FINAL REPORT
JRW-5/2

Aqueous aerobic biodegradation test on ***50302P***

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Table of contents

1 Identification of the test 3
1.1 General information 3
1.2 Study personnel 4
1.3 Study schedule 4
1.4 Archiving 4
2 Confidentiality statement 5
3 GLP compliance statement 5
4 Quality assurance audit statement 5
5 Summary and conclusions 6
6 Introduction 7
6.1 Purpose and principle of the test method 7
6.2 Standard followed 7
7 Materials and methods 8
7.1 Reference and test item 8
7.2 General procedure 8
7.3 Analytical methods 9
8 Results 12
8.1 Test conditions and set-up 12
8.2 Analyses of inoculum, reference and test item 12
8.3 Biodegradation percentages 13
8.3.1 Biodegradation based on O_2 consumption 13
8.3.2 Biodegradation based on CO_2 production 17

1 Identification of the test

1.1 General information

Project number

JRW-5/2

Sponsor

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Reference item

Cellulose

Test item

50302P

Test duration

56 days

1.2 Study personnel

Study Director:	Olive Nkundwakazi
Replacement Study Director(s):	Eveline Beeckman
	Sylvie Denis
Study Director(s) QA:	Lander De Zutter
	Lynn Serbruyns
	Michela Siotto
	Wouter Thys

1.3 Study schedule

Starting date study:	March 25, 2024
Starting date experiments:	March 27, 2024
Starting date of incubation:	March 27, 2024
Completion date of incubation:	May 22, 2024
Test duration:	56 days
Completion date of experiments:	May 24, 2024
Completion date study:	June 7, 2024
Revision date:	October 4, 2024

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 Normec OWS nv. These records include notebooks, study plan, study report, samples of test item and specimens. They will be stored in a file coded:

JRW-5/2

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

After seven (7) years, all data and records will be destroyed unless a written request is received from the sponsor to return it. Additives (concentration < 10% in final product) and liquids will be stored for 2 years unless a written request for storage up to 7 years is received from the sponsor.

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.

Lynn Serbruyns
Department Manager
Biodegradation

3 GLP compliance statement

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

Olive Nkundwakazi
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 October 7, 2024.

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

Michela Siotto
Study Director QA

5 Summary and conclusions

The aerobic biodegradation of test item 50302P was evaluated in an aqueous aerobic biodegradation test using sludge inoculum without any pre-adaptation to the test item according to ISO 14851 (2019). The test was performed in triplicate and the incubation temperature was continuously kept at 21°C ± 1°C. The total test duration was 56 days and the final biodegradation percentages are based on CO_2 production.

According to the ISO 14851 (2019) standard, the test is considered valid if a) the degree of biodegradation of the reference material is > 60% at the end of the test, b) the oxygen consumption of the control at the end of the test is not exceeding an upper limit value obtained by experience (this value depends on the amount of inoculum and is for example about 60 mg O_2/l in the case of 30 mg TSS/l of sludge) and c) the oxygen consumption of the three controls and of the three test item replicates is within 20% of the mean at the plateau phase or at the end of the test. The results show that the requirements were fulfilled. At the end of the test (56 days) a biodegradation of 91.5% ± 1.0% was obtained for reference item cellulose. The total O_2 consumption of the controls after 56 days of testing was 17.7 ± 1.4 mg O_2/l medium, which is within the prescribed range for the used inoculum (28.2 mg TSS/l). Moreover, a deviation from the mean of respectively 7.7% and 5.5% was calculated for the control and test item reactors at the end of the test.

Test item 50302P started to degrade at a moderate rate. After 14 days, the test item was degraded by 27.8%. Thereafter, the biodegradation rate slightly increased and after 28 days a value of 71.1% was reached. At the end of the test (56 days) an absolute biodegradation of 83.5% ± 2.2% was measured. On a relative basis, compared to reference item cellulose, a biodegradation of 91.3% was calculated.

A test item is considered to meet the biodegradation requirement if 90% of the organic carbon in the whole item or for each constituent, which is present in the material at a concentration of more than 1% (by dry mass), is converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. The requirement needs to be reached within a maximum test duration of 1 year. From the results it can be concluded that test item 50302P fulfills the requirement on biodegradation as defined by the *OK Compost HOME certification scheme* of TÜV AUSTRIA Belgium.

6 Introduction

6.1 Purpose and principle of the test method

The aqueous biodegradation test determines the biodegradation of a test item under laboratory conditions by a consortium of bacteria from different sources or a conditioned sludge pre-exposed to the test item or structure-related component. The test material is brought into a chemically defined (mineral) liquid medium, essentially free of other organic carbon sources, and spiked with micro-organisms.

During the aerobic biodegradation of organic materials in an aqueous medium, oxygen is consumed and carbon is converted to gaseous, mineral C (under the form of carbon dioxide, CO_2). Part of the organic material is assimilated for cell growth. KOH solution traps the CO_2 released and the induced pressure-drop is directly related to the consumed oxygen and hence to the biodegradation of the test item.

The amount of biodegradation based on O_2 consumption is expressed as the ratio of the BOD (corrected for the control) to the Theoretical Oxygen Demand (ThOD) or Chemical Oxygen Demand (COD) of the used test item. The biodegradation based on CO_2 production is calculated as the percentage of solid carbon of the test compound which has been converted to gaseous, mineral C under the form of CO_2.

The test is considered as valid if:

- The percentage of biodegradation for the reference item is more than 60% at the end of the test;
- The oxygen consumption of the control is not exceeding an upper limit value obtained by experience (this value depends on the amount of inoculum and is for example about 60 mg O_2/l in the case of 30 mg TSS/l of sludge);
- The oxygen consumption of the three control reactors and of the three test reactors is within 20% of the mean at the plateau phase or at the end of the test.

A test item is considered to meet the biodegradation requirement if 90% of the organic carbon in the whole item or for each constituent, which is present in the material at a concentration of more than 1% (by dry mass), is converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. The results of this test can be used for certification in line with *OK Compost HOME certification scheme* of TÜV AUSTRIA Belgium.

The maximum allowed test duration determined by the standards is 1 year.

6.2 Standard followed

- ISO 14851 *Determination of the ultimate aerobic biodegradability of plastic materials in an aqueous medium - Method by measuring the oxygen demand in a closed respirometer* (2019).

7 Materials and methods

7.1 Reference and test item

Reference item

Name:	Cellulose
Purity:	Native cellulose powder for thin layer chromatography (Avicel)
Physical form:	Powder
Color:	White
Batch number:	K54514731231
Expiration date:	September 2031
Brand:	Merck Art. Nr. 2331
Storage conditions:	Room temperature in the dark

Test item

Name:	50302P
Description:	Nonwoven
Grammage:	60gsm
Code:	23.34.7-8
Color:	Beige
Sample preparation:	Cryogenically milled (< 1 mm)
Storage conditions:	Room temperature in the dark

7.2 General procedure

The source of micro-organisms (inoculum) is a mixture of activated sludge, obtained from different wastewater treatment plants. The sites treat wastewater from domestic and/or industrial origin. The different sludges were sieved over an 80 µm sieve and mixed in equal parts. The final inoculum was obtained after removal of supernatant liquid and replacement with mineral medium used for the removal of soluble components, after which the inoculum is aerated for a few hours.

