FINAL REPORT
DRA-3/1

Aerobic biodegradation under controlled composting conditions of **MBR16070601**

Author: Lynn Serbruyns
Sponsor: BiologiQ, Inc.
2400 East 25th St
Idaho Falls
ID 83404
UNITED STATES

Table of contents

1 Identification of the test

1.1 General information

Project number

DRA-3/1

Sponsor

BiologiQ, Inc.
2400 East 25th St
Idaho Falls
ID 83404
UNITED STATES

Sponsor contact

Mr. Don Allen
Don.Allen@BiologiQ.com
Phone: +1 208 357 9653

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

Contact in the United States

Mr. Richard Tillinger
richard.tillinger@ows.be
Phone: +1 937 829 6888

Mrs. Norma McDonald
norma.mcdonald@ows.be
Phone: +1 513 535 6760

Test item

MBR16070601

Reference item

Cellulose

Test duration

105 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:	August 23rd, 2016
Starting date experiments:	August 23rd, 2016
Starting date of incubation under dynamic conditions:	August 23rd, 2016
Completion date of incubation under dynamic conditions:	October 11th, 2016
Test duration dynamic phase:	49 days
Starting date of incubation under static conditions:	October 11th, 2016
Completion date of incubation under static conditions:	December 6th, 2016
Test duration static phase:	56 days
Completion date of experiments:	January 11th, 2017
Completion date study:	January 27th, 2017
Total test duration:	105 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:

DRA-3/1

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).

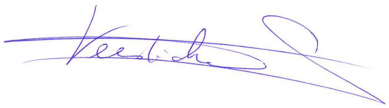
Lynn Serbruyns
Study Director

4 Quality assurance audit statement

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

A quality control was executed on ~~.Feb-~~02-2017

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 MBR16070601 was evaluated in a controlled composting test according to ISO 14855-1 (2012). The incubation temperature was continuously kept at 58°C ± 2°C. After 49 days the test was converted from dynamic to static composting conditions. The total test duration was 105 days.

According to the norm ISO 14855-1 (2012) a CO_2 production between 50 mg and 150 mg CO_2/g VS should be measured for the controls during the first 10 days of the test. After 10 days a background activity of 67 mg CO_2/g VS was measured, which indicates the good quality of the inoculum.

The biodegradation of reference item cellulose started immediately at a good rate. After 15 days cellulose was already degraded by 60.7%. From then on biodegradation rate slowed down, resulting in a biodegradation of 78.3% ± 7.9% after 45 days. Biodegradation continued more or less at the same rate and after 105 days (end of the test) a final biodegradation of 90.9% ± 4.5% was measured. The test is considered valid if after 45 days the biodegradation percentage of the reference item is more than 70% and if the standard deviation of the biodegradation percentage of the reference item is less than 20% at the end of the test. Both requirements were fulfilled.

The biodegradation of test item MBR16070601 started immediately at a good rate and after 28 days a biodegradation of 63.9% ± 3.9% was already reached. From then on biodegradation rate gradually slowed down, resulting at the end of the test (105 days) in a biodegradation of 85.4% ± 3.8%, or 93.9% relative to reference item cellulose.

The American standard ASTM D6400 *Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2012) and the international standard ISO 17088 *Specifications for compostable plastics* (2012) stipulate that 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), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. Organic constituents present at levels between 1 to 10% shall be tested individually. According to the European standard EN 13432 *Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging* (2000) a material can only be called biodegradable when the percentage of biodegradation is at least 90% in total or 90% of the maximum degradation of a suitable reference item after a plateau has been reached for both reference and test item. The maximum allowed test duration by the standards on industrial composting is 180 days. As the material does not contain constituents in a concentration between 1% and 10%, it can be concluded that test item MBR16070601 does fulfill the biodegradation requirement of these standards and is completely biodegradable within 105 days of testing under the given aerobic conditions.

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

The controlled composting biodegradation test is an optimized simulation of an intensive aerobic composting process where the biodegradability of a test item under dry, aerobic conditions is determined. The inoculum consists of stabilized and mature compost derived from the organic fraction of municipal solid waste. The test item is mixed with the inoculum and introduced into static reactor vessels where it is intensively composted under optimum oxygen, temperature and moisture conditions.

During the aerobic biodegradation of organic materials, a mixture of gases (principally carbon dioxide and water) are the final decomposition products while part of the organic material will be assimilated for cell growth. The carbon dioxide production is continuously monitored and integrated to determine the carbon dioxide production rate and the cumulative carbon dioxide production.

After determining the carbon content of the test item, 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.

6.2 Standard followed

- ISO 14855-1 *Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide* (2012).

7 Materials and methods

7.1 Test and reference item

Test item

Name:	MBR16070601
Description:	Pellets
Colour:	Off-white
Batch number:	MBR16070601
Sample preparation:	Cryogenically milled till dimensions between 1 and 4 mm

Reference item

Name:	Cellulose
Purity:	Native cellulose powder for thin layer chromatography (Avicel)
Physical form:	Powder
Colour:	White
Batch number:	K45989631508
Expiration date:	February 2021
Brand:	Merck Art. Nr. 2331

7.2 General procedure

7.2.1 Composting under dynamic conditions

The inoculum is derived from the organic fraction of municipal solid waste, which is stabilized further and matured in a composting bin at the laboratory under controlled aeration conditions (see Figure 1). Before use the mature compost is sieved on a screen of 5 mm and the fine fraction is used as the inoculum. It is recommended that the compost inoculum has a total solids content of roughly 50-55% and a volatile solids concentration of more than 30% on dry solids.

