Industrial compostability testing program on GS 270 (3.59 mm) and Evaluation of aerobic biodegradation under controlled composting conditions of Film MBR15120101 & Film MBR15121703 According to ASTM D6400 (2012), EN 13432 (2000) & ISO 17088 (2012)

- Executive summary
- Material characteristics
- Aerobic biodegradation test under controlled composting conditions at elevated temperature
- Pilot-scale composting test
- Ecotoxicity tests
  - Barley plant growth test
  - Cress test
Executive summary
EXECUTIVE SUMMARY
DRA-1

Industrial compostability testing program on GS 270 (3.59 mm)

and

Evaluation of aerobic biodegradation under controlled composting conditions of Film MBR15120101 & Film MBR15121703

According to


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On February 2, 2016 a compostability testing program was initiated on GS 270 in a thickness of 3.59 mm in line with the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012).

GS 270 was evaluated on material characteristics and disintegration including effects on the biological treatment process and effect on quality of compost. Biodegradability was not evaluated on GS 270 as such, but on Film MBR15120101 and Film MBR15121703, which contain both GS 270 in a concentration between 27% and 30%.

Film MBR15120101 consists of 30% GS 270, 67% PBAT and 3% Bynel 41E710, while Film MBR15121703 consists of 27% GS 270, 70% PBAT and 3% S/MA EF80P (as declared by the sponsor).

The detailed test results are given in the various reports.

Material characteristics
The volatile solids concentration, heavy metals content and fluorine content of GS 270 are reported in report R-DRA-1/1. The test material fulfills all requirements on volatile solids, heavy metals and fluorine as stipulated by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012).

Biodegradation under aerobic conditions
Samples Film MBR15120101 and Film MBR15121703, which contain GS 270 in a concentration of 30% and 27%, respectively, were tested for biodegradation in an aerobic biodegradation test under controlled composting conditions at elevated temperature (58°C) according to ISO 14855-1 (2012)1 (see report R-DRA-1/5). After 45 days a plateau in biodegradation was reached at a level of 90.9% ± 6.0% and 96.0% ± 4.3% for samples Film MBR15120101 and Film MBR15121703, respectively. On a relative basis, compared to suitable reference substrate cellulose, a biodegradation percentage of 108.3% and 114.4%, respectively, was calculated. Therefore it can be concluded that the 90% biodegradation requirement as defined by EN 13432 (2000) was fulfilled for both samples.

Taking into account the high dosage of GS 270 in the films and the complete biodegradation of these products, it can be derived that GS 270 is completely biodegradable under controlled composting conditions at 58°C.

In order to be able to conclude that Film MBR15120101 and Film MBR15121703 fulfill also the biodegradation requirements of ASTM D6400 (2012) and ISO 17088 (2012), the biodegradability of the 3% constituents (Bynel 41E710 in Film MBR15120101 and S/MA EF80P in Film MBR15121703) should be tested individually.

---

1 ISO 14855-1 Determination of the ultimate aerobic biodegradability of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide (2012)
Pilot-scale composting test
The quantitative disintegration of GS 270 in a thickness of 3.59 mm was evaluated in a pilot-scale aerobic composting test according to ISO 16929 (2013)² (see report R-DRA-1/2). The disintegration of the test item proceeded very swiftly. Already after 3 weeks of composting the test item seemed completely disappeared. After 12 weeks of composting 100% complete disintegration was obtained. Material GS 270 (3.59 mm) easily fulfills the 90% pass level as required by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012).

Compost quality – Plant toxicity tests
The addition of 10% GS 270 at start of the composting did not cause a negative effect on compost quality (including chemical parameters and toxicity tests) (see reports R-DRA-1/2 (pilot-scale composting test), R-DRA-1/3 (barley plant growth test) and R-DRA-1/4 (cress test)). Material GS 270 does fulfill the requirements on compost quality as stipulated by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012).

GENERAL CONCLUSION
As a general conclusion it can be stated that GS 270 in a thickness of 3.59 mm does fulfill the evaluation criteria for material characteristics, biodegradation, disintegration and compost quality, which are outlined in the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012). GS 270 (3.59 mm) can concluded to be fully compostable in industrial processes.

Moreover based on the results of the biodegradation test, it can be concluded that Film MBR15120101 and Film MBR15121703 do both fulfill the evaluation criteria for biodegradation, which are outlined in EN 13432 (2000). In order to be able to conclude that Film MBR15120101 and Film MBR15121703 fulfill also the biodegradation requirements of ASTM D6400 (2012) and ISO 17088 (2012), the biodegradability of the 3% constituents (Bynel 41E710 in Film MBR15120101 and S/MA EF80P in Film MBR15121703) should be tested individually.

Gent, June 15th, 2016

Bruno De Wilde
Lab Manager, OWS nv

³ ISO 17088 prescribes that the concentrations of regulated metals and other toxic substances in the product shall not exceed the limits specific to the country where the final product will be placed on the market or disposed.
Material characteristics of GS 270

Report R-DRA-1/1
FINAL REPORT
DRA-1/1

Material characteristics of
GS 270

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1 Identification of the test

Project number
DRA-1/1

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Test item
A visual presentation of test material GS 270 is given in Figure 1.

Figure 1. Visual presentation of test material GS 270
2 Introduction

2.1 Volatile solids content

The international standard ISO 17088 Specifications for compostable plastics (2012) and the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) prescribe a minimum volatile solids content of 50% on total solids (TS).

The total solids or dry matter content 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 total solids content is given in percent on wet weight.

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 total solids.

2.2 Heavy metals and fluorine

The American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the Canadian standard CAN/BNQ 0017-088 Specifications for compostable plastics (2010) and the European norm EN 13432 (2000) define limit levels for heavy metals and fluorine. ISO 17088 (2012) prescribes that the concentrations of regulated metals and other toxic substances in the product shall not exceed the limits specific to the country where the final product will be placed on the market or disposed of. The analyses are executed by an external lab. The limit values and test procedures are given in Table 2.

3 Results

3.1 Volatile solids content

The total solids content (TS), the moisture content, the volatile solids content (VS) on total solids and the ash content on total solids of the test material are shown in Table 1. ISO 17088 (2012) and EN 13432 (2000) prescribe a minimum volatile solids content of 50% on TS. Test material GS 270 with a volatile solids content of 99.9% on TS easily fulfills this requirement.

Table 1. Total solids content, moisture content, volatile solids content and ash content of the test material

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>GS 270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS, %)</td>
<td>95.1</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>4.9</td>
</tr>
<tr>
<td>Volatile solids (VS, % on TS)</td>
<td>99.9</td>
</tr>
<tr>
<td>Ash content (% on TS)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

3.2 Heavy metals and fluorine

The heavy metals content and the fluorine content of test item GS 270 are given in Table 2, together with the limit values as prescribed by ASTM D6400 (2012), CAN/BNQ 0017-088 (2010) and EN 13432 (2000). All values lay well below the maximum levels as prescribed by the standards.
4 Conclusions

From the results it can be concluded that GS 270 fulfills the requirements on material characteristics (volatile solids, heavy metals and fluorine) as defined by ASTM D6400 (2012), ISO 17088 (2012) and EN 13432 (2000).

Gent, March 9th, 2016

Nike Mortier  
Study Director

Bruno De Wilde  
Lab Manager
Table 2. Heavy metals and fluorine content (ppm on total solids)

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Limit values</th>
<th>Test procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>USA**</td>
<td>Canada</td>
</tr>
<tr>
<td>Heavy metals*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>&lt; 10</td>
<td>&lt; 1400</td>
</tr>
<tr>
<td>Cu</td>
<td>&lt; 1</td>
<td>&lt; 750</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt; 1</td>
<td>&lt; 210</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 0.1</td>
<td>&lt; 19.5</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 1</td>
<td>&lt; 150</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt; 0.1</td>
<td>&lt; 8.5</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>Mo</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>Se</td>
<td>&lt; 0.75</td>
<td>&lt; 50</td>
</tr>
<tr>
<td>As</td>
<td>&lt; 1</td>
<td>&lt; 20.5</td>
</tr>
<tr>
<td>Co</td>
<td>&lt; 1</td>
<td>-</td>
</tr>
<tr>
<td>Fluorine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>&lt; 10</td>
<td>-</td>
</tr>
</tbody>
</table>

* Microwave digestion was executed on the sample according to DIN EN 13657, before the analysis of the heavy metals
** Maximum levels for USA (according to ASTM D6400 (2012) heavy metals content must be less than 50% of those prescribed for sludges or composts in the country where the product is sold)
Aerobic biodegradation test under controlled composting conditions at elevated temperature on Film MBR15120101 & Film MBR15121703

Report R-DRA-1/5
Aerobic biodegradation under controlled composting conditions of
Film MBR15120101
Film MBR15121703

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1 Identification of the test

1.1 General information

Project number
DRA-1/5

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Test items
Film MBR15120101
Film MBR15121703

Reference item
Cellulose

Test duration
45 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: March 8\textsuperscript{th}, 2016
Starting date experiments: March 8\textsuperscript{th}, 2016
Starting date of incubation: March 10\textsuperscript{th}, 2016
Completion date of incubation: April 24\textsuperscript{th}, 2016
Completion date of experiments: May 11\textsuperscript{th}, 2016
Completion date study: May 26\textsuperscript{th}, 2016
Total test duration: 45 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-1/5

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 Jun-06-2016

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 items Film MBR15120101 and Film MBR15121703 was evaluated in a controlled composting test according to ISO 14855-1 (2012). The incubation temperature was continuously kept at 58°C ± 2°C. The total test duration was 45 days.

