EXECUTIVE SUMMARY
BH-22

Industrial and home compostability testing program on
Unbleached bagasse tableware
(thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side))

According to AS 4736 (2006) and AS 5810 (2010)

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Biodegradation

As test material Unbleached bagasse tableware is produced from a material of natural origin (Unbleached sugarcane pulp board), which is not chemically modified, and as the test material does not contain additional organic constituents in a concentration above 1% (dry weight) and the total sum of these organic constituents without determined biodegradability does not exceed 5% (as declared by the sponsor), no biodegradability testing is required. Therefore, it can be concluded that material Unbleached sugarcane pulp board does fulfill the biodegradability requirements of AS 4736 (2006) and AS 5810 (2010).

Disintegration at elevated temperatures

The quantitative disintegration of material Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) (version 1) (see report R-BH-22/2) was evaluated in a pilot-scale aerobic composting test according to ISO 16929 (2013)[1]. After 12 weeks of composting 100% complete disintegration was obtained. Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) fulfilled easily the 90% pass level for disintegration as stipulated by AS 4736 (2006).

Disintegration at ambient temperature

Material Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) was qualitatively evaluated for disintegration during composting at ambient temperature. The test item showed already complete disintegration within 8 weeks of composting, which is much less than the maximum prescribed test duration of 26 weeks (see report R-BH-22/5). Consequently, sufficient disintegration is demonstrated for the evaluated material according to AS 5810 (2010).

Compost quality – Toxicity tests

The addition of 10% Unbleached bagasse tableware (version 1) at start of the composting did not cause a negative effect on the chemical compost parameters and germination and growth of barley plants (see reports R-BH-22/2 (pilot-scale composting test) and R-BH-22/3 (barley plant growth test).

The addition of 10% Unbleached bagasse tableware (version 2 with reduced concentration NEUSIZE AK-15) at start of the composting did not cause a negative effect on the chemical compost parameters and germination and growth of cress plants (see reports R-BH-22/7 (pilot-scale composting test) and R-BH-22/8 (cress test)).

The addition of 10% Unbleached bagasse tableware (version 3 with reduced concentration NEUSIZE AK-15) at start of the composting did not cause a negative effect on the chemical compost parameters and survival and mean weight of earthworms (see reports R-BH-22/9 (pilot-scale composting test) and R-BH-22/10 (earthworm acute toxicity test)).

[1] ISO 16929 *Plastics – Determination of the Degree of Disintegration of Plastic Materials under Defined Composting Conditions in a Pilot-Scale Test* (2013)

General conclusion

As a general conclusion it can be stated that, in our opinion, Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) (version with reduced concentration NEUSIZE AK-15 till 0.83%) does fulfill the evaluation criteria for material characteristics, biodegradation, disintegration and compost quality, which are outlined in the Australian standard AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006) and the Australian standard AS 5810 *Biodegradable plastics – Biodegradable plastics suitable for home composting* (2010). Unbleached bagasse tableware (0.92 mm (bottom), 1.04 mm (side); 629 g/m² (bottom), 476 g/m² (side)) (version with reduced concentration NEUSIZE AK-15 till 0.83%) can be considered fully compostable under industrial and home composting conditions.

Gent, September 9th, 2020

Bruno De Wilde
Lab Manager

Material characteristics

Report R-BH-22/1

FINAL REPORT
BH-22/1

Material characteristics of
Unbleached bagasse tableware

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1 Identification of the test

Project number

BH-22/1

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Test item

A visual presentation of test item Unbleached bagasse tableware is given in Figure 1.

Figure 1. Visual presentation of test item Unbleached bagasse tableware

2 Introduction

2.1 Volatile solids content

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), the French standard NF T51-800 *Plastics - Specifications for plastics suitable for home composting* (2015), the Canadian standard CAN/BNQ 0017-088 *Specifications for compostable plastics* (2010) and the international standard ISO 18606 *Packaging and the environment - Organic recycling* (2013) 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 'M_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 'M_010. Determination of organic matter and carbon content'. The results are given in percent on total solids.

2.2 Heavy metals and fluorine

The European norm EN 13432 (2000), the French standard NF T51-800 (2015), the American standard ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and the Canadian standard CAN/BNQ 0017-088 (2010) define limit levels for heavy metals and fluorine. The international standard ISO 18606 (2013) 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 item are shown in Table 1. EN 13432 (2000), NF T51-800 (2015), CAN/BNQ 0017-088 (2010) and ISO 18606 (2013) prescribe a minimum volatile solids content of 50% on TS. Test item Unbleached bagasse tableware with a volatile solids content of 98.7% on TS easily fulfills this requirement.

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

Characteristics	Unbleached bagasse tableware
Total solids (TS, %)	96.5
Moisture content (%)	3.5
Volatile solids (VS, % on TS)	98.7
Ash content (% on TS)	1.3

3.2 Heavy metals and fluorine

The heavy metals content and the fluorine content of test item Unbleached bagasse tableware are given in Table 2, together with the limit values as prescribed by EN 13432 (2000), NF T51-800 (2015), ASTM D6868 (2017) and CAN/BNQ 0017-088 (2010). All values lay well below the maximum levels as prescribed by the standards.

4 Conclusions

From the results it can be concluded that Unbleached bagasse tableware fulfills the requirements on material characteristics (volatile solids, heavy metals and fluorine) as defined by EN 13432 (2000), NF T51-800 (2015), ASTM D6868 (2017), CAN/BNQ 0017-088 (2010) and ISO 18606 (2013).

Gent, February 12th, 2019

Lies Fougnies
Technician Analyses

Bruno De Wilde
Lab Manager

Table 2. Heavy metals and fluorine content (ppm on total solids)

Analysis	Unbleached bagasse tableware	Limit values				Test procedure
		Europe EN 13432 (2000)	France NF T51-800 (2015)	USA** ASTM D6868 (2017)	Canada CAN/BNQ 0017-088 (2010)	
Heavy metals*						
Zn	< 10	≤ 150	≤ 150	< 1400	< 463	DIN EN ISO 17294-2
Cu	1.3	≤ 50	≤ 50	< 750	< 189	DIN EN ISO 17294-2
Ni	< 1	≤ 25	≤ 25	< 210	< 45	DIN EN ISO 17294-2
Cd	< 0.1	≤ 0.5	≤ 0.5	< 19.5	< 5	DIN EN ISO 17294-2
Pb	< 1	≤ 50	≤ 50	< 150	< 125	DIN EN ISO 17294-2
Hg	< 0.1	≤ 0.5	≤ 0.5	< 8.5	< 1	DIN EN ISO 12846
Cr	1.6	≤ 50	≤ 50	-	< 265	DIN EN ISO 17294-2
Mo	< 1	≤ 1	≤ 1	-	< 5	DIN EN ISO 17294-2
Se	< 0.75	≤ 0.75	≤ 0.75	< 50	< 4	DIN EN ISO 17294-2
As	< 1	≤ 5	≤ 5	< 20.5	< 19	DIN EN ISO 17294-2
Co	< 1	-	≤ 38	-	< 38	DIN EN ISO 17294-2
Fluorine						
F	17	≤ 100	≤ 100	-	-	DIN 51723 mod.

** 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 D6868 (2017) heavy metals content must be less than 50% of those prescribed for sludges or composts in the country where the product is sold)*

Pilot-scale composting test: quantitative disintegration and compost production

Report R-BH-22/2

FINAL REPORT
BH-22/2

Pilot-scale composting + sieving test for measurement of disintegration on **Unbleached bagasse tableware** **(thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side))**

and for production of compost for subsequent ecotoxicity testing on **Unbleached bagasse tableware**

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1 Identification of the test

1.1 General information

Project number

BH-22/2

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Test item

Unbleached bagasse tableware:

- Thickness: 0.92 mm (bottom), 1.04 mm (side)
- Grammage: 629 g/m² (bottom), 476 g/m² (side)

1.2 Study personnel

Study Director:	Kwok Kuen Chow
Replacement Study Director:	Nike Mortier
Study Director Quality Assurance:	Steven Verstichel

1.3 Study schedule

Study initiation date:	January 24th, 2019
Proposed experimental starting date:	January 24th, 2019
Proposed starting date of incubation:	January 31st, 2019
Proposed completion date of incubation:	April 25th, 2019
Proposed experimental completion date:	June 4th, 2019
Proposed study completion date:	June 21st, 2019

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:

BH-22/2

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).

Kwok Kuen Chow
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 Aug-07-2019

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

Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) was evaluated for disintegration in a pilot-scale aerobic composting test simulating industrial composting processes according to ISO 16929 (2013). At start-up 10% Unbleached bagasse tableware was added to biowaste: 1% Unbleached bagasse tableware (diameter top: 21.3 cm, diameter bottom: 11.0 cm, height: 6.5 cm), cut into 4 pieces, and 9% milled Unbleached bagasse tableware. The 1% test item concentration was used for the determination and evaluation of the disintegration, while the extra 9% milled test material was necessary to cover subsequent ecotoxicity testing on compost residuals of Unbleached bagasse tableware. 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 1.5 weeks and above or around 40°C during the entire test. Furthermore, the temperature did not exceed 75°C during the test, except some days during the first 1.1 week of composting for both test bins with maximum values of 76.6°C and 78.4°C, respectively. However, immediate action was undertaken when the 75°C limit was exceeded and the temperature decreased. The biowaste at start showed a pH of 5.8. After 1.7 weeks of composting the pH had increased till above 8.1 for all series. During the further test period the pH remained above 8.6. The oxygen concentration remained always above 10%, except once after 3 weeks of composting in the combined control bin with an oxygen concentration of 9.1%. However immediate action was undertaken to adjust the oxygen concentration. As such, good aerobic conditions were guaranteed during the test.

The disintegration of Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, proceeded very well. After 2 weeks of composting the test material was abundantly present in the test bins but small tears were noticed in the majority of the test material. One week later all test item pieces had fallen apart into pieces of variable size. After 5 weeks of composting a considerable amount of test material could still easily be retrieved from the test bin. The disintegration proceeded and after 8 weeks of composting the amount and size of the remaining pieces had significantly reduced. Only a few tiny pieces of the tableware could be found in the test bin. After 12 weeks of testing (= end of the test) all test material had disappeared from the test bins.

At the end of the composting test, the whole contents of the test bins were used for sieving, sorting, further isolation and analyses. The contents of the test bins were 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. Not a single test item pieces could be retrieved from the different sieving fractions (2 - 5 mm, 5 - 10 mm, > 10 mm). Disintegration is defined as a size reduction to < 2 mm. According to EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and ISO 18606 *Packaging and the environment - Organic recycling* (2013) less than 10% of the material added at start 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 Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)).

Table 1. Disintegration of Unbleached bagasse tableware

Test item	Thickness (mm)	Grammage (g/m^2)	Disintegration (%)	Requirement (%)
Unbleached bagasse tableware	Bottom: 0.92 Side: 1.04	Bottom: 629 Side: 476	**100**	≥ 90

The quality of the composts to which 10% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 9.3 and 9.0 was measured for the control composts and the test composts, respectively. A somewhat lower average electrical conductivity (salt content) was found in the test composts (3360 µS/cm) when compared to the control composts (4120 µS/cm). A low salt content is beneficial for the compost quality. At the end of the test the NH_4^+-N levels were reduced for all replicates, while the NO_x^--N content had increased till an average value of 95 mg NO_x^--N/l and 247 mg NO_x^--N/l for the control composts and the test composts, respectively. This indicates that the nitrification had started and was proceeding. Rather similar average N was measured in the control and the test composts. A somewhat higher average P, K and Mg level was obtained for the control composts when compared to the test composts. Furthermore, a comparable average density was found for the control composts (0.424 kg/l) and test composts (0.464 kg/l). The C/N ratio was 9 for all the composts. A higher average volatile solids degradation was measured for the test series compared to the control series, indicating that the test material was degrading. Moreover, a high volatile solids degradation was measured for all replicates, indicating that the composting process had proceeded well.

In conclusion it can be stated that Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m^2 (bottom), 476 g/m^2 (side)) does easily fulfil the 90% disintegration requirement stipulated by EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013). Even in a higher thickness or grammage the material has the potential to fulfil the disintegration requirement. Moreover, no negative effect on the composting process and on the (physico-chemical) quality of the produced compost was observed, when adding 10% Test Unbleached bagasse tableware 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 mm, 5 mm 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

- ISO 16929 *Plastics – Determination of the Degree of Disintegration of Plastic Materials under Defined Composting Conditions in a Pilot-Scale Test* (2013)

7 Materials and methods

7.1 Test item

Name:	Unbleached bagasse tableware
Description:	Paperboard bowl (Figure 1)
Colour:	Beige
Dimension:	Height: 6.5 cm Diameter (top): 21.3 cm Diameter (bottom): 11.0 cm
Grammage:	Bottom: 629 g/m^2, side: 476 g/m^2
Thickness:	Bottom: 0.92 mm, side: 1.04 mm
Total solids (TS):	95.8%
Volatile solids (VS):	98.8% on TS
Sample preparation:	Cut into 4 pieces (for determination of disintegration) Milled (< 4 mm) (for production of compost of subsequent ecotoxicity testing)
Storage conditions:	Room temperature in the dark

Figure 1. Visual presentation of Unbleached bagasse tableware, top (left), side (middle), bottom (right)

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.

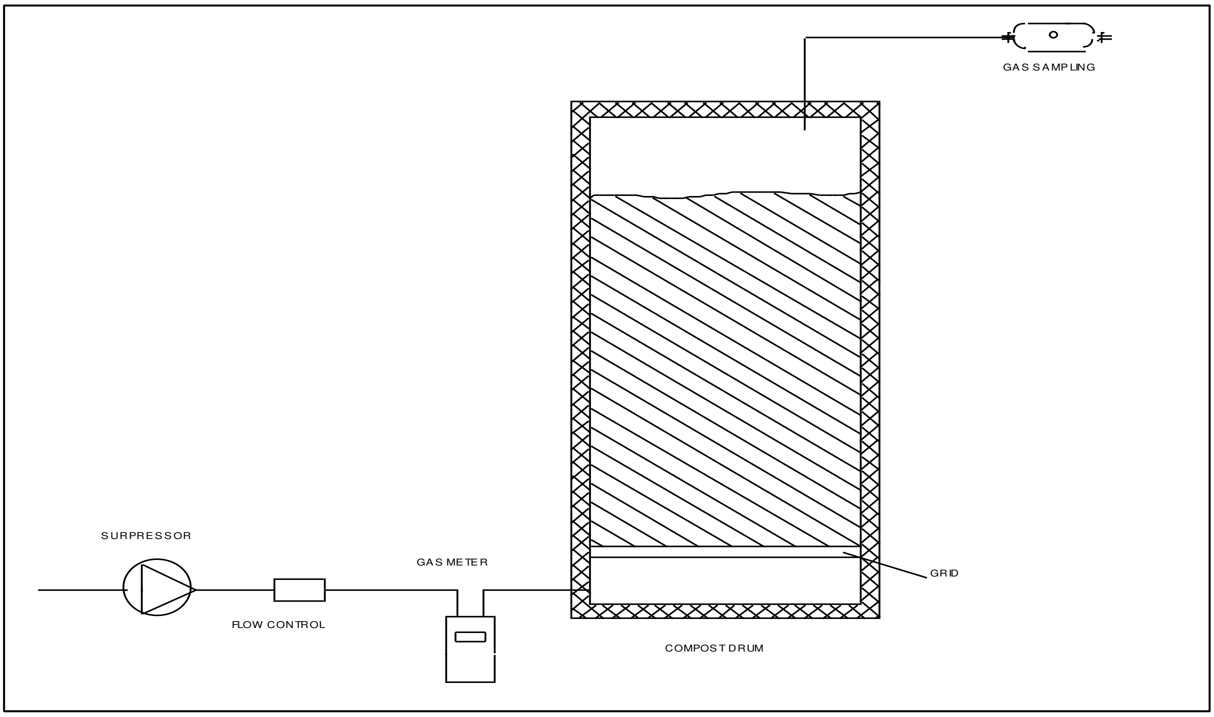

Figure 2. Set-up pilot-scale aerobic composting test

7.3 Analytical methods

Ammonium - nitrogen (NH_4^+-N)

This analysis is performed as described in 'M_054. Determination of ammonium nitrogen by a discrete analyser system and spectrophotometric detection'. The ammonium-N is determined in an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Ammonia reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitroprusside at around pH 12.6 to form a blue compound. The absorbance of this compound is measured spectrophotometrically at the wavelength 660 nm and is related to the ammonia concentration by means of a calibration curve. The results are given in g per l wet weight.

Dry matter or total solids

The dry matter is determined by drying at 105°C for at least 14 hours and weighing, as described in 'M_009. Determination of moisture content'. The dry matter is given in percent on wet weight.

Gas composition

The gas analyses are performed on a PerkinElmer gas chromatograph with CTRI column as described in 'I_235. Manual TotalChrom software'. The gas chromatograph is calibrated with a standard gas mixture consisting of 10% O_2, 20% CO_2, 30% N_2 and 40% CH_4. Every day gas analyses are executed; the gas chromatograph is validated. The results are given in percent.

Grammage

After an acclimatization period of 24 hours at 30% relative humidity and 24 hours at 23°C and 50% relative humidity (ISO 187), the grammage determination is performed according to ISO 536 *Paper and board – Determination of grammage* (2012). Circular pieces are cut with an automatic cutting machine and weighed with an analytical balance. An external laboratory executes the analysis.

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

This analysis is done as described in 'M_055. Determination of total oxidized nitrogen by a discrete analyser system and spectrophotometric detection'. The determination is performed on an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Nitrate is reduced to nitrite by hydrazine under alkaline conditions. The total nitrite ions are then reacted with sulphanilamide and N-1-napthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance is measured at 540 nm and is related to the Total Oxidized Nitrogen (TON) concentration by means of a calibration curve. The results are given in mg 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 'M_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 'M_057. 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 'M_001. Determination of rotting degree – Self-heating test in a Dewar vessel'.

Salt content (electrical conductivity, E.C.)

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 'M_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 'M_057. Preparation of extracts and 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 micrometre (accuracy of 0.1 µm) according to ISO 534 *Paper and board – Determination of thickness, density and specific volume* (2011). An external laboratory executes the analysis.

Total magnesium (Mg)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total Mg content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total Mg content is expressed as g Mg per kg total solids.

Total nitrogen (N)

This analysis is done as described in 'M_039. Determination of total nitrogen after dry combustion – dumas method'. By combusting the sample at 950°C – 1200°C and adding a controlled extra dose of oxygen for a short time, the nitrogen components will oxidize to nitrogen oxides (NOx). In the presence of a CuO catalyst and a copper reducer the nitrogen oxides are converted to N_2. The formed N_2 is measured by a Thermal Conductivity Detector (TCD). The results are given in g per kg total solids.

Total phosphorus (P)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total P content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total P content is expressed as g P per kg total solids.

Total potassium (K)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total P content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total K content is expressed as g K per kg total solids.

Volatile fatty acids (VFA)

The volatile fatty acids are determined as described in ‘M_035. Determination of volatile fatty acids by gas chromatography and flame ionization detector’. 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 ‘M_057. Preparation of extracts and solutions’. 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 FFAP of 30 m. The carrier gas is H_2. A mixture with precise concentrations of eight reference volatile fatty acids is used for calibration while 2-methyl-caproic acid is used as an internal standard. The results are given in g per l wet weight.

Volatile solids - ash

The volatile solids and ash contents are determined by heating the dried sample at 550°C for at least 4 hours and weighing, as described in ‘M_010. Determination of organic matter and carbon content’. The results are given in percent on dry matter.

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 ‘M_011. Determination of volumetric density’.

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 and grammage of test item

The results of the thickness and grammage measurements on the bottom and the side of Unbleached bagasse tableware are given in Table 2. A visual presentation of the measuring points is shown in Figure 3. The measured thicknesses and grammages of the test item are taken into account for the disintegration result obtained in this study.

Table 2. Thickness and grammage of Unbleached bagasse tableware

Unbleached bagasse tableware	Measured value (AVG ± SD)	Minimum value	Maximum value
Thickness (mm)			
Bottom	0.92 ± 0.07	0.81	1.03
Side	1.04 ± 0.12	0.85	1.20
Grammage (g/m²)			
Bottom	629 ± 38	541	694
Side	476 ± 16	453	495

With AVG = average and SD = standard deviation.

