beaker of KOH and are used to correct for the CO_2 in the air in the headspace of the vessel. The total test duration was 782 days.

Table 1. Test set-up soil biodegradation test

RN	Test series	Inoculum (g)	Item (g)
0	Technical control	-	-
1	Control	500	-
2	Cellulose	499	1.0
3	Weathered plastic film	500	1.0
00	Technical control	-	-
4	Control	500	-
5	Cellulose	499	1.0
6	Weathered plastic film	499	1.0
000	Technical control	-	-
7	Control	500	-
8	Cellulose	499	1.0
9	Weathered plastic film	500	1.0

RN = reactor number

8.2 Analyses of inoculum, test and reference item

The inoculum was a mixture of natural soils collected from a sandy field in Lokeren and 2 types of forest in Moerbeke (all located in Belgium). The mixture consisted of 1/3 field soil and 2/3 forest soil (equally divided). Before use, the soils were sieved over a 2 mm screen to remove stones and other inert materials, recognizable roots and other plant debris, and thoroughly mixed.

The characteristics of the soil inoculum are given in Table 2. The inoculum should have a water content between 40% and 60% of the total water holding capacity and a pH between 6.0 and 8.0. A total solids content of 78.2% was obtained. This corresponds with a moisture content on dry matter of 27.8% or 53.8% of the total water holding capacity. The inoculum showed an optimal pH of 7.6, while a C/N ratio of 10 was measured, ensuring a sufficient nitrogen presence.

The reference and test item were analyzed for total solids (TS), volatile solids (VS) and total organic carbon content (TOC) (see Table 3).

Table 2. Characteristics of the inoculum

Characteristics	Inoculum
Total solids (TS, %)	78.2
Moisture content (%)	21.8
Volatile solids (VS, % on TS)	6.5
Ash content (% on TS)	93.5
pH	7.6
Electrical conductivity (EC, µS/cm)	163
Total water holding capacity (WHC_{tot}, % on TS)	51.7
Total N (g/kg TS)	3.2
C/N	10

Table 3. Total solids (TS), volatile solids (VS) and total organic carbon (TOC) content of the reference and test item

Test item	TS (%)	VS (% on TS)	TOC (%)
Cellulose	97.4	99.7	41.8
Weathered plastic film	94.9	99.8	62.3

8.3 CO_2 production

The total cumulative CO_2 production for each reactor at the end of the test (782 days) is given in Table 4. Also the net cumulative CO_2 production of the reference and test item is given in mg per g of test item. Figures 2 up to 4 show the evolution of the total cumulative CO_2 production.

According to ASTM D5988 (2012) the test is considered valid when the deviation of the CO_2 production of the control reactors is less than 20% of the mean at the plateau phase or at the end of the test. After 782 days (end of test) a significantly lower value of 0.6% was obtained, demonstrating the good precision of the test.

Table 4. CO_2 production at the end of the test (782 days)

RN	Test series	Total CO_2 (mg)	Net CO_2 (mg/g test item)
1	Control	4908	-
2	Cellulose	6566	1693
3	Weathered plastic film	6496	1625
4	Control	4851	-
5	Cellulose	6422	1549
6	Weathered plastic film	6181	1308
7	Control	4879	-
8	Cellulose	6446	1574
9	Weathered plastic film	6328	1456