According to the norm ISO 14855-1 (2012) a CO₂ production between 50 mg and 150 mg CO₂/g VS should be measured for the controls during the first 10 days of the test. After 10 days a background activity of 63 mg CO₂/g VS was measured, which indicates the good quality of the inoculum.

The biodegradation of reference item cellulose started almost immediately at a good rate. After 15 days cellulose was already degraded by 70.6%. From then on a slower biodegradation rate was maintained and at the end of the test (45 days) a biodegradation percentage of 83.9% ± 1.3% 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 clearly fulfilled.

The biodegradation of test items Film MBR15120101 and Film MBR15121703 proceeded well throughout the test. A high biodegradation rate was observed during the first 10 days, after which biodegradation slowed down and levelled off. At the end of the test (45 days) an absolute biodegradation of 90.9% ± 6.0% and 96.0% ± 4.3% was measured for Film MBR15120101 and Film MBR15121703, respectively. On a relative basis, compared to suitable reference substrate cellulose, a biodegradation percentage of 108.3% and 114.4% was calculated, respectively.

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 international standard ISO 17088 Specifications for compostable plastics (2012) and the American standard ASTM D 6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (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. The maximum allowed test duration by the standards on industrial compostability is 180 days. From these results it can be concluded that test items Film MBR15120101 and Film MBR15121703 fulfilled the biodegradation requirement of EN 13432 (2000) within 45 days of testing. In order to fulfill the requirements of ASTM D6400 (2012) and ISO 17088 (2012) the biodegradability of the 3% components (Bynel 41E710 in Film MBR15120101, S/MA EF80P in Film MBR15121703) should be tested individually.

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 1
Name: Film MBR15120101
Description: Film
Colour: Off-white
Batch number: MBR15120101
Production date: 1 December 2015
Production process: Film blowing
Sample preparation: Cryogenically milled (< 1 mm)

Test item 2
Name: Film MBR15121703
Description: Film
Colour: Off-white
Batch number: MBR15121703
Production date: 17 December 2015
Production process: Film blowing
Sample preparation: Cryogenically milled (< 1 mm)

Reference item
Name: Cellulose
Purity: Native cellulose powder for thin layer chromatography (Avicel)
Physical form: Powder
Colour: White
Batch number: K44173131346
Expiration date: January 2020
Brand: Merck Art. Nr. 2331

7.2 General procedure

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 CO₂ is produced.

The gas leaving each individual reactor is continuously analysed on regular intervals for CO₂ and O₂ concentration. Also the flow rate is measured regularly. Likewise the cumulative CO₂ 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 CO₂. 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
7.3 Analytical methods

Ammonium - nitrogen (NH₄⁺ -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% O₂, 6% CO₂, 79% N₂. Every day gas analyses were executed the gas chromatograph is validated. The results are given in per cent.

Nitrate and nitrite - nitrogen (NOₓ⁻ -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.
Total nitrogen (N)
This analysis is done as described in 'METH L.005. Determination of total nitrogen'. In the presence of a catalysing agent (K₂SO₄-mixture) and under boiling conditions (380°C – 395°C) with a mixture of sulphuric acid-salicylic acid bound nitrogen is converted into the salt (NH₄)₂SO₄. 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 H₂. 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 12 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 items Film MBR15120101 and Film MBR15121703 were previously reduced in size (cryogenically milled until < 1 mm). The total test duration was 45 days.

<table>
<thead>
<tr>
<th>RN</th>
<th>Test series</th>
<th>Inoculum (g)</th>
<th>Item (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1198</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Cellulose</td>
<td>1197</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Film MBR15120101</td>
<td>1199</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Film MBR15121703</td>
<td>1198</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>1199</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Cellulose</td>
<td>1197</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Film MBR15120101</td>
<td>1198</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Film MBR15121703</td>
<td>1198</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>1199</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Cellulose</td>
<td>1198</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Film MBR15120101</td>
<td>1198</td>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>Film MBR15121703</td>
<td>1199</td>
<td>80</td>
<td>0</td>
</tr>
</tbody>
</table>

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 dry 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 fulfilled. The inoculum showed a total solids content of 53.2% and a volatile solids content of 31.0% on TS. Moreover a pH of 7.8 was measured.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS, %)</td>
<td>53.2</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>46.8</td>
</tr>
<tr>
<td>Volatile solids (VS, % on TS)</td>
<td>31.0</td>
</tr>
<tr>
<td>Ash content (% on TS)</td>
<td>69.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.8</td>
</tr>
<tr>
<td>Electrical conductivity (EC, μS/cm)</td>
<td>2920</td>
</tr>
<tr>
<td>Volatile fatty acids (VFA, g/l)</td>
<td>b.r.</td>
</tr>
<tr>
<td>Total N (g/kg TS)</td>
<td>18.6</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/l)</td>
<td>38.2</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/l)</td>
<td>331</td>
</tr>
<tr>
<td>C/N</td>
<td>8</td>
</tr>
</tbody>
</table>

b.r. = below reporting limit:
reporting limit: VFA = 0.3 g/l
According to the norm ISO 14855-1 (2012) a CO₂ production between 50 mg and 150 mg CO₂/g VS should be measured for the controls during the first 10 days of the test. After 10 days a background activity of 63 mg CO₂/g VS was measured, which indicates the good quality of the inoculum.

The reference and test items 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 items

<table>
<thead>
<tr>
<th>Test item</th>
<th>TS (%)</th>
<th>VS (% on TS)</th>
<th>TOC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>97.1</td>
<td>100.0</td>
<td>42.1</td>
</tr>
<tr>
<td>Film MBR15120101</td>
<td>96.5</td>
<td>100.0</td>
<td>55.3</td>
</tr>
<tr>
<td>Film MBR15121703</td>
<td>94.1</td>
<td>100.0</td>
<td>53.6</td>
</tr>
</tbody>
</table>

8.3 CO₂ production

The total cumulative CO₂ production for each reactor at the end of the test (45 days) is given in Table 4. Also the net cumulative CO₂ production of the reference and test items is given in g absolute and in mg per g of test item. Figures 3 up to 6 show the evolution of the total cumulative CO₂ production.

Table 4. CO₂ production at the end of the test (45 days)

<table>
<thead>
<tr>
<th>RN</th>
<th>Test series</th>
<th>Total CO₂ (g)</th>
<th>Net CO₂ (g)</th>
<th>Net CO₂ (mg/g test item)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>34.2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Cellulose</td>
<td>135.5</td>
<td>103.5</td>
<td>1290</td>
</tr>
<tr>
<td>3</td>
<td>Film MBR15120101</td>
<td>168.7</td>
<td>136.6</td>
<td>1706</td>
</tr>
<tr>
<td>4</td>
<td>Film MBR15121703</td>
<td>175.8</td>
<td>143.8</td>
<td>1797</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>32.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cellulose</td>
<td>138.0</td>
<td>105.9</td>
<td>1316</td>
</tr>
<tr>
<td>7</td>
<td>Film MBR15120101</td>
<td>182.5</td>
<td>150.4</td>
<td>1875</td>
</tr>
<tr>
<td>8</td>
<td>Film MBR15121703</td>
<td>189.5</td>
<td>157.4</td>
<td>1965</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>30.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Cellulose</td>
<td>134.4</td>
<td>102.4</td>
<td>1276</td>
</tr>
<tr>
<td>11</td>
<td>Film MBR15120101</td>
<td>188.3</td>
<td>156.3</td>
<td>1944</td>
</tr>
<tr>
<td>12</td>
<td>Film MBR15121703</td>
<td>183.7</td>
<td>151.6</td>
<td>1896</td>
</tr>
</tbody>
</table>
Figure 3. Total CO$_2$ production of the control reactors

Figure 4. Total CO$_2$ production of cellulose reactors
Figure 5. Total CO₂ production of Film MBR15120101 reactors

Figure 6. Total CO₂ production of Film MBR15121703 reactors
8.4 Biodegradation percentages

The results on the calculation of the biodegradation percentages after 45 days (end of test) 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 up to 10 represent the biodegradation of the separate replicates.