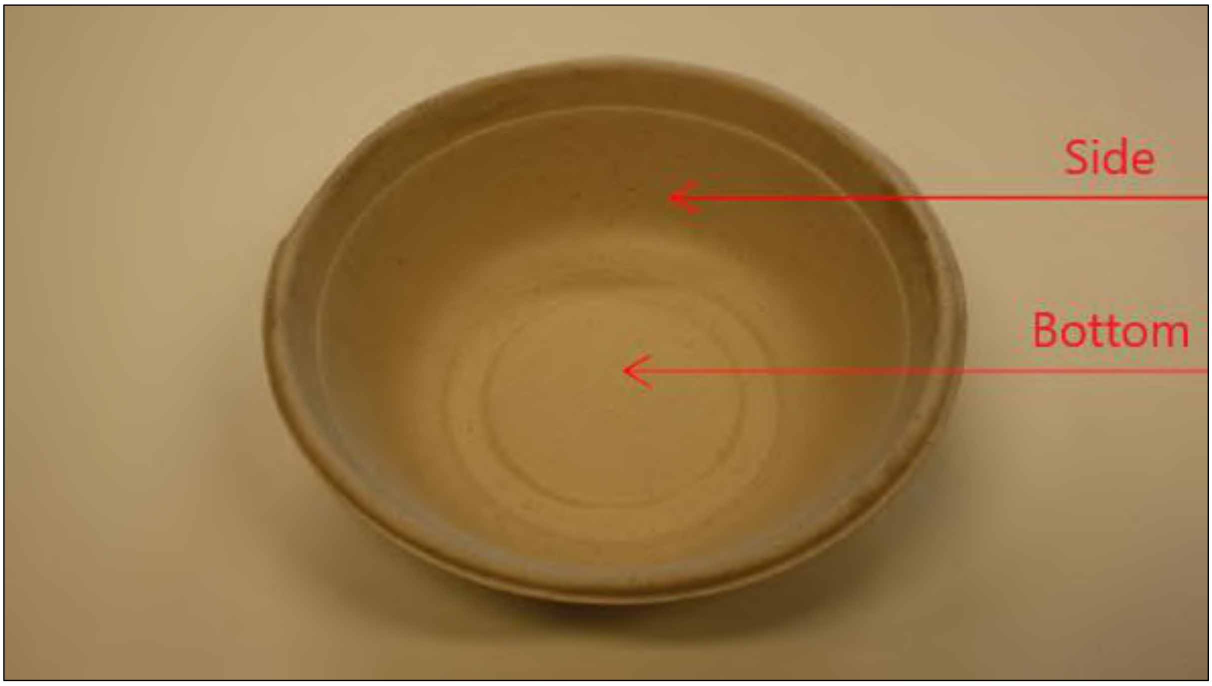

Figure 3. Measurement points on Unbleached bagasse tableware (Side and Bottom)

8.2 Test conditions and set-up

Four composting bins with a total volume of 200 l each were started. The control bins (BH-22/2-01 and BH-22/2-02) contained only biowaste, while the test bins (BH-22/2-03 and BH-22/2-04) contained also 10% Unbleached bagasse tableware: 1% Unbleached bagasse tableware, cut into 4 pieces (diameter top: 21.3 cm; diameter bottom: 11.0 cm; height: 6.5 cm) and 9% milled Unbleached bagasse tableware (< 4 mm). The 1% test item concentration was used for the determination and evaluation of the disintegration, while the extra 9% milled test material was necessary to cover subsequent ecotoxicity testing on compost residual of Unbleached bagasse tableware. 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

Composition	Control bins		Test bins	
	BH-22/2 01	BH-22/2 02	BH-22/2 03	BH-22/2 04
VGF (kg)	55.2	55.2	55.2	55.2
Structural material (kg)	6.0	6.0	6.0	6.0
Unbleached bagasse tableware (cut into 4 pieces) (kg)	-	-	0.62	0.62
Unbleached bagasse tableware (milled < 4 mm) (kg)	-	-	5.51	5.51
% Unbleached bagasse tableware (cut into 4 pieces), on biowaste	-	-	1.0	1.0
% Unbleached bagasse tableware (milled < 4 mm), on biowaste	-	-	9.0	9.0

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 Erembodegem, 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 fulfilled. The biowaste contained a moisture content of 71.2% and a volatile solids content of 77.8% on TS. At start-up a pH of 5.8 was measured. Furthermore, the C/N ratio of the biowaste at start should preferably be between 20 and 30. A somewhat lower C/N ratio of 18 was found for the biowaste. The somewhat lower C/N ratio of the biowaste did not really hinder the test. A low C/N ratio results from a high level of nitrogen in the biowaste (e.g. due to many proteins). This can lead to NH_3 emission (and odour) and eventually slow or difficult nitrification towards the end of the composting cycle. It must be noted that mainly a high C/N ratio can be disadvantageous for the composting process, as this is indicative for N deficiency. The test bins with 10% Unbleached bagasse tableware showed a higher and optimal C/N ratio of 26 due to the addition of 10% Unbleached bagasse tableware with a high carbon content and a low nitrogen content. The high addition of test material is required according to EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013).

Table 4. Characteristics of VGF and structural material

Characteristics	VGF	Structural material
Total solids (TS, %)	26.9	46.7
Moisture content (%)	73.1	53.3
Volatile solids (VS, % on TS)	74.5	95.4
Ash content (% on TS)	25.5	4.6
pH	5.8	-
Electrical conductivity (EC, µS/cm)	3740	-
Volatile fatty acids (VFA, g/l)	2.8	-
NO_x^--N (mg/l)	b.r.	-
NH_4^+-N (mg/l)	246	-
Total N (g/kg TS)	22.8	14.2
C/N	16	34

b.r. = below reporting limit
Reporting limit: NO_x^--N = 10 mg/l

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

Characteristics	Biowaste (= VGF + structural material)	Biowaste + 10% Unbleached bagasse tableware
Total solids (TS, %)	28.8	34.9
Moisture content (%)	71.2	65.1
Volatile solids (VS, % on TS)	77.8	83.1
Ash content (% on TS)	22.2	16.9
Total N (g/kg TS)	21.4	16.2
C/N	18	26

8.4 Temperature profile and analyses exhaust air

Figure 4 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 and did not exceed the 75°C limit, except between 4 and 6 days of composting and again after 9 days in test bin BH-22/2-03 with a maximum value of 76.9°C and between 5 and 6 days of composting in test bin BH-22/2-04 with a maximum value of 78.4°C. However, action was undertaken and the temperature decreased. The temperature remained above 60°C during at least 1.5 week in all bins. It was noticed that the temperature exceeded the 65°C limit after the first week several times between 1.1 and 1.8 weeks for the control bins with a maximum value of 69.3°C (BH-22/2-01) and 70.2°C (BH-22/2-02) and several times between 1.1 week and 3.8 weeks for the test bins with a maximum value of 76.6°C (BH-22/2-03) and 73.7°C (BH-22/2-04). The elevated temperatures in the test bins 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 this test and that the test material degraded well, sufficient microbial diversity was guaranteed in spite of the fact that the temperature exceeded the 65°C limit. After one week of composting control bin BH-22/2-02 was placed in an incubation room at 45°C to ensure high temperatures. One day later the same was done for control bin BH-22/2-01, while both test bins were placed in the incubation room at 45°C after 1.6 weeks of composting. Furthermore, after 2.8 weeks of composting the contents of control bins BH-22/2-01 and BH-22/2-02 were combined into a one bin, separated by a net. The same was done for the contents of test bins BH-22/2-03 and BH-22/2-04 (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 combination of the bins resulted in a temperature increase in both combined bins. Elevated temperatures during the composting process were also caused by the turning of the contents of the bins, during which air channels and fungal flakes 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 or around 40°C during the entire test. It was noticed that the temperature in the test bins was higher when compared to the control bins between 1 and 9 weeks. This indicated that the test material in a high load of 10% was degrading.

Figure 5 shows the CO_2 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 CO_2 production gradually decreased. 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 CO_2 production rate in the test bins was generally higher than that in the control bins during the period between 1 and 3 weeks of composting and between 4 and 8 weeks of composting.

The oxygen concentration of the exhaust air is given in Figure 6. The oxygen concentration remained always above 10%, except once after 3 weeks of composting for the combined control bin BH-22/2-01&02 with an oxygen concentration of 9.1%. However immediate action was undertaken to adjust the oxygen concentration. As such, good aerobic conditions were guaranteed during the test.

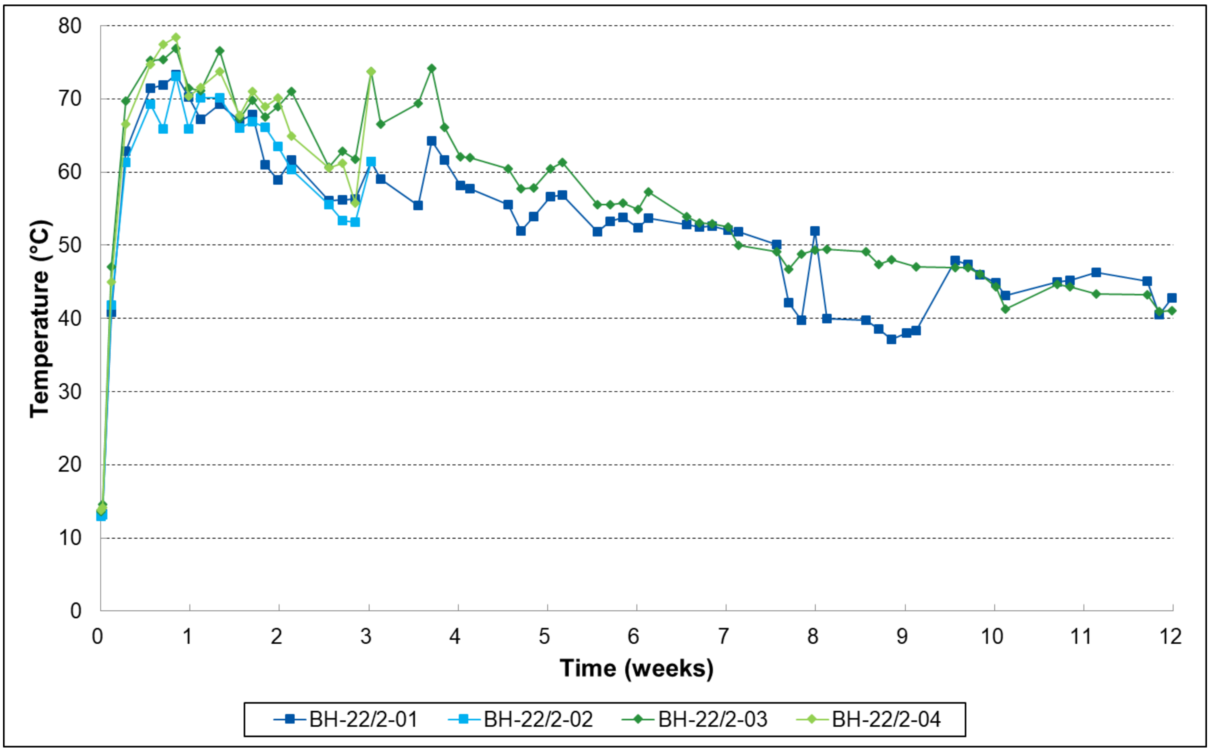

Figure 4. Temperature evolution during the composting test

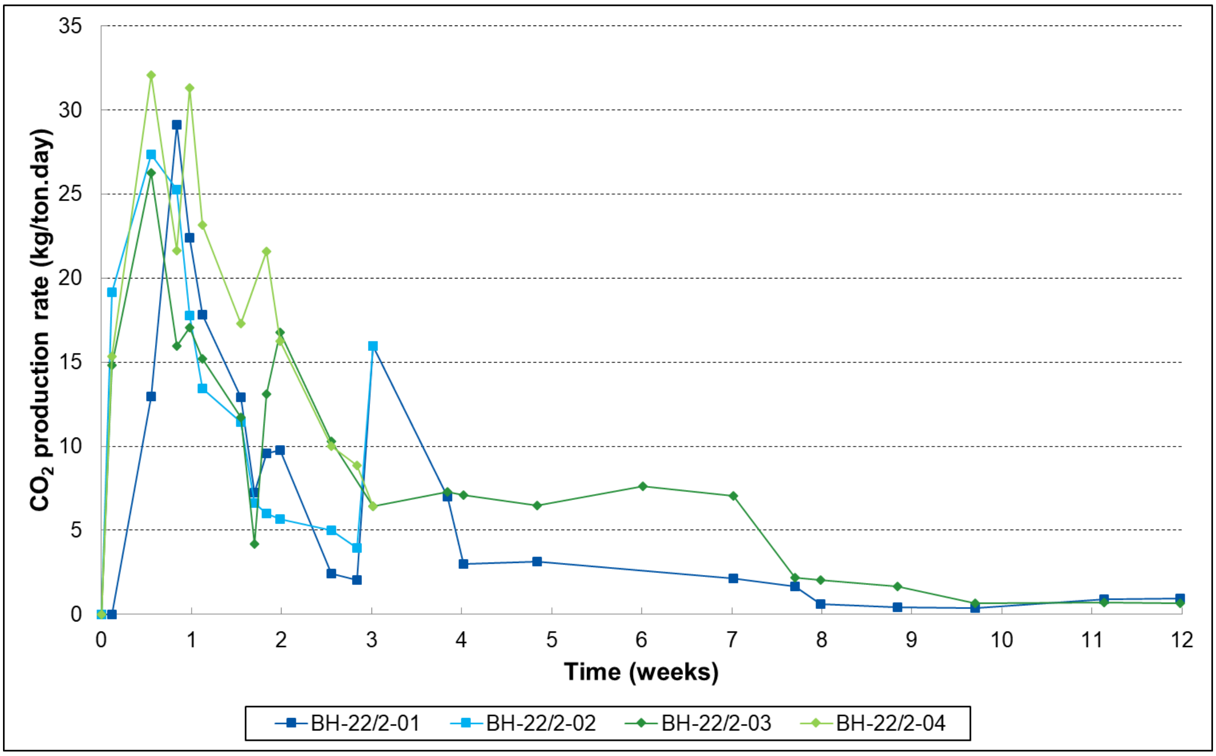

Figure 5. CO_2 production rate during the composting test (individual measurements at regular points in time)

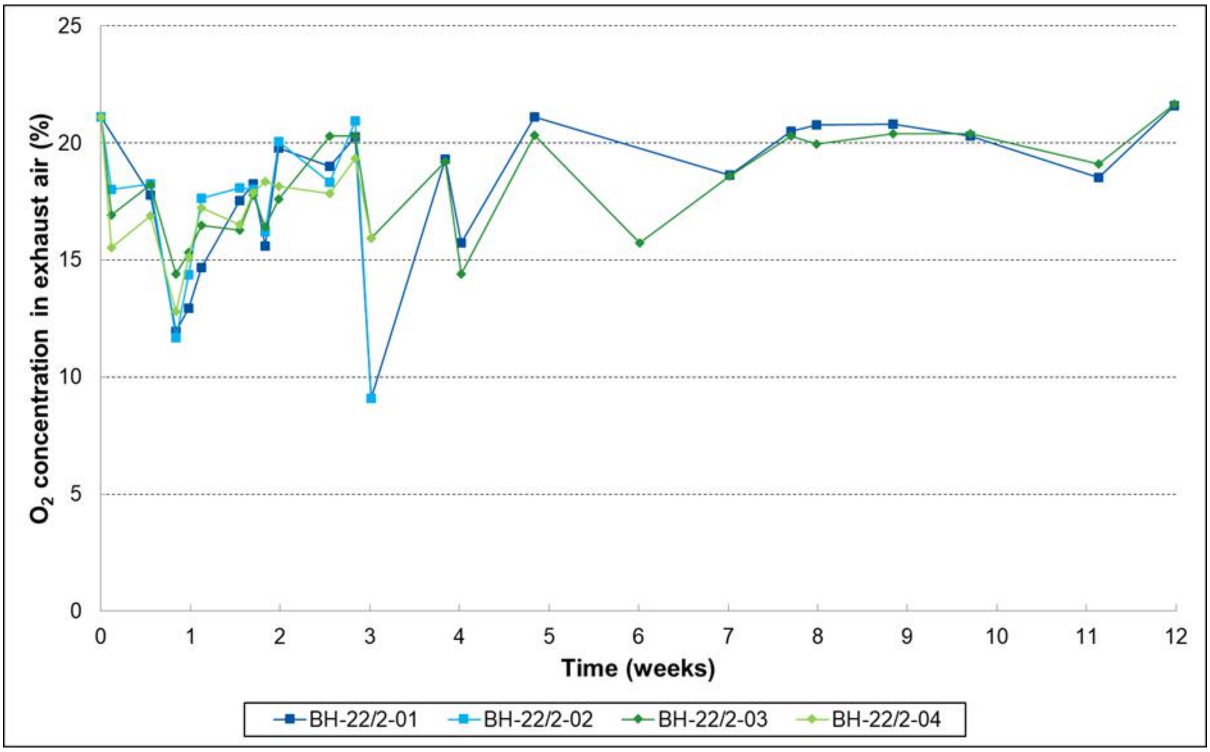

Figure 6. O_2 concentration in the exhaust air during the composting test

8.5 Evolution of pH, NH_4^+-N and NO_x^- -N

Figure 7 shows the evolution of the pH during the composting cycle, while Figures 8 and 9 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 pH of 5.8. After 1.7 weeks of composting the pH had increased till above 8.1 for all series. During the further test period the pH remained above 8.6. At the end of the test (after 12 weeks) an average pH of 9.3 and 9.0 was measured for the control composts and the test composts, respectively.

The biowaste at start contained an ammonium content of 246 mg NH_4^+-N/l. A fluctuation of the ammonium content was observed during the test. However, after 10 weeks of composting a decrease of the NH_4^+-N content was noticed for both control replicates. For the two test replicates a decrease was only noticed at the end of the test. After 12 weeks of composting (= end of the test) an average NH_4^+-N content of 25.6 mg NH_4^+-N/l and 57.2 mg NH_4^+-N/l was found for the control replicates and the test replicates, respectively.

After 10 weeks of composting the NO_x^- -N concentration of both control replicates had increased, while the NO_x^--N content of the test replicates only saw an increase at the end of the test. At the end of the test an average NO_x^- -N content of 95 mg NO_x^- -N/l and 247 mg NO_x^- -N/l was found for the control replicates and the test replicates, respectively.

At the end of the test the NH_4^+-N levels were reduced for all replicates, while the NO_x^- -N content had increased. This indicates that the nitrification process had started and was proceeding.