The test and reference item are mixed with the inoculum in a ratio of roughly 1 to 1.5 parts of total solids to 6 parts of total solids and introduced into the reactors. These reactors are closed airtight and put into the incubators (see Figure 2). The temperature of the reactors is continuously controlled and kept at 58°C ± 2°C.

Pressurized dry air is sent over a gas flow controller, which regulates very precisely the flow rate and blown into the composting vessel at the bottom through a porous plate. Through biodegradation solid carbon of the test compound is converted and CO_2 is produced.

The gas leaving each individual reactor is continuously analysed on regular intervals for CO_2 and O_2 concentration. Also the flow rate is measured regularly. Likewise the cumulative CO_2 production can be determined. The percentage of biodegradation is determined as the percentage of solid carbon of the test compound that is converted to gaseous, mineral C under the form of CO_2. More details on the procedure for the particular test reported, are given in the study plan.

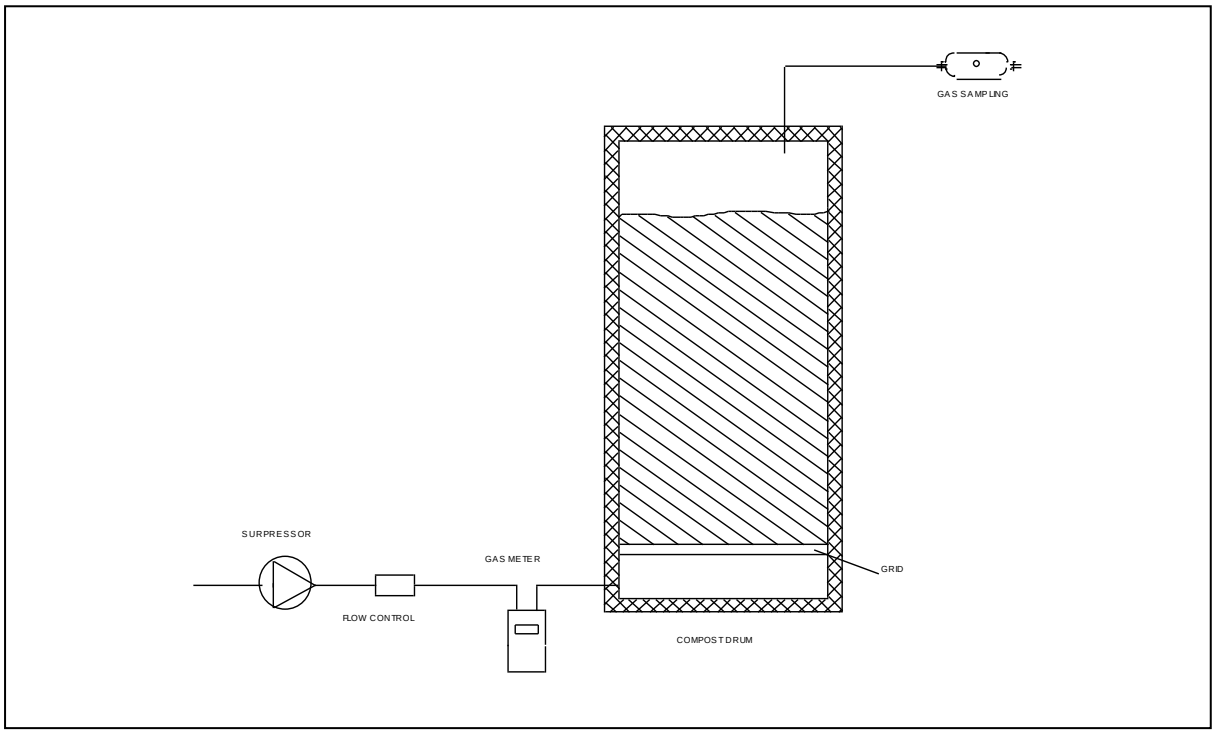


Figure 1. Set-up composting bin for compost maturation

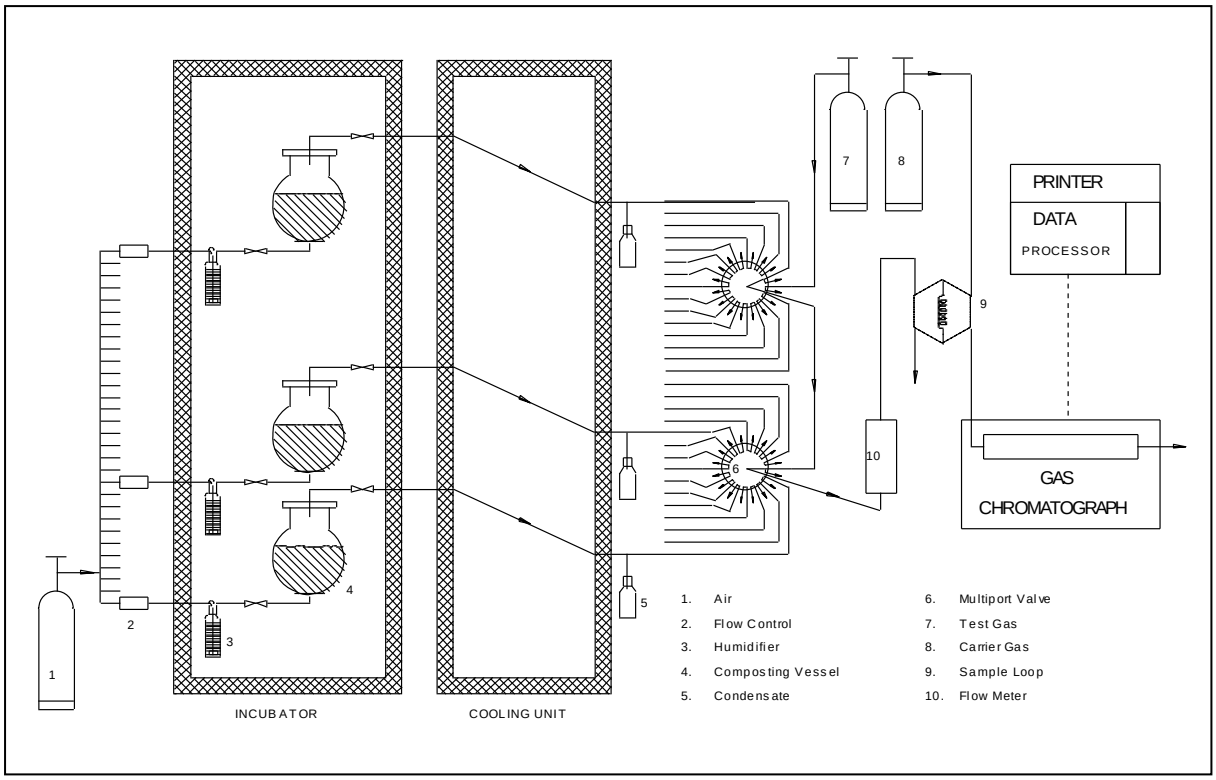


Figure 2. Set-up controlled composting test

7.2.2 Composting under static conditions

After 49 days of composting under dynamic conditions a homogeneous compost sample (about 100 g) of each reactor mixture was further composted under static conditions. The sample is put in a beaker and placed in a reactor, which can be closed airtight (Figure 3). The reactor also contains a beaker with KOH to absorb the carbon dioxide, released during the composting process. The two beakers are put on a perforated plate. A same amount of water is put on the bottom of the reactors in order to prevent a drying out of the compost sample.