At the start of the test, each reactor is filled with the same amount of mineral medium. A precise amount of inoculum (1-5%) is added to each reactor to obtain a test medium with a concentration of approximately 30 mg suspended solids/l. The reference and test item are added directly to the reactors. After filling of the reactors, KOH solution is added to the rubber carriers, OXITOP-OC heads are connected and the reactors are put on an inductive stirrer (see Figure 1). A magnetic rod keeps the reference item, test item and the growing biomass into suspension throughout the test. The vessels are aerated for 15 minutes in the incubator before closing and initiating the actual incubation period for biodegradation. This final aeration period is needed to equilibrate the final mixture and to stabilize the temperature. The reactors are incubated at a constant temperature (21°C ± 1°C) in the dark for a period of minimum 28 days.

During the test, the KOH solution absorbs the CO_2 produced. This absorption causes a pressure drop inside the reactors which can be translated to a given O_2 consumption. The Biological Oxygen Demand (BOD) is continuously analysed on regular intervals (every 4 hours). The percentage of biodegradation based on O_2 consumption is expressed as the ratio of the BOD (corrected for the control) to the Theoretical Oxygen Demand (ThOD) or Chemical Oxygen Demand (COD) of the used test item.

At regular intervals (every two weeks) the amount of CO_2 produced is determined by titration of the KOH solution. The biodegradation based on CO_2 production is calculated as the percentage of solid carbon of the test compound which has been converted to gaseous, mineral C under the form of CO_2.

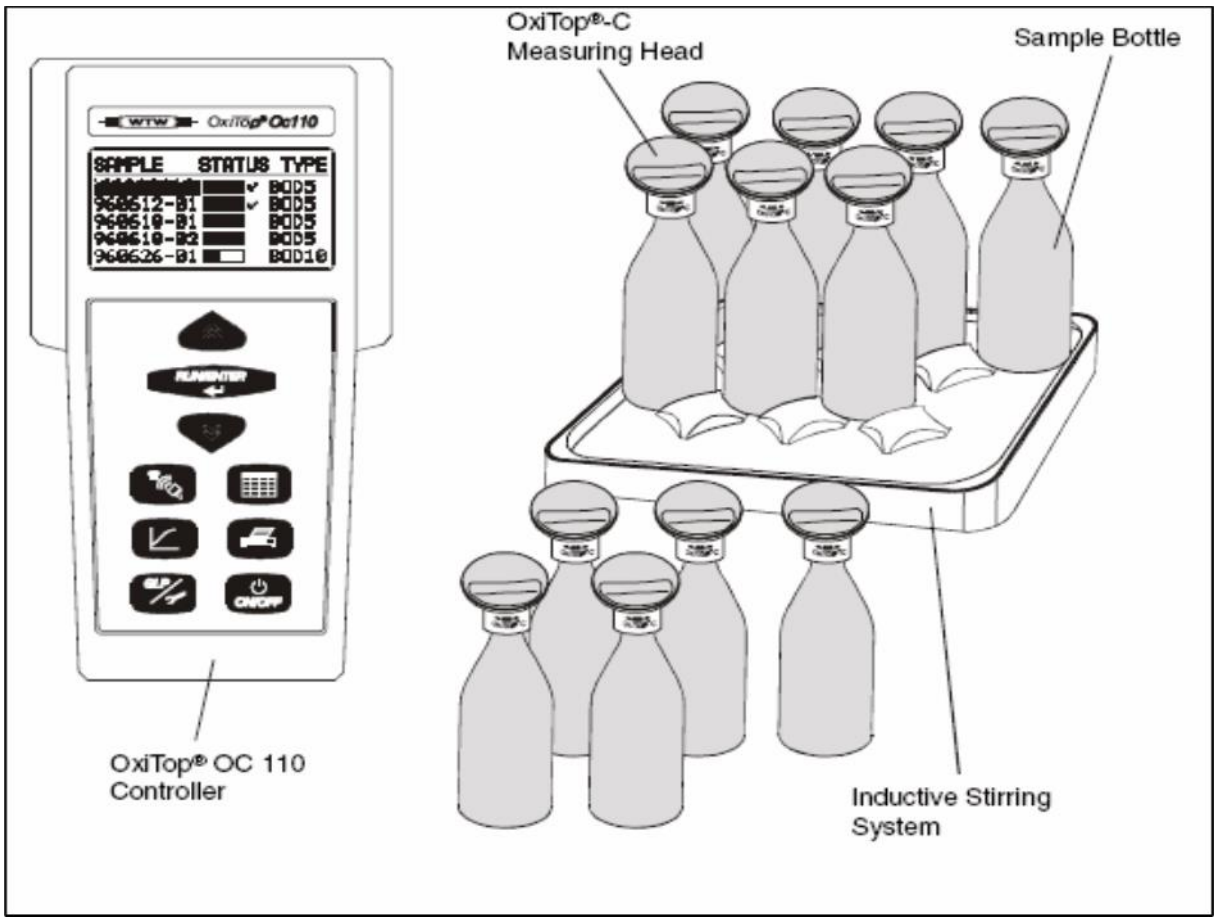

Figure 1. Set-up aqueous biodegradation test

7.3 Analytical methods

Ammonium - nitrogen (NH_4^+-N)

This analysis is done as described in 'M_054. Determination of ammonium nitrogen by a discrete analyzer system and spectrophotometric detection'. The determination is performed on the aqueous sample after filtration through a syringe filter (pore size = 1.20 µm). Ammonia in the sample reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitroprusside at around pH 12.6 to form a blue compound. The absorbance of this compound is measured spectrophotometrically at the wavelength 660 nm and is related to the ammonia concentration by means of a calibration curve. The results are given in mg per l wet weight.

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 'M_009. Determination of moisture content'. The dry matter is given in percent on wet weight.

Elemental analysis

The elemental analysis is performed by an external lab. CHN is conducted according to DIN 51732 (2014). The results are expressed in percent.

Kjeldahl nitrogen (Kj-N)

This analysis is done as described in 'M_036. Determination of Kjeldahl nitrogen'. In the presence of a catalysing agent (K_2SO_4-Se-mixture) and under boiling conditions (380°C) with a mixture of sulphuric 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.

Nitrate and nitrite - nitrogen (NO_x^--N)

This analysis is done as described in 'M_055. Determination of total oxidized nitrogen by a discrete analyzer system and spectrophotometric detection'. The determination is performed on the aqueous sample after filtration through a syringe filter (pore size = 1.20 µm). Nitrate in the sample is reduced to nitrite by hydrazine under alkaline conditions. The total nitrite ions are then reacted with sulphanilamide and N-1-napthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance is measured at 540 nm and is related to the Total Oxidized Nitrogen (NO_x^--N) concentration by means of a calibration curve. In order to measure only nitrite in the sample the reduction of nitrate by hydrazine is omitted. The concentration of nitrate can then be calculated by subtracting the concentration of nitrite from the concentration of total oxidized nitrogen. The results are given in mg per l wet weight.

Oxidized Nitrogen (NO_x^--N) is also detected with a colorimetric, semi-quantitative test. MQuant® nitrate test strips from Sigma-Aldrich are used. The strips have a detection area which, in contact with the liquid sample, changes color depending on the nitrate and nitrite concentration. The presence of the two species is visually determined by comparing the color of the reaction area of the test strip with a color scale. The method has a detection limit of 10 mg NO_3^- per liter.

pH

The pH is measured directly on the aqueous sample with a pH meter after calibration with standard buffer solutions (pH = 4.0, pH = 7.0 and pH = 10.0), as described in 'M_006. Determination of pH and electrical conductivity'.

Theoretical oxygen demand (ThOD)

The ThOD is calculated from the chemical formula or based on the elemental analysis of the test material, according to the formula described in ISO 14851 (2019), Annex A. The results are given in g/g.

Titration

The amount of CO_2 captured in a 3N KOH solution (with the formation of K_2CO_3), is determined titrimetrically with 0.05N HCl. 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 results are given in ml. 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).