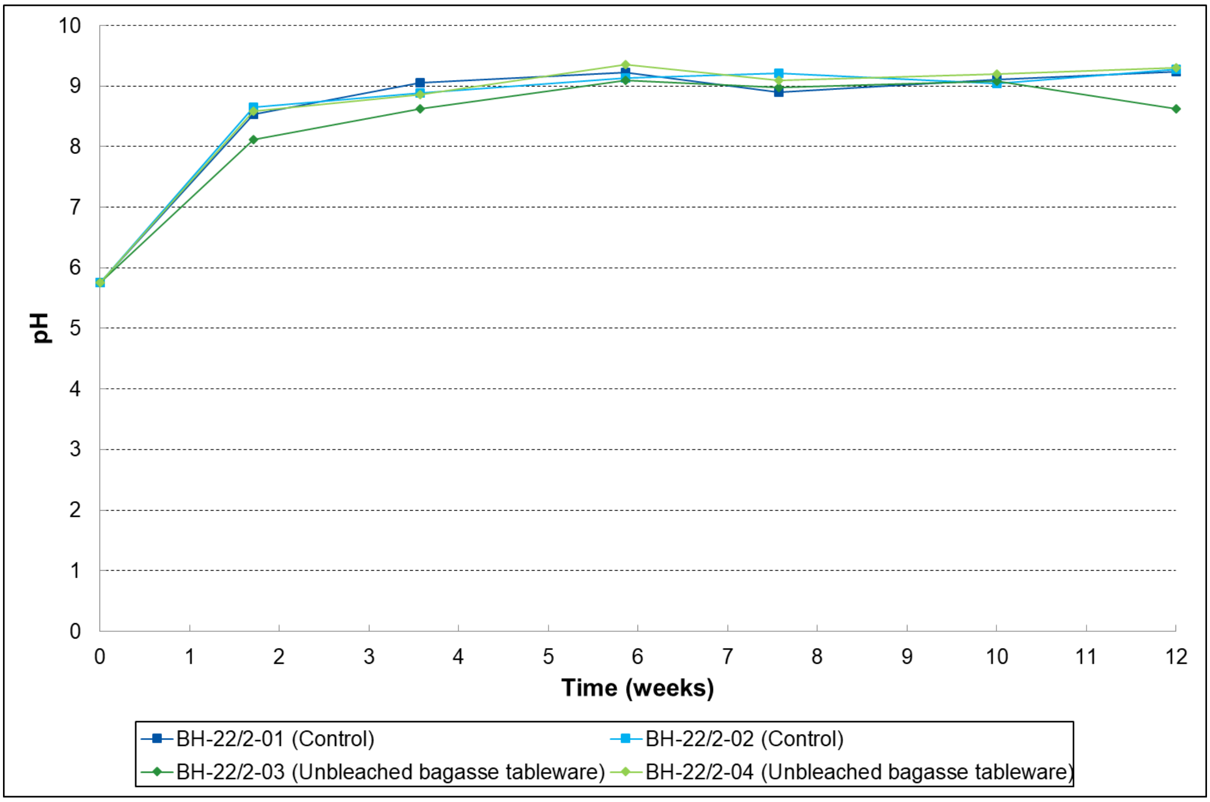

Figure 7. Evolution of pH during composting cycle

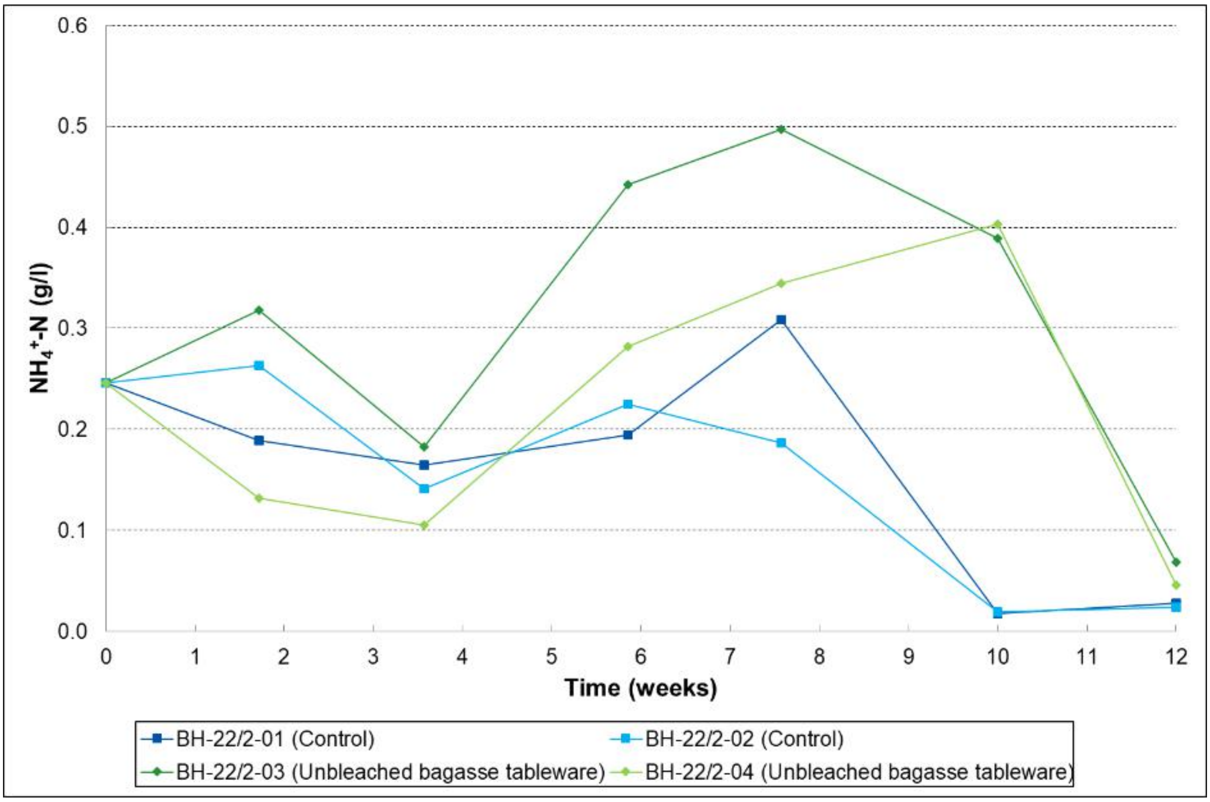

Figure 8. Trend of NH_4^+-N during composting cycle

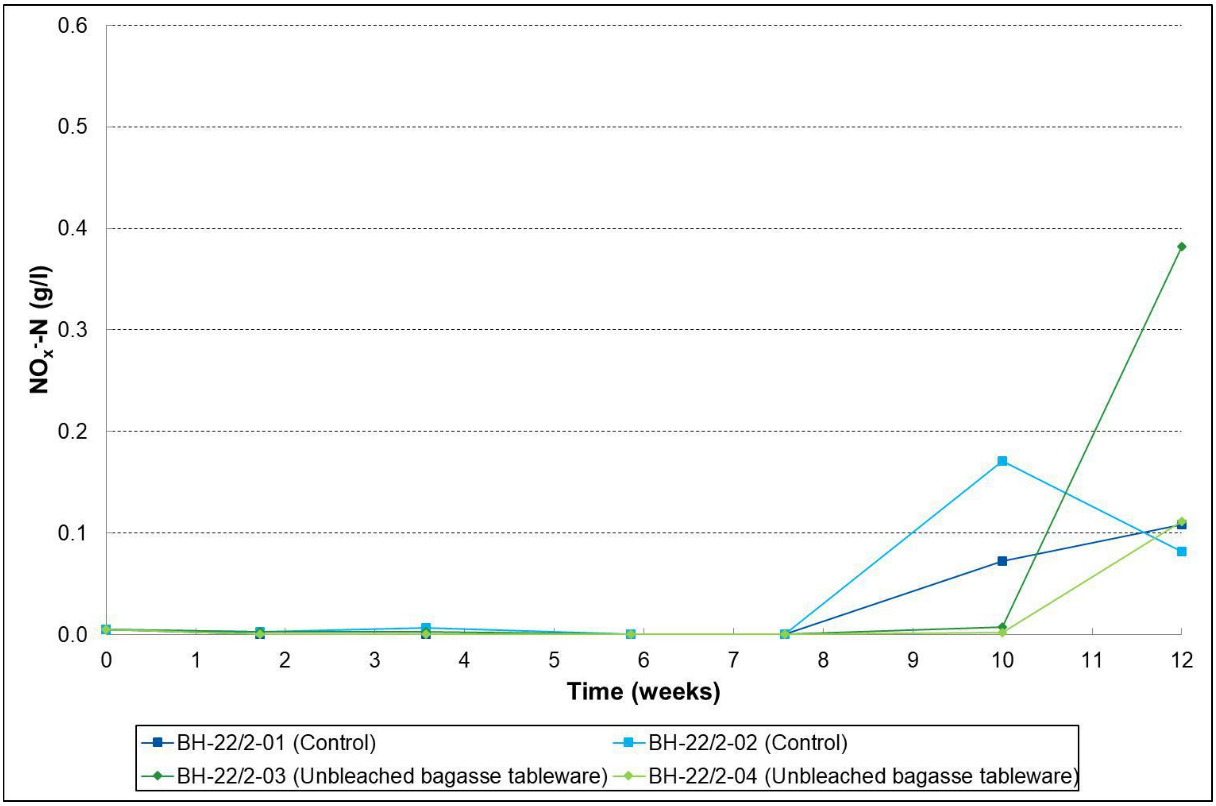

Figure 9. Trend of NO_x^- -N during composting cycle

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 Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) proceeded very well. Figure 10 shows a visual presentation of the contents of a test bin with 1% Unbleached bagasse tableware, cut into 4 pieces, and 9% milled (< 4 mm) after 2 weeks of composting. The test material was abundantly present in the test bins. However, small tears were noticed in the majority of the test material. One week later all test item pieces had fallen apart into pieces of variable size (Figure 11). The disintegration proceeded and after 4 weeks of composting the size of the test item pieces had further reduced (Figure 12). One week later a considerable amount of test material could easily be retrieved from the test bin. The pieces had reduced in size to an average of approximately 4 cm × 6 cm (Figure 13). After 8 weeks the number and size of the remaining pieces had significantly reduced. Only a few tiny pieces of the tableware could be found in the test bin (Figure 14). The disintegration proceeded and at the end of the test (after 12 weeks of composting) not a single piece of Unbleached bagasse tableware could be retrieved from the test composts. Figure 15 gives a visual comparison of the < 10 mm compost fraction of control and test compost at the end of the test. No visual distinction was observed between control and test composts.

Figure 10. Visual presentation of the contents of a test bin with 1% Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, and 9% Unbleached bagasse tableware (milled < 4 mm) after 2 weeks of composting

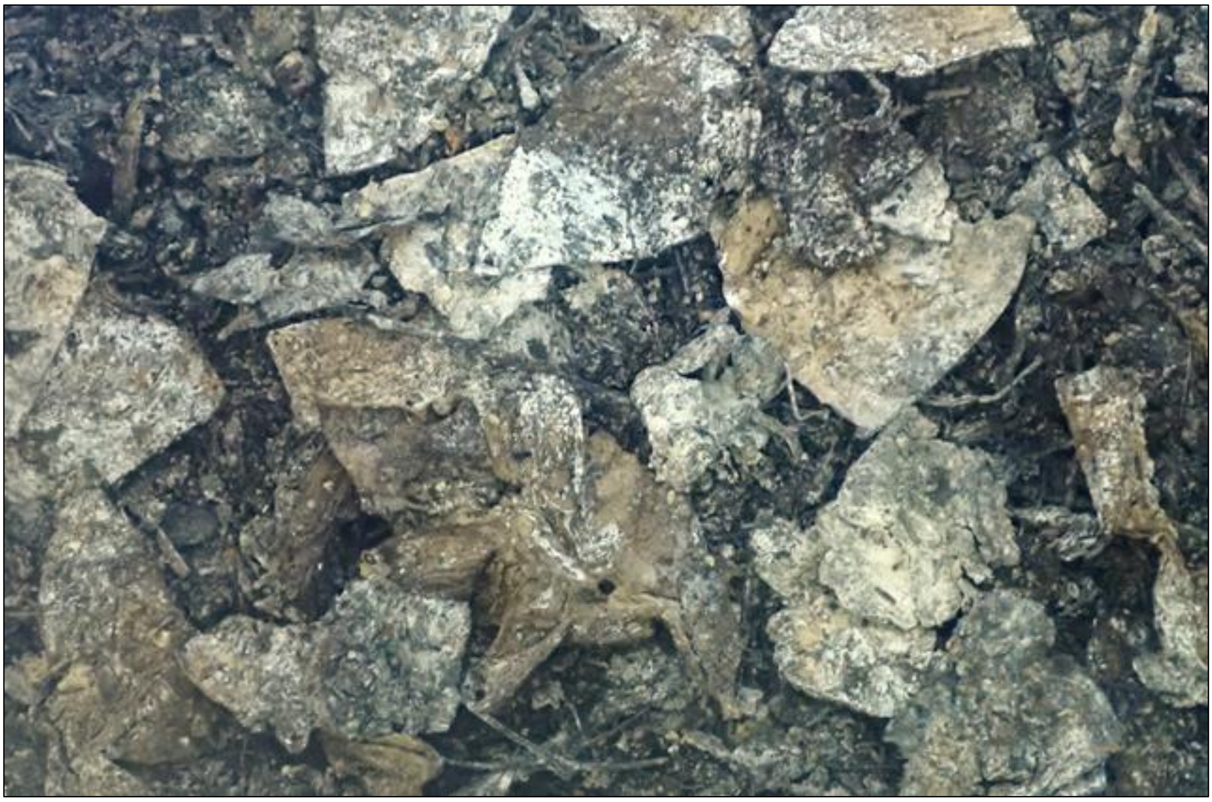

Figure 11. Visual comparison between test item Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, at start and after 3 weeks of composting

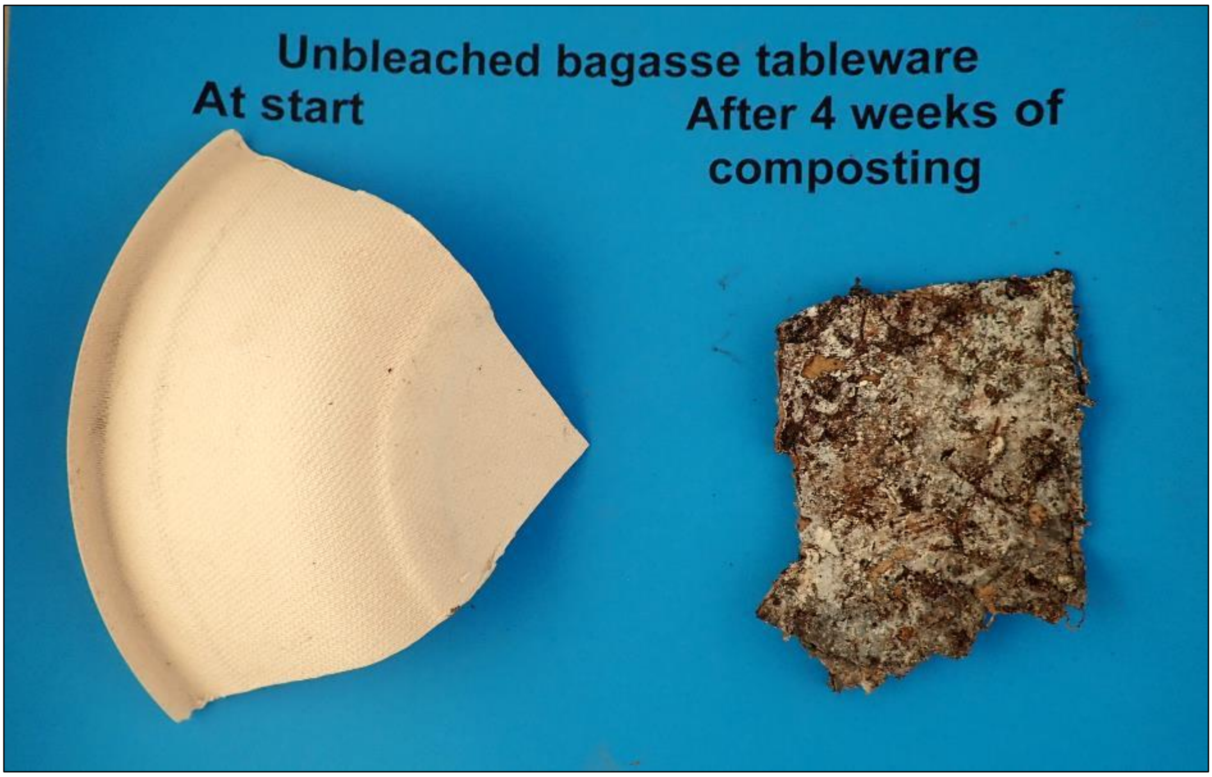

Figure 12. Visual comparison between test item Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, at start and after 4 weeks of composting

Figure 13. Visual comparison between test item Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, at start and after 5 weeks of composting

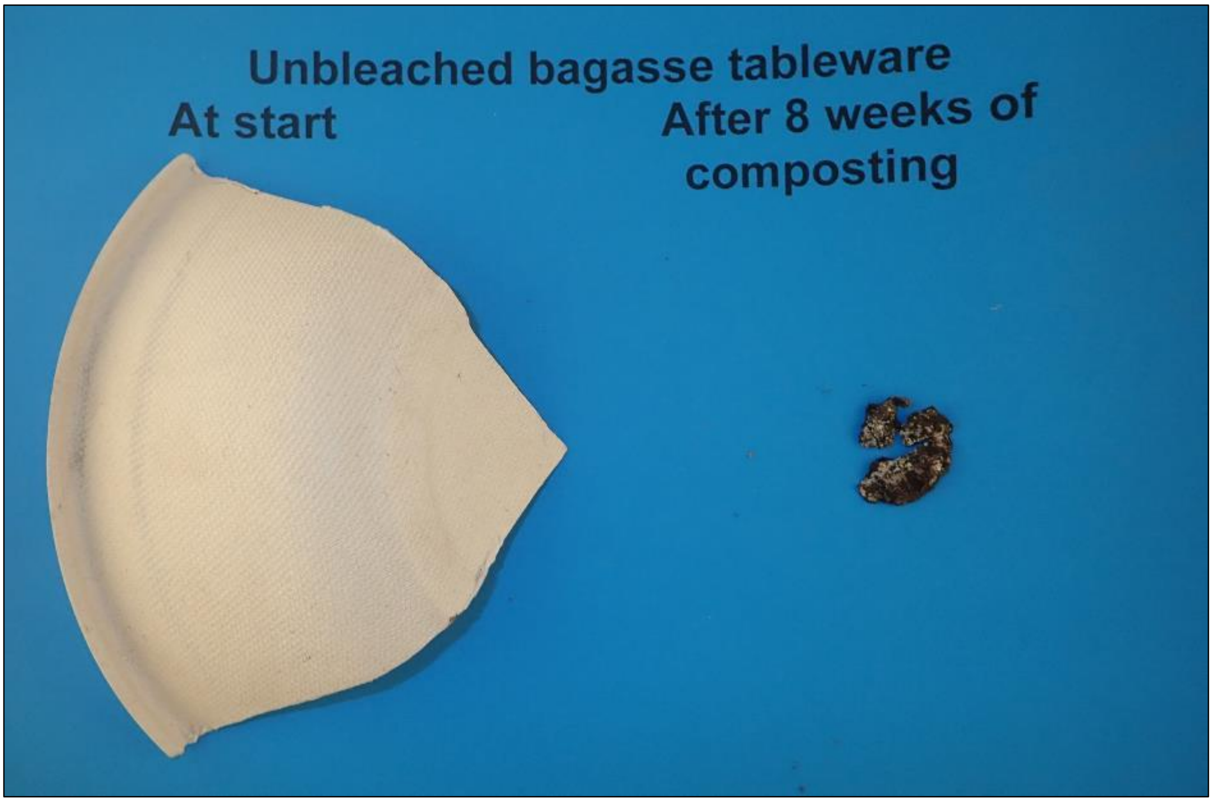

Figure 14. Visual comparison between test item Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)), cut into 4 pieces, at start and after 8 weeks of composting

Figure 15. Visual comparison between the < 10 mm fraction of control compost and test compost obtained after 12 weeks of composting

8.7 Sieving - disintegration

At the end of the composting test (after 12 weeks), the whole contents of the test bins were 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) no residuals of test item Unbleached bagasse tableware could be retrieved. 100% disintegration was established for test material Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)). According to EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and ISO 18606 *Packaging and the environment - Organic recycling* (2013) 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 Unbleached bagasse tableware (thickness: 0.92 mm (bottom), 1.04 mm (side); grammage: 629 g/m² (bottom), 476 g/m² (side)) easily fulfils the disintegration criterion in a pilot-scale composting test as prescribed by EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013).

8.8 Chemical analyses

At the end of the composting test, the whole contents of the bins were 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% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 9.3 and 9.0 was measured for the control composts and the test composts, respectively. A somewhat lower average electrical conductivity (salt content) was found in the test composts (3360 µS/cm) when compared to the control composts (4120 µS/cm). A low salt content is beneficial for the compost quality. At the end of the test the NH_4^+-N levels were reduced for all replicates, while the NO_x^- -N content had increased till an average value of 95 mg NO_x^- -N/l and 247 mg NO_x^- -N/l for the control composts and the test composts, respectively. This indicates that the nitrification had started and was proceeding. Rather similar average N was measured in the control and the test composts. A somewhat higher average P, K and Mg level was obtained for the control composts when compared to the test composts. Furthermore, a comparable average density was found for the control composts (0.424 kg/l) and test composts (0.464 kg/l). The C/N ratio was 9 for all the composts.

A comparable volatile solids content was measured for the < 10 mm fraction of the test composts and the control composts (Table 6). A higher average volatile solids degradation was measured for the test series compared to the control series (Table 7), indicating that the test material was degrading. Moreover, a high volatile solids degradation was measured for all replicates, indicating that the composting process had proceeded well.