Three technical controls are included in the test set-up. The technical control has the same composition as the other reactors, except that no beaker with compost sample is added. The reactors are stored in an incubator at 58°C ± 2°C.

The CO_2 production is determined by titration. The percentage of biodegradation is 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. After each titration, a new beaker with KOH is put into the reactor. At the same time the compost sample is stirred and moistened if needed.

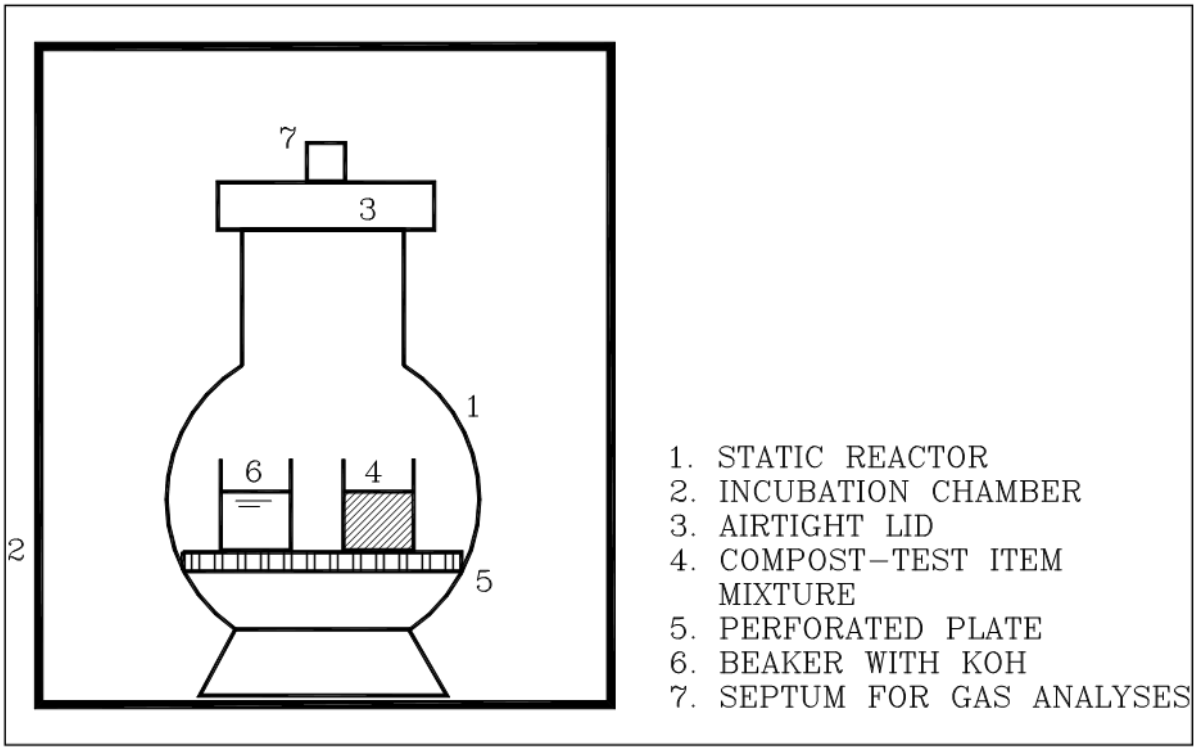


Figure 3. Set-up controlled composting test under static conditions

7.3 Analytical methods

Ammonium - nitrogen (NH_4^+-N)

This analysis is done as described in 'METH L.016. Determination of ammonia-nitrogen by FIA (spectrometric detection)'. The ammonium-N is determined in an aqueous extract (5 parts of demineralised water versus 1 part of sample; see METH L.012). The sample containing ammonium ions is injected into a continuous carrier stream by means of an injection valve and is mixed with a continuously streaming flow of an alkaline solution. The gaseous ammonia formed is separated through a diffusion cell from the solution over a hydrophobic semi permeable membrane and taken up by a streaming recipient flow containing a pH indicator. Due to the resulting pH shift, the indicator solution will change its colour which is measured continuously in the flow photometer at 590 nm. The results are given in g 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 'METH L.009. Determination of moisture content'. The dry matter is given in percent on wet weight.