Total organic carbon (TOC)

The total organic carbon content is determined by subtracting the total inorganic carbon content from the total carbon content as described in 'M_017. Determination of total organic carbon - total carbon after dry combustion and inorganic carbon after acidification'.

The total carbon content is determined using a high temperature (950°C to 1200°C) combustion method. The formed CO_2 is measured with infrared (IR) detection. Total inorganic carbon is measured by acidification of the sample and heating at 150°C. The sample is first incinerated in a muffle furnace at 550°C. The leftover ashes are subsequently acidified. The released CO_2 is determined by IR detection. The results are given in percent on wet weight.

Total suspended solids (TSS) & volatile suspended solids (VSS)

This analysis is done as described in 'M_019. Determination of suspended solids by filtration through glass-fibre filter'. A known quantity of sample is filtered through a glass fibre filter using under pressure created by a vacuum pump. After filtration the filter is washed with water in order to remove soluble solids. The filter is then dried at 105°C and the amount of dry residue is weighed. Afterwards the filter is incinerated at 550°C and the amount of ash residue is weighed. The results are given in g/l.

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 'M_010. Determination of organic matter and carbon content'. The results are given in percent on dry matter.

Weight determination

During the test, several balances are used, with an accuracy of 0.1 mg for the determination of dry and volatile matter and weighing of the reference and test item, and an accuracy of 0.01 g for weighing of the mineral medium and inoculum.

8 Results

8.1 Test conditions and set-up

A set of 9 equal reactor vessels with a total volume of 500 ml each was used. Each reactor was filled with 250 g of test medium, consisting of 245 g of mineral medium and 5 g of inoculum. Reference item cellulose was added as powder, while test item 50302P was previously reduced in size (cryogenically milled until < 1 mm). The test set-up is given in Table 1. Also, allylthiourea was added to each reactor at start and every 4 weeks during the test to prevent nitrification. After the addition of the reference and test item, the reactors were put on an inductive stirrer. A magnetic rod kept the reference item, test item and growing biomass into suspension throughout the test. The reactors were incubated at a constant temperature of 21°C ± 1°C in the dark. The total test duration was 56 days.

Table 1. Test set-up aqueous biodegradation test

RN	Test series	Min. medium (g)	Inoculum (g)	Item (mg)
1	Control	245	5	-
2	Control	245	5	-
3	Control	245	5	-
4	Cellulose	245	5	24.9
5	Cellulose	245	5	25.1
6	Cellulose	245	5	24.9
7	50302P	245	5	25.1
8	50302P	245	5	24.9
9	50302P	245	5	25.0

RN = reactor number

8.2 Analyses of inoculum, reference and test item

The inoculum consisted of a homogeneous mixture of activated sludge from 3 different sewage-treatment plants (Destelbergen, Gent and Landegem) treating domestic and/or industrial wastewater. After filtration over an 80 µm sieve, mixing in equal parts, decantation of the supernatant and replacement with mineral medium, the final sludge inoculum was obtained. This inoculum was actively aerated for three hours. The test medium was obtained by adding 5 g of sludge inoculum to 245 g of mineral medium. The characteristics of the inoculum and test medium are given in Table 2.

Table 2. Characteristics of inoculum and test medium

Characteristics	Result
Inoculum	
Total suspended solids (TSS, g/l)	1.41
Volatile suspended solids (VSS, g/l)	0.99
Volatile suspended solids (VSS, % on TSS)	69.6
Kjeldahl-N (mg/l)	90
Test medium	
pH	7.0
NH_4^+-N (mg/l)	b.r
NO_x^--N (mg/l)	2.5

b.r. = below reporting limit:
reporting limit: NH_4^+-N = 0.25 mg/l

According to ISO 14851 (2019) the oxygen consumption of the control at the end of the test should not exceed an upper limiting value obtained by experience (this value depends on the amount of inoculum and is, for example about 60 mg O_2/l in the case of 30 mg TSS/l of sludge). At the start of the test a total suspended solids content of 28.2 mg/l and a volatile suspended solids content of 19.7 mg/l was obtained in the test medium. At the end of the

test (56 days) the total O_2 consumption of the controls was 17.7 ± 1.4 mg O_2/l medium, which is within the prescribed range for the used inoculum.

The total solids (TS), volatile solids (VS), theoretical oxygen demand (ThOD, calculated from elemental analysis), total organic carbon content (TOC) and theoretical amount of evolved carbon dioxide ($ThCO_2$) of the reference and test item are summarised in Table 3.

Table 3. TS, VS, ThOD, TOC and $ThCO_2$ of reference and test item

Item	TS (%)	VS (% on TS)	ThOD (mg/g)	TOC (%)	$ThCO_2$ (mg/g)
Cellulose	97.8	99.9	1127	42.4	1555
50302P	96.5	95.6	1594	51.7	1896

8.3 Biodegradation percentages

8.3.1 Biodegradation based on O_2 consumption

Biodegradation was determined by measuring the amount of O_2 consumption throughout the test. The calculation of the biodegradation percentages is based on the net oxygen consumption (after subtraction of the oxygen consumed in the control reactors) in the reference or test reactor and on the ThOD added to each reactor. At the end of the test (56 days) all vessels were checked on the presence of nitrate and nitrite by means of nitrate/nitrite strips. No nitrate or nitrite was detected, so no correction for O_2 consumption due to nitrification needed to be made.

Table 4 shows the ThOD (theoretical oxygen demand), net O_2 consumption and biodegradation percentage of reference and test item at the end of the test (56 days). The evolution of the cumulative O_2 consumption of the control, reference and test item is represented in Figure 2 up to Figure 4. Figure 5 shows the evolution of the average biodegradation of reference and test item (based on O_2 consumption), while Figure 6 and Figure 7 show the biodegradation of the replicates.

Table 4. ThOD, net O_2 consumption and biodegradation after 56 days

Test series	ThOD (mg/l)	Net O_2 (mg/l)	Biodegradation (%)		
			AVG	SD	REL
Cellulose	112.5	108.5	96.5	1.9	100.0
50302P	159.4	147.0	92.2	5.5	95.6

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

The biodegradation of reference item cellulose started after a lag phase of approximately 2 days and proceeded at a good rate. After 13 days cellulose was degraded by 61.1%. The biodegradation rate gradually decreased and at the end of the test (56 days) a plateau in biodegradation was reached at a level of 96.5% ± 1.9%. According to ISO 14851 (2019) the test is considered valid if at the end of the test the biodegradation percentage of the reference item is more than 60%. This requirement was fulfilled.

Test item 50302P started to degrade after a lag of approximately 9 days. The biodegradation proceeded at a good rate and after 23 days, a value of 73.0% was achieved. The test item continued to degrade at a gradually decreasing rate and at the end of the test (56 days) a plateau was reached at a level of 92.2% ± 5.5%. On a relative basis, compared to reference item cellulose, a biodegradation of 95.6% was calculated.

The test is considered valid if the oxygen consumption of the three controls and of the three test item replicates is within 20% of the mean at the plateau phase or at the end of the test.

At the end of the test (56 days) a deviation from the mean of 7.7% and 5.5% was obtained. The requirement was fulfilled.