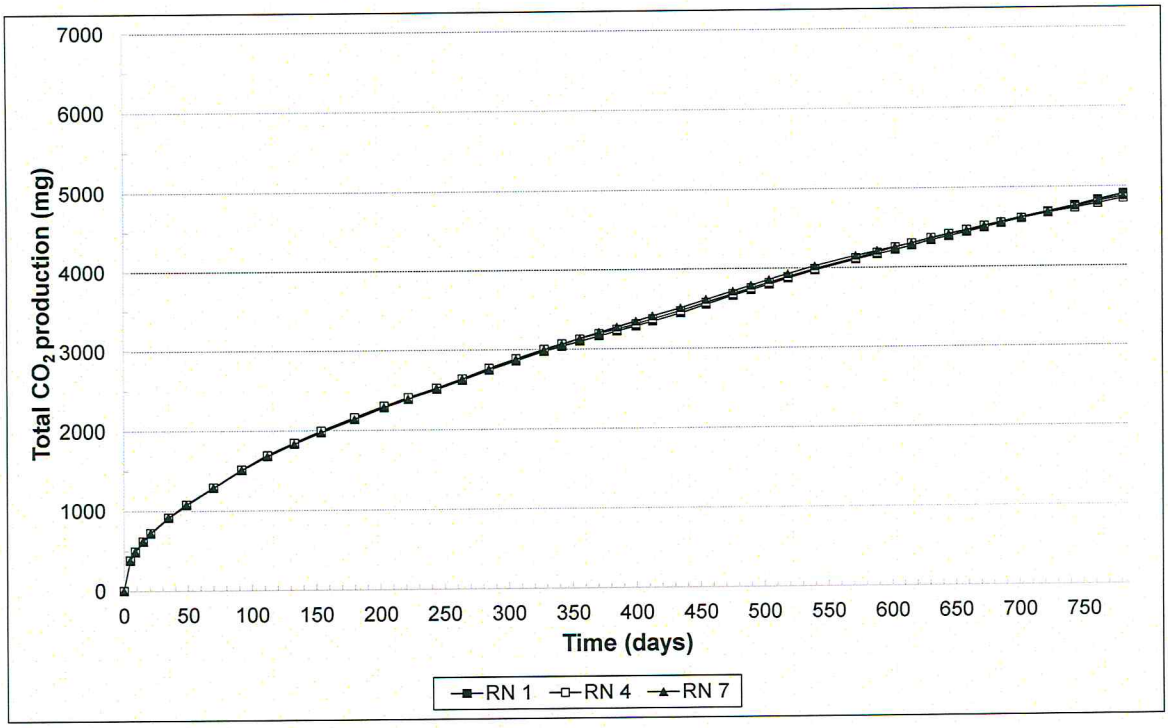

Figure 2. Total CO_2 production of control reactors

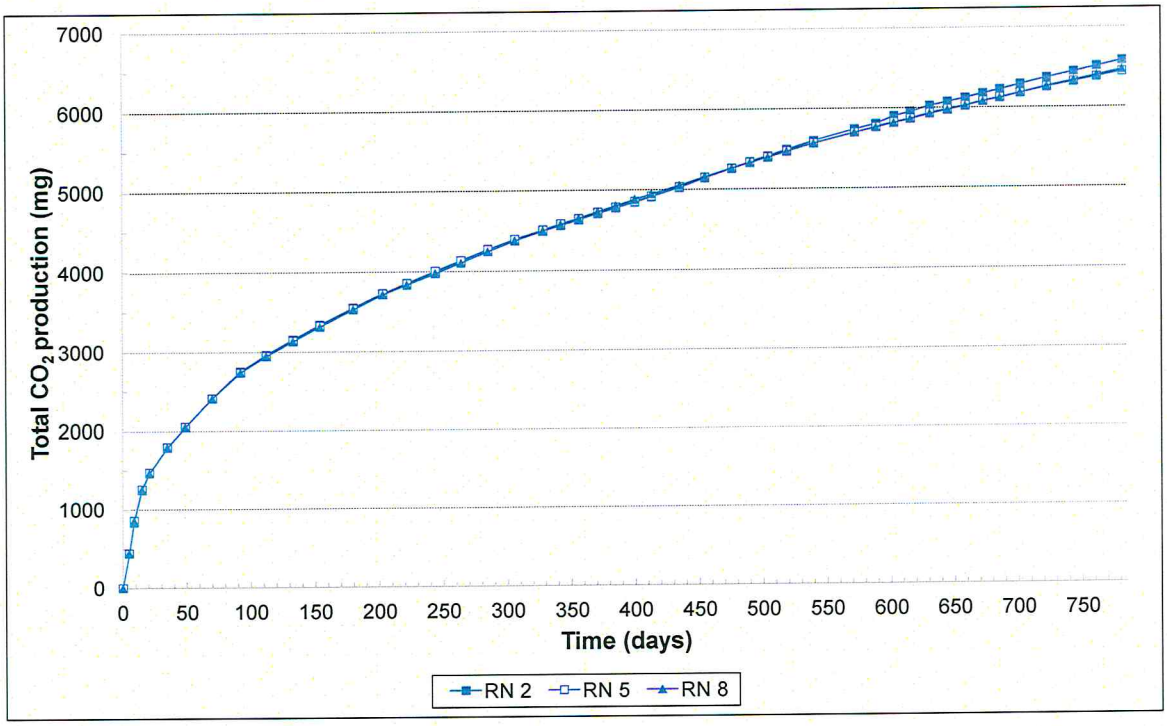

Figure 3. Total CO_2 production of cellulose reactors

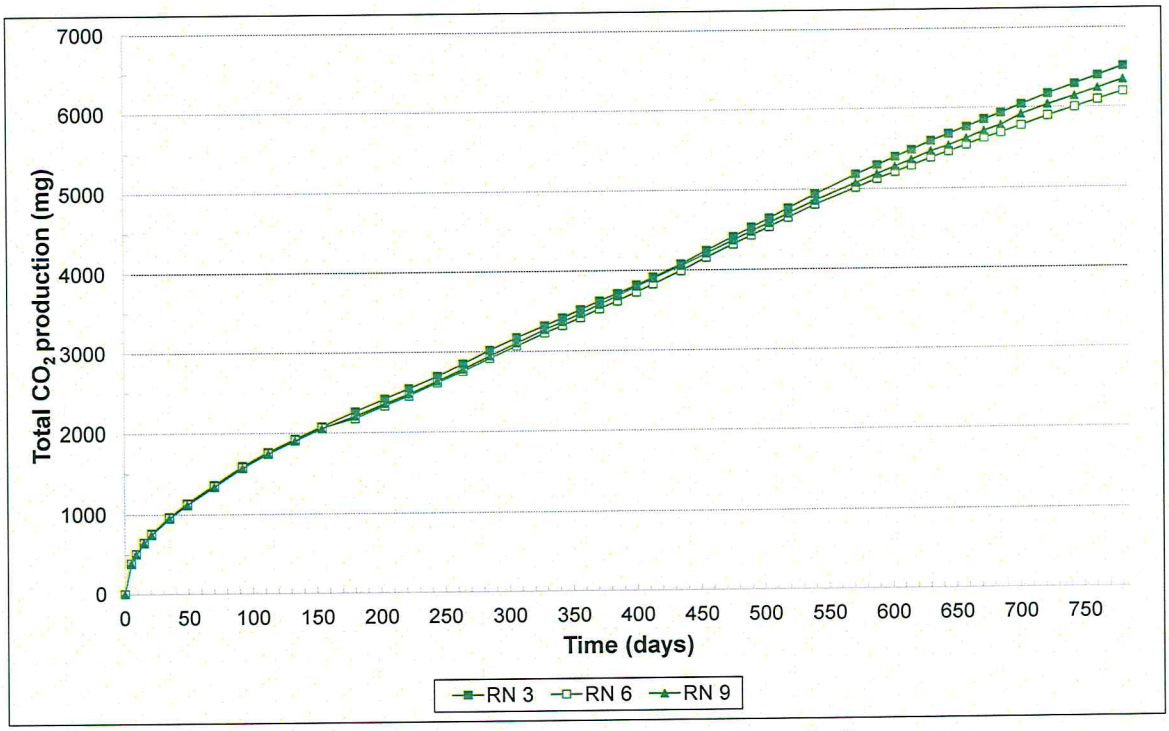

Figure 4. Total CO_2 production of Weathered plastic film reactors

8.4 Biodegradation percentages

The results on the calculation of the biodegradation percentages at the end of the test (782 days) are summarized in Table 5. The percentages are determined by the ratio of gaseous carbon, which is found back under the form of carbon dioxide at the end of the incubation period, to the original amount of carbon input. Figure 5 shows the evolution of the average biodegradation percentages of reference and test item, while Figures 6 and 7 represent the biodegradation of the separate replicates.