Gas composition

The gas analyses are performed on a PerkinElmer gas chromatograph with CTRI column as described in 'INST L.435. Manual TotalChrom software'. The gas chromatograph is calibrated with a standard gas mixture consisting of 15% O_2, 6% CO_2, 79% N_2. Every day gas analyses were executed the gas chromatograph is validated. The results are given in per cent.

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

This analysis is done as described in 'METH L.017. Determination of total oxidized nitrogen by FIA (spectrometric detection)'. The determination is performed on an aqueous extract (5 parts of demineralised water versus 1 part of sample; see METH L.012). The sample containing nitrite/nitrate ions is fed into a continuously flowing buffer solution (carrier stream) by means of an injection valve. Nitrate in the sample is reduced to nitrite in a cadmium reductor. On the addition of an acidic sulphanilamide solution, nitrite initially present and nitrite formed from reduction of nitrate will form a diazo compound. This compound is coupled with N-(1-naphtyl)-ethylene diamine dihydrochloride (NED) to form a purple azo dye. This azo dye is measured at 540 nm. The results are given in g per l 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 (2014). 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 ISO 16948 (2015). The results are given in per cent.

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.

Volatile fatty acids (VFA)

The volatile fatty acids are determined as described in 'METH L.203 Determination of volatile fatty acids'. The sample is diluted with water and centrifuged to remove the suspended solids. Afterwards ether is added and the acids are extracted by centrifugation. The actual analysis is done by gas chromatography. The gas chromatograph is a Clarus 480. The column used is a Stabilwax of 30 m. The carrier gas is H_2. A mixture with precise concentrations of eight reference volatile fatty acids is used for calibration while 2-methyl-caproic acid is used as an internal standard. The results are given in g per l wet weight.

Weight determination

During the test 3 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. 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 test item. A Robbe Low Profile balance (max. 300 kg; d = 50 g) was used for weighing of the biowaste and the compost bins.

8 Results

8.1 Test conditions and set-up

A set of 9 equal composting vessels with a total volume of 4 l each was used, incubated at a constant temperature of 58°C ± 2°C. The test set-up is given in Table 1. Reference material cellulose was added as powder, while test item MBR16070601 was previously reduced in size (cryogenically milled till dimensions between 1 and 4 mm, as requested by the sponsor). After 49 days the test was converted from dynamic (active aeration) to static (passive aeration) composting conditions. The total test duration was 105 days.

Table 1. Test set-up controlled composting test

RN	Test series	Inoculum (g)	Item (g)	Water (g)
1	Control	1200	0	0
2	Cellulose	1200	80	0
3	MBR16070601	1200	80	0
4	Control	1200	0	0
5	Cellulose	1200	80	0
6	MBR16070601	1200	80	0
7	Control	1200	0	0
8	Cellulose	1200	80	0
9	MBR16070601	1200	80	0

RN = reactor number

8.2 Analyses of inoculum, test and reference item

The inoculum was derived from the organic fraction of municipal solid waste. The waste was stabilized and aerated in a composting bin at the laboratory under controlled conditions for more than 20 weeks. Before use the compost was sieved through 5 mm. The characteristics of the inoculum used at start are given in Table 2.

The inoculum should have a total solids (TS) content between 50% and 55% and a volatile solids content (VS) on TS of more than 30%. Moreover the pH should be between 7.0 and 9.0. As can be seen from Table 2 these requirements were largely fulfilled. The inoculum showed a total solids content of 56.2%, a volatile solids content of 33.2% on TS and a pH of 8.2. The total solids content was just above the optimal range, but on an empirical basis a good moisture content was still obtained. The compost inoculum must feel somewhat sticky and have some free water available when pressed by hand.

Table 2. Characteristics of the inoculum

Characteristics	Inoculum
Total solids (TS, %)	56.2
Moisture content (%)	43.8
Volatile solids (VS, % on TS)	33.2
Ash content (% on TS)	66.8
pH	8.2
Electrical conductivity (EC, µS/cm)	2900
Volatile fatty acids (VFA, g/l)	b.r.
Total N (g/kg TS)	25.8
NH_4^+-N (mg/l)	14.2
NO_x^--N (mg/l)	515
C/N	6

b.r. = below reporting limit: reporting limit VFA = 0.3 g/l

According to the norm ISO 14855-1 (2012) a CO_2 production between 50 mg and 150 mg CO_2/g VS should be measured for the controls during the first 10 days of the test. After 10 days a background activity of 67 mg CO_2/g VS was measured, which indicates the good quality of the inoculum.

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

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	97.0	100.0	42.7
MBR16070601	96.5	100.0	54.6

8.3 CO_2 production

The total cumulative CO_2 production for each reactor at conversion (gas chromatograph data until 48 days) and at the end of the test (105 days) is given in Table 4. Also the net cumulative CO_2 production of the reference and test item is given in g absolute and in mg per g of test item. Figures 4 up to 6 show the evolution of the total cumulative CO_2 production.