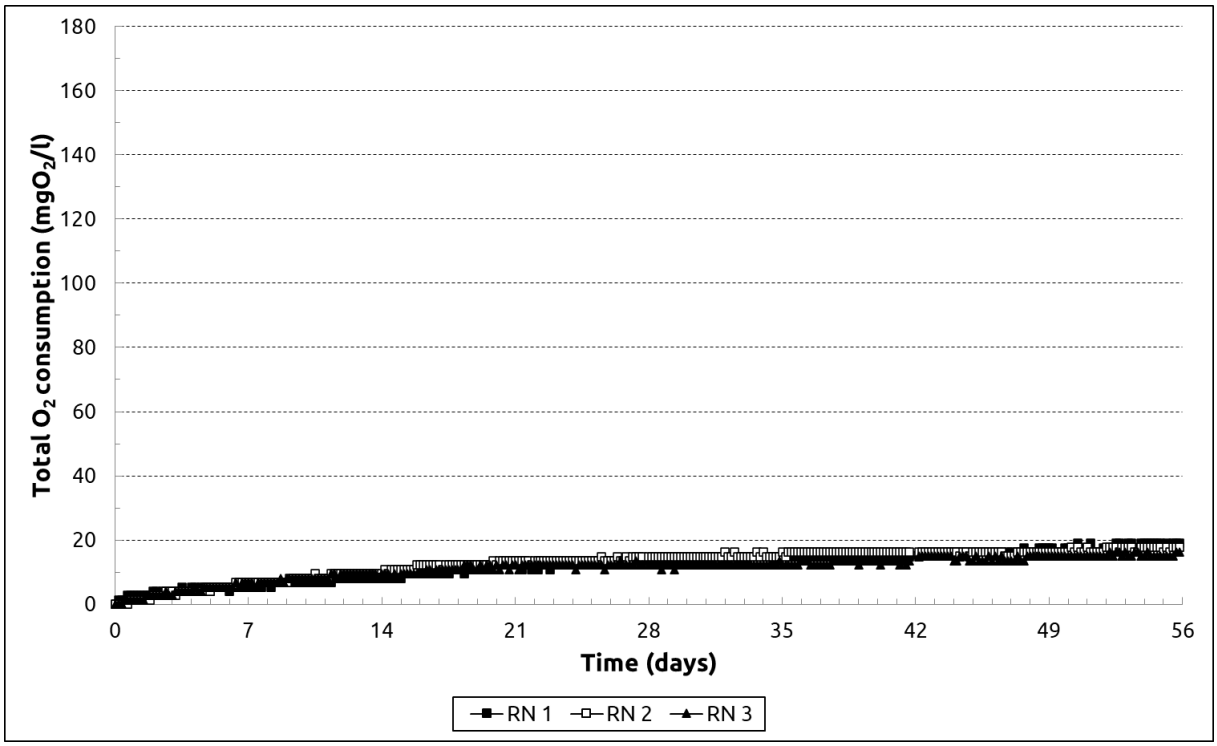

Figure 2. Total O_2 consumption of control reactors

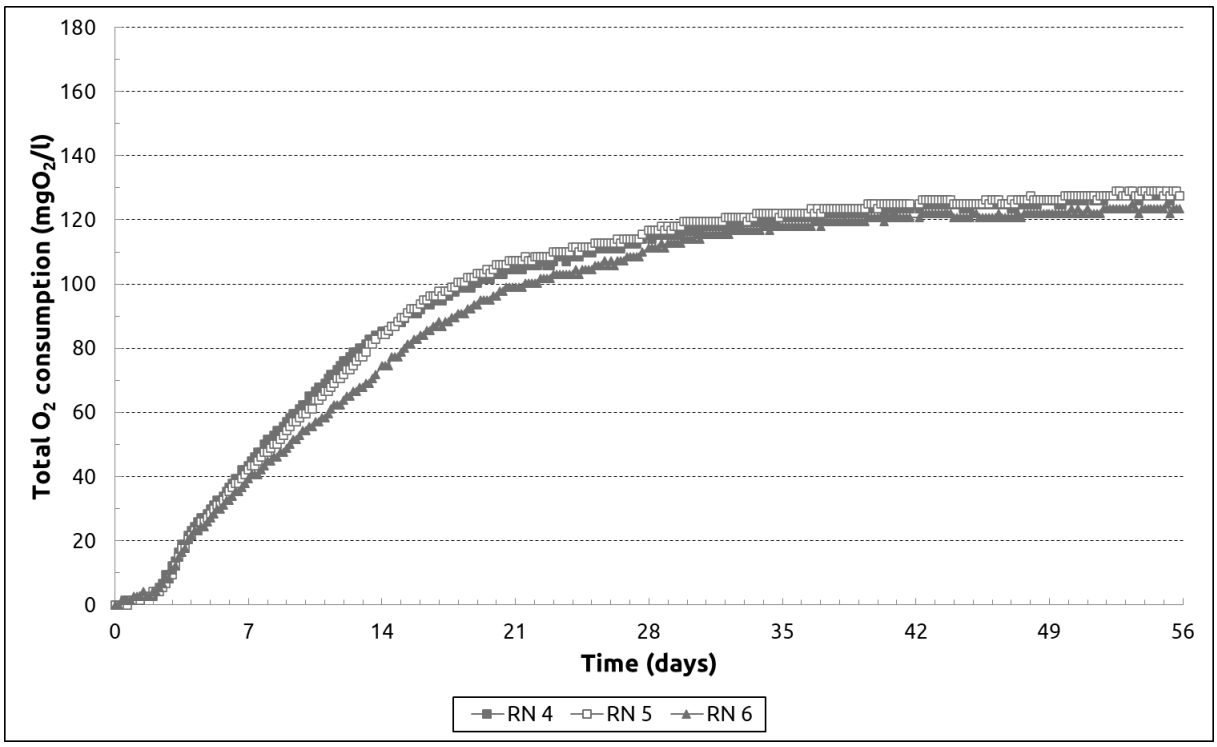

Figure 3. Total O_2 consumption of cellulose reactors

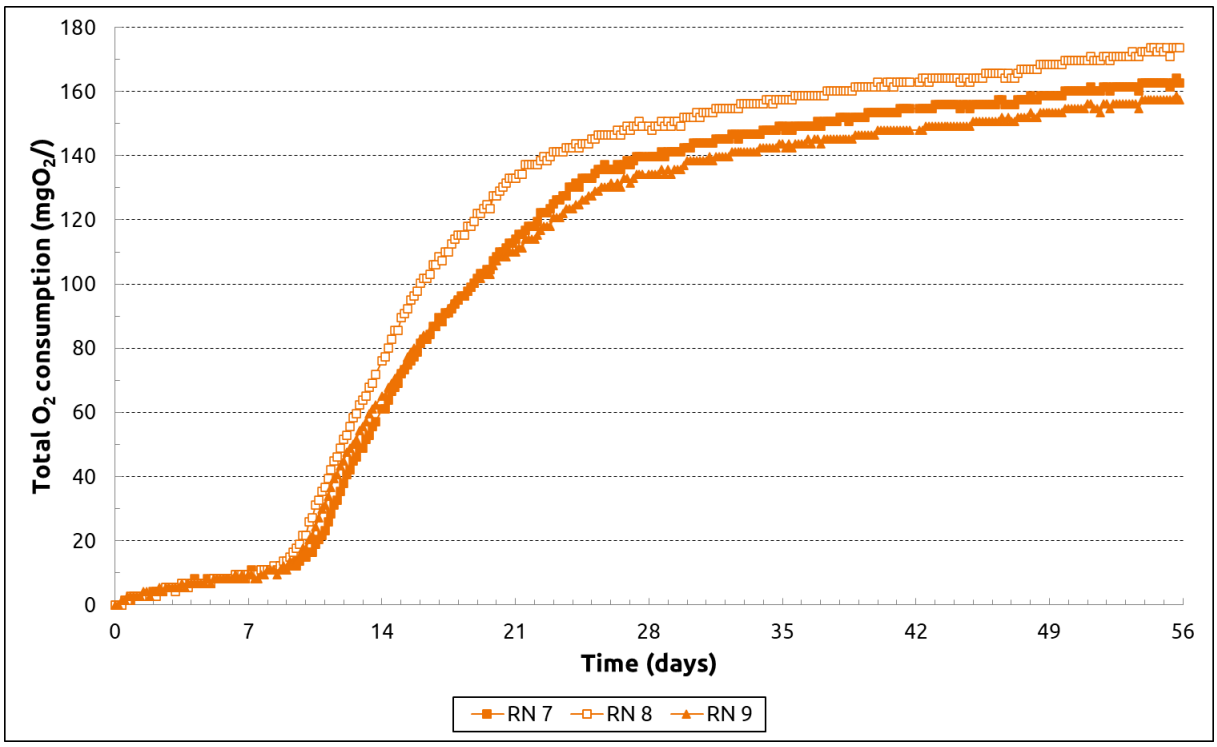

Figure 4. Total O_2 consumption of 50302P reactors