Table 6. Chemical analysis of the compost fractions after 12 weeks of composting

Parameter	Control composts		Test composts	
	BH-22/2 01	BH-22/2 02	BH-22/2 03	BH-22/2 04
> 10 mm fraction				
Total solids (TS, %)	65.3	61.4	45.2	46.0
Volatile solids (VS, % on TS)	63.1	67.4	65.1	69.0
Ash (% on TS)	36.9	32.6	34.9	31.0
< 10 mm fraction				
Total solids (TS, %)	65.9	60.3	47.3	48.0
Volatile solids (VS, % on TS)	55.7	60.7	60.5	60.4
Ash (% on TS)	44.3	39.3	39.5	39.6
pH	9.2	9.3	8.6	9.3
Volatile fatty acids (VFA, g/l)	b.r.	b.r.	b.r.	b.r.
Total N (g/kg TS)	31.7	32.8	33.3	35.4
Total P (g/kg TS)	9.26	7.94	7.59	6.89
Total K (g/kg TS)	25.3	26.2	22.6	23.1
Total Mg (g/kg TS)	6.63	6.01	5.02	5.33
NH_4^+-N (mg/l)	27.4	23.9	68.5	45.9
NO_x^--N (mg/l)	108	81.9	382	111
Electrical conductivity (µS/cm)	4800	3430	3430	3280
Rottegrad	V	V	V	V
Density (kg/l)	0.451	0.397	0.465	0.463
C/N	9	9	9	9

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

Table 7. Volatile solids degradation for the different test series

Test series	Volatile solids degradation	
	Average %	%
Control composts	69.3	
BH-22/2-01		70.8
BH-22/2-02		67.9
Test composts	75.9	
BH-22/2-03		74.8
BH-22/2-04		77.1

Qualitative disintegration test in compost at ambient temperature

Report R-BH-22/5

FINAL REPORT
BH-22/5

Evaluation of disintegration during composting at ambient temperature of **Unbleached bagasse tableware**

(Thickness: bottom: 0.92 mm; sidewall: 1.04 mm)
(Grammage: bottom: 629 g/m²; sidewall: 476 g/m²)

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3.2 Test conditions and set-up 5
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1 Identification of the test

Project number

BH-22/5

Conditions

The test was performed under screening conditions

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Test item

Unbleached bagasse tableware (Figure 1)

- Thickness
  - Bottom: 0.92 mm
  - Sidewall: 1.04 mm
- Grammage
  - Bottom: 629 g/m²
  - Sidewall: 476 g/m²
- Dimensions
  - Height: 6.5 cm
  - Diameter (top): 21.3 cm
  - Diameter (bottom): 11 cm

Figure 1. Visual presentation of Unbleached bagasse tableware, top (left), side (middle), bottom (right)

2 Introduction

The purpose of this test is to evaluate the disintegration of a material at ambient temperature in compost. During home composting the high temperatures (> 50°C), obtained during industrial composting processes, are mostly not reached. Therefore a material must demonstrate sufficient disintegration at ambient temperature before it can be allowed in home composting. The test item is mixed with compost and incubated at 28°C in the dark. The maximum test duration during which disintegration should be demonstrated is 26 weeks. Regularly the moisture content is verified and adjusted when needed. At the same time the compost is manually stirred and the test item is visually monitored. The test set-up is based with some modifications, however, on the international standard ISO 20200 *Plastics — Determination of the degree of disintegration of plastic materials under simulated composting conditions in a laboratory-scale test* (2015).

3 Results

3.1 Thickness and grammage of the test item

The results of the thickness and grammage measurements on test item Unbleached bagasse tableware are given in Table 1. The measured thicknesses and grammages of the test item are taken into account for the disintegration result obtained in this study.

Table 1. Thickness and grammage of the test item

Unbleached bagasse tableware		Value (AVG ± SD)	Minimum value	Maximum value
Thickness (µm)*	Bottom	921 ± 71	805	1030
	Sidewall	1035 ± 122	846	1198
Grammage (g/m²)**	Bottom	629 ± 38	541	694
	Sidewall	476 ± 16	453	495

With AVG = average and SD = standard deviation.
* ISO 534 *Paper and board – Determination of thickness, density and specific volume* (2011)
** ISO 536 *Paper and board – Determination of grammage* (2012)

3.2 Test conditions and set-up

The disintegration of Unbleached bagasse tableware was evaluated during 8 weeks of composting at ambient temperature. The test item was cut into 2.5 cm × 2.5 cm pieces, mixed with compost and incubated at 28°C ± 2°C in the dark. The compost consisted of a 80/20 mixture of < 10 mm mature compost with an age of 19 weeks and fresh milled Vegetable, Garden and Fruit waste (VGF), respectively. The compost was regularly stirred and moistened if needed. At the same time the visual appearance of the test material was evaluated.

3.3 Visual perceptions

The disintegration of Unbleached bagasse tableware (Thickness: bottom: 0.92 mm; sidewall: 1.04 mm – Grammage: bottom: 629 g/m²; sidewall: 476 g/m²), cut into 2.5 cm × 2.5 cm pieces, has proceeded very well. Figure 2 shows a visual comparison between Unbleached bagasse tableware, cut into 2.5 cm × 2.5 cm pieces, at start and after an incubation period of 2 weeks at ambient temperature. After 2 weeks of composting at ambient temperature tiny tears were observed in the 2.5 cm × 2.5 cm test item pieces and some fungal growth was present on the surface of the test item pieces. Two weeks later the 2.5 cm × 2.5 cm test item pieces had become fragile and the major part of the test item pieces had fallen apart into smaller test item pieces (Figure 3). Moreover, it was noticed that the colour of the test item pieces had become dark brown. The disintegration proceeded and after 8 weeks of composting at ambient temperature all test item pieces were disappeared and no pieces could be retrieved from the composting reactors (Figure 4).

Because of the good results, the test was already stopped after 8 weeks instead of the standard duration of 26 weeks.

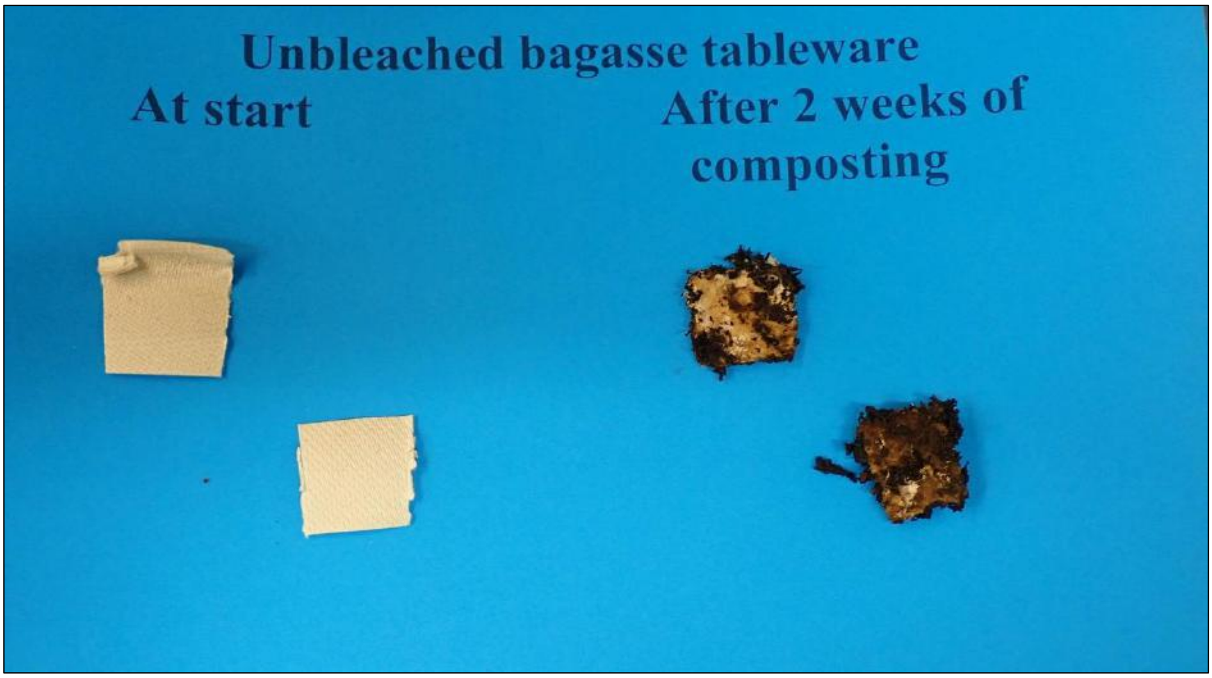

Figure 2. Visual comparison between Unbleached bagasse tableware, cut into 2.5 cm × 2.5 cm pieces, at start and after 2 weeks of composting

Figure 3. Visual comparison between Unbleached bagasse tableware, cut into 2.5 cm × 2.5 cm pieces, at start and after 4 weeks of composting

Figure 4. Visual presentation of a composting reactor with Unbleached bagasse tableware after 8 weeks of incubation at ambient temperature

4 Conclusion

The disintegration of test material Unbleached bagasse tableware (Thickness: bottom: 0.92 mm; sidewall: 1.04 mm – Grammage: bottom: 629 g/m²; sidewall: 476 g/m²) was evaluated during 8 weeks of composting at ambient temperature. The test item was cut into 2.5 cm × 2.5 cm pieces, mixed with compost and incubated at 28°C ± 2°C in the dark. At the end of the composting test, the disintegration was evaluated.

The disintegration of the test item has proceeded very well. Already after 8 weeks of composting the test material had completely disappeared, which is much less than the maximum prescribed test duration of 26 weeks.

The French standard specification NF T51-800 *Plastics – Specifications for plastics suitable for home composting* (2015) and the OK compost HOME certification scheme of TÜV AUSTRIA Belgium stipulate that, when a material has passed the 90% disintegration requirement in a quantitative test according to ISO 16929 *Plastics – Determination of the Degree of Disintegration of Plastic Materials under Defined Composting Conditions in a Pilot-Scale Test* (2013), it is enough to demonstrate sufficient disintegration for home composting in a qualitative test, based on ISO 20200, at ambient temperature (20°C – 30°C). According to the Australian standard specification AS 5810 *Biodegradable plastics – Biodegradable plastics suitable for home composting* (2010) the criterion for evaluation of disintegration is that no more than 10% w/w (dry weight) of the original dry weight of the test material fails to pass through a 2 mm fraction sieve. Any remaining residue shall not be distinguishable from the other material in the compost at 500 mm as observed by the naked eye.

Unbleached bagasse tableware has reached the 90% disintegration requirement of EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000) (see report BH-22/2). Therefore and as sufficient proof of disintegration was obtained in this disintegration test at ambient temperature, it can be concluded that test material Unbleached bagasse tableware (Thickness: bottom: 0.92 mm; sidewall: 1.04 mm – Grammage: bottom: 629 g/m²; sidewall: 476 g/m²) is, in our opinion, for the requirement of disintegration, eligible for OK compost HOME certification and complies with NF T51-800 (2015) and AS 5810 (2010).

Gent, March 27th, 2019

Bruno De Wilde (Authentication) Digitaal ondertekend door Bruno De Wilde (Authentication) Datum: 2019.05.07 15:01:17 +02'00'

Johanna Camerlinck
Study Director

Bruno De Wilde
Lab Manager

Ecotoxicity tests

Barley plant growth test on compost residuals of Unbleached bagasse tableware

Report R-BH-22/3

FINAL REPORT
BH-22/3

Ecotoxicity test – Barley plant growth test on compost residuals of
Unbleached bagasse tableware

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1 Identification of the test

1.1 General information

Project number

BH-22/3

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Test item

Unbleached bagasse tableware compost.

Reference item

Blank compost without any addition.

1.2 Study personnel

Study Director:	Sieglinde Debruyne
Replacement Study Director:	Nike Mortier
Study Director QA:	Inez Monteny

1.3 Study schedule

Study initiation date:	May 14th, 2019
Study completion date:	June 4th, 2019
Experimental starting date:	May 15th, 2019
Starting date of incubation:	May 21st, 2019
Completion date of incubation:	May 31st, 2019
Duration of incubation:	10 days
Experimental completion date:	June 3rd, 2019

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:

BH-22/3

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).

Sieglinde Debruyne
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 Aug-07-2019

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

Inez Monteny
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 Unbleached bagasse tableware was added in a 10% concentration to biowaste at start of the composting test. The pilot-scale composting test is reported in report R-BH-22/2.

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 was executed according to the following standards: 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), the American standard ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and the international standard ISO 18606 *Packaging and the environment - Organic recycling* (2013). The test was stopped after 10 days, which is within the prescribed time interval of *'Methodenbuch 1998, Kapitel II: 5. Pflanzen-verträglichkeit – Bundesgütegemein-schaft Kompost e.V.'*.

According to EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013) the germination rate and the plant biomass (on fresh weight basis or on dry weight basis) of the test compost should be more than 90% of those from the corresponding blank compost. The 90% pass level was reached for both the germination rate and the plant biomass (on fresh weight basis and on dry weight basis) of both mixtures of the test compost. Therefore, it can be concluded that the requirements of EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013) on ecotoxicity are fulfilled for barley plants.

In conclusion, it can be stated that, after composting Unbleached bagasse tableware 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 European norm EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), the American standard ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and the international standard ISO 18606 *Packaging and the environment - Organic recycling* (2013). The time duration was based on *'Methodenbuch 1998, Kapitel II: 5. Pflanzen-verträglichkeit – Bundesgütegemein-schaft Kompost e.V.'*.

7 Materials and methods

7.1 Test items

The test items are compost samples obtained at the end of the pilot-scale composting test BH-22/2.

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 BH-22/2-01 and BH-22/2-02.
Test compost:	Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from bins BH-22/2-03 and BH-22/2-04 to which 10% Unbleached bagasse tableware (1% cut into 6 pieces and 9% milled (< 4 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 demi 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 'M_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 BH-22/2, 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

Treatment	Weight	
	Ref. Sub. (g/pot)	Compost (g/pot)
3 x Blank compost (25%)	172.5	57.5
3 x Blank compost (50%)	115.0	115.0
3 x Test compost (25%)	172.5	57.5
3 x Test compost (50%)	115.0	115.0

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 99%, which is above the recommended value of 90%.

8.2 Germination and yield

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 (%)

Test series	Germination rate (%)	
	AVG	SD
Blank compost 25%	92.7	8.1
Blank compost 50%	98.7	2.3
Test compost 25%	98.0	2.0
Test compost 50%	96.7	3.1

With AVG = average, SD = standard deviation.

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

Test series	Fresh weight yield (g)	
	AVG	SD
Blank compost 25%	11.63	0.81
Blank compost 50%	10.10	0.29
Test compost 25%	12.55	0.45
Test compost 50%	10.09	0.32
Test series	**Dry weight yield (g)**	
	AVG	**SD**
Blank compost 25%	0.92	0.06
Blank compost 50%	0.92	0.02
Test compost 25%	0.95	0.05
Test compost 50%	0.89	0.02

With AVG = average, SD = standard deviation.

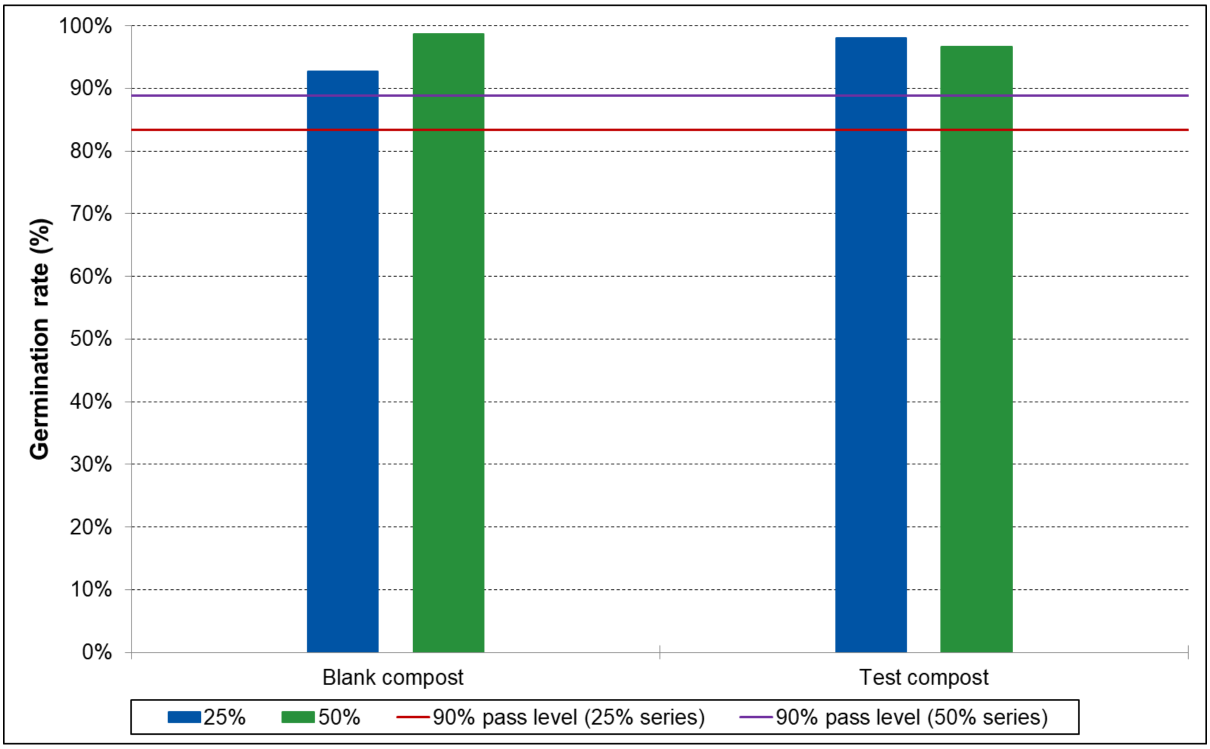

Figure 1. Average germination rate
(as percentage to the total amount of seeds added at start)

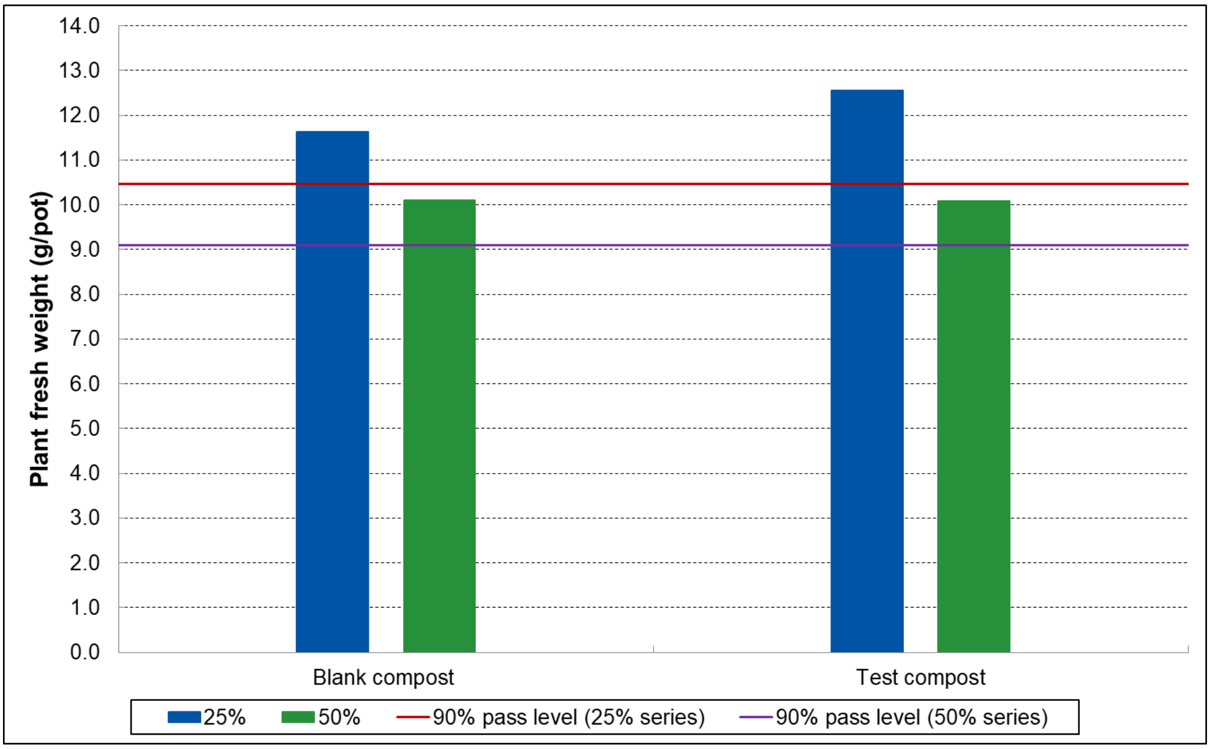

Figure 2. Absolute plant fresh weight (g/pot)

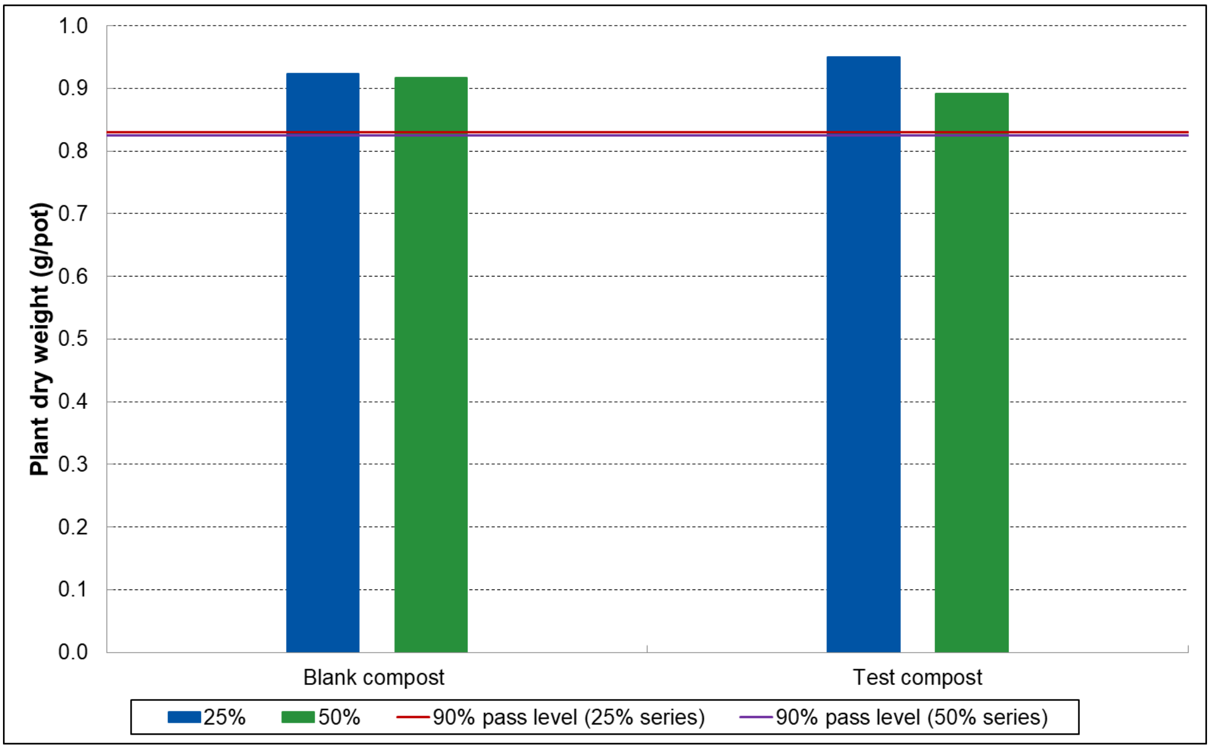

Figure 3. Absolute plant dry weight (g/pot)

According to EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and ISO 18606 *Packaging and the environment - Organic recycling* (2013) 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.