Table 4. CO_2 production after 48 and 105 days

RN	Test series	Total CO_2 (g)	Net CO_2	
			(g)	(mg/g test item)
After 48 days				
1	Control	45.3	-	-
2	Cellulose	150.7	105.0	1316
3	MBR16070601	161.0	115.3	1442
4	Control	47.9	-	-
5	Cellulose	132.8	87.1	1091
6	MBR16070601	177.9	132.2	1650
7	Control	43.9	-	-
8	Cellulose	149.0	103.3	1293
9	MBR16070601	162.7	117.0	1462
After 105 days				
1	Control	80.7	-	-
2	Cellulose	195.8	115.4	1446
3	MBR16070601	212.4	132.0	1650
4	Control	74.9	-	-
5	Cellulose	187.6	107.2	1342
6	MBR16070601	224.1	143.7	1794
7	Control	85.5	-	-
8	Cellulose	198.3	117.9	1476
9	MBR16070601	214.7	134.4	1679

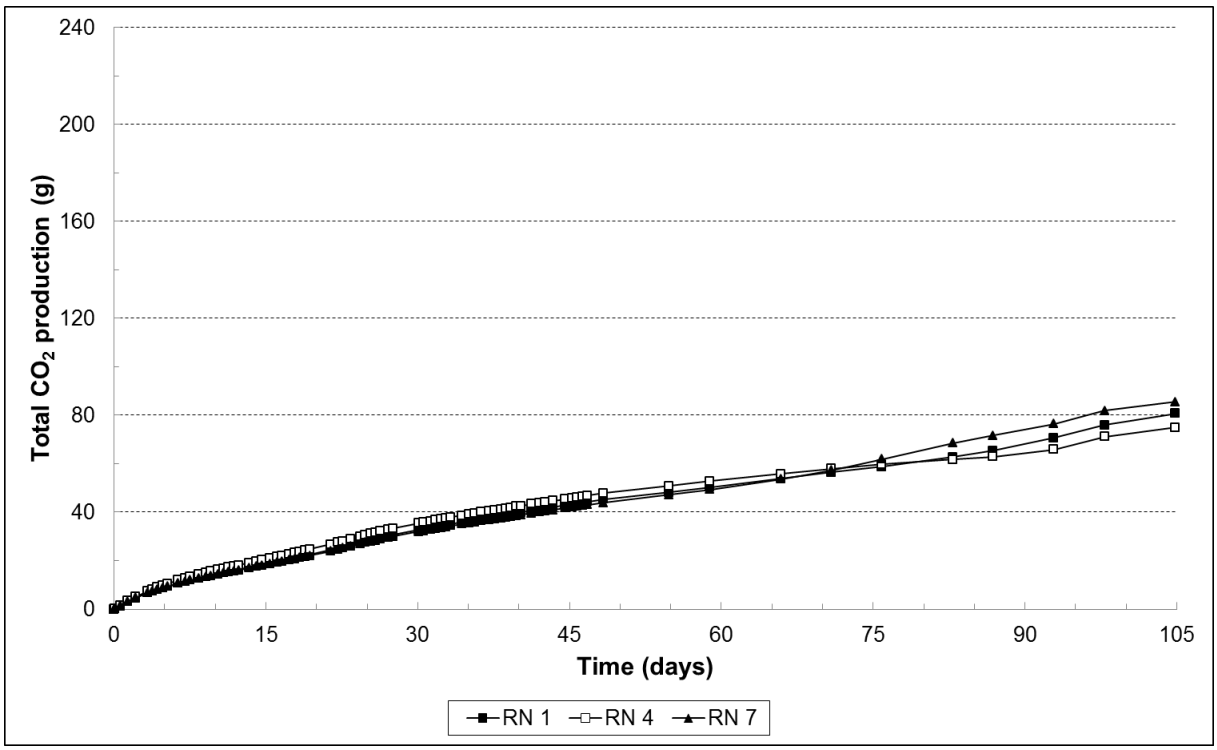


Figure 4. Total CO_2 production of the control reactors

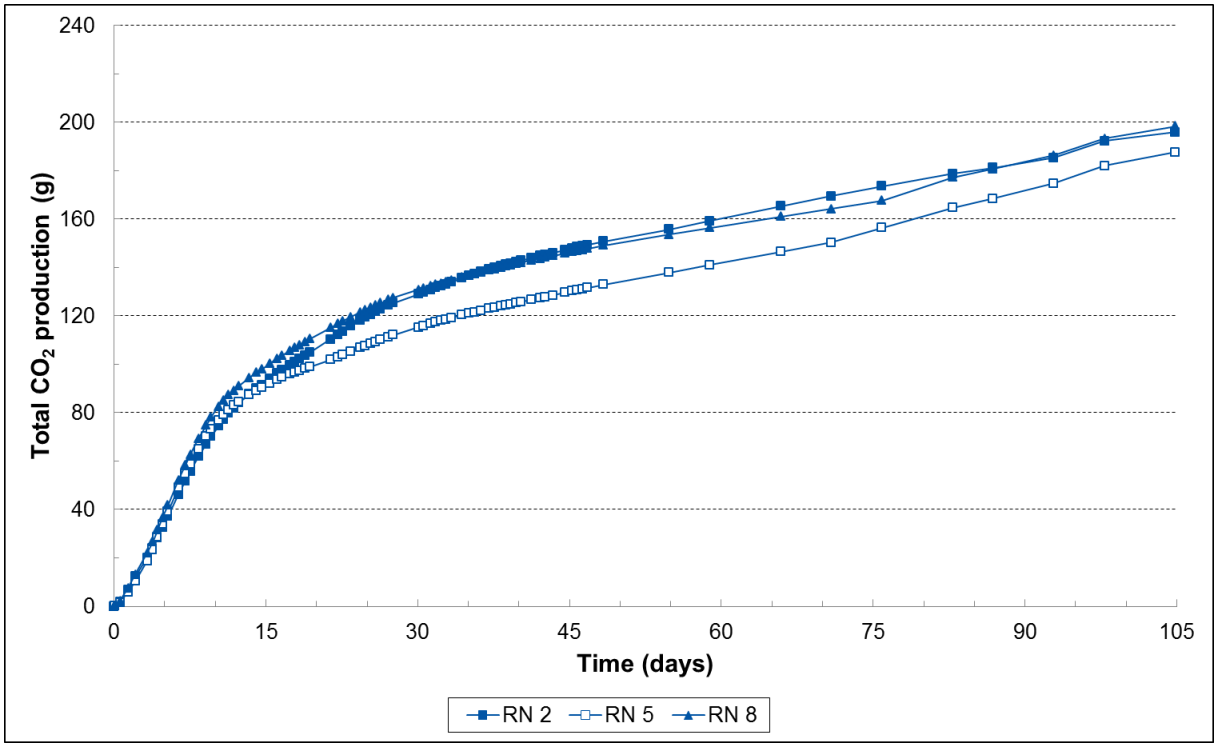


Figure 5. Total CO_2 production of cellulose reactors

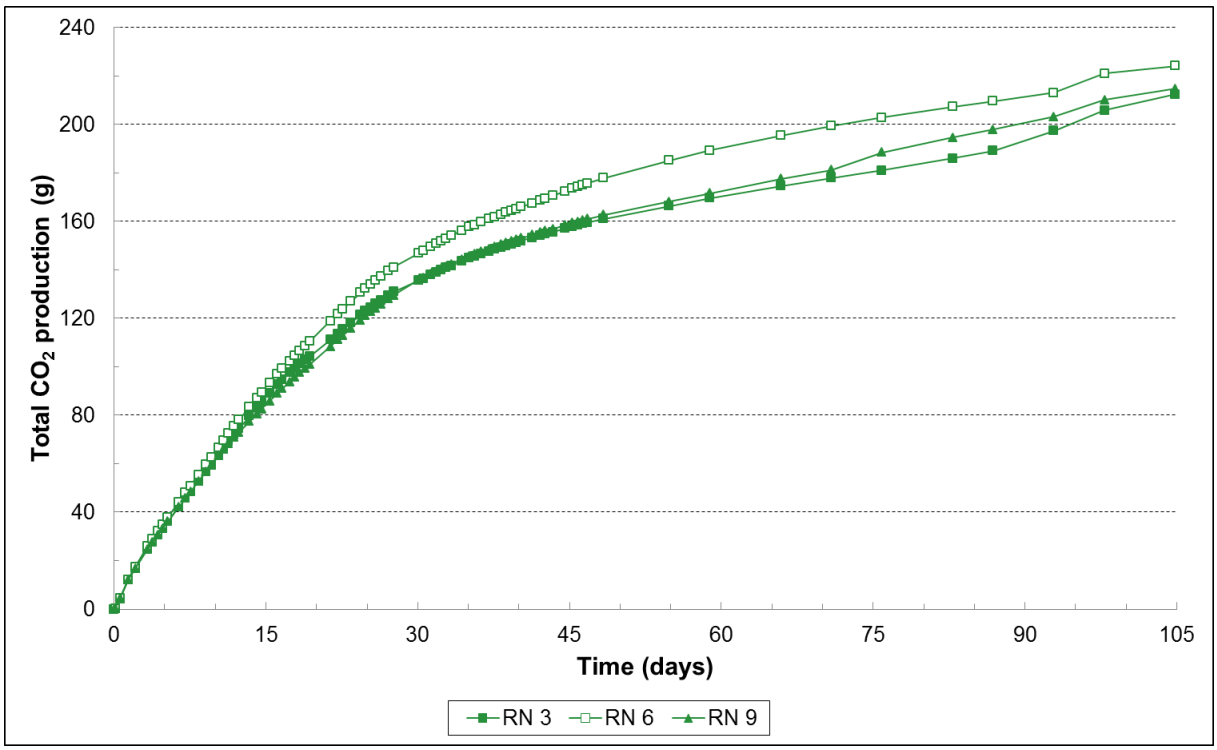


Figure 6. Total CO_2 production of MBR16070601 reactors

8.4 Biodegradation percentages

The results on the calculation of the biodegradation percentages at conversion (gas chromatograph data until 48 days) and at the end of the test (105 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 7 shows the evolution of the average biodegradation percentages, while Figures 8 and 9 represent the biodegradation of the separate replicates.

Table 5. Biodegradation percentages after 48 and 105 days