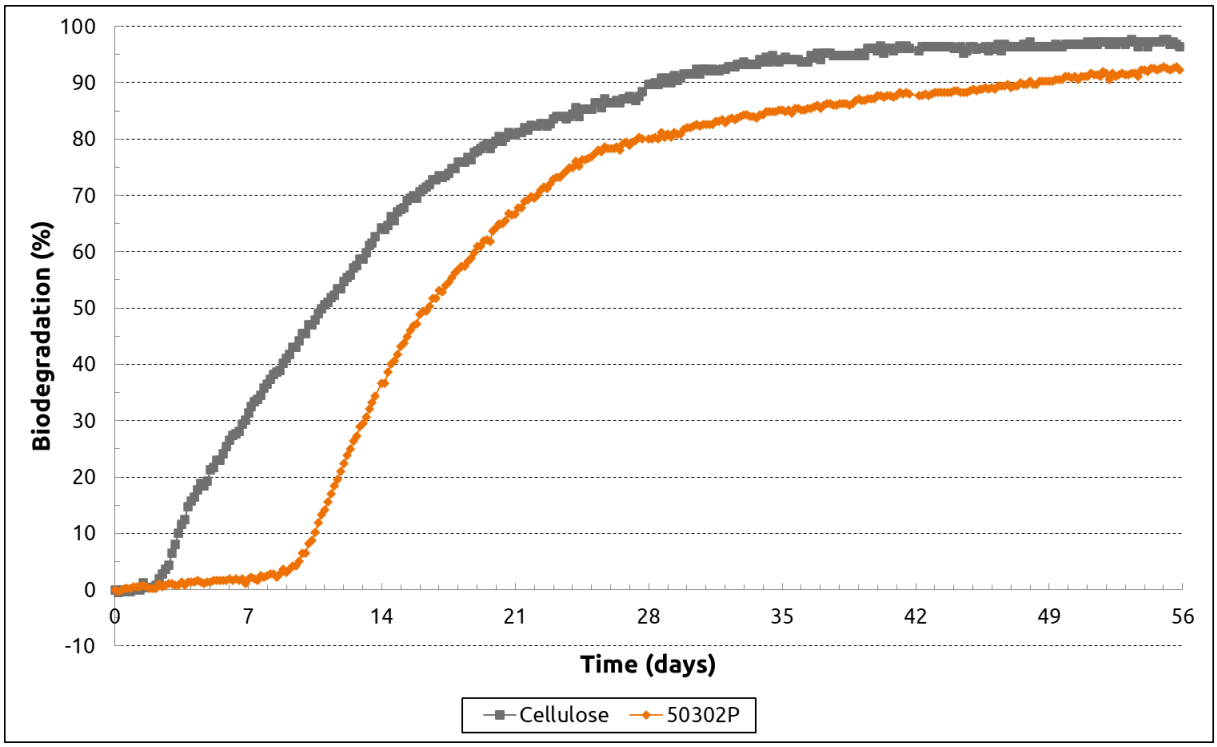

Figure 5. Evolution of the average biodegradation percentage of reference and test item (based on O_2 consumption)

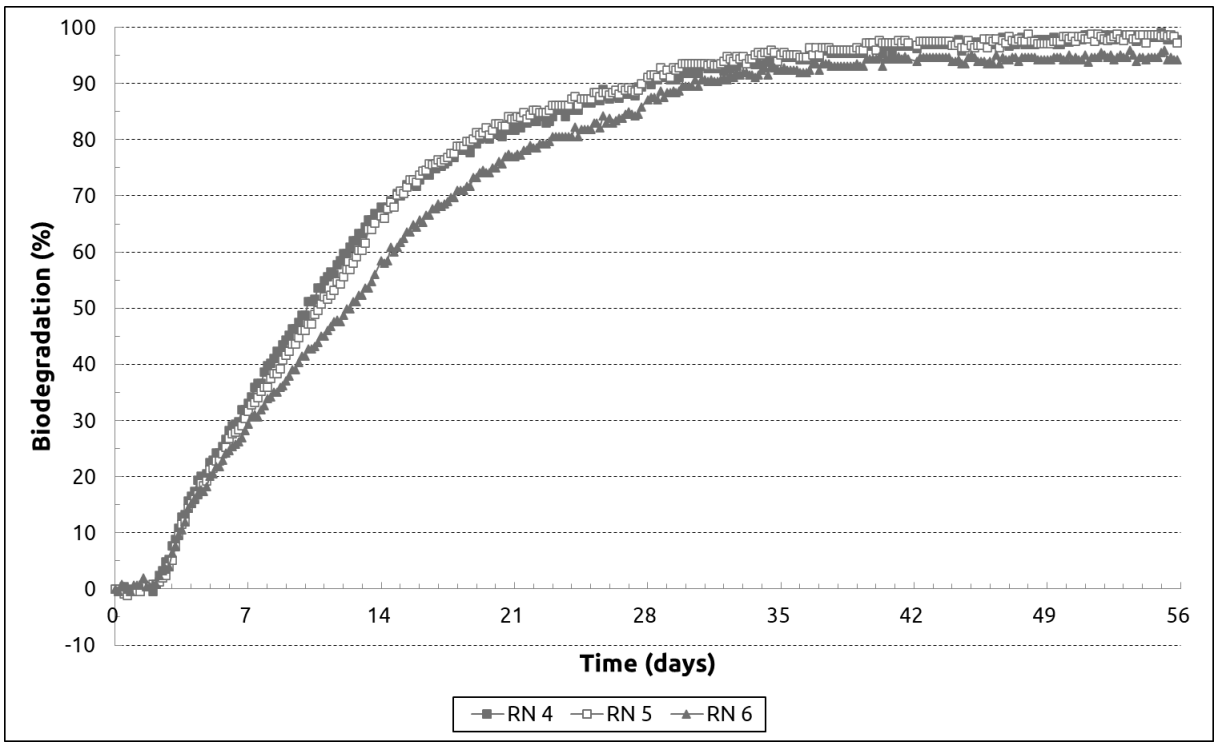

Figure 6. Evolution of the biodegradation percentage of replicates of cellulose (based on O_2 consumption)