The 90% pass level was reached for both the germination rate and the plant biomass (on fresh weight basis and on dry weight basis) of both mixtures of the test compost (see Table 4). Therefore, it can be stated that the requirements of EN 13432 (2000), ASTM D6868 (2017) and ISO 18606 (2013) 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

Test series	Germination	Fresh weight plant yield	Dry weight plant yield
Test compost 25%	106	108	103
Test compost 50%	98	100	97

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 Unbleached bagasse tableware in a 10% concentration, no residuals are left behind that 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

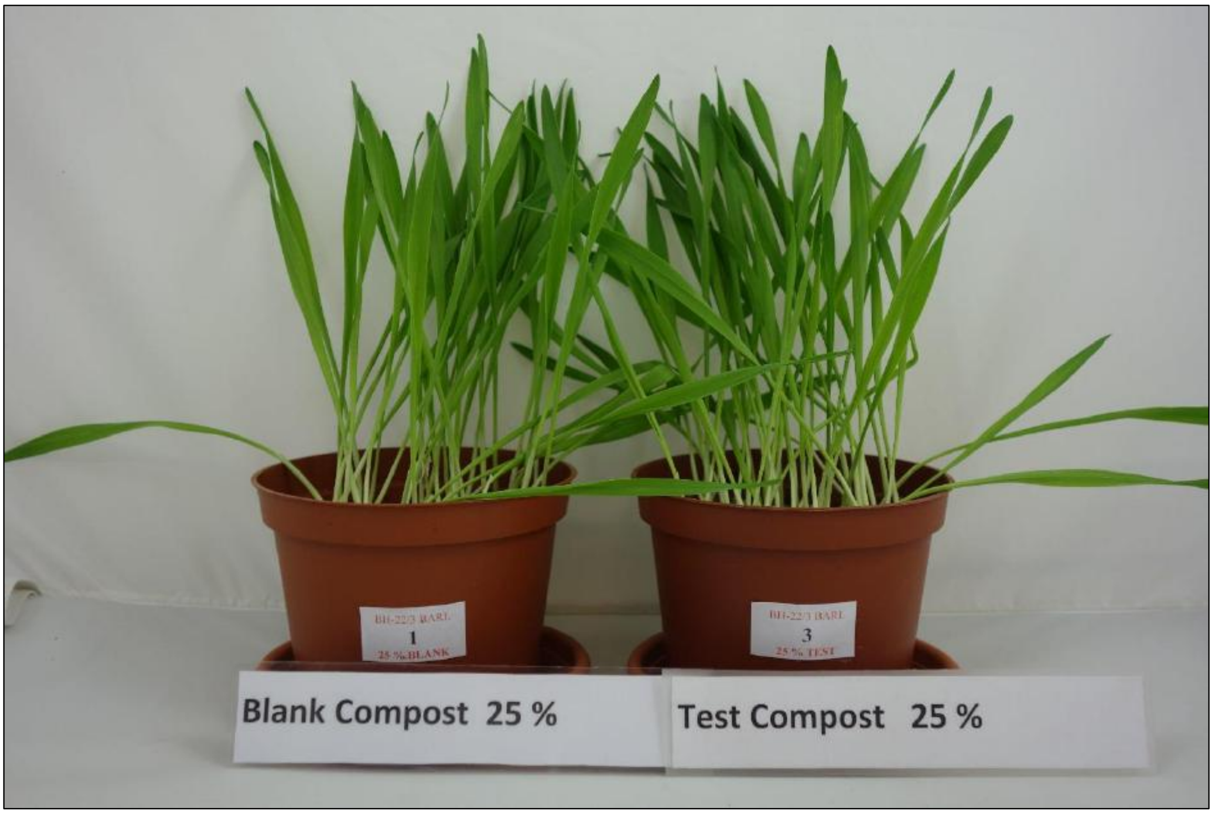

Figure 5. Detailed barley plant growth after an incubation period of 8 days (from left to right): 25% series of blank compost and test compost

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

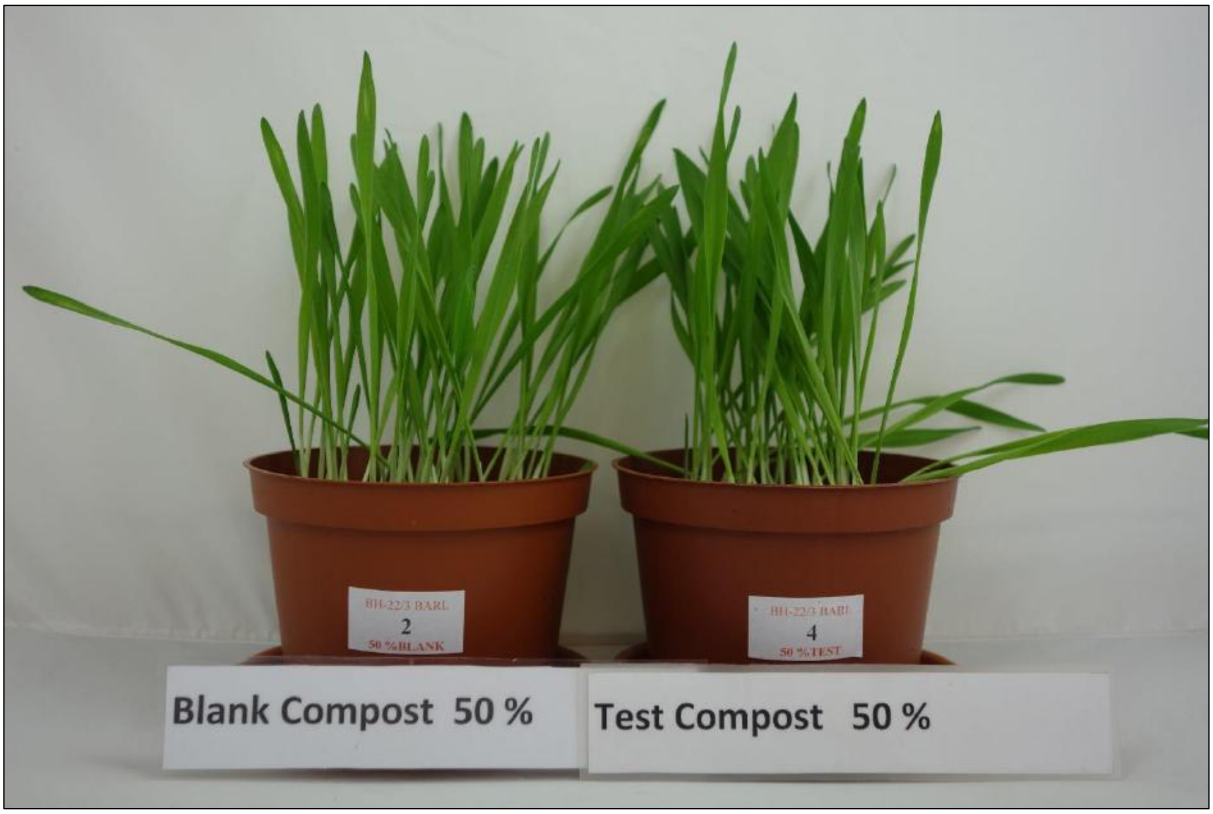

Figure 7. Detailed barley plant growth after an incubation period of 8 days (from left to right): 50% series of blank compost and test compost

Pilot-scale composting test for the production of compost for subsequent ecotoxicity testing

Report R-BH-22/7

FINAL REPORT
BH-22/7

Pilot-scale composting test for production of compost for subsequent ecotoxicity testing on **Unbleached bagasse tableware**

Author: Max Minne
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1 Identification of the test

1.1 General information

Project number

BH-22/7

Sponsor

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Test item

Unbleached bagasse tableware

1.2 Study personnel

Study Director:	Max Minne
Replacement Study Director:	Kwok Kuen Chow
Study Director QA:	Steven Verstichel

1.3 Study schedule

Study initiation date:	August 23rd, 2019
Experimental starting date:	August 23rd, 2019
Starting date of incubation:	August 29th, 2019
Completion date of incubation:	November 21st, 2019
Experimental completion date:	December 17th, 2019
Study completion date:	December 18th, 2019

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:

BH-22/7

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).

p.p. Kwok Kuen Chow

Max Minne
Study Director

4 Quality assurance audit statement

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

A quality control was executed on .Feb-10-2020

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

In this pilot-scale composting test according to ISO 16929 (2013), simulating industrial composting processes, compost was produced for subsequent ecotoxicity tests on Unbleached bagasse tableware. Test method ISO 16929 (2013) prescribes that a test item shall be added in a concentration of 10% to biowaste in order to prepare compost for subsequent toxicity tests. Therefore, test item Unbleached bagasse tableware (milled; < 4 mm) was added in a concentration of 10% to biowaste at start of the pilot-scale composting test. 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 over a mesh size of 10 mm.

The composting test was done under optimum composting conditions. The operational parameters showed that the test was valid. The temperature in the bins remained above 60°C during more than one week. Moreover, the temperature did not exceed the 75°C limit. The temperature in all bins remained well above or around 40°C during the entire test. The pH of the biowaste at start was 5.3 and after 1.7 weeks of composting the pH had already increased till above 8.1 for all test series. During the further test period the pH remained above 7.2. The oxygen concentration remained always above 10%, as such good aerobic conditions were guaranteed during the test.

The quality of the composts to which 10% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 8.2 and 7.8 was measured for the control composts and the test composts, respectively. Higher, but still normal salt levels were found in the test composts when compared to the control composts. At the end of the test low NH_4^+-N levels were found in all composts, while the NO_x^- -N content had increased. After 12 weeks an average NO_x^- -N content of 318 mg NO_x^- -N/l (control composts) and 605 mg NO_x^- -N/l (test composts) was measured. This indicates that the nitrification process had started and was proceeding well. A rather comparable average P, K and Mg content was obtained for the control composts and the test composts, while a lower average N level was noted for the test composts compared to the control composts. An average density of 0.558 kg/l and 0.561 kg/l was found for the control composts and the test composts, respectively. The C/N ratio varied between 8 and 11. Moreover, a higher average volatile solids degradation was measured for the test series compared to the control series, indicating that the test material was degrading. The high average volatile solids degradation for all series, demonstrates that the composting process has proceeded well.

In conclusion it can be stated that no negative effect on the composting process and on the (physico-chemical) quality of the produced compost was observed, when adding 10% Unbleached bagasse tableware at start of the composting process.

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 10 mm. 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

- ISO 16929 *Plastics – Determination of the Degree of Disintegration of Plastic Materials under Defined Composting Conditions in a Pilot-Scale Test* (2013)

7 Materials and methods

7.1 Test item

<u>Name</u>: Unbleached bagasse tableware
<u>Description</u>: Paperboard bowl (Figure 1)
<u>Colour</u>: Light beige
<u>Sample preparation</u>: Milled (< 4 mm)
<u>Storage conditions</u>: At room temperature in the dark

Figure 1. Visual presentation of test item Unbleached bagasse tableware

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.

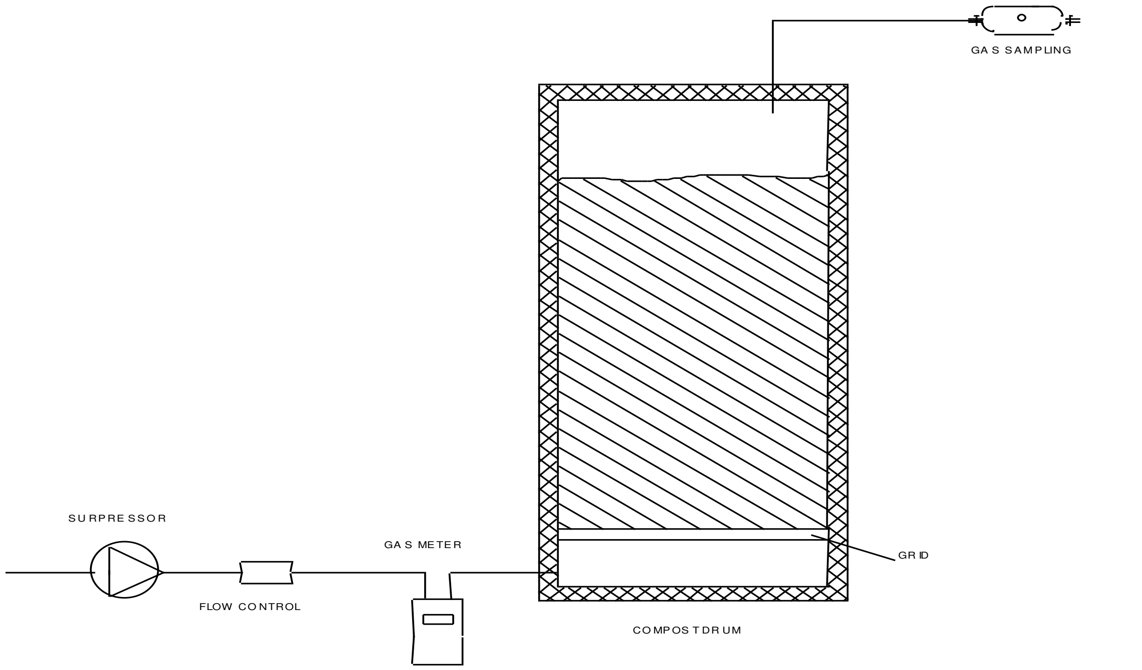

Figure 2. Set-up pilot-scale aerobic composting test

7.3 Analytical methods

Ammonium - nitrogen (NH_4^+-N)

This analysis is performed as described in 'M_054. Determination of ammonium nitrogen by a discrete analyser system and spectrophotometric detection'. The ammonium-N is determined in an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Ammonia reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitroprusside at around pH 12.6 to form a blue compound. The absorbance of this compound is measured spectrophotometrically at the wavelength 660 nm and is related to the ammonia concentration by means of a calibration curve. The results are given in 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 'M_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 'I_435. Manual TotalChrom software'. The gas chromatograph is calibrated with a standard gas mixture consisting of 10% O_2, 20% CO_2, 30% N_2 and 40% CH_4. Every day gas analyses were executed the gas chromatograph is validated. The results are given in per cent.

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

This analysis is done as described in 'M_055. Determination of total oxidized nitrogen by a discrete analyser system and spectrophotometric detection'. The determination is performed on an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Nitrate is reduced to nitrite by hydrazine under alkaline conditions. The total nitrite ions are then reacted with sulphanilamide and N-1-napthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance is measured at 540 nm and is related to the Total Oxidized Nitrogen (TON) concentration by means of a calibration curve. The results are given in mg 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 'M_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 'M_057. Extraction of water and potassium chloride soluble nutrients and elements'.

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 'M_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 'M_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 'M_057. Extraction of water and potassium chloride soluble nutrients and elements'. The results are given in µS/cm.

Total magnesium (Mg)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total Mg content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total Mg content is expressed as g Mg per kg total solids.

Total nitrogen (N)

This analysis is done as described in 'M_039. Determination of total organic carbon and total nitrogen – Method by total carbon, total nitrogen and inorganic carbon combustion'. By combusting the sample at 950°C – 1200°C and adding a controlled extra dose of oxygen for a short time, the nitrogen components will oxidize to nitrogen oxides (NO_x). In the presence of a CuO catalyst and a copper reducer the nitrogen oxides are converted to N_2. The formed N_2 is measured by a Thermal Conductivity Detector (TCD). The results are given in g per kg total solids.

Total phosphorus (P)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total P content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total P content is expressed as g P per kg total solids.

Total potassium (K)

This analysis is done as described in 'M_053. Determination of selected elements by inductively coupled plasma optical emission spectrometry'. The total K content of the compost is determined by inductively coupled plasma optical emission spectrometry (ICP-OES) after extraction with aqua regia according to 'M_048. Extraction of Aqua Regia Soluble Elements'. The total K content is expressed as g K per kg total solids.

Volatile fatty acids (VFA)

The volatile fatty acids are determined as described in 'M_035. Determination of volatile fatty acids by gas chromatography and flame ionization detector'. 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 'M_057. Preparation of extracts and analysis solutions' 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 FFAP of 30 m. The carrier gas is H_2. A mixture with precise concentrations of eight reference volatile fatty acids is used for calibration while 2-methyl-caproic acid is used as an internal standard. The results are given in g per l wet weight.

Volatile solids (VS) - ash

The volatile solids and ash contents are determined by heating the dried sample at 550°C for at least 4 hours and weighing, as described in 'M_010. Determination of organic matter and carbon content'. The results are given in percent on dry matter.

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 'M_011. Determination of volumetric density'.

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

Four composting bins with a total volume of 200 l each were started: two control bins (BH-22/7-01 and BH-22/7-02) and two test bins (BH-22/7-03 and BH-22/7-04). The control bins contained only biowaste, while the test bins contained also 10% Unbleached bagasse tableware (milled < 4 mm). The 10% Unbleached bagasse tableware was necessary to cover subsequent ecotoxicity tests on Unbleached bagasse tableware.

The exact test set-up is given in Table 1. 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 1. Test set-up

Composition	Control bins		Test bins	
	BH-22/7-01	BH-22/7-02	BH-22/7-03	BH-22/7-04
VGF (kg)	55.2	55.2	55.2	55.2
Structural material (kg)	6.0	6.0	6.0	6.0
Unbleached bagasse tableware, milled (< 4 mm) (kg)	-	-	6.12	6.12
% Unbleached bagasse tableware on biowaste	-	-	10.0	10.0

8.2 Analyses 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 Erembodegem, Belgium. The characteristics of VGF and structural material are given in Table 2. Table 3 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 2 and 3 it can be seen that these requirements were fulfilled. The biowaste contained a moisture content of 66.2% and a volatile solids content of 71.3% on TS. At start-up a pH of 5.3 was measured. Furthermore, the C/N ratio of the biowaste at start should preferably be between 20 and 30. An optimal C/N ratio of 23 was obtained for the biowaste. The biowaste with 10% Unbleached bagasse tableware showed a higher C/N ratio of 32. This was due to the addition of 10% test material with a high carbon content and a low nitrogen content, resulting in a higher C/N ratio. The high addition is prescribed by ISO 16929 (2013), EN 13432 (2000), AS 4736 (2006), ASTM D6868 (2019) and ISO 18606 (2013).