Test series	Average C_{input} (g)	Average $C_{gaseous}$ (g)	Biodegradation (%)			95% CL
			AVG	SD	REL	
After 48 days						
Cellulose	34.1	26.9	78.8	7.9	100.0	12.9
MBR16070601	43.7	33.1	75.9	5.8	96.2	9.5
After 105 days						
Cellulose	34.1	31.0	90.9	4.5	100.0	9.9
MBR16070601	43.7	37.3	85.4	3.8	93.9	8.1

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

The biodegradation of reference item cellulose started immediately at a good rate. After 15 days cellulose was already degraded by 60.7%. From then on biodegradation rate slowed down, resulting in a biodegradation of 78.3% ± 7.9% after 45 days. Biodegradation continued more or less at the same rate and after 105 days (end of the test) a final biodegradation of 90.9% ± 4.5% was measured. The test is considered valid if after 45 days the biodegradation percentage of the reference item is more than 70% and if the standard deviation of the biodegradation percentage of the reference item is less than 20% at the end of the test. Both requirements were fulfilled.

The biodegradation of test item MBR16070601 started immediately at a good rate and after 28 days a biodegradation of 63.9% ± 3.9% was already reached. From then on biodegradation rate gradually slowed down, resulting at the end of the test (105 days) in a biodegradation of 85.4% ± 3.8%, or 93.9% relative to reference item cellulose.