Table 5. Biodegradation percentages at the end of the test (782 days)

Test series	Average C_{input} (mg)	Average $C_{gaseous}$ (mg)	Biodegradation (%)			95% CL
			AVG	SD	REL	
Cellulose	417	438	104.9	5.0	100.0	8.6
Weathered plastic film	622	399	64.0	6.9	61.1	11.2

With AVG = average, SD = standard deviation, REL = relative biodegradation and CL = confidence limits

The biodegradation of reference item cellulose started almost immediately and proceeded at a good rate. After 70 days cellulose was already degraded by 73.1%. At the end of the test (after 782 days) a plateau in biodegradation was reached at a level of 104.9% ± 5.0%. The test is considered valid when the degree of biodegradation of the reference material cellulose is more than 70% at the plateau phase or at the end of the test. This requirement was clearly fulfilled. The biodegradation percentage above 100% can be explained by a synergistic effect, also called priming. A priming effect occurs if the soil inoculum in the test reactor is producing more CO_2 than the soil inoculum in the control reactors. This results in a net CO_2 production that is not exclusively coming from the test item and, in case of readily degradable products, in a biodegradation percentage of more than 100%.

The biodegradation of Weathered plastic film started after a lag phase of about 200 days. After 400 days a biodegradation of 20.5% was measured. The biodegradation proceeded at a constant moderate rate and after 782 days a biodegradation of 64.0% ± 6.9% was

reached. On a relative basis, with cellulose as the suitable reference substrate, a biodegradation of 61.1% was calculated. Moreover biodegradation of Weathered plastic film was still proceeding.

The American standard ASTM D6954 *Standard Guide for Exposing and Testing Plastics that Degrade in the Environment by a Combination of Oxidation and Biodegradation* (2013) stipulates that for products consisting of a single polymer (homopolymers or random copolymers), 60% of the organic carbon must be converted to carbon dioxide before ending the test. The 60% biodegradation requirement was reached for test item Weathered plastic film.