Table 2. Characteristics of VGF and structural material

Characteristics	VGF	Structural material
Total solids (TS, %)	30.7	62.9
Moisture content (%)	69.3	37.1
Volatile solids (VS, % on TS)	66.3	93.8
Ash content (% on TS)	33.7	6.2
pH	5.3	-
Electrical conductivity (EC, µS/cm)	2720	-
Volatile fatty acids (VFA, g/l)	3.6	-
NO_x^--N (mg/l)	b.r.	-
NH_4^+-N (mg/l)	408	-
Total N (g/kg TS)	17.7	4.6
C/N	19	103

b.r. = below reporting limit
Reporting limit: NO_X^--N = 10 mg/l

Table 3. Characteristics of the biowaste and biowaste with test item

Characteristics	Biowaste (= VGF + structural material)	Biowaste + 10% test item
Total solids (TS, %)	33.8	39.6
Moisture content (%)	66.2	60.4
Volatile solids (VS, % on TS)	71.3	77.4
Ash content (% on TS)	28.7	22.6
Total N (g/kg TS)	15.3	12.1
C/N	23	32

8.3 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 for all bins and remained below 75°C. The temperature remained above 60°C during at least 1 week, but exceeded the 65°C limit in the test bins after the first week of composting. The higher temperatures in the test bins were caused by the degradation of the test material that was added in a 10% concentration at start. Taken into account the good degradation of the test material and the fact that the composting process proceeded well during this test, sufficient microbial diversity was guaranteed in spite of the fact that the temperature exceeded the 65°C limit in the test bins after the first week. The control bins were placed in an incubation room at 45°C after 6 days of composting. Two days later the same was done for test bin BH-22/7-04 and after 12 days of composting also test bin BH-22/7-03 was placed in the incubation room. After 2.5 weeks of composting the contents of the control bins (BH-22/7-01 and BH-22/7-02) were 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 BH-22/7-03 and BH-22/7-04. The combination of the contents of the bins resulted in a significant temperature increase. Elevated temperatures during the composting process were also caused by the turning of the contents of the bins, during which air channels and fungal flakes 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 or around 40°C during the entire test.

Figure 4 shows the CO_2 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 CO_2 production gradually decreased. From week 1 till week 4 a higher CO_2 production was measured in the test bins compared to the control bins, which was in line with the higher temperatures and indicates that the test material was degrading. At the end of the test a low activity was found for all test series, indicating that the composting process was completed.

The oxygen concentration of the exhaust air is given in Figure 5. The oxygen concentration remained always above 10%. Good aerobic conditions were guaranteed during the test.

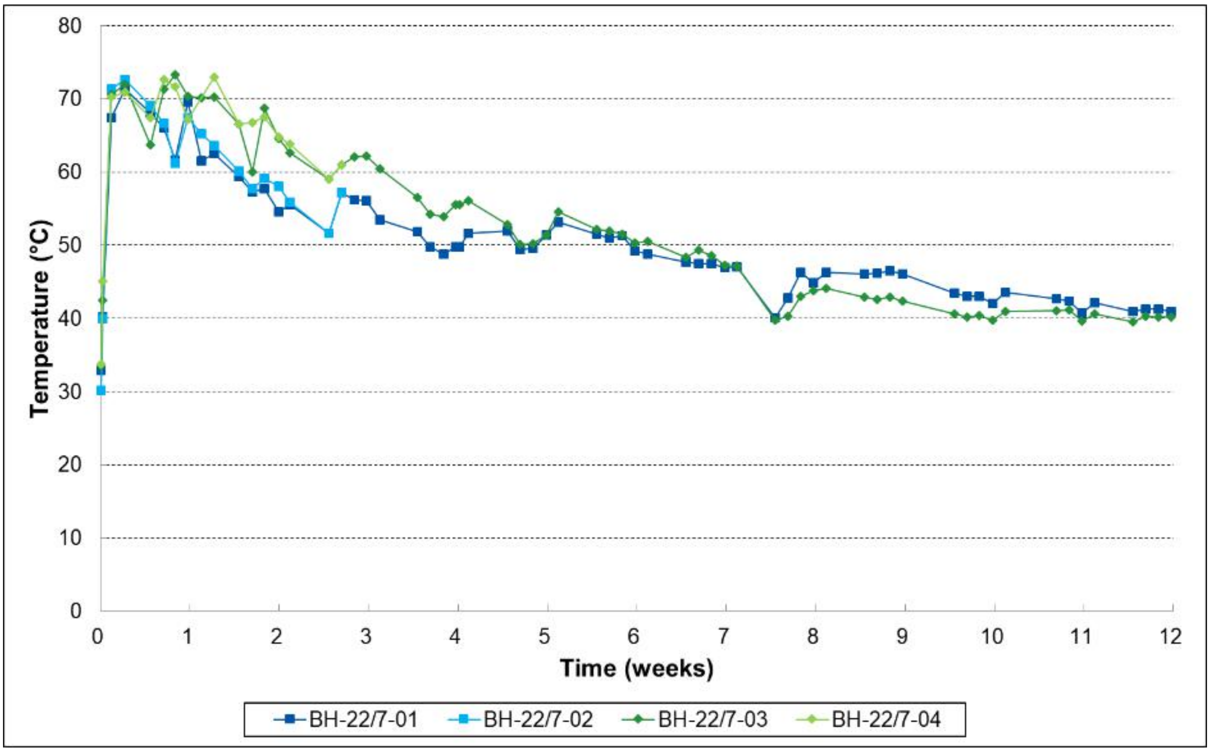

Figure 3. Temperature evolution during the composting test

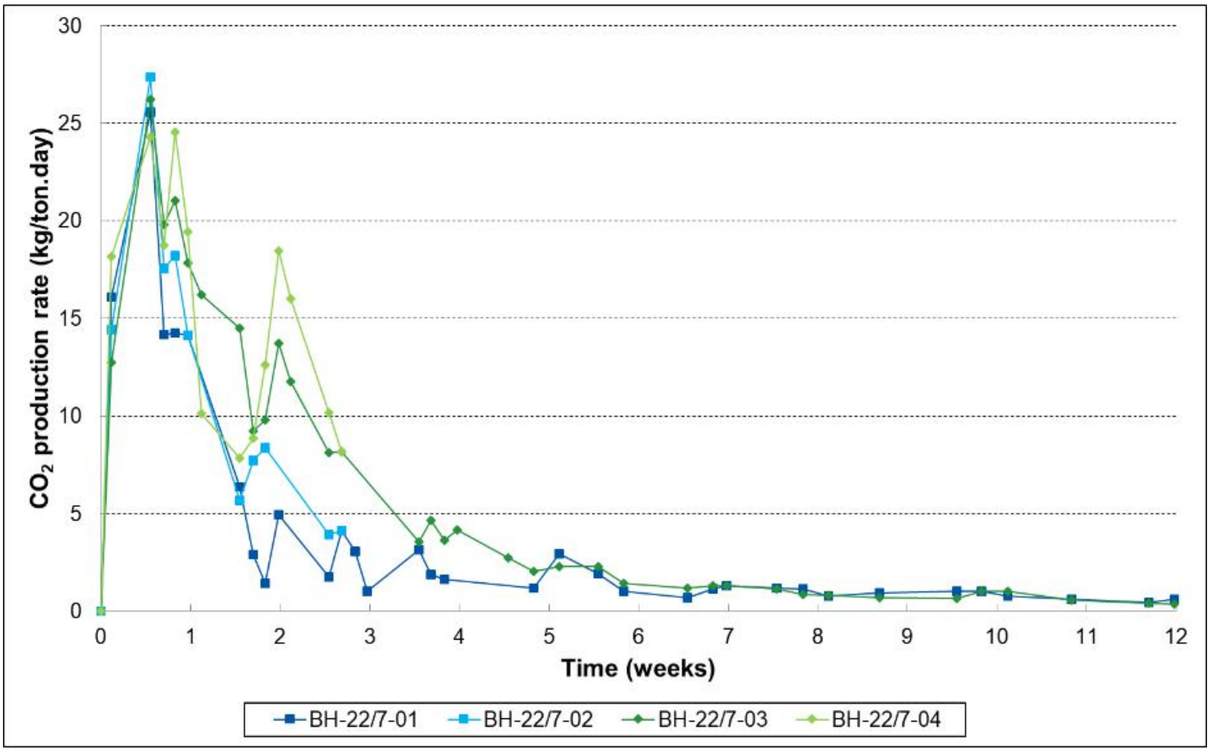

Figure 4. CO_2 production rate during the composting test (individual measurements at regular points in time)

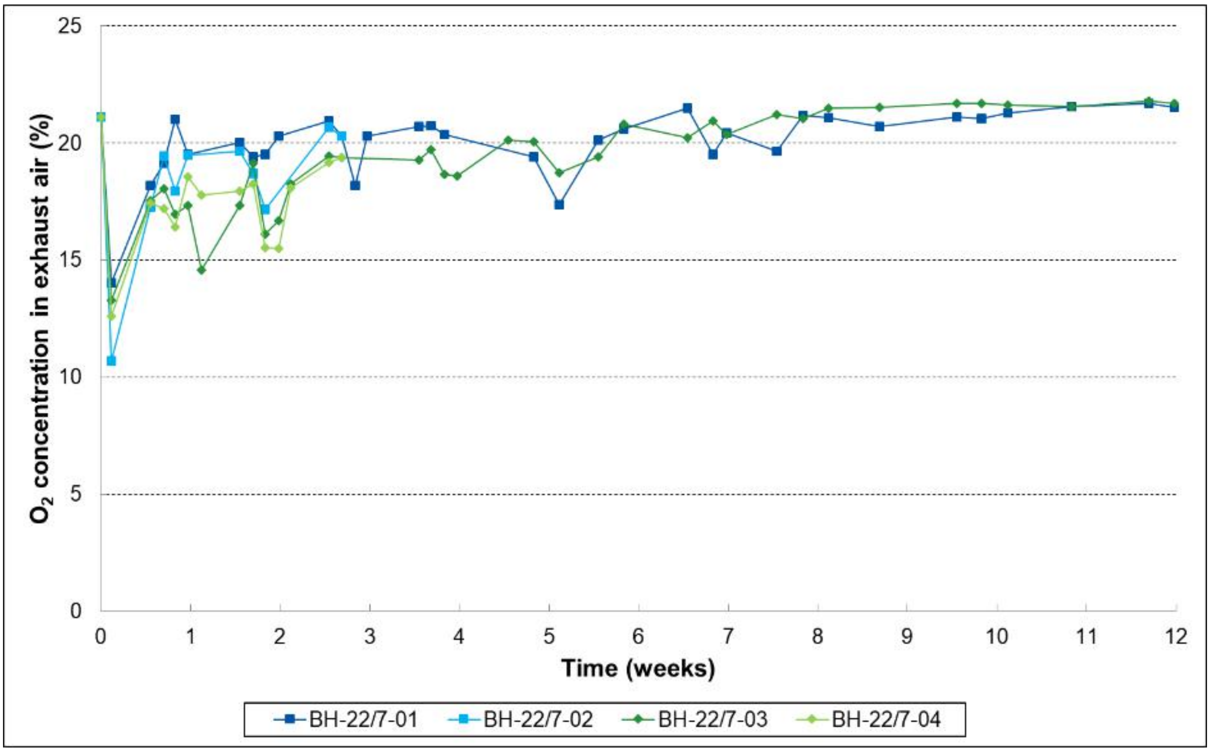

Figure 5. O_2 concentration in the exhaust air during the composting test

8.4 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 pH of 5.3 and after 1.7 weeks of composting the pH had already increased till above 8.1 for all test series. During the further test period the pH remained above 7.2. At the end of the test (after 12 weeks) an average pH of 8.2 and 7.8 was measured for the control composts and the test composts, respectively.

The biowaste at start contained an ammonium content of 408 mg NH_4^+-N/l. An initial decrease of the ammonium content was observed in all replicates, followed by an increase. After 5.9 weeks low ammonium levels (< 20 mg NH_4^+-N/l) were obtained for both test replicates. These low ammonium levels were maintained till the end of the test. The ammonium content of the control replicates decreased somewhat slower, but at the end of the test also low ammonium levels were measured.

After 5.9 weeks of composting an increase of the NO_x^- -N concentration was noticed in both test replicates followed by both control bins 1.7 weeks later. At the end of the test an average NO_x^- -N content of 318 mg NO_x^- -N/l (control composts) and 605 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 NO_x^- -N content had increased. This indicates that the nitrification process had started and was proceeding well.

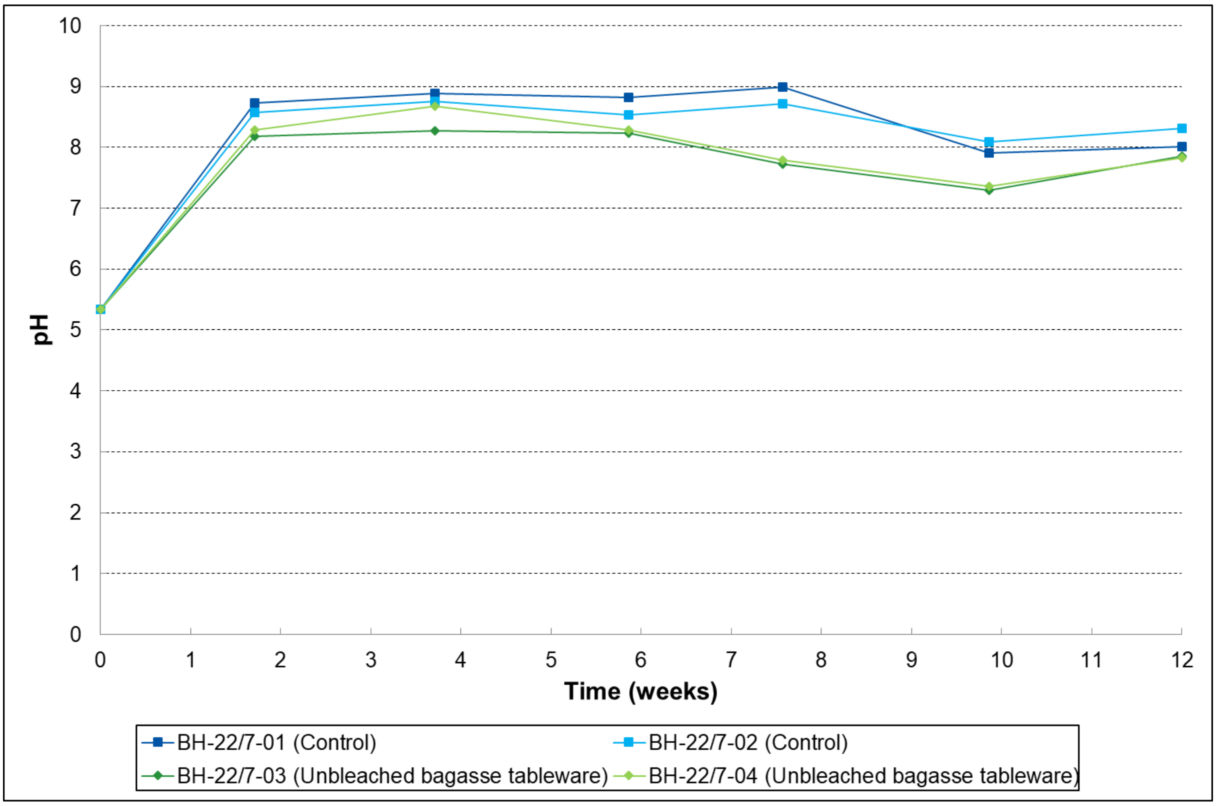

Figure 6. Evolution of pH during composting cycle

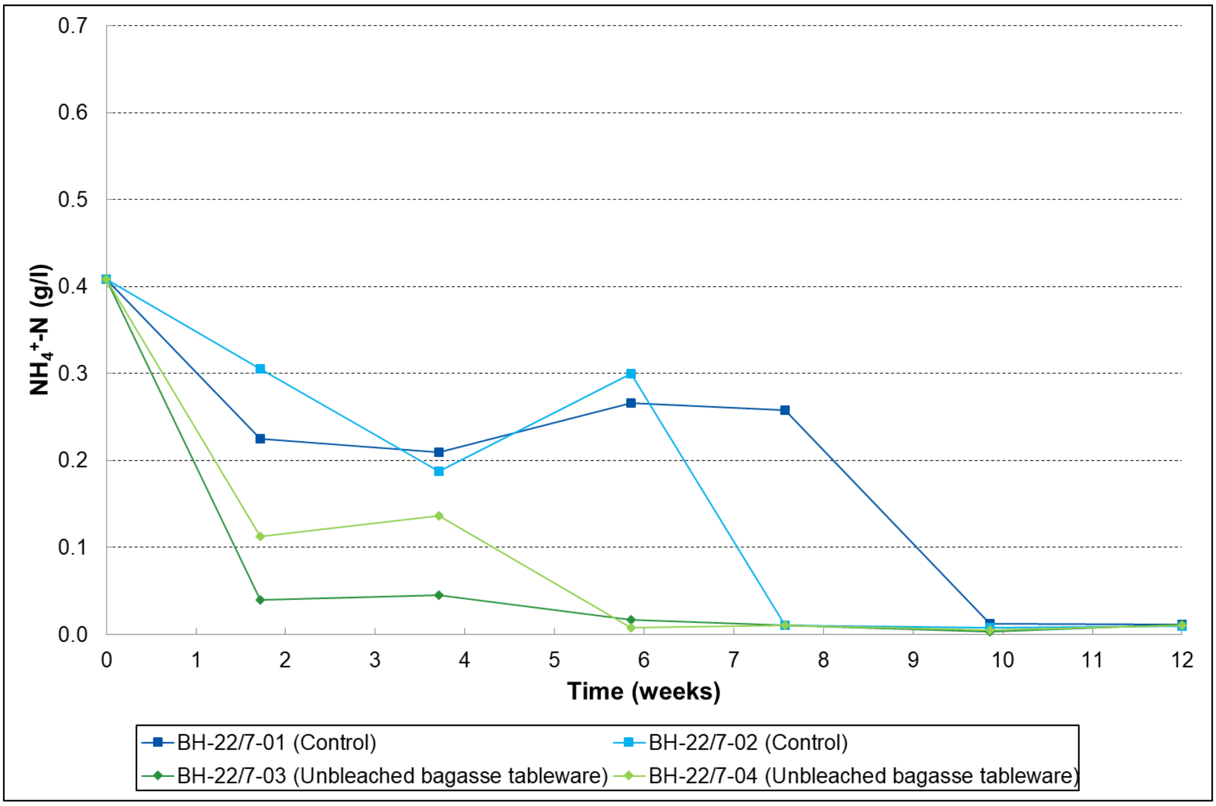

Figure 7. Trend of NH_4^+-N during composting cycle

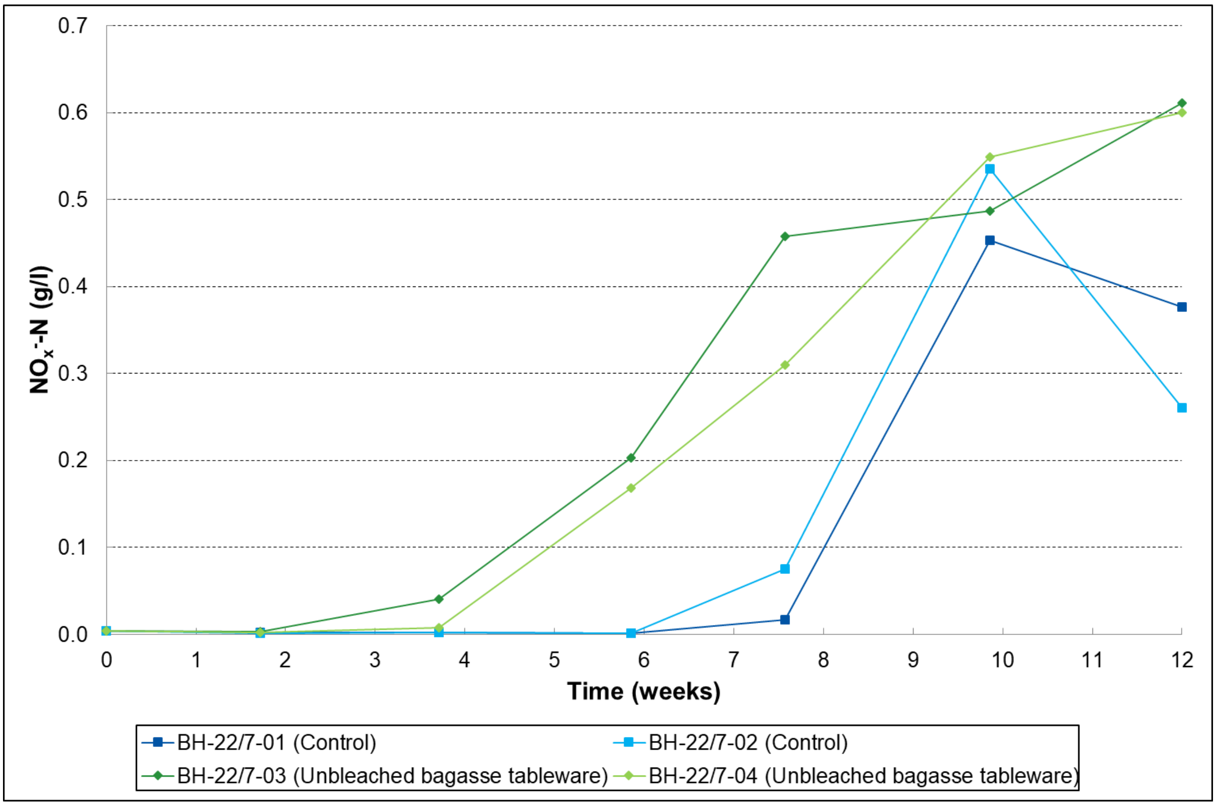

Figure 8. Trend of NO_x^- -N during composting cycle

8.5 Chemical analyses

At the end of the composting test, the whole contents of the bins were 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 4.