The American standard ASTM D6400 *Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2012) and the international standard ISO 17088 *Specifications for compostable plastics* (2012) stipulate that 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), shall be converted to carbon dioxide by the end of the test period when compared to the positive control or in the absolute. Organic constituents present at levels between 1 to 10% shall be tested individually. According to the European standard EN 13432 *Requirements for packaging recoverable through composting and biodegradation – Test scheme and evaluation criteria for the final acceptance of packaging* (2000) a material can only be called biodegradable when the percentage of biodegradation is at least 90% in total or 90% of the maximum degradation of a suitable reference item after a plateau has been reached for both reference and test item. The maximum allowed test duration by the standards on industrial composting is 180 days. As the material does not contain constituents in a concentration between 1% and 10%, it can be concluded that test item MBR16070601 does fulfill the biodegradation requirement of these standards and is completely biodegradable within 105 days of testing under the given aerobic conditions.

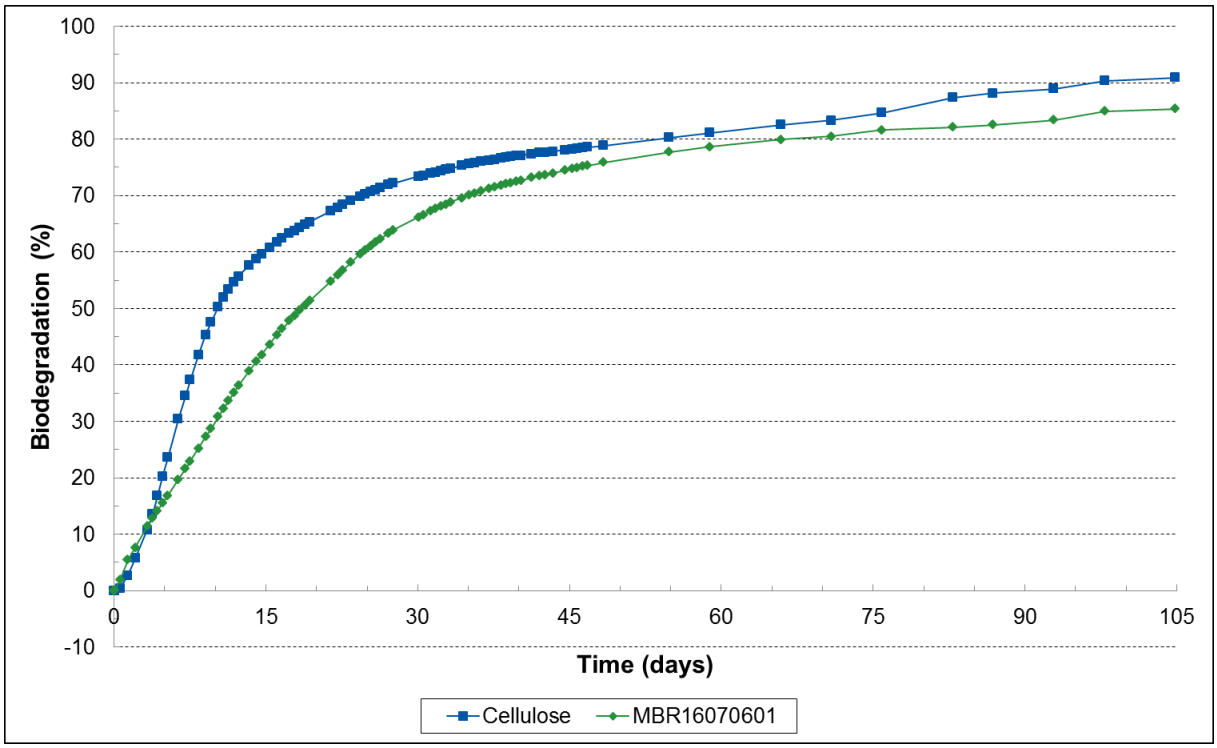


Figure 7. Evolution of the biodegradation percentage of reference and test item

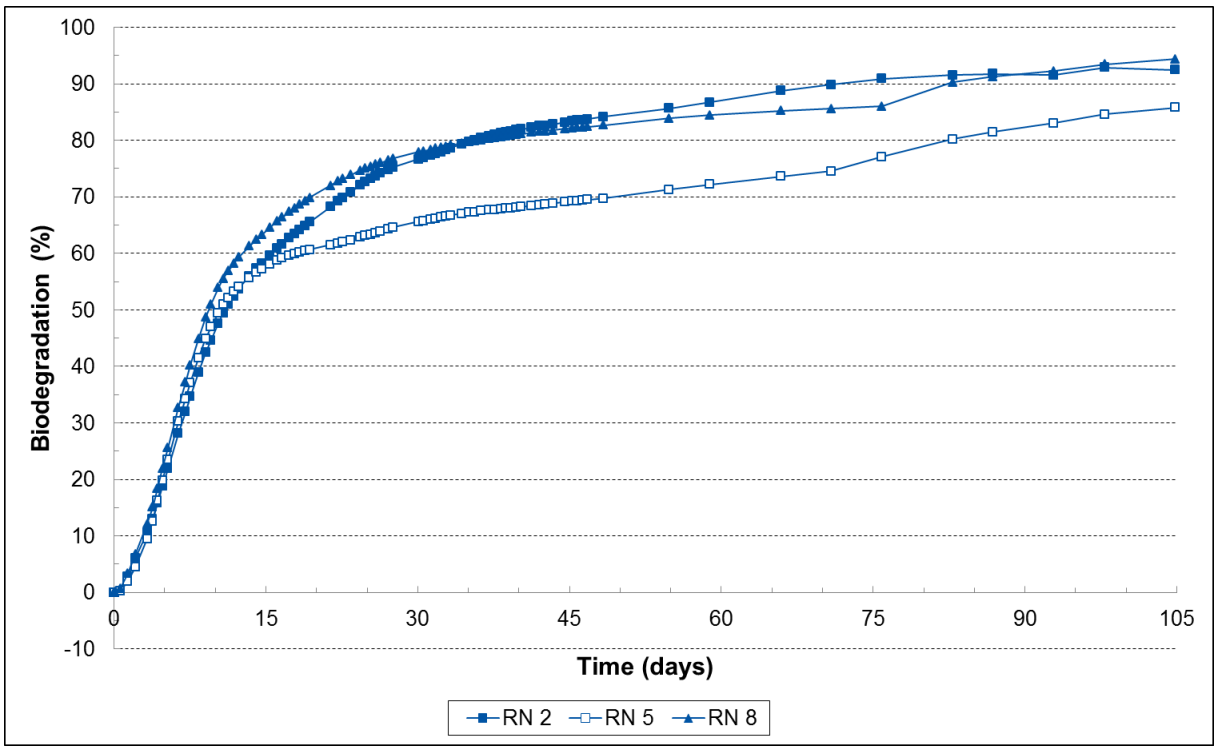