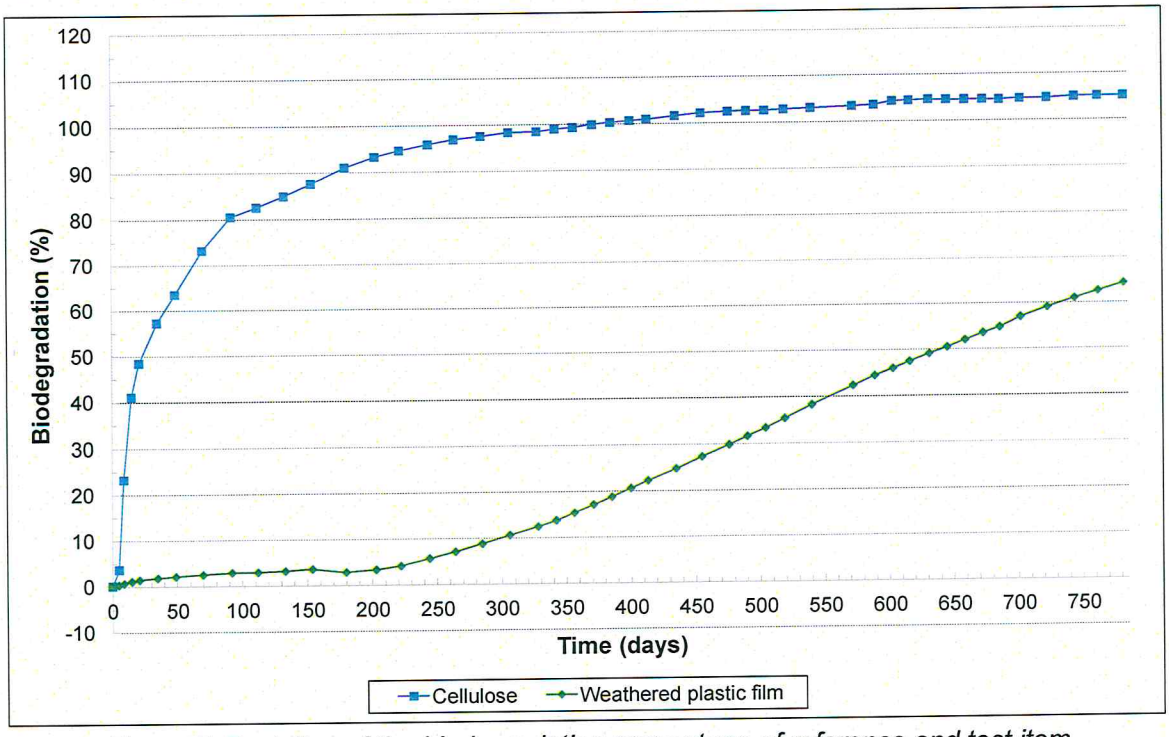

Figure 5. Evolution of the biodegradation percentage of reference and test item

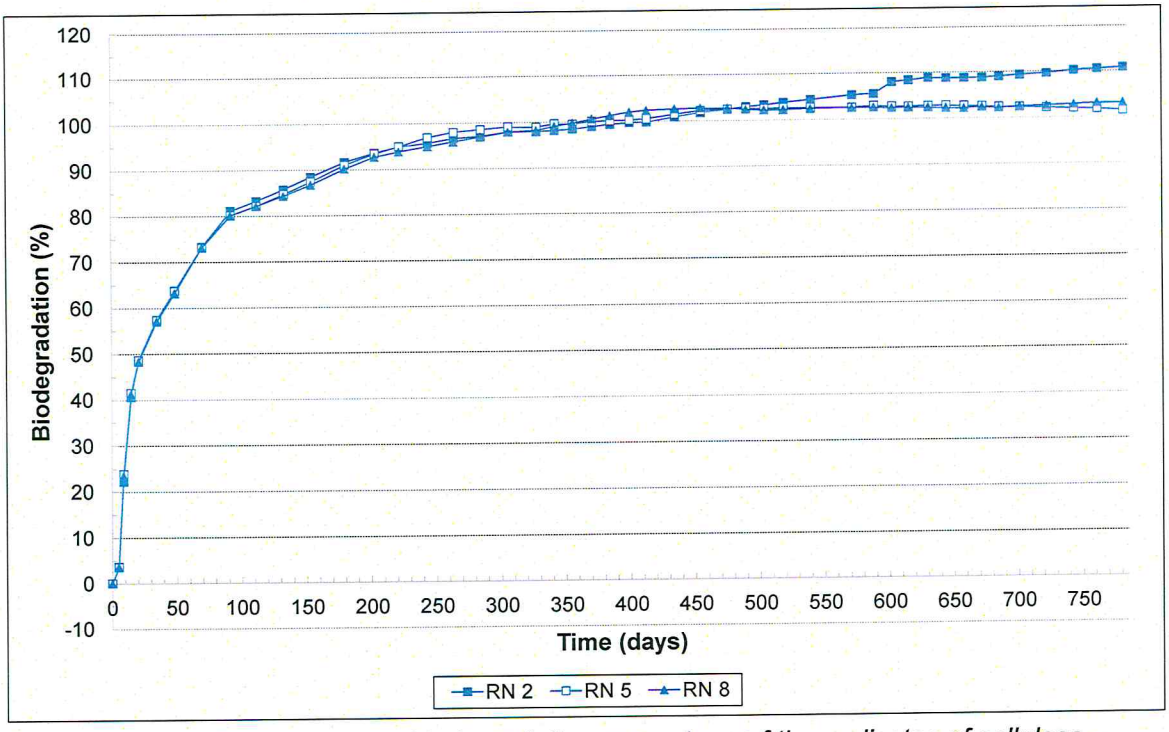

Figure 6. Evolution of the biodegradation percentage of the replicates of cellulose