Table 5. Biodegradation percentages at the end of the test (45 days)

<table>
<thead>
<tr>
<th>Test series</th>
<th>Average $C_{\text{input}}$ (g)</th>
<th>Average $C_{\text{gaseous}}$ (g)</th>
<th>Biodegradation (%)</th>
<th>95% CL AVG</th>
<th>SD</th>
<th>REL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>33.8</td>
<td>28.3</td>
<td>83.9</td>
<td>1.3</td>
<td>100.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Film MBR15120101</td>
<td>44.3</td>
<td>40.3</td>
<td>90.9</td>
<td>6.0</td>
<td>108.3</td>
<td>10.1</td>
</tr>
<tr>
<td>Film MBR15121703</td>
<td>42.9</td>
<td>41.2</td>
<td>96.0</td>
<td>4.3</td>
<td>114.4</td>
<td>7.3</td>
</tr>
</tbody>
</table>

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

The biodegradation of reference item cellulose started almost immediately at a good rate. After 15 days cellulose was already degraded by 70.6%. From then on a slower biodegradation rate was maintained and at the end of the test (45 days) a biodegradation percentage of 83.9% ± 1.3% 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 clearly fulfilled.

The biodegradation of test items Film MBR15120101 and Film MBR15121703 proceeded well throughout the test. A high biodegradation rate was observed during the first 10 days, after which biodegradation slowed down and levelled off. At the end of the test (45 days) an absolute biodegradation of 90.9% ± 6.0% and 96.0% ± 4.3% was measured for Film MBR15120101 and Film MBR15121703, respectively. On a relative basis, compared to suitable reference substrate cellulose, a biodegradation percentage of 108.3% and 114.4% was calculated, respectively.

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 international standard ISO 17088 Specifications for compostable plastics (2012) and the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (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. The maximum allowed test duration by the standards on industrial compostability is 180 days. From these results it can be concluded that test items Film MBR15120101 and Film MBR15121703 fulfilled the biodegradation requirement of EN 13432 (2000) within 45 days of testing. In order to fulfill the requirements of ASTM D6400 (2012) and ISO 17088 (2012) the biodegradability of the 3% components (Bynel 41E710 in Film MBR15120101, S/MA EF80P in Film MBR15121703) should be tested individually.
Figure 7. Evolution of the biodegradation percentage of reference and test items

Figure 8. Evolution of the biodegradation percentage of the replicates of cellulose
Figure 9. Evolution of the biodegradation percentage of the replicates of Film MBR15120101

Figure 10. Evolution of the biodegradation percentage of the replicates of Film MBR15121703
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. Fungal growth was observed in the cellulose reactors during the first three weeks of testing. After 40 days reference item cellulose was no longer visible. The test reactors also showed good structure and moisture conditions. Fungal growth was observed from the second until the fifth week. After 33 days test items Film MBR15120101 and Film MBR15121703 could no longer be discerned from the compost inoculum.

At the end of the test the different test series were analyzed for total solids (TS), volatile solids (VS) and pH (see Table 6). After 45 days a comparable volatile solids content was measured for the test item reactors compared to the control and cellulose reactors, confirming the complete biodegradation of test items Film MBR15120101 and Film MBR15121703.

<table>
<thead>
<tr>
<th>Test series</th>
<th>TS (%)</th>
<th>VS (% on TS)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>54.5</td>
<td>30.5</td>
<td>7.6</td>
</tr>
<tr>
<td>Cellulose</td>
<td>54.2</td>
<td>29.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Film MBR15120101</td>
<td>53.9</td>
<td>29.0</td>
<td>7.9</td>
</tr>
<tr>
<td>Film MBR15121703</td>
<td>52.4</td>
<td>29.1</td>
<td>7.9</td>
</tr>
</tbody>
</table>
Pilot-scale composting test: quantitative disintegration and compost production of GS 270

Report R-DRA-1/2
FINAL REPORT
DRA-1/2

Pilot-scale composting + sieving test for measurement of disintegration on GS 270 (Thickness: 3.59 mm)

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Sponsor: BiologIQ, Inc.
2400 East 25th St
Idaho Falls
ID 83404
UNITED STATES
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1 Identification of the test

1.1 General information

Project number
DRA-1/2

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Test item
GS 270:
- Plates (thickness: 3.59 mm): for quantitative evaluation of disintegration
- Pellets: for production of compost for subsequent ecotoxicity testing
1.2 Study personnel

Study Director: Jan-Willem Decraene
Replacement Study Director: Nike Mortier
Study Director QA: Steven Verstichel

1.3 Study schedule

Study initiation date: February 11\textsuperscript{th}, 2016
Experimental starting date: February 11\textsuperscript{th}, 2016
Starting date of incubation: February 18\textsuperscript{th}, 2016
Completion date of incubation: May 12\textsuperscript{th}, 2016
Experimental completion date: June 20\textsuperscript{th}, 2016
Study completion date: June 20\textsuperscript{th}, 2016

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-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).

_______________________
Jan-Willem Decraene
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 Jun-17-2016

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

Plastic plate sample GS 270 in a thickness of 3.59 mm was evaluated for disintegration in a pilot-scale aerobic composting test simulating industrial composting processes according to ISO 16929 (2013). At start-up 10% test material was added to biowaste: 1% GS 270 film (3.59 mm), added as such (= 7.5 x 7.5 cm pieces), and 9% milled GS 270 pellets (< 2 mm). The 1% test item concentration was used for the determination and evaluation of the disintegration of GS 270 (3.59 mm). The extra 9% milled test material was necessary for subsequent ecotoxicity testing. The control vessels consisted of pure biowaste. The test was performed in duplicate and lasted 12 weeks. At the end of the composting test, the compost was sieved and disintegration was evaluated.

The composting test was done under optimum composting conditions. The operational parameters showed that the test was valid. In every bin, the temperature remained above 60°C during at least 1 week and above 40°C during the entire test period. Furthermore the temperature did not exceed 75°C during the test, except shortly after 5 and 12 days of composting for control bin DRA-1/2-02 and test bin DRA-1/2-04, respectively with a value of 75.9°C and 76.2°C. Each time the limit was exceeded, action was undertaken to establish lower temperatures. At start-up a rather low pH of 4.8 was measured. However, after 1.6 weeks the pH was increased till at least 5.7 and reached values above 8.5 after 3.6 weeks. The pH remained above 8.0 during the further test period. The oxygen concentration stayed above 10%. As such, good aerobic conditions were guaranteed during the test.

The disintegration of the 7.5 x 7.5 cm GS 270 (3.59 mm) plates proceeded very swiftly. Already after 4 days of composting the major part of the plates had fallen apart into pieces of variable size. Three days later all plates had fallen apart into pieces with an average size of approximately 2 x 2 cm. The disintegration went on and after 2 weeks of composting only a few small pieces of GS 270 could be retrieved. By far the major part had already disappeared. One week later all test material seemed completely disintegrated. This was confirmed at the end of the test (= after 12 weeks). Not a single piece of GS 270 (3.59 mm) could be retrieved from the test composts.

At the end of the composting test the whole content of the bins was used for sieving, sorting, further isolation and analyses. The content of the test bins was sieved over 10 mm, 5 mm and 2 mm, after which a homogeneous sample of all compost fractions > 2 mm was manually selected and a mass balance was performed. Disintegration is defined as a size reduction to < 2 mm. According to the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012) less than 10% of the material may remain present in the > 2 mm fraction after 12 weeks of composting. As can be seen from Table 1, 100% disintegration was obtained for GS 270 in a thickness of 3.59 mm. Consequently, it can be concluded that the 90% pass level as required by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) was easily reached.

<table>
<thead>
<tr>
<th>Table 1. Disintegration of GS 270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test item</td>
</tr>
<tr>
<td>GS 270</td>
</tr>
</tbody>
</table>
The quality of the composts to which 10% GS 270 was added at start of the composting cycle was equally good compared to the control composts. The volatile fatty acid concentration remained below 500 mg/kg in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. A normal average pH of 8.6 and 8.7 was measured for the control composts and test composts, respectively. A somewhat lower average salt level was found in the test composts (2060 µS/cm) when compared to the control composts (2270 µS/cm). A low salt content is beneficial for the compost quality. At the end of the test low NH₄⁺-N levels were obtained and an increase in the NO₃⁻-N content was observed for all series. After 12 weeks an average NO₃⁻-N content of 227 mg NO₃⁻-N/l and 217 mg NO₃⁻-N/l was found for the control composts and the test composts, respectively. This indicates that the nitrification process had started and was proceeding well. A similar nutrients content (P, K and Mg) content was obtained for the control and test composts, while a higher N content was observed for the test composts.