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 4 it can be seen that these requirements were fulfilled for all compost series.

The quality of the composts to which 10% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 8.2 and 7.8 was measured for the control composts and the test composts, respectively. Higher, but still normal salt levels were found in the test composts when compared to the control composts. At the end of the test low NH_4^+-N levels were found in all composts, while the NO_x^- -N content had increased. After 12 weeks an average NO_x^- -N content of 318 mg NO_x^- -N/l (control composts) and 605 mg NO_x^- -N/l (test composts) was measured. This indicates that the nitrification process had started and was proceeding well. A rather comparable average P, K and Mg content was obtained for the control composts and the test composts, while a lower average N level was noted for the test composts compared to the control composts. An average density of 0.558 kg/l and 0.561 kg/l was found for the control composts and the test composts, respectively. The C/N ratio varied between 8 and 11.

A higher average volatile solids content was measured for the < 10 mm fraction of the control composts when compared to the test composts. Moreover, a higher average volatile solids degradation was measured for the test series (Table 5), indicating that the test material was degrading. The high average volatile solids degradation for all series, demonstrates that the composting process has proceeded well.

Table 4. Chemical analysis of the compost fractions after 12 weeks of composting

Parameter	Control composts		Test composts	
	BH-22/7-01	BH-22/7-02	BH-22/7-03	BH-22/7-04
> 10 mm fraction				
Total solids (TS, %)	66.2	60.7	69.2	66.1
Volatile solids (VS, % on TS)	49.1	49.3	43.0	51.9
Ash (% on TS)	50.9	50.7	57.0	48.1
< 10 mm fraction				
Total solids (TS, %)	70.4	65.6	67.0	69.5
Volatile solids (VS, % on TS)	48.7	43.9	31.7	32.9
Ash (% on TS)	51.3	56.1	68.3	67.1
pH	8.0	8.3	7.9	7.8
Volatile fatty acids (VFA, g/l)	b.r.	b.r.	b.r.	b.r.
Total N (g/kg TS)	25.4	20.3	16.4	19.6
Total P (g/kg TS)	5.01	3.94	4.05	3.86
Total K (g/kg TS)	15.6	13.7	13.8	12.2
Total Mg (g/kg TS)	5.11	4.07	5.36	4.74
NH_4^+-N (mg/l)	11	b.r.	11	11
NO_x^--N (mg/l)	376	261	611	600
Electrical conductivity (µS/cm)	2990	3200	3410	3680
Rottegrad	V	V	V	V
Density (kg/l)	0.537	0.578	0.568	0.554
C/N	10	11	10	8

b.r. = below reporting limit
Reporting limit: VFA = 0.3 g/l
NH_4^+-N = 10 mg/l

Table 5. Volatile solids degradation for the different test series

Test series	Volatile solids degradation	
	Average %	%
Control composts		52.5
BH-22/7-01	51.4	
BH-22/7-02	53.6	
Test composts		69.2
BH-22/7-03	69.3	
BH-22/7-04	69.1	

Ecotoxicity tests

Cress test
on compost residuals of Unbleached bagasse tableware

Report R-BH-22/8

FINAL REPORT
BH-22/8

Ecotoxicity test – Cress test on compost residuals of **Unbleached bagasse tableware**

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1 Identification of the test

1.1 General information

Project number

BH-22/8

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Test item

Unbleached bagasse tableware compost

Reference item

Blank compost without any addition

1.2 Study personnel

Study Director:	Nike Mortier
Replacement Study Director:	Steven Verstichel
Study Director QA:	Inez Monteny

1.3 Study schedule

Study initiation date:	December 9th, 2019
Study completion date:	December 30th, 2019
Experimental starting date:	December 10th, 2019
Starting date of incubation:	December 12th, 2019
Completion date of incubation:	December 26th, 2019
Duration of incubation:	14 days
Experimental completion date:	December 30th, 2019

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:

BH-22/8

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.

p.p. Sam Deconinck

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 Dec-31-2019

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

Inez Monteny
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 Unbleached bagasse tableware was added in a 10% concentration to biowaste at start of the composting test. The pilot-scale composting test is reported in report R-BH-22/7.

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 was executed according to the following standards: 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), the Australian standard AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006), the American standard ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and the international standard ISO 18606 *Packaging and the environment - Organic recycling* (2013).

According to EN 13432 (2000), AS 4736 (2006), ASTM D6868 (2017) and ISO 18606 (2013) the germination rate and the plant biomass (on fresh weight basis or on dry weight basis) of the test compost should be more than 90% of those from the corresponding blank compost. The 90% pass level was reached for both the germination rate and the plant biomass (on fresh weight basis and on dry weight basis) of both mixtures of the test compost. Therefore, it can be concluded that the requirements of EN 13432 (2000), AS 4736 (2006), ASTM D6868 (2017) and ISO 18606 (2013) on ecotoxicity are fulfilled for cress plants.

In conclusion, it can be stated that, after composting Unbleached bagasse tableware 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 European norm EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), the Australian standard AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006), the American standard ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and the international standard ISO 18606 *Packaging and the environment - Organic recycling* (2013).

7 Materials and methods

7.1 Test items

The test items are compost samples obtained at the end of the pilot-scale composting test BH-22/7.

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 BH-22/7-01 and BH-22/7-02.

Test compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from bins BH-22/7-03 and BH-22/7-04 to which 10% Unbleached bagasse tableware (milled (< 4 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 ‘M_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 BH-22/7, 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 cress test

Treatment	Weight	
	Ref. Sub. (g/pot)	Compost (g/pot)
3 x Blank compost (25%)	172.5	57.5
3 x Blank compost (50%)	115.0	115.0
3 x Test compost (25%)	172.5	57.5
3 x Test compost (50%)	115.0	115.0

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 98%, which is well above the recommended value of 90%.

8.2 Germination and yield

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 (%)

Test series	Germination rate (%)	
	AVG	SD
Blank compost 25%	98.3	0.6
Blank compost 50%	99.0	1.0
Test compost 25%	98.3	1.2
Test compost 50%	97.7	2.1

With AVG = average, SD = standard deviation.

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

Test series	Fresh weight yield (g)	
	AVG	SD
Blank compost 25%	7.95	0.14
Blank compost 50%	6.37	0.29
Test compost 25%	7.70	0.11
Test compost 50%	6.21	0.17
Test series	**Dry weight yield (g)**	
	AVG	**SD**
Blank compost 25%	0.38	0.01
Blank compost 50%	0.37	0.01
Test compost 25%	0.37	0.01
Test compost 50%	0.36	0.02

With AVG = average, SD = standard deviation.

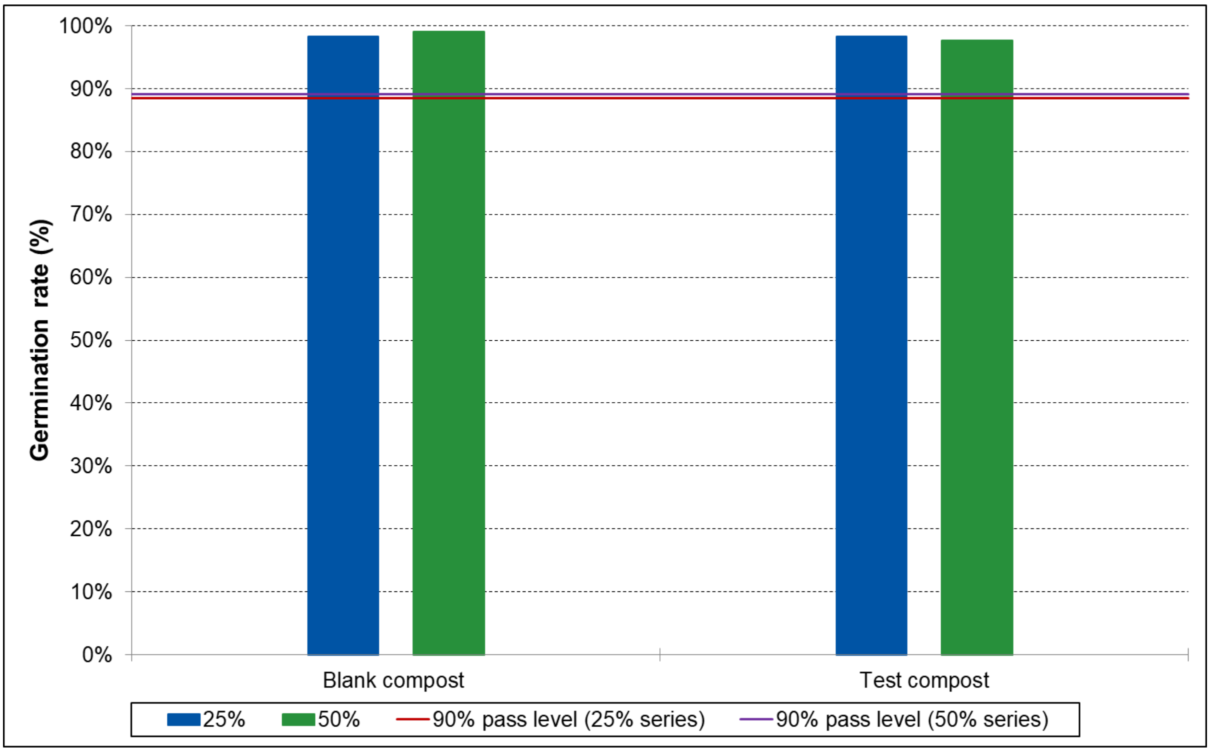

Figure 1. Average germination rate
(as percentage to the total amount of seeds added at start)

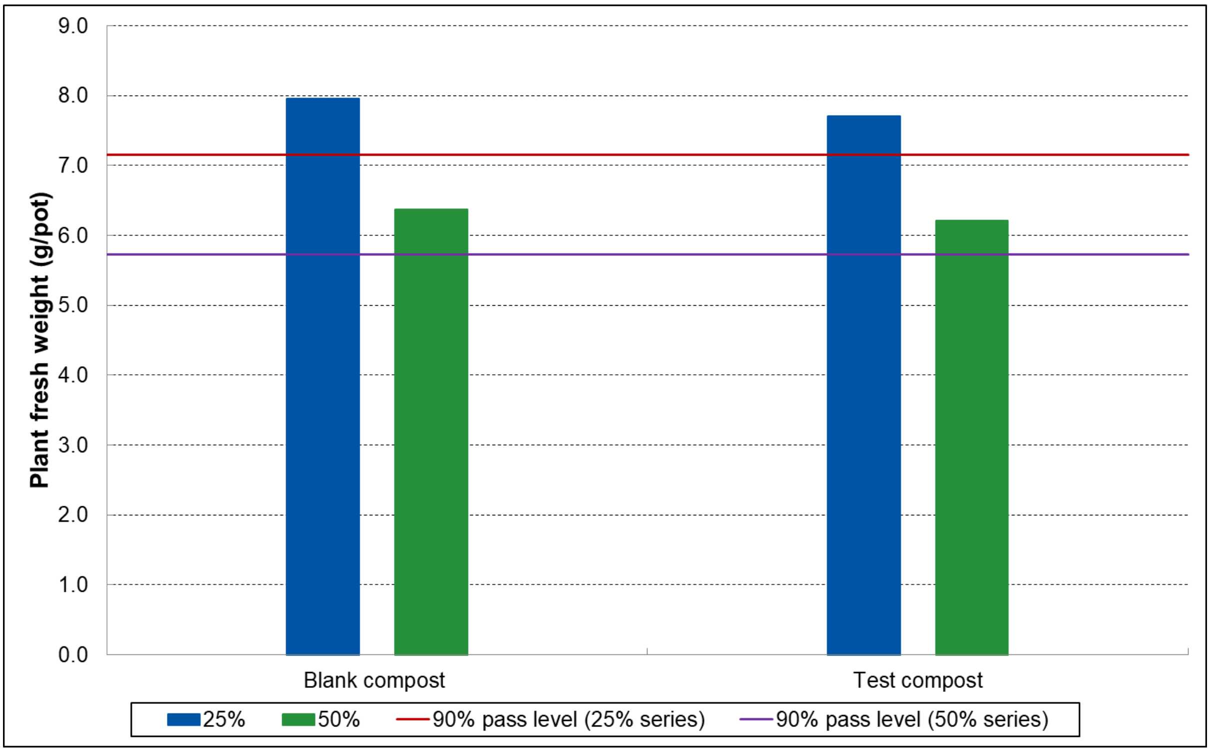

Figure 2. Absolute plant fresh weight (g/pot)

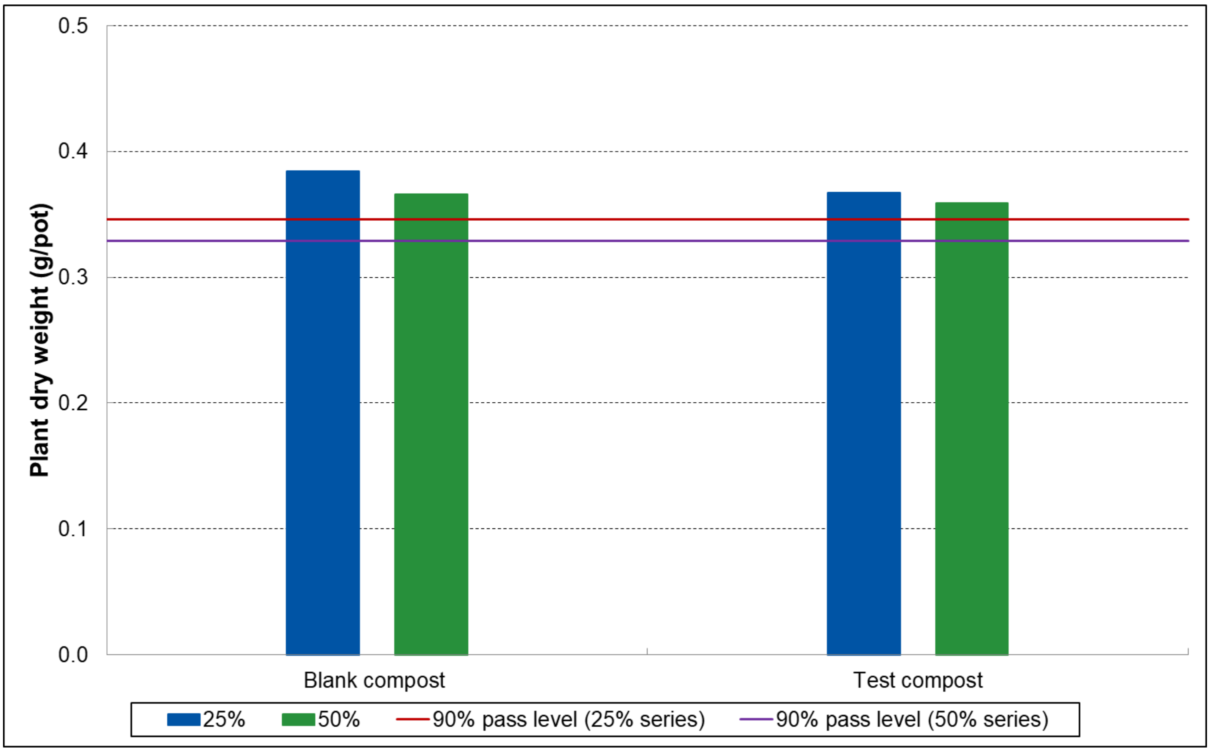

Figure 3. Absolute plant dry weight (g/pot)

According to EN 13432 *Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging* (2000), AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006), ASTM D6868 *Standard Specification for Labeling of End Items that Incorporate Plastics and Polymers as Coatings or Additives with Paper and Other Substrates Designed to be Aerobically Composted in Municipal or Industrial Facilities* (2017) and ISO 18606 *Packaging and the environment - Organic recycling* (2013) 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.

The 90% pass level was reached for both the germination rate and the plant biomass (on fresh weight basis and on dry weight basis) of both mixtures of the test compost (see Table 4). Therefore, it can be stated that the requirements of EN 13432 (2000), AS 4736 (2006), ASTM D6868 (2017) and ISO 18606 (2013) 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

Test series	Germination	Fresh weight plant yield	Dry weight plant yield
Test compost 25%	100	97	96
Test compost 50%	99	98	98

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 Unbleached bagasse tableware in a 10% concentration, no residuals are left behind that 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 7 days (from bottom to top): 25% series of blank compost and test compost

Figure 5. Detailed cress plant growth after an incubation period of 7 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 7 days (from bottom to top): 50% series of blank compost and test compost

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

Pilot-scale composting test for the production of compost for subsequent ecotoxicity testing

Report R-BH-22/9

FINAL REPORT
BH-22/9
Pilot-scale composting test for production of compost for subsequent ecotoxicity testing on **Unbleached bagasse tableware**

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1 Identification of the test

1.1 General information

Project number

BH-22/9

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Test item

Unbleached bagasse tableware

1.2 Study personnel

Study Director:	Kwok Kuen Chow
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Study Director QA:	Steven Verstichel
	Eveline Beeckman

1.3 Study schedule

Study initiation date:	April 14th, 2020
Proposed experimental starting date:	April 14th, 2020
Proposed starting date of incubation:	April 23rd, 2020
Proposed completion date of incubation:	July 16th, 2020
Experimental completion date:	August 14th, 2020
Study completion date:	August 28th, 2020

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:

BH-22/9

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).

p.p. Krystle De Preester

Kwok Kuen Chow
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 Sep-09-2020

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

In this pilot-scale composting test according to ISO 16929 (2019), simulating industrial composting processes, compost was produced for subsequent ecotoxicity tests on Unbleached bagasse tableware. Test method ISO 16929 (2019) prescribes that a test item shall be added in a concentration of 10% to biowaste in order to prepare compost for subsequent toxicity tests. Therefore, test item Unbleached bagasse tableware, milled (< 4 mm) was added in a concentration of 10% to biowaste at start of the pilot-scale composting test. 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 over a mesh size of 10 mm.

The operational parameters showed that the test was valid. The temperature profile showed an initial thermophilic phase and a mesophilic continuation, which is representative for industrial composting. The oxygen concentration remained always above 10%. As such, good aerobic conditions were guaranteed during the test. The pH of the biowaste at start was 6.3 and after 1.7 weeks of composting the pH had already increased till above 7.9 for all test series. During the further test period the pH remained above 7.3.

The quality of the composts to which 10% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 8.0 and 7.7 was measured for the control composts and the test composts, respectively. Lower average salt levels were found in the test composts (2340 µS/cm) when compared to the control composts (2670 µS/cm). A low salt content is beneficial for the compost quality. At the end of the test low NH_4^+-N levels were found in all composts, while the NO_x^--N content had increased. After 12 weeks an average NO_x^--N content of 317 mg NO_x^--N/l and 518 mg NO_x^--N/l was measured for control composts and test composts, respectively. This indicates that the nitrification process had started and was proceeding well. A rather comparable average nutrients (nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg)) content was obtained for the control composts and the test composts. An average density of 0.543 kg/l and 0.521 kg/l was found for the control composts and the test composts, respectively. The C/N ratio varied between 7 and 9. A comparable volatile solids content was measured for the < 10 mm fraction of the control composts and the test composts. A higher average volatile solids degradation was measured for the test series when compared to the control series, indicating that the test material was degrading. Moreover, a high average volatile solids degradation was obtained for all series, demonstrating that the composting process has proceeded well.

In conclusion it can be stated that no negative effect on the composting process and on the (physico-chemical) quality of the produced compost was observed, when adding 10% Unbleached bagasse tableware at start of the composting process.

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 10 mm. 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

- ISO 16929 *Plastics – Determination of the Degree of Disintegration of Plastic Materials under Defined Composting Conditions in a Pilot-Scale Test* (2019)

7 Materials and methods

7.1 Test item

Name:	Unbleached bagasse tableware
Description:	Paper bowl (Figure 1)
Colour:	White
Total solids (TS):	96.1%
Volatile solids (VS):	97.9% on TS
Sample preparation:	Milled (< 4 mm)
Storage conditions:	Room temperature in the dark

Figure 1. Visual presentation of test item Unbleached bagasse tableware

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.