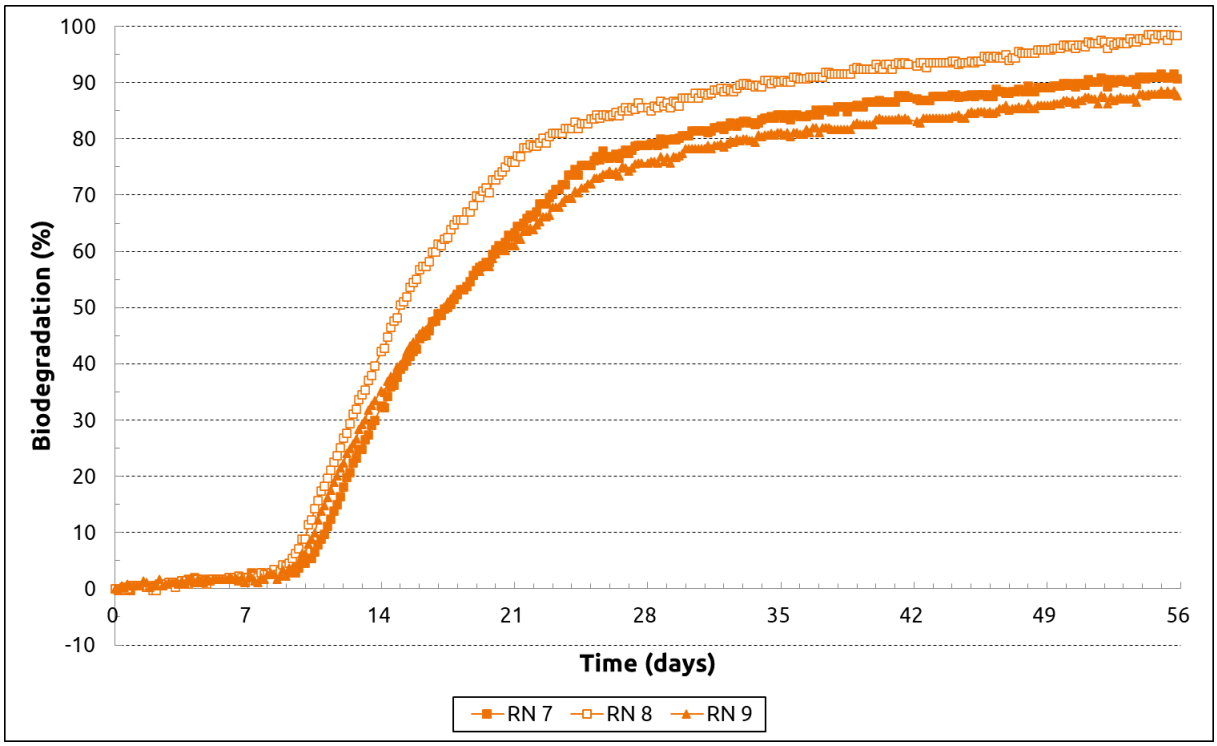

Figure 7. Evolution of the biodegradation percentage of replicates of 50302P (based on O_2 consumption)

8.3.2 Biodegradation based on CO_2 production

The biodegradation was also determined by measuring the amount of CO_2 that had been captured in the KOH solution during the test.

Table 5 shows the $ThCO_2$ (= theoretical CO_2 production based on the % organic C and input of the test item), net CO_2 production and biodegradation percentage of reference and test item at the end of the test (56 days). A visual presentation of the cumulative CO_2 production of the control, reference and test item is given in Figure 8 up to Figure 10. Figure 11 shows the evolution of the average biodegradation of reference and test item (based on CO_2 production), while Figure 12 and Figure 13 show the biodegradation of the replicates.

Table 5. $ThCO_2$, net CO_2 production and biodegradation after 56 days

Test series	$ThCO_2$ (mg)	Net CO_2 (mg)	Biodegradation (%)			95% CL
			AVG	SD	REL	
Cellulose	38.8	35.5	91.5	1.0	100.0	1.6
50302P	47.4	39.6	83.5	2.2	91.3	3.1

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

The biodegradation of reference item cellulose started at a good rate. After 14 days cellulose was degraded by 62.4%. The biodegradation rate gradually decreased and at the end of the test (56 days) a plateau in biodegradation was reached at a level of 91.5% ±1.0%. According to ISO 14851 (2019) the test is considered valid if at the end of the test the biodegradation percentage of the reference item is more than 60%. This requirement was fulfilled.

Test item 50302P started to degrade at a moderate rate. After 14 days, a percentage of 27.8% was obtained. Thereafter, the biodegradation rate slightly increased and after 28 days a value of 71.1% was reached. At the end of the test (56 days) an absolute biodegradation of 83.5% ± 2.2% was measured and biodegradation was still slowly progressing. On a relative basis, compared to reference item cellulose, a biodegradation of 91.3% was calculated.

A test item is considered to meet the biodegradation requirement if 90% of the organic carbon in the whole item or for each constituent, which is present in the material at a concentration of more than 1% (by dry mass), is converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. The requirement needs to be reached within a maximum test duration of 1 year. From the results it can be concluded that test item 50302P fulfills the requirement on biodegradation as defined by the *OK Compost HOME certification scheme* of TÜV AUSTRIA Belgium.