A lower average density was found for the test composts (0.454 kg/l) when compared to the control composts (0.520 kg/l). The C/N ratio varied between 8 and 10. A high average volatile solids degradation was observed for all test series. This indicates that the composting process has proceeded well for all composts. Moreover, a higher average volatile solids decrease was measured for the test composts when compared to the control composts, indicating that the test material was degrading.

In conclusion it can be stated that GS 270 (3.59 mm) does easily fulfill the 90% disintegration requirement stipulated by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012). Even a higher thickness has the potential to pass the 90% disintegration requirement. Moreover, no negative effect on the composting process and on the (physicochemical) quality of the produced compost was observed, when adding 10% GS 270 at start of the composting.
6 Introduction

6.1 Purpose and principle of test method

The composting bin test simulates as closely as possible a real and complete composting process in pilot-scale composting bins of 200 l. The test item is mixed with the organic fraction of fresh, pre-treated municipal solid waste (biowaste) and introduced in an insulated composting bin after which composting spontaneously starts. Like in full-scale composting, inoculation and temperature increase happen spontaneously. The composting process is directed through aeration and moisture content. The temperature and exhaust gas composition are regularly monitored. The composting process is continued till fully stabilized compost is obtained (3 months).

At the end of the composting process, the compost is sieved by means of a vibrating sieve over 2, 5 and 10 mm. Disintegration is evaluated very precisely by manual selection. If possible a mass balance is calculated on the basis of wet and dry weight. The compost obtained at the end of the composting process can be used for further measurements such as chemical and physical analyses and ecotoxicity tests.

The test is considered valid only if:

- The maximum temperature during composting is above 60°C and remains below 75°C;
- The daily temperature remains above 60°C during at least 1 week and above 40°C during at least 4 weeks;
- The pH increases to above 7.0 during the test and does not fall below 5.0;
- After 12 weeks the blank compost has Rottegrad IV - V and a volatile fatty acids content of less than 500 mg/kg.

More details about the test procedure are given in the study plan.

6.2 Standard followed

7 Materials and methods

7.1 Test items

For measurement of disintegration:

Test item 1

Name: GS 270 (Figure 1)
Description: Compression molded plastic plate
Colour: Brown
Batch number: Batch 317
Production date: 20-27 January 2016
Thickness: 3.59 mm ± 0.07 mm
Total solids (TS): 95.1%
Volatile solids (VS): 99.9% on TS
Sample preparation: Added as such: 7.5 × 7.5 cm

For compost production for subsequent ecotoxicity testing:

Test item 2

Name: GS 270 (Figure 1)
Description: Plastic resin pellet
Colour: Light brown
Batch number: Batch 317
Production date: 5 November 2015
Total solids (TS): 95.0%
Volatile solids (VS): 99.9% on TS
Sample preparation: Cryogenically milled (< 2 mm)

Figure 1. Visual presentation of GS 270 (left: plate, right: pellets)
7.2 General procedure

The fresh biowaste is derived from the organic fraction of municipal solid waste after a source-separated collection. The test item is mixed with the biowaste, which is used as carrier matrix, and composted in a pilot-scale composting unit (Figure 2). At the end of the composting test the compost is sieved and disintegration is evaluated. More details on the procedure for the particular test reported are given in the study plan.

![Figure 2. Set-up pilot-scale aerobic composting test](image)

7.3 Analytical methods

Ammonium - nitrogen (NH₄⁺-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 10% O₂, 20% CO₂, 30% N₂ and 40% CH₄. Every day gas analyses were executed the gas chromatograph is validated. The results are given in per cent.

Nitrate and nitrite - nitrogen (NOₓ-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’.

Rottegrad
The ‘Rottegrad’ or maturity of the compost is determined by measuring the self-heating capacity of the compost. A precise volume of compost is placed in a ‘Dewar’ vessel after which the temperature is left to increase spontaneously. The maximum temperature reached is a measure of the stability. More details on the test procedure are given in the ‘METH L.001. Determination of rotting degree – Self-heating test in a Dewar vessel’.

Salt content (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.

Thickness
After an acclimatization period of 24 hours at 23°C and 50% relative humidity, 10 points are measured on the test item. The measurement is executed on a universal bench micrometer (accuracy of 0.1 μm) according to ISO 4593 Plastics – Film and sheeting – Determination of thickness by mechanical scanning (2009). An external laboratory executes the analysis.

Volumetric density
The volumetric density is determined by filling a 1 l cylinder and measuring the weight after compression with a 650 g plunger for 180 s. This is repeated three times. The exact procedure is described in ‘METH L.011. Determination of volumetric density’.
Total magnesium (Mg)
This analysis is done as described in ‘METH L.020. Determination of total and extractable K, Mg and Ca’. The total Mg content of the compost is determined by Atomic Absorption Spectrophotometry (AAS) after extraction with aqua regia. The total Mg content is expressed as MgO in % on wet weight basis. The results are given in g per kg total solids.

Total nitrogen (N)
This analysis is done as described in ‘METH L.005. Determination of total nitrogen’. In the presence of a catalysing agent (K₂SO₄-mixture) and under boiling conditions (380°C – 395°C) with a mixture of sulphuric acid-salicylic acid bound nitrogen is converted into the salt (NH₄)₂SO₄. 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 phosphorus (P)
This analysis is done as described in ‘METH L.014. Determination of phosphorus’. The total P content of the compost is determined by spectrophotometry after extraction with aqua regia. The results are given in g per kg total solids.

Total potassium (K)
This analysis is done as described in ‘METH L.020. Determination of total and extractable K, Mg and Ca’. The total K content of the compost is determined by Atomic Emission Spectroscopy (AES) after extraction with aqua regia. The total K content is expressed as K₂O in % on wet weight basis. The results are given in g per kg total solids.

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 H₂. 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.

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 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 Thickness of test item

The result of the thickness measurements on test item GS 270 is given in Table 2. The measured thickness of the test item is taken into account for the disintegration result obtained in this study.

Table 2. Thickness of test item

<table>
<thead>
<tr>
<th>Test item</th>
<th>Measured thickness (mm) (AVG ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GS 270</td>
<td>3.59 ± 0.07</td>
</tr>
</tbody>
</table>

With AVG = average and SD = standard deviation.

8.2 Test conditions and set-up

Four composting bins with a total volume of 200 l each were started. The control bins (DRA-1/2-01 and DRA-1/2-02) contained only biowaste, while the test bins (DRA-1/2-03 and DRA-1/2-04) contained also test material: 1% GS 270 (3.59 mm), added as such (= 7.5 × 7.5 cm pieces), and 9% milled GS 270 pellets (< 2 mm). The 1% test item concentration was used for the determination and evaluation of the disintegration of the test item. The extra 9% milled test material was necessary for subsequent ecotoxicity testing. Moreover, the film sample was placed in slide frames in order to evaluate the disintegration visually. The exact test set-up is given in Table 3. The biowaste consisted of VGF (Vegetable, Garden and Fruit waste) to which 11% extra structural material was added in order to obtain optimal composting conditions. At start-up, all vessels were filled to the top of the bin.

Table 3. Test set-up

<table>
<thead>
<tr>
<th>Composition</th>
<th>Control bins</th>
<th>Test bins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DRA-1/2-01</td>
<td>DRA-1/2-02</td>
</tr>
<tr>
<td>VGF (kg)</td>
<td>55.2</td>
<td>55.2</td>
</tr>
<tr>
<td>Structural material (kg)</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>GS 270, added as such (kg)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GS 270, milled (kg)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% GS 270, added as such, on biowaste</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>% GS 270, milled, on biowaste</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
8.3 Analyses of biowaste

The fresh biowaste was derived from the separately collected organic fraction of municipal solid waste, which was obtained from the biowaste composting plant of Schendelbeke, Belgium. The characteristics of VGF and structural material are given in Table 4. Table 5 shows the characteristics of the mixtures in the composting bins.

The biowaste at start (= VGF + structural material) should have a moisture content and a volatile solids content on total solids (TS) of more than 50% and a pH above 5. From Tables 4 and 5 it can be seen that these requirements were largely fulfilled. The biowaste contained a moisture content of 67.3% and a volatile solids content of 72.0% on TS. At start-up a rather low pH of 4.8 was measured. However, after 1.6 weeks the pH was increased till at least 5.7 and reached values above 8.5 after 3.6 weeks of composting. Furthermore the C/N ratio of the biowaste at start should preferably be between 20 and 30. An optimal C/N ratio of 26 was obtained for the biowaste. The test bins with 10% test material showed a higher C/N ratio of 37 due to the addition of 10% test material with a high carbon and a low nitrogen content. The high addition of test material is required by ISO 16929 (2013), ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012).