Figure 8. Evolution of the biodegradation percentage of the replicates of cellulose

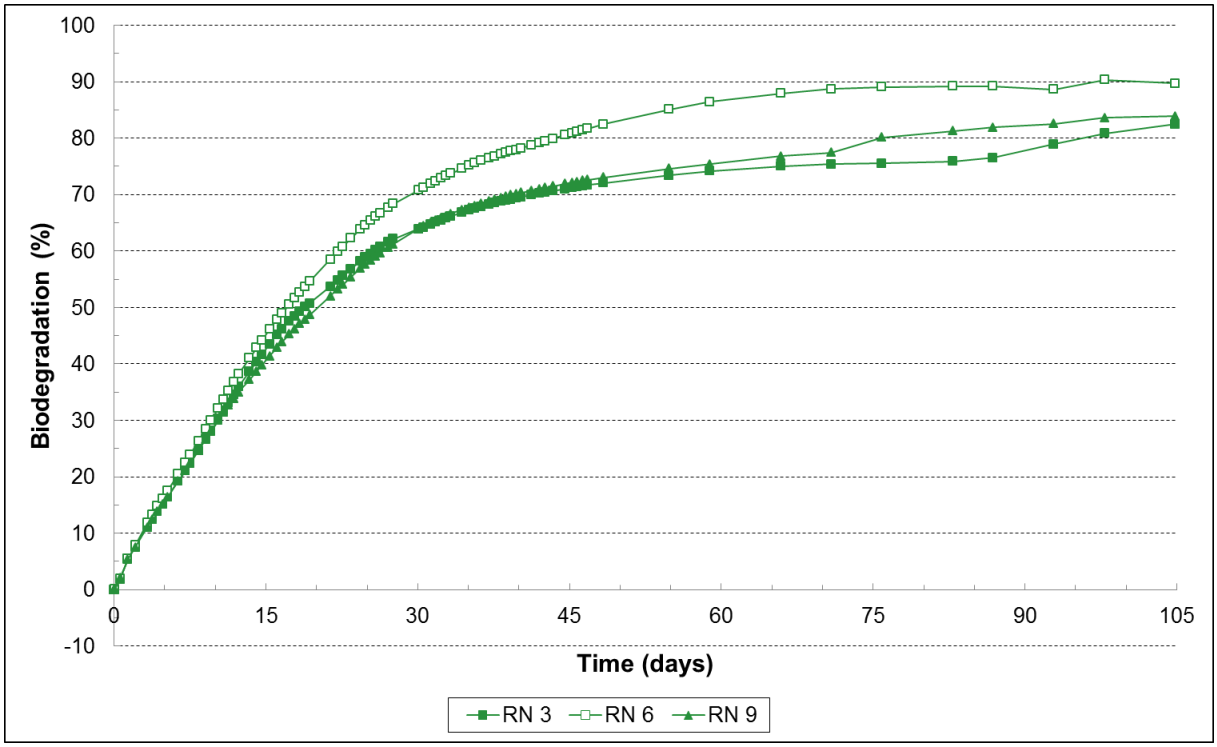


Figure 9. Evolution of the biodegradation percentage of the replicates of MBR16070601

8.5 Visual perceptions and analyses at end of test

Once every week 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.

Control and cellulose showed a good structure and moisture content throughout the test. Some fungal growth was observed in the cellulose reactors during the second week of incubation and after 5 weeks reference item cellulose was no longer visible. The test item reactors also showed good structure and moisture conditions. Fungal growth was detected during the first 5 weeks of incubation and at the end of the dynamic phase (49 days) test item MBR16070601 was no longer visible.

At the conversion from dynamic to static conditions (49 days) and at the end of the test (105 days) the different test series were analyzed for total solids (TS), volatile solids (VS) and pH (see Table 6). At the conversion and at the end of the test the volatile solids content of the test item reactors was lower than that of the control reactors confirming the complete biodegradation of test item MBR16070601.

Table 6. pH, TS and VS content at conversion and after 105 days (end of test)

Test series	TS (%)	VS (% on TS)	pH
After 49 days			
Control	58.0	32.0	7.9
Cellulose	57.6	29.8	8.7
MBR16070601	58.6	30.6	8.8
After 105 days			
Control	57.9	29.7	8.0
Cellulose	59.5	27.9	8.7
MBR16070601	61.9	26.7	8.9