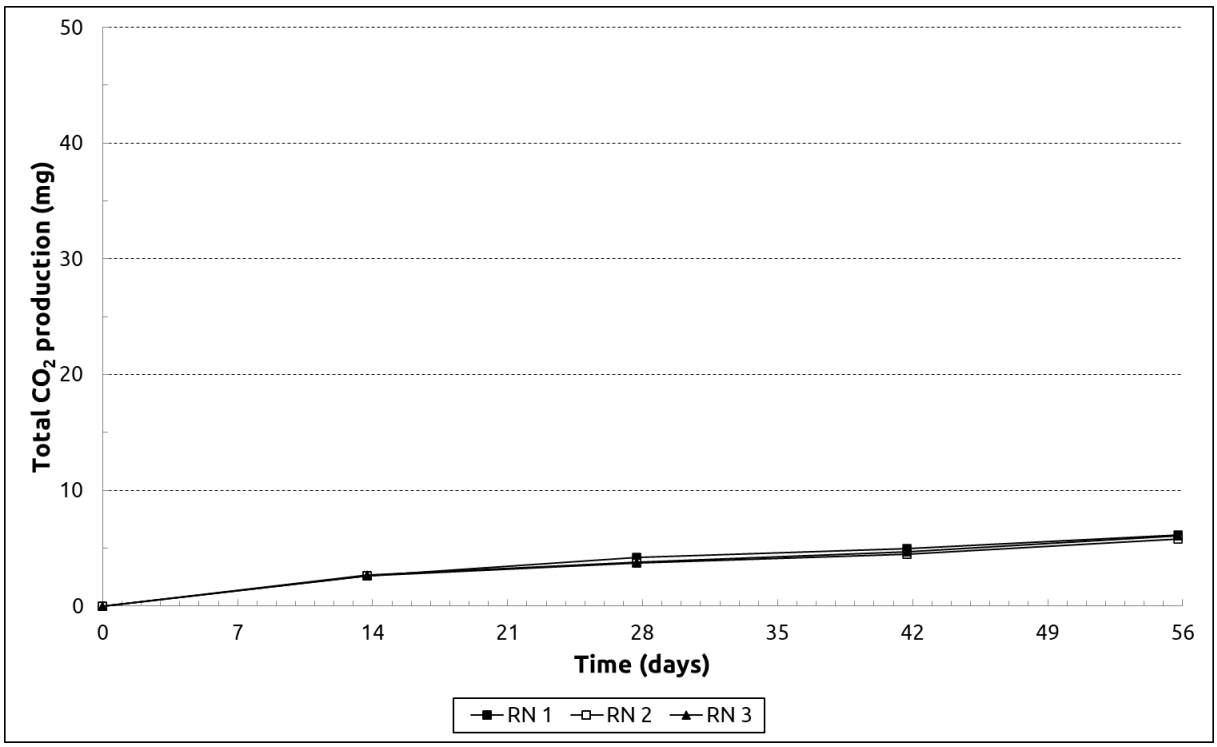

Figure 8. Total CO_2 production of control reactors

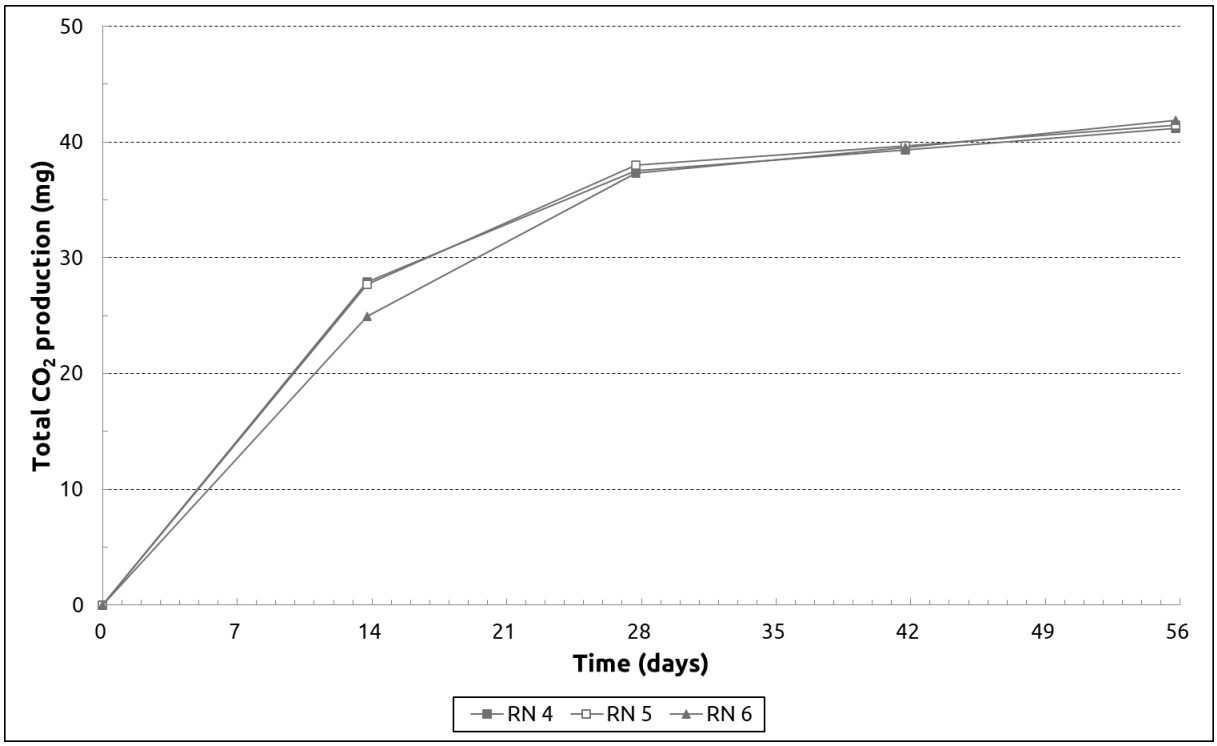

Figure 9. Total CO_2 production of cellulose reactors

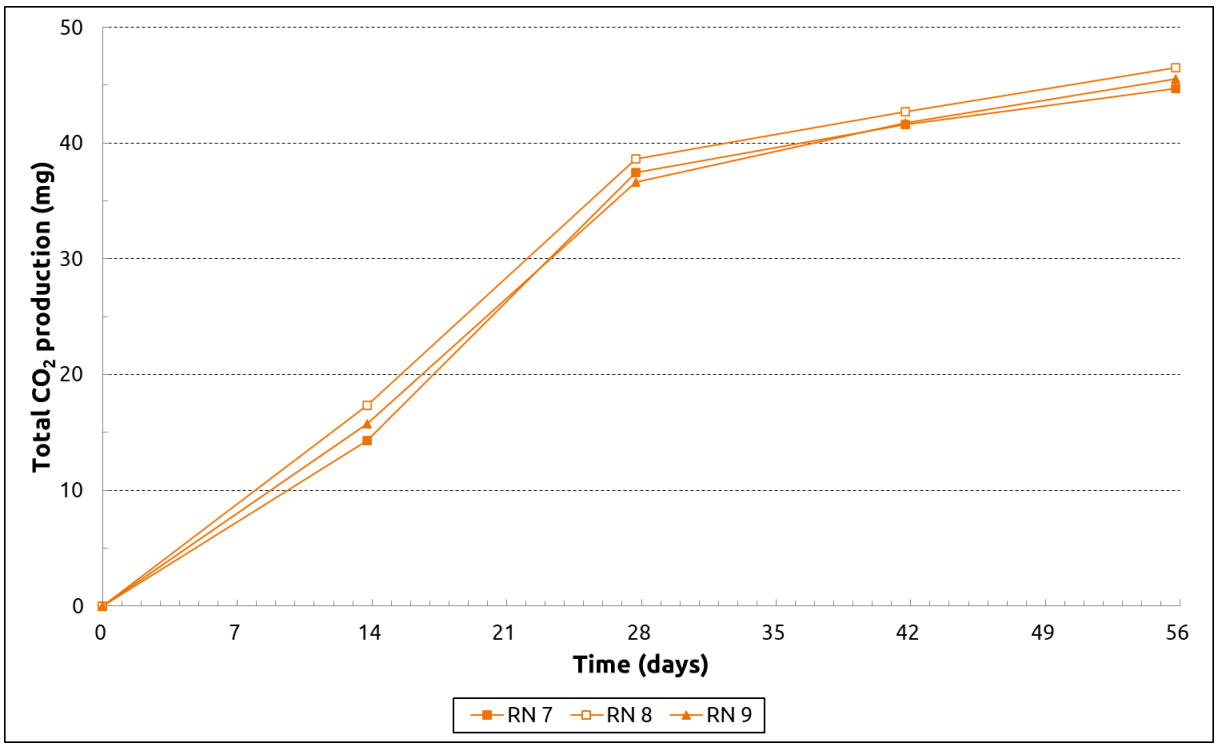

Figure 10. Total CO_2 production of 50302P reactors

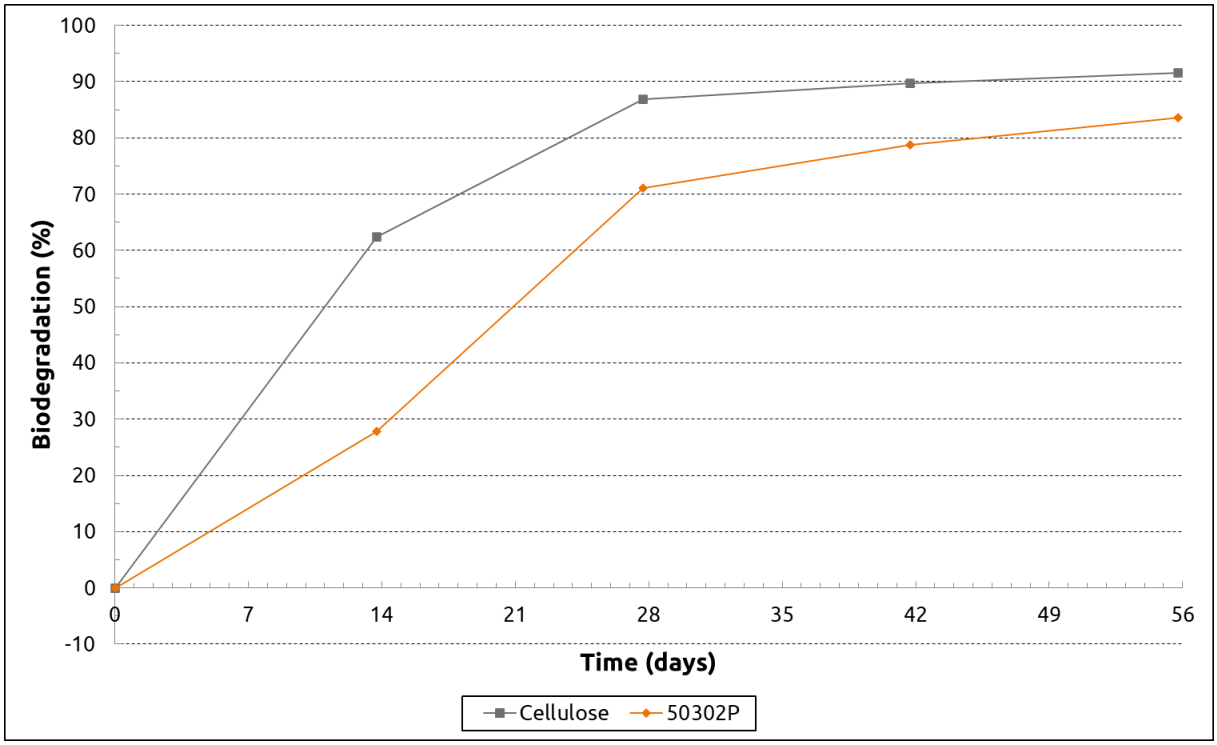