Table 4. Characteristics of VGF and structural material

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>VGF</th>
<th>Structural material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS, %)</td>
<td>31.0</td>
<td>48.8</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>69.0</td>
<td>51.2</td>
</tr>
<tr>
<td>Volatile solids (VS, % on TS)</td>
<td>68.1</td>
<td>95.0</td>
</tr>
<tr>
<td>Ash content (% on TS)</td>
<td>31.9</td>
<td>5.0</td>
</tr>
<tr>
<td>pH</td>
<td>4.8</td>
<td>-</td>
</tr>
<tr>
<td>Electrical conductivity (EC, μS/cm)</td>
<td>2370</td>
<td>-</td>
</tr>
<tr>
<td>Volatile fatty acids (VFA, g/l)</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/l)</td>
<td>13</td>
<td>-</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/l)</td>
<td>240</td>
<td>-</td>
</tr>
<tr>
<td>Total N (g/kg TS)</td>
<td>14.5</td>
<td>8.9</td>
</tr>
<tr>
<td>C/N</td>
<td>24</td>
<td>53</td>
</tr>
</tbody>
</table>

Table 5. Characteristics of the biowaste and biowaste with test item

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Biowaste (= VGF + structural material)</th>
<th>Biowaste + 10% test material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total solids (TS, %)</td>
<td>32.7</td>
<td>38.4</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>67.3</td>
<td>61.6</td>
</tr>
<tr>
<td>Volatile solids (VS, % on TS)</td>
<td>72.0</td>
<td>78.3</td>
</tr>
<tr>
<td>Ash content (% on TS)</td>
<td>28.0</td>
<td>21.7</td>
</tr>
<tr>
<td>Total N (g/kg TS)</td>
<td>13.7</td>
<td>10.7</td>
</tr>
<tr>
<td>C/N</td>
<td>26</td>
<td>37</td>
</tr>
</tbody>
</table>
8.4 Temperature profile and analyses exhaust air

Figure 3 shows the temperature evolution during the composting test. According to ISO 16929 (2013) the test is considered valid if in the composting bins the maximum temperature during composting is above 60°C and remains below 75°C during the first week and below 65°C thereafter in order to ensure that the microbial diversity is not reduced. Furthermore, the daily temperature should remain above 60°C during at least 1 week and above 40°C during at least 4 consecutive weeks.

These requirements were largely fulfilled. After start-up the temperature increased almost immediately till above 60°C in both control bins DRA-1/2-01 and DRA-1/2-02. The temperature increase in test bins DRA-1/2-03 and DRA-1/2-04 was somewhat delayed. Two days after start-up both test bins were placed in an incubation room at 45°C to ensure high temperatures. As a result, a temperature increase till above 60°C was obtained in both test bins after 5 days of composting. Control bins DRA-1/2-01 and DRA-1/2-02 were also placed in an incubation room at 45°C after 8 days of composting. The temperature in all bins did not exceed the 75°C limit, except shortly after 5 and 12 days of composting for control bin DRA-1/2-02 and test bin DRA-1/2-04, respectively with a value of 75.9°C and 76.2°C. Each time the limit was exceeded, action was undertaken to establish lower temperatures: both test bins were placed for 24 hours at ambient temperature after 12 days of composting. It was noticed that the temperature exceeded the 65°C limit in the test bins between 1.1 and 3.7 weeks of composting, while only once in control bin DRA-1/2-01 after 1.3 weeks of composting. The elevated temperatures were mainly caused by a high microbial activity due to the degradation of the test material that was added in a high load of 10%. Taken into account that the composting process proceeded well during the complete testing period, sufficient microbial diversity was guaranteed in spite of the fact that the temperature exceeded 65°C. After 2.7 weeks of composting the content of control bins DRA-1/2-01 and DRA-1/2-02 was combined into one bin, separated by a net. This was done in order to compensate for the volume reduction, which naturally occurs during the composting, and to maintain optimal composting conditions. The same was done for test bins DRA-1/2-03 and DRA-1/2-04. Elevated temperatures during the composting process were also caused by the turning of the content of the bins, during which air channels and fungal flocks were broken up and moisture, microbiota and substrate were divided evenly. As such optimal composting conditions were re-established, resulting in a higher activity and a temperature increase. The temperature remained above 40°C during the entire test period. It was noticed that the temperature in the test bins with 10% test material was higher when compared to the control bins in the period between approximately 1 and 12 weeks of composting. This indicates that the test material in a high load of 10% was degrading.

Figure 4 shows the CO₂ production rate during the composting test (individual measurements at regular points in time), which is representative for biological activity. After start-up a high activity was measured for the control and test bins, after which the CO₂ production gradually decreased. An additional peak in the CO₂ production rate was observed after 2.7 weeks of composting in all bins. This corresponds with the somewhat higher temperature and is due to the combination of the content of the bins, resulting in a higher activity. At the end of the test a low activity was found for all test series, indicating that the composting process was completed. It was noticed that the CO₂ production rate in the test bins was generally higher when compared to the control bins in the period between approximately 1 and 8 weeks of composting, corresponding with the higher temperatures. This also indicates that the test material was degrading.

The oxygen concentration of the exhaust air is given in Figure 5. The oxygen concentration remained always above 10%. As such, good aerobic conditions were guaranteed during the test.
Figure 3. Temperature evolution during the composting test

Figure 4. CO₂ production rate during the composting test (individual measurements at regular points in time)
Figure 5. $O_2$ concentration in the exhaust air during the composting test
8.5 Evolution of pH, NH$_4^+$-N and NO$_x^-$-N

Figure 6 shows the evolution of the pH during the composting cycle, while Figures 7 and 8 give the trend in NH$_4^+$-N, respectively NO$_x^-$-N for the different bins.

According to the international standard ISO 16929 (2013) the pH should increase till a value above 7 during composting and not fall below 5. The biowaste at start showed a rather low pH of 4.8. However, after 1.6 weeks of composting the pH was already increased till above 8.6 and 5.7 for the control and the test replicates, respectively. The pH of the test bins further increased and after 3.6 weeks the pH was increased till above 8.5 for all test series. The pH remained above 8.0 during the further test period for all replicates. At the end of the test (after 12 weeks) an average pH of 8.6 and 8.7 was measured for the control composts and the test composts, respectively.

The biowaste at start contained an ammonium content of 240 mg NH$_4^+$-N/l. After an initial increase of the ammonium content in the control replicates, the NH$_4^+$-N levels decreased in all replicates. After 7.7 weeks of composting low ammonium levels (< 20 mg NH$_4^+$-N/l) were obtained in all replicates. These low ammonium levels were maintained till the end of the test.

Between 5.9 and 7.7 weeks of composting an increase of the NO$_x^-$-N concentration was observed for all replicates. The NO$_x^-$-N content further increased and at the end of the test an average NO$_x^-$-N content of 227 mg NO$_x^-$-N/l (control composts) and 217 mg NO$_x^-$-N/l (test composts) was found.

At the end of the test low NH$_4^+$-N levels were obtained for all replicates, while the average NO$_x^-$-N content had increased. This indicates that the nitrification process had started and was proceeding well.
Figure 7. Trend of NH$_4^+$-N during composting cycle

Figure 8. Trend of NO$_x$-N during composting cycle
8.6 Visual perceptions

The mixtures in the composting bins were regularly turned by hand, during which the disintegration of the test item was carefully examined. The disintegration of GS 270 (3.59 mm), added as such (= 7.5 × 7.5 cm plates), proceeded very swiftly. Figure 9 shows a visual presentation of the content of a test bin with 10% GS 270 (1% added as 7.5 × 7.5 cm pieces and 9% milled) after 4 days of composting. The major part of the plates had fallen apart into pieces of variable size. It was noticed that the test material had already become very weak. Moreover, the colour of the plates had become light brown opaque. Three days later all plates had fallen apart into pieces with an average size of approximately 2 × 2 cm (Figure 10). The disintegration went on and after 2 weeks of composting only a few small pieces of GS 270 could be retrieved (Figure 11). By far the major part had already disappeared. One week later all test material seemed completely disintegrated. This was confirmed at the end of the test (= after 12 weeks). Not a single piece of GS 270 (3.59 mm) could be retrieved from the test composts. Figure 12 gives a visual comparison of the < 10 mm compost fraction of control and GS 270 compost at the end of the test. No visual distinction was observed between control and test composts.