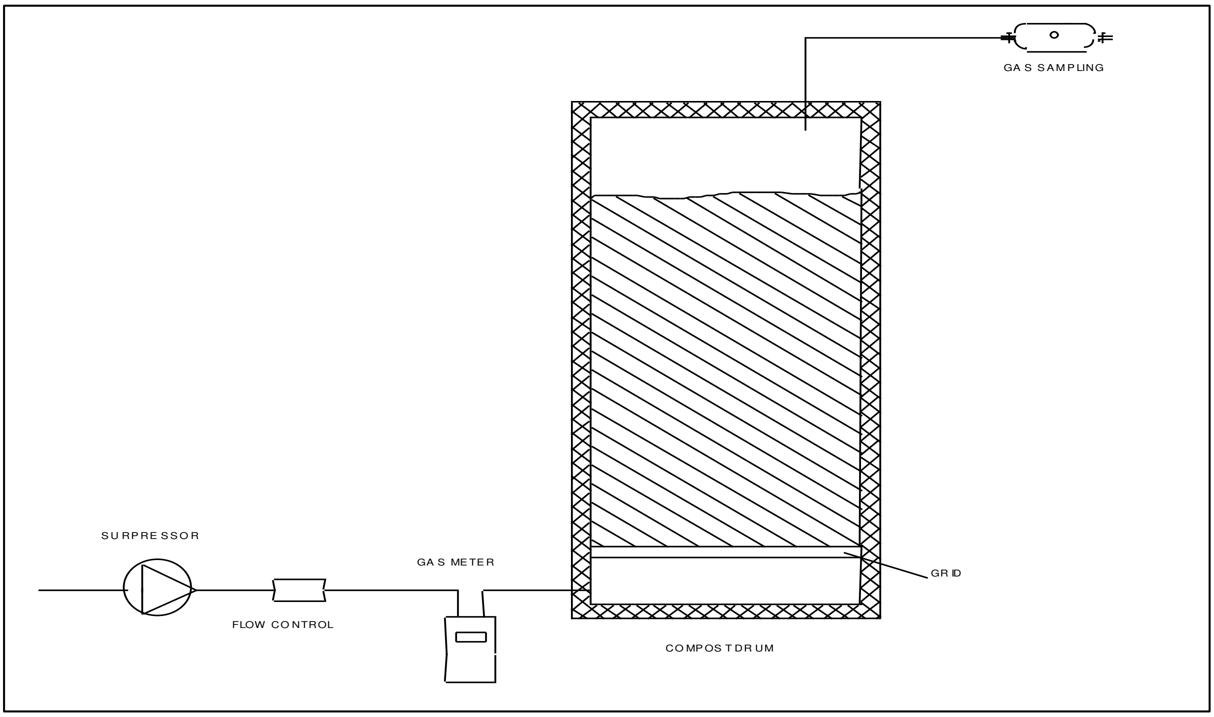

Figure 2. Set-up pilot-scale aerobic composting test

7.3 Analytical methods

Ammonium - nitrogen (NH_4^+-N)

This analysis is performed as described in 'M_054. Determination of ammonium nitrogen by a discrete analyser system and spectrophotometric detection'. The ammonium-N is determined in an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Ammonia reacts with hypochlorite ions generated by the alkaline hydrolysis of sodium dichloroisocyanurate to form monochloramine. This reacts with salicylate ions in the presence of sodium nitroprusside at around pH 12.6 to form a blue compound. The absorbance of this compound is measured spectrophotometrically at the wavelength 660 nm and is related to the ammonia concentration by means of a calibration curve. The results are given in 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 'M_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 'I_435. Manual TotalChrom software'. The gas chromatograph is calibrated with a standard gas mixture consisting of 10% O_2, 20% CO_2, 30% N_2 and 40% CH_4. Every day gas analyses were executed the gas chromatograph is validated. The results are given in per cent.

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

This analysis is done as described in ‘M_055. Determination of total oxidized nitrogen by a discrete analyser system and spectrophotometric detection’. The determination is performed on an aqueous extract (5 parts of demineralised water versus 1 part of sample; see M_057). Nitrate is reduced to nitrite by hydrazine under alkaline conditions. The total nitrite ions are then reacted with sulphanilamide and N-1-napthylethylenediamine dihydrochloride under acidic conditions to form a pink azo-dye. The absorbance is measured at 540 nm and is related to the Total Oxidized Nitrogen (TON) concentration by means of a calibration curve. The results are given in mg 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 ‘M_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 ‘M_057. Extraction of water and potassium chloride soluble nutrients and elements’.

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 ‘M_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 ‘M_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 ‘M_057. Extraction of water and potassium chloride soluble nutrients and elements’. The results are given in µS/cm.

Total nitrogen (N)

This analysis is done as described in ‘M_039. Determination of total organic carbon and total nitrogen – Method by total carbon, total nitrogen and inorganic carbon combustion’. By combusting the sample at 950°C – 1200°C and adding a controlled extra dose of oxygen for a short time, the nitrogen components will oxidize to nitrogen oxides (NO_x). In the presence of a CuO catalyst and a copper reducer the nitrogen oxides are converted to N_2. The formed N_2 is measured by a Thermal Conductivity Detector (TCD). The results are given in g per kg total solids.

Total phosphorus (P), potassium (K) and magnesium (Mg)

This analysis is done as described in ‘M_053. Determination of Aqua Regia Soluble Elements by Inductively Coupled Plasma Optical Emission Spectrometry’. The content of these elements in the compost is determined after extraction with aqua regia according to ‘M_048. Extraction of Aqua Regia Soluble Elements’. The total P, K and Mg content is expressed as g per kg total solids.

Volatile fatty acids (VFA)

The volatile fatty acids are determined as described in 'M_035. Determination of volatile fatty acids by gas chromatography and flame ionization detector'. 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 'M_057. Preparation of extracts and analysis solutions' 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 FFAP of 30 m. The carrier gas is H_2. A mixture with precise concentrations of eight reference volatile fatty acids is used for calibration while 2-methyl-caproic acid is used as an internal standard. The results are given in g per l wet weight.

Volatile solids (VS) - ash

The volatile solids and ash contents are determined by heating the dried sample at 550°C for at least 4 hours and weighing, as described in 'M_010. Determination of organic matter and carbon content'. The results are given in percent on dry matter.

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 'M_011. Determination of volumetric density'.

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

Four composting bins with a total volume of 200 l each were started: two control bins (BH-22/9-01 and BH-22/9-02) and two test bins (BH-22/9-03 and BH-22/9-04). The control bins contained only biowaste, while the test bins contained also 10% Unbleached bagasse tableware (milled, < 4 mm). The 10% Unbleached bagasse tableware was necessary to cover subsequent ecotoxicity tests on Unbleached bagasse tableware.

The exact test set-up is given in Table 1. 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 1. Test set-up

Composition	Control bins		Test bins	
	BH-22/9-01	BH-22/9-02	BH-22/9-03	BH-22/9-04
VGF (kg)	55.2	55.2	55.2	55.2
Structural material (kg)	6.0	6.0	6.0	6.0
Unbleached bagasse tableware, milled (< 4 mm) (kg)	-	-	6.12	6.12
% Unbleached bagasse tableware on biowaste	-	-	10.0	10.0

8.2 Analyses 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 Erembodegem, Belgium. The characteristics of VGF and structural material are given in Table 2. Table 3 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 2 and 3 it can be seen that these requirements were fulfilled. The biowaste contained a moisture content of 63.9% and a volatile solids content of 59.5% on TS. At start-up a pH of 6.3 was measured. Furthermore, the C/N ratio of the biowaste at start should preferably be between 20 and 30. A somewhat lower C/N ratio of 19 was obtained for the biowaste. The somewhat lower C/N ratio of the biowaste did not really hinder the test. A low C/N ratio results from a high level of nitrogen in the biowaste (e.g. due to many proteins). This can lead to NH_3 emission (and odour) and eventually slow or difficult nitrification towards the end of the composting cycle. It must be noted that mainly a high C/N ratio can be disadvantageous for the composting process, as this is indicative for N deficiency. The test bins with 10% test material showed a higher and optimal C/N ratio of 27 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 (2019), EN 13432 (2000), AS 4736 (2006), ASTM D6868 (2019) and ISO 18606 (2013).

Table 2. Characteristics of VGF and structural material

Characteristics	VGF	Structural material
Total solids (TS, %)	34.2	53.4
Moisture content (%)	65.8	46.6
Volatile solids (VS, % on TS)	53.6	94.1
Ash content (% on TS)	46.4	5.9
pH	6.3	-
Electrical conductivity (EC, µS/cm)	1560	-
Volatile fatty acids (VFA, g/l)	1.7	-
NO_x^--N (mg/l)	b.r.	-
NH_4^+-N (mg/l)	206	-
Total N (g/kg TS)	16.4	11.2
C/N	16	42

b.r. = below reporting limit
Reporting limit: NO_X^--N = 10 mg/l

Table 3. Characteristics of the biowaste and biowaste with test item

Characteristics	Biowaste (= VGF + structural material)	Biowaste + 10% test item
Total solids (TS, %)	36.1	41.5
Moisture content (%)	63.9	58.5
Volatile solids (VS, % on TS)	59.5	67.6
Ash content (% on TS)	40.5	32.4
Total N (g/kg TS)	15.7	12.6
C/N	19	27

8.3 Temperature profile and analyses exhaust air

Figure 3 shows the temperature evolution during the composting process. According to ISO 16929 (2019) the test is considered valid if following temperature profile is obtained:

- Days 2–7: between 60°C and 75°C;
- Days 8–28: between 55 (±5)°C and 65 (±5)°C;
- Days 29–56: between 50 (±5)°C and 60 (±5)°C;
- Days 57–84: below 45°C.

As can be seen from Figure 3 these requirements were largely fulfilled. After start-up the temperature increased almost immediately till above 60°C and stayed below 75°C. Afterwards all bins remained between the temperature criteria, except after 9 days of composting when the 70°C limit was exceeded for both test bins with a maximum value of 72.6°C. However, immediate action was taken and the temperature was decreased. After 8 days of composting control bin BH-22/9-02 was placed in an incubation room at 42°C to ensure high temperatures and one day later the same was done for all other bins. After 7.8 weeks of composting all bins were placed in an incubation room at 37°C to ensure the 45°C limit was not exceeded during the last 4 weeks of composting. Furthermore, after 2.7 weeks of composting the contents of the control bins (BH-22/9-01 and BH-22/9-02) were 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. At the same moment the contents of the test bins (BH-22/9-03 and BH-22/9-04) were also combined into one bin, separated by a net. The combination of the bins resulted in a temperature increase. Elevated temperatures during the composting process were also caused by the turning of the contents of the bins, during which air channels and fungal flakes 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 profile showed an initial thermophilic phase and a mesophilic continuation, which is representative for industrial composting and therefore it can be concluded that the temperature conditions were fulfilled. It was noticed that the temperature in the test bins with 10% test material was higher when compared to the control bins during almost the entire period between 1.3 and 4.6 weeks of composting. This indicates that the test material was degrading.

Figure 4 shows the CO_2 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 CO_2 production gradually decreased. 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 CO_2 production rate in the test bins with 10% test material was higher when compared to the control bins in the period between 1.3 and 3.5 weeks of composting. This is in line with the temperature measurements and confirms 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%. Good aerobic conditions were guaranteed during the test.

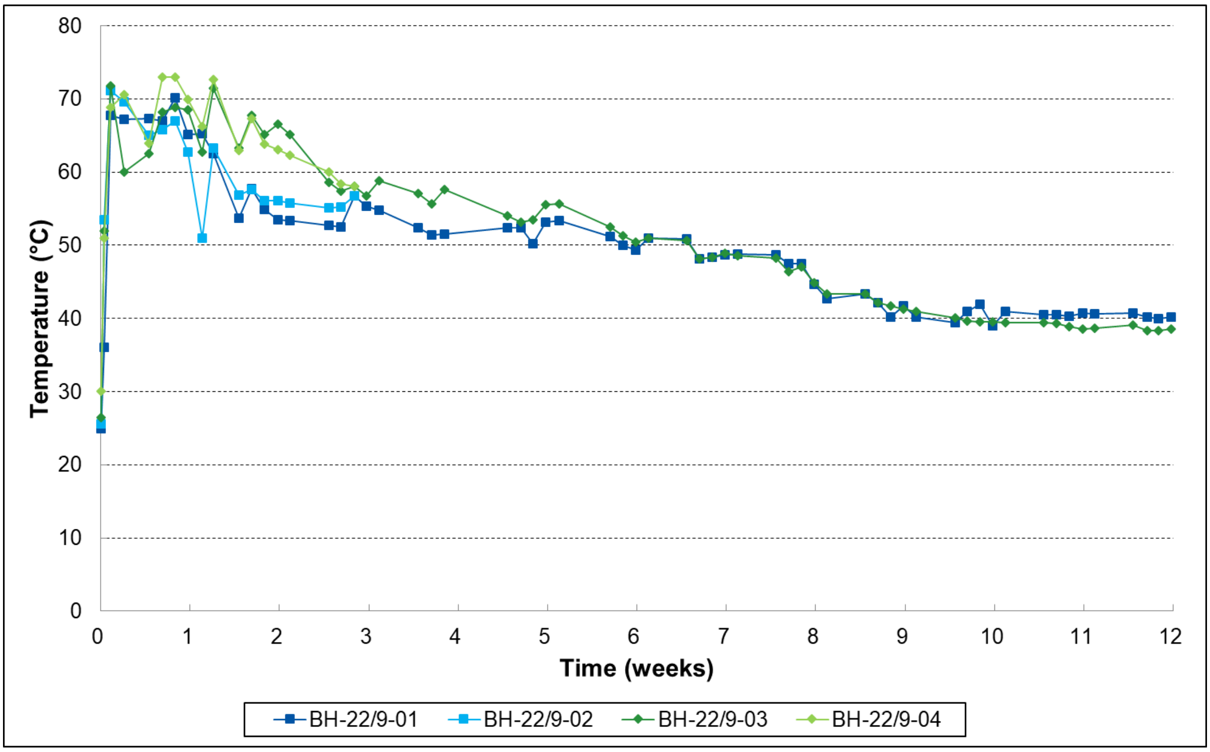

Figure 3. Temperature evolution during the composting test

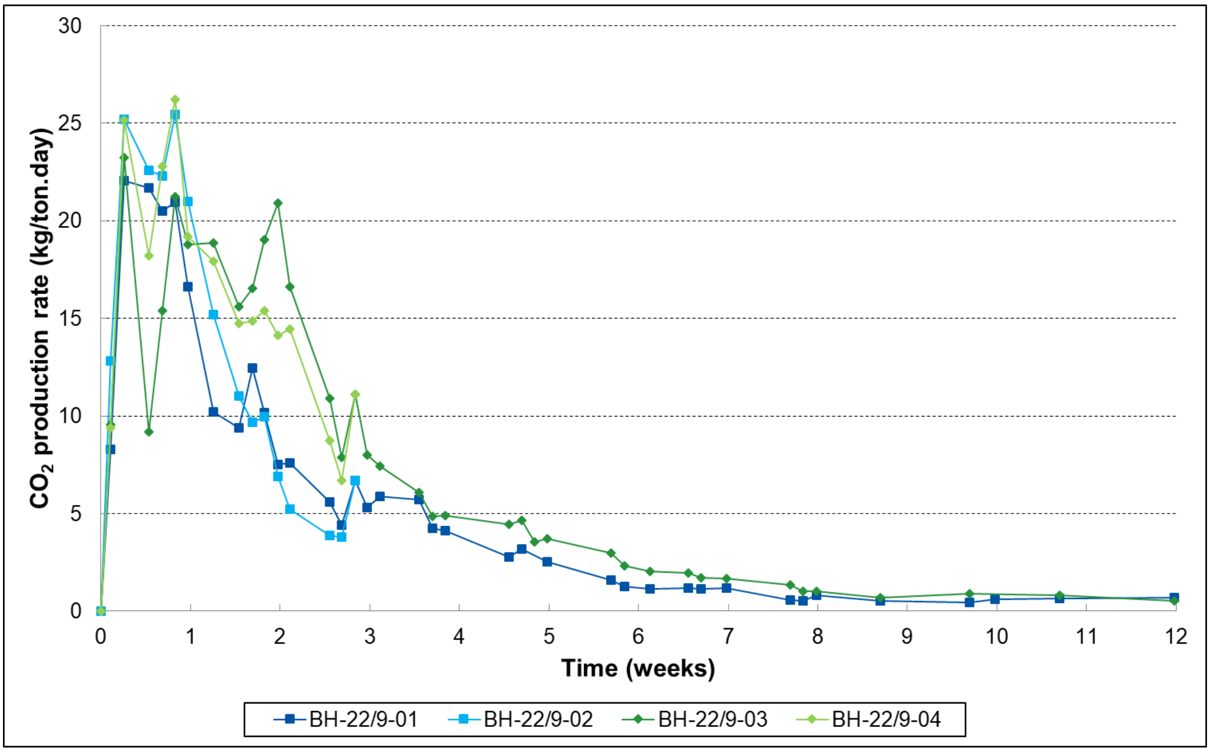

Figure 4. CO_2 production rate during the composting test (individual measurements at regular points in time)

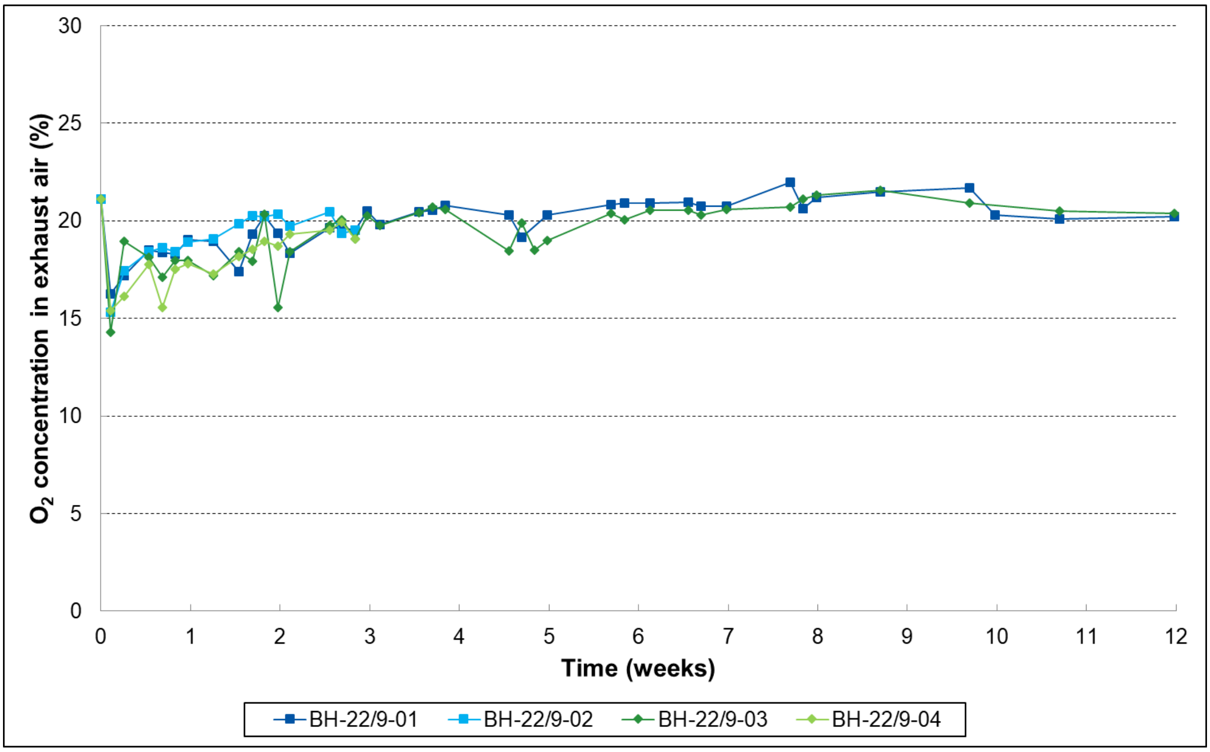

Figure 5. O_2 concentration in the exhaust air during the composting test

8.4 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 (2019) the pH should increase till a value above 7 during composting and not fall below 5. The biowaste at start showed a pH of 6.3 and after 1.7 weeks of composting the pH had already increased till above 7.9 for all test series. During the further test period the pH remained above 7.3. At the end of the test (after 12 weeks) an average pH of 8.0 and 7.7 was measured for the control composts and the test composts, respectively.

The biowaste at