Figure 11. Evolution of the average biodegradation percentage of reference and test item (based on CO_2 production)

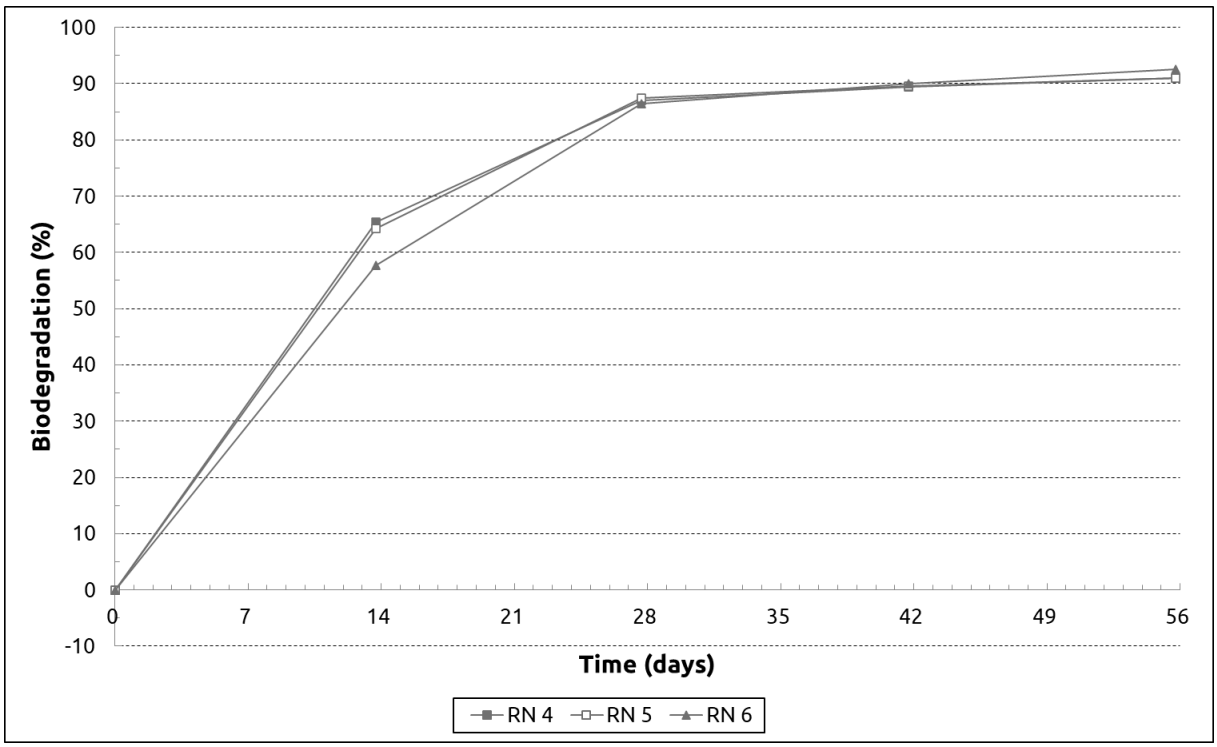

Figure 12. Evolution of the biodegradation percentage of replicates of cellulose (based on CO_2 production)

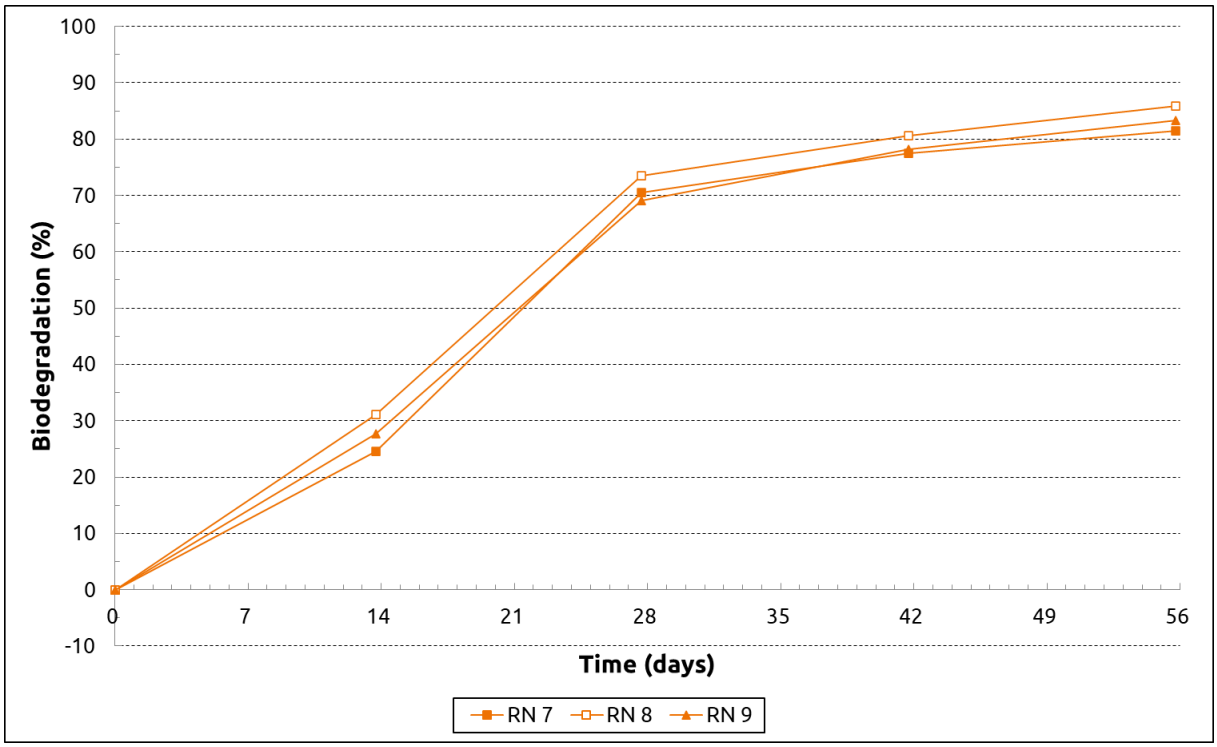

Figure 13. Evolution of the biodegradation percentage of replicates of 50302P (based on CO_2 production)