Figure 9. Visual presentation of the content of a test bin with 1% GS 270 (3.59 mm), added as 7.5 × 7.5 cm pieces, and 9% milled GS 270 after 4 days of composting
Figure 10. Visual comparison between test item GS 270 (3.59 mm), added as 7.5 × 7.5 cm pieces, at start and after 1 week of composting

Figure 11. Visual comparison between test item GS 270 (3.59 mm), added as 7.5 × 7.5 cm pieces, at start and after 2 weeks of composting
8.7 Sieving - disintegration

At the end of the composting test (after 12 weeks), the whole content of the test bins was used for sieving, sorting, further isolation and analyses. Disintegration is defined as a size reduction to < 2 mm. After carefully selecting all fractions (2 - 5 mm, 5 - 10 mm, > 10 mm) not a single piece of GS 270 could be retrieved. 100% complete disintegration was established for test material GS 270 in a thickness of 3.59 mm. According to the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012) less than 10% of the material added at start may remain present in the > 2 mm fraction after 12 weeks of composting. From the results it can be concluded that test material GS 270 in a thickness of 3.59 mm easily fulfills the disintegration criterion in a pilot-scale composting test as prescribed by ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012). Even a higher thickness has the potential to pass the 90% disintegration requirement.
8.8 Chemical analyses

At the end of the composting test, the whole content of the bins was sieved over a mesh size of 10 mm. The > 10 mm fraction was analysed for total solids and volatile solids content. The overall compost quality is determined by the analyses performed on the < 10 mm fraction. The results of all these analyses are given in Table 6.

To ensure a completion of the normal composting process, the blank biowaste control must have a Rottegrad of IV or V and volatile fatty acids content lower than 500 mg/kg at the end of the test. From Table 6 it can be seen that these requirements were fulfilled for control and test composts.

The quality of the composts to which 10% GS 270 was added at start of the composting cycle was equally good compared to the control composts. The volatile fatty acid concentration remained below 500 mg/kg in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. A normal average pH of 8.6 and 8.7 was measured for the control composts and test composts, respectively. A somewhat lower average salt level was found in the test composts (2060 μS/cm) when compared to the control composts (2270 μS/cm). A low salt content is beneficial for the compost quality. At the end of the test low NH₄⁺-N levels were obtained and an increase in the NOₓ⁻-N content was observed for all series. After 12 weeks an average NOₓ⁻-N content of 227 mg NOₓ⁻-N/l and 217 mg NOₓ⁻-N/l was found for the control composts and the test composts, respectively. This indicates that the nitrification process had started and was proceeding well. A similar nutrients content (P, K and Mg) content was obtained for the control and test composts, while a higher N content was observed for the test composts. A lower average density was found for the test composts (0.454 kg/l) when compared to the control composts (0.520 kg/l). The C/N ratio varied between 8 and 10.

A somewhat higher volatile solids content was measured for the < 10 mm fraction of the test composts with 10% test material when compared to the control composts. However, a higher average volatile solids decrease was measured for the test composts when compared to the control composts, indicating that the test material was degrading (Table 7). Moreover, a high average volatile solids decrease was measured for all series indicating that the composting process has proceeded well.
Table 6. Chemical analysis of the compost fractions after 12 weeks of composting

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control composts</th>
<th>10% GS 270 composts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DRA-1/2 01</td>
<td>DRA-1/2 02</td>
</tr>
<tr>
<td>&gt; 10 mm fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total solids (TS, %)</td>
<td>47.3</td>
<td>49.5</td>
</tr>
<tr>
<td>Volatile solids (VS, % on TS)</td>
<td>58.8</td>
<td>55.7</td>
</tr>
<tr>
<td>Ash (% on TS)</td>
<td>41.2</td>
<td>44.3</td>
</tr>
<tr>
<td>&lt; 10 mm fraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Solids (TS, %)</td>
<td>50.2</td>
<td>49.1</td>
</tr>
<tr>
<td>Volatile Solids (VS, % on TS)</td>
<td>47.2</td>
<td>48.8</td>
</tr>
<tr>
<td>Ash (% on TS)</td>
<td>52.8</td>
<td>51.2</td>
</tr>
<tr>
<td>pH</td>
<td>8.5</td>
<td>8.7</td>
</tr>
<tr>
<td>Volatile fatty acids (VFA, g/l)</td>
<td>b.r.</td>
<td>b.r.</td>
</tr>
<tr>
<td>Total N (g/kg TS)</td>
<td>24.7</td>
<td>23.8</td>
</tr>
<tr>
<td>Total P (g/kg TS)</td>
<td>4.5</td>
<td>4.4</td>
</tr>
<tr>
<td>Total K (g/kg TS)</td>
<td>15.2</td>
<td>16.2</td>
</tr>
<tr>
<td>Total Mg (g/kg TS)</td>
<td>4.9</td>
<td>5.0</td>
</tr>
<tr>
<td>NH$_4$+-N (mg/l)</td>
<td>b.r.</td>
<td>b.r.</td>
</tr>
<tr>
<td>NO$_x$-N (mg/l)</td>
<td>239</td>
<td>216</td>
</tr>
<tr>
<td>Electrical Conductivity (μS/cm)</td>
<td>2120</td>
<td>2410</td>
</tr>
<tr>
<td>Rottegrad</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Density (kg/l)</td>
<td>0.508</td>
<td>0.533</td>
</tr>
<tr>
<td>C/N</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

* b.r. = below reporting limit

Reporting limit: VFA = 0.3 g/l
NH$_4$+-N = 10.0 mg/l

Table 7. Volatile solids degradation for the different test series

<table>
<thead>
<tr>
<th>Test series</th>
<th>Volatile solids degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Control composts</td>
<td>69.1</td>
</tr>
<tr>
<td>DRA-1/2-01</td>
<td>67.8</td>
</tr>
<tr>
<td>DRA-1/2-02</td>
<td>70.4</td>
</tr>
<tr>
<td>10% GS 270 composts</td>
<td>75.8</td>
</tr>
<tr>
<td>DRA-1/2-03</td>
<td>75.3</td>
</tr>
<tr>
<td>DRA-1/2-04</td>
<td>76.3</td>
</tr>
</tbody>
</table>
Ecotoxicity tests

Barley plant growth test on compost residuals of GS 270

Report R-DRA-1/3
Ecotoxicity test – Barley plant growth test on compost residuals of GS 270

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8.1 Test conditions and set-up ................................................................. 10  
8.2 Results and discussion ................................................................. 11
1 Identification of the test

1.1 General information

Project number
DRA-1/3

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Test item
GS 270 compost

Reference item
Blank compost without any addition
1.2 Study personnel

Study Director: Nike Mortier
Replacement Study Director: Michela Siotto
Study Director QA: Steven Verstichel

1.3 Study schedule

Study initiation date: May 20th, 2016
Study completion date: June 9th, 2016
Experimental starting date: May 23rd, 2016
Starting date of incubation: May 24th, 2016
Completion date of incubation: June 3rd, 2016
Duration of incubation: 10 days
Experimental completion date: June 6th, 2016

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-1/3

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).

___________________
Nike Mortier
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 Jun-17-2016

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

A barley plant growth test, which is representative for monocotyledonous plants, was performed on the test compost, obtained at the end of a pilot-scale composting test in which test item GS 270 was added in a 10% concentration to biowaste at start of the composting test. The pilot-scale composting test is reported in report R-DRA-1/2.

The blank compost and the test compost were both tested in 2 mixing ratios of compost and reference substrate: (1) 75% reference substrate & 25% compost and (2) 50% reference substrate & 50% compost on weight basis.

The test is executed according to the following standards: the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012). The test was stopped after 10 days, which is within the prescribed time interval of ‘Methodenbuch 1998, Kapitel II: 5. Pflanzen-verträglichkeit – Bundesgütegemeinschaft Kompost e.V.’.

According to ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) the germination rate and the plant biomass of the test compost should be more than 90% of those from the corresponding blank compost. These criteria were easily reached for both the germination rate and the plant biomass of both mixtures of the test compost. Therefore it can be concluded that the requirements of ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) on ecotoxicity are fulfilled for barley plants.

In conclusion, it can be stated that, after composting GS 270 in a 10% concentration, no residuals were left such as metabolites, undegraded components and inorganic components that exert a negative influence on the germination and growth of barley plants.
6 Introduction

6.1 Purpose and principle of test method

The barley plant growth test is applied after a preceding composting test. The compost produced at the end of the composting test may contain residuals of the original test item such as metabolites, undegraded components and inorganic components. The purpose of the barley plant growth test is to evaluate any toxic effect of the test compost containing the test item residuals in comparison to blank compost to which no reference or test item was added at the start of the preceding composting test. The barley plant is chosen as representative of monocotyledonous plants.

The test includes germination and growth of barley in mixtures of reference substrate and compost. At the end of the test the fresh and dry weight of the plants is determined for each test series and compared. Also the germination rate is measured.

6.2 Standards followed

The test is executed in line with the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012). The time duration was based on ‘Methodenbuch 199, Kapitel II: 5. Pflanzen-verträglichkeit – Bundesgütegemein-schaft Kompost e.V.’.
7 Materials and methods

7.1 Test item

The test items are compost samples obtained at the end of the pilot-scale composting test DRA-1/2.