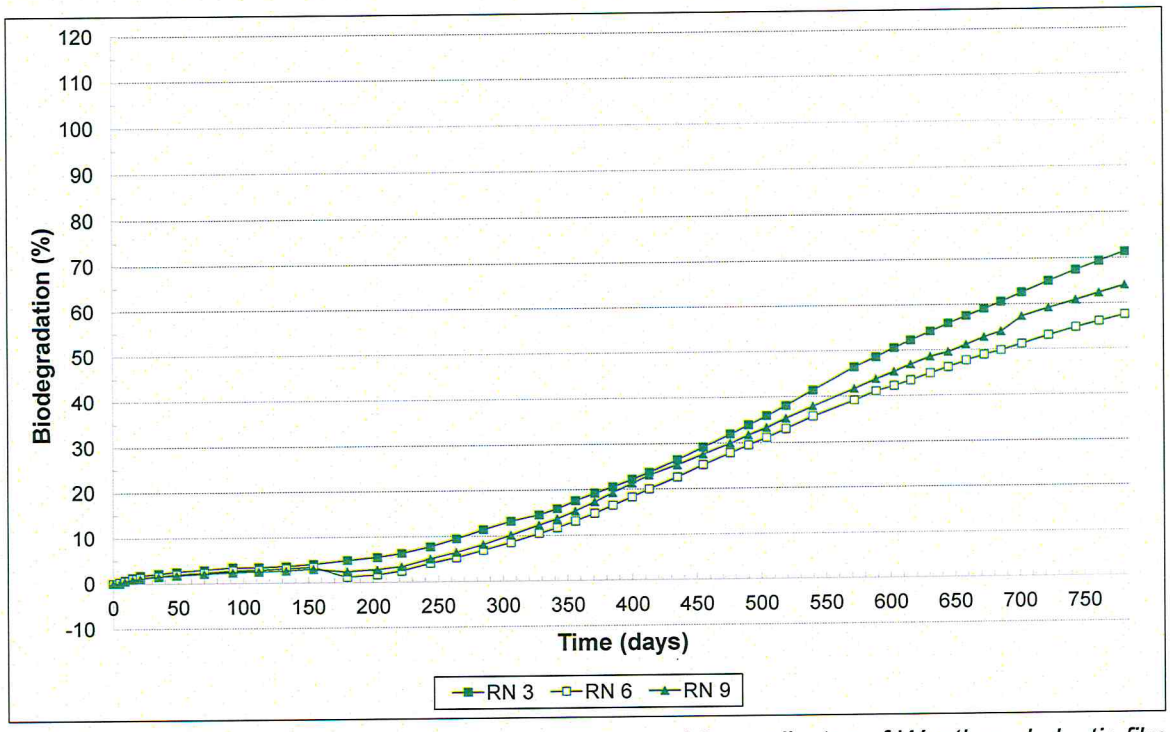

Figure 7. Evolution of the biodegradation percentage of the replicates of Weathered plastic film

8.5 Visual perceptions and analyses at end of test

At the end of the soil biodegradability test the different test series were examined for total solids (TS), volatile solids (VS) and pH (see Table 6). These results show that optimal moisture and pH conditions were obtained at the end of the test.

During the incubation, at each time of shaking, the reactors were inspected visually for several aspects such as moisture content, structure of the mixture, development of fungi and visual appearance of the test item. Good structure and moisture conditions were maintained throughout the test. No fungal growth was observed. At the end of the test (782 days) test item Weathered plastic film was not visible anymore.

Table 6. pH, TS and VS content at the end of the test (782 days)

Test series	TS (%)	VS (% on TS)	pH
Control	80.7	6.1	7.8
Cellulose	80.4	6.1	7.9
Weathered plastic film	80.4	6.3	7.9