Blank compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from the bins with no test item, namely bins DRA-1/2-01 and DRA-1/2-02.

Test compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from bins DRA-1/2-03 and DRA-1/2-04 to which 10% GS 270 (1% 7.5 x 7.5 cm pieces + 9% milled (< 2 mm)) was added at start of the composting.

7.2 General procedure

The barley plant growth test is performed in flower pots of 500 ml, containing a mixture of compost and reference substrate. Each compost is tested in 2 mixing ratios of compost and reference substrate: (1) 75% reference substrate & 25% compost and (2) 50% reference substrate & 50% compost on weight basis. Each mixture is tested in 3 replicates.

At the start of the test, each flower pot is filled with at least 200 g of compost/reference substrate mixture and 100 ml demineralized water is added. Subsequently, 50 barley seeds are put on top of the mixture and covered with a thin layer of siliceous sand. Finally, an extra amount of demineralized water can be added to assure optimal moisture content.

After the flower pots have been completely prepared, they are covered with a glass plate and incubated at a constant temperature of 20°C ± 2°C in the dark.

After germination, the plate is removed and the pots are exposed to a light intensity of at least 3000 lux during at least 12 hours per day. During the test, extra water is added if needed, and visual perceptions are noted. In order to avoid side effects, the position of each pot is changed during the testing period, according to a logical rotation scheme.

The test is finished after 11 (± 1) days. At the end of the test the total fresh and dry weight of the above-soil plant material is determined for each flower pot separately. Also the germination rate is measured.

The toxicity of possible residuals of the test item is evaluated by comparing the results on germination and plant yield of test compost to blank compost. More details on the procedure for the particular test reported, are given in the Study Plan.
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.

Germinative capacity
5 ml of demineralised water is added to a petri dish with filter paper on top of a cotton layer. Twenty barley seeds are put on top of the filter paper. A second filter paper is put on top of the seeds. The petri dish is sealed with parafilm and left in the dark at room temperature. After 5 days the number of germinated seeds is counted. The germination is given in % on the amount of seeds at start. The germinative capacity is tested in 5 replicates.

Weight determination
During the test, several balances are used, with an accuracy of 0.1 mg for the determination of dry matter and weighing of the plants, and an accuracy of 0.01 g for weighing the compost and reference substrate.
8 Results

8.1 Test conditions and set-up

The composts, obtained at the end of the pilot-scale composting DRA-1/2, were thoroughly mixed prior to use.

In total 12 flower pots were used. The mixtures of reference substrate and compost are given on weight basis. Table 1 describes the test set-up.

Table 1. Test set-up barley plant growth test

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ref. Sub. (g/pot)</td>
<td>Compost (g/pot)</td>
</tr>
<tr>
<td>3 x Blank compost (25%)</td>
<td>172.5</td>
<td>57.5</td>
</tr>
<tr>
<td>3 x Blank compost (50%)</td>
<td>115.0</td>
<td>115.0</td>
</tr>
<tr>
<td>3 x Test compost (25%)</td>
<td>172.5</td>
<td>57.5</td>
</tr>
<tr>
<td>3 x Test compost (50%)</td>
<td>115.0</td>
<td>115.0</td>
</tr>
</tbody>
</table>

The used reference substrate is ‘Einheitserde O’ (EEO), which is produced by Einheitserdewerk Hameln A. Stangenberg GmbH, Kiebitzweg 3, 31789 Hameln in Germany.

The seeds are barley seeds 'Barke non treated' and are derived from AVEVE, Tiensestraat 300, 3400 Landen, Belgium. The seeds were examined for their germinative capacity. The germinative capacity was 97%, which is above the recommended value of 90%.
8.2 Results and discussion

The test was stopped after 10 days, which was within the prescribed time duration. Table 2 represents the average germination rate of the different test series as a percentage of the total amount of seeds added at start. The relative germination rate is also shown in Figure 1. Table 3 shows the average fresh and dry weight yield (of above-soil plant parts) for each test series, as well as the standard deviation. The results are shown in Figure 2 and Figure 3.

Table 2. Germination rate of barley (%)

<table>
<thead>
<tr>
<th>Test series</th>
<th>Germination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
</tr>
<tr>
<td>Blank compost 25%</td>
<td>98.7</td>
</tr>
<tr>
<td>Blank compost 50%</td>
<td>94.7</td>
</tr>
<tr>
<td>Test compost 25%</td>
<td>97.3</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>98.0</td>
</tr>
</tbody>
</table>

With AVG = average, SD = standard deviation.

Table 3. Absolute fresh and dry weight yield of barley plants

<table>
<thead>
<tr>
<th>Test series</th>
<th>Fresh Weight Yield (g)</th>
<th>Dry Weight Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
<td>SD</td>
</tr>
<tr>
<td>Blank compost 25%</td>
<td>12.74</td>
<td>0.29</td>
</tr>
<tr>
<td>Blank compost 50%</td>
<td>12.33</td>
<td>0.46</td>
</tr>
<tr>
<td>Test compost 25%</td>
<td>12.82</td>
<td>0.36</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>13.21</td>
<td>0.37</td>
</tr>
</tbody>
</table>

With AVG = average, SD = standard deviation.
Figure 1. Average germination rate
(as percentage to the total amount of seeds added at start)

Figure 2. Absolute plant fresh weight (g/pot)
According to ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and ISO 17088 Specifications for compostable plastics (2012) the germination rate and the plant biomass (on fresh weight basis or on dry weight basis) in the test compost should be more than 90% of those in the corresponding blank compost.

These criteria were easily reached for both the germination rate and the plant biomass of both mixtures of the test compost (see Table 4). Therefore, it can be stated that the requirements of ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) on ecotoxicity are fulfilled for barley plants.

Table 4. Germination and plant yield of the test compost as a percentage of the corresponding mixture of blank compost

<table>
<thead>
<tr>
<th>Test series</th>
<th>Germination</th>
<th>Fresh weight plant yield</th>
<th>Dry weight plant yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test compost 25%</td>
<td>99</td>
<td>101</td>
<td>103</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>104</td>
<td>107</td>
<td>108</td>
</tr>
</tbody>
</table>

Figures 4 to 7 give a visual presentation of the plant growth of the barley plants in the 25% series (Figures 4 & 5) and the 50% series (Figures 6 & 7) of the compost/reference substrate mixtures. No signs of chlorosis and necrosis were seen for the different compost mixtures.

As a general conclusion it can be stated that after composting GS 270 in a 10% concentration, no residuals are left behind, that would exert a negative effect on the emergence and growth of barley plants.
Figure 4. Overview of the barley plant growth after an incubation period of 8 days (from bottom to top): 25% series of blank compost and test compost

Figure 5. Detailed barley plant growth after an incubation period of 8 days (from left to right): 25% series of blank compost and test compost
Figure 6. Overview of the barley plant growth after an incubation period of 8 days (from bottom to top): 50% series of blank compost and test compost

Figure 7. Detailed barley plant growth after an incubation period of 8 days (from left to right): 50% series of blank compost and test compost
Ecotoxicity tests
Cress test
on compost residuals of GS 270
Report R-DRA-1/4
FINAL REPORT
DRA-1/4

Ecotoxicity test – Cress test on compost residuals of GS 270

Author: Nike Mortier
Sponsor: BiologIQ, Inc.
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UNITED STATES
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3 GLP compliance statement ........................................................... 5

4 Quality assurance audit statement ................................................ 5

5 Summary and conclusions .......................................................... 6

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1 Identification of the test

1.1 General information

Project number
DRA-1/4

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Mrs. Norma McDonald
norma.mcdonald@ows.be

Phone: +1 513 535 6760

Test item
GS 270 compost

Reference item
Blank compost without any addition
1.2 Study personnel

Study Director:          Nike Mortier
Replacement Study Director:    Michela Siotto
Study Director QA:    Steven Verstichel

1.3 Study schedule

Study initiation date:  May 20th, 2016
Study completion date:  June 9th, 2016

Experimental starting date:  May 23rd, 2016
Starting date of incubation:  May 24th, 2016
Completion date of incubation:  June 7th, 2016
Duration of incubation:    14 days
Experimental completion date:    June 9th, 2016

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-1/4

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).

___________________
Nike Mortier
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 Jun-17-2016

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

A cress test, which is representative for dicotyledonous plants, was performed on the test compost, obtained at the end of a pilot-scale composting test in which test item GS 270 was added in a 10% concentration to biowaste at start of the composting test. The pilot-scale composting test is reported in report R-DRA-1/2.

The blank compost and the test compost were both tested in 2 mixing ratios of compost and reference substrate: (1) 75% reference substrate & 25% compost and (2) 50% reference substrate & 50% compost on weight basis.

The test is executed according to the following standards: the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012).

According to ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) the germination rate and the plant biomass of the test compost should be more than 90% of those from the corresponding blank compost. These criteria were easily reached for both the germination rate and the plant biomass of both mixtures of the test compost. Therefore it can be concluded that the requirements of ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) on ecotoxicity are fulfilled for cress plants.

In conclusion, it can be stated that, after composting GS 270 in a 10% concentration, no residuals were left such as metabolites, undegraded components and inorganic components that exert a negative influence on the germination and growth of cress plants.
6 Introduction

6.1 Purpose and principle of test method

The cress test is applied after a preceding composting test. The compost produced at the end of the composting test can eventually contain residuals of the original test item such as metabolites, undegraded components and inorganic components. The purpose of the cress test is to evaluate any toxic effect of the test compost containing the test item residuals in comparison to blank compost to which no reference or test item was added at the start of the preceding composting test. The cress plant is chosen as a representative for dicotyledonous plants and for its sensitive germination.

The test involves germination and growth of cress in the test compost. At the end of the test the fresh and dry weight of the plants are determined for each test series and compared. Also the germination rate is measured.

6.2 Standards followed

The test is executed in line with the American standard ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), the European norm EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and the international standard ISO 17088 Specifications for compostable plastics (2012).
7 Materials and methods

7.1 Test item

The test items are compost samples obtained at the end of the pilot-scale composting test DRA-1/2.

Blank compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from the bins with no test item, namely bins DRA-1/2-01 and DRA-1/2-02.

Test compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from bins DRA-1/2-03 and DRA-1/2-04 to which 10% GS 270 (1% 7.5 x 7.5 cm pieces + 9% milled (< 2 mm)) was added at start of the composting.

7.2 General procedure

The cress test is done in flower pots of 500 ml, containing a mixture of compost and reference substrate. Each compost is tested in 2 mixing ratios of compost and reference substrate: (1) 75% reference substrate & 25% compost and (2) 50% reference substrate & 50% compost on weight basis. Each mixture is tested in 3 replicates.

At the start of the test, each flower pot is filled with at least 200 g of compost/reference substrate mixture and 100 ml demi water is added. Subsequently, 100 cress seeds are put on top of the mixture and covered with a thin layer of siliceous sand. Finally, an extra amount of demineralized water can be added to assure optimal moisture content.

After the flower pots have been completely prepared, they are covered with a glass plate and incubated at a constant temperature of 20°C ± 2°C in the dark.

After germination, the plate is removed and the pots are exposed to a light intensity of at least 3000 lux during at least 12 hours per day. During the test, extra water is added if needed, and visual perceptions are noted. In order to avoid side effects, the position of each pot is changed a few times during the testing period, according to a logical rotation scheme.

The test is finished 14 days (± 2 days) after 50% of the control seedlings have emerged. At the end of the test the total fresh and dry weight of the above-soil plant material is determined for each flower pot separately. Also the germination rate is measured.

The toxicity of possible residuals of the test item is evaluated by comparing the results on germination and plant yield of test compost to blank compost. More details on the procedure for the particular test reported, are given in the Study Plan.
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.

Germinative capacity
5 ml of demineralised water is added to a petri dish with filter paper on top of a cotton layer. Twenty cress seeds are put on top of the filter paper. A second filter paper is put on top of the seeds. The petri dish is sealed with parafilm and left in the dark at room temperature. After 5 days the number of germinated seeds is counted. The germination is given in % on the amount of seeds at start. The germinative capacity is tested in 5 replicates.

Weight determination
During the test, several balances are used, with an accuracy of 0.1 mg for the determination of dry matter and weighing of the plants, and an accuracy of 0.01 g for weighing the compost and reference substrate.
8 Results

8.1 Test conditions and set-up

The composts, obtained at the end of the pilot-scale composting test DRA-1/2, were thoroughly mixed prior to use.

In total 12 flower pots were used. The mixtures of reference substrate and compost are given on weight basis. Table 1 describes the test set-up.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight</th>
<th>Ref. Sub. (g/pot)</th>
<th>Compost (g/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x Blank compost (25%)</td>
<td></td>
<td>172.5</td>
<td>57.5</td>
</tr>
<tr>
<td>3 x Blank compost (50%)</td>
<td></td>
<td>115.0</td>
<td>115.0</td>
</tr>
<tr>
<td>3 x Test compost (25%)</td>
<td></td>
<td>172.5</td>
<td>57.5</td>
</tr>
<tr>
<td>3 x Test compost (50%)</td>
<td></td>
<td>115.0</td>
<td>115.0</td>
</tr>
</tbody>
</table>

The used reference substrate is ‘Einheitserde O’ (EEO), which is produced by Einheitserdewerk Hameln A. Stangenberg GmbH, Kiebitzweg 3, 31789 Hameln in Germany.

The seeds are cress seeds type 'large-leaved' and are derived from AVEVE, Pantserschipstraat 6, 9000 Gent, Belgium. The cress seeds were examined for their germinative capacity. The germinative capacity was 95%, which is well above the recommended value of 90%.
8.2 Results and discussion

The test was stopped 12 days after 50% of the control seedlings have emerged. Table 2 represents the average germination rate of the different test series as a percentage of the total amount of seeds added at start. The relative germination rate is also shown in Figure 1. Table 3 shows the average fresh and dry weight yield (of above-soil plant parts) for each test series, as well as the standard deviation. The results are shown in Figure 2 and Figure 3.

Table 2. Germination rate of cress (%)

<table>
<thead>
<tr>
<th>Test series</th>
<th>Germination rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
</tr>
<tr>
<td>Blank compost 25%</td>
<td>98.0</td>
</tr>
<tr>
<td>Blank compost 50%</td>
<td>97.0</td>
</tr>
<tr>
<td>Test compost 25%</td>
<td>98.0</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>97.7</td>
</tr>
</tbody>
</table>

*With AVG = average, SD = standard deviation.*

Table 3. Absolute fresh and dry weight yield of cress plants

<table>
<thead>
<tr>
<th>Test series</th>
<th>Fresh Weight Yield (g)</th>
<th>Dry Weight Yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
<td>SD</td>
</tr>
<tr>
<td>Blank compost 25%</td>
<td>8.84</td>
<td>0.50</td>
</tr>
<tr>
<td>Blank compost 50%</td>
<td>7.77</td>
<td>0.37</td>
</tr>
<tr>
<td>Test compost 25%</td>
<td>9.21</td>
<td>0.53</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>7.70</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*With AVG = average, SD = standard deviation.*
Figure 1. Average germination rate
(as percentage to the total amount of seeds added at start)

Figure 2. Absolute plant fresh weight (g/pot)
According to ASTM D6400 Standard Specification for Labeling of Plastics Designed to be Aerobically Composted in Municipal or Industrial Facilities (2012), EN 13432 Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging (2000) and ISO 17088 Specifications for compostable plastics (2012) the germination rate and the plant biomass (on fresh weight basis or on dry weight basis) in the test compost should be more than 90% of those in the corresponding blank compost.

These criteria were easily reached for both the germination rate and the plant biomass of both mixtures of the test compost (see Table 4). Therefore, it can be stated that the requirements of ASTM D6400 (2012), EN 13432 (2000) and ISO 17088 (2012) on ecotoxicity are fulfilled for cress plants.

**Table 4. Germination and plant yield of the test compost as a percentage of the corresponding mixture of blank compost**

<table>
<thead>
<tr>
<th>Test series</th>
<th>Germination</th>
<th>Fresh weight plant yield</th>
<th>Dry weight plant yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test compost 25%</td>
<td>100</td>
<td>104</td>
<td>102</td>
</tr>
<tr>
<td>Test compost 50%</td>
<td>101</td>
<td>99</td>
<td>97</td>
</tr>
</tbody>
</table>

Figures 4 to 7 give a visual presentation of the plant growth of the cress plants in the 25% series (Figures 4 & 5) and the 50% series (Figures 6 & 7) of the compost/reference substrate mixtures. No signs of chlorosis and necrosis were seen for the different compost mixtures.

As a general conclusion it can be stated that after composting GS 270 in a 10% concentration, no residuals are left behind, that would exert a negative effect on the emergence and growth of cress plants.
Figure 4. Overview of the cress plant growth after an incubation period of 13 days (from bottom to top): 25% series of blank compost and test compost.

Figure 5. Detailed cress plant growth after an incubation period of 13 days (from left to right): 25% series of blank compost and test compost.
Figure 6. Overview of the cress plant growth after an incubation period of 13 days (from bottom to top): 50% series of blank compost and test compost

Figure 7. Detailed cress plant growth after an incubation period of 13 days (from left to right): 50% series of blank compost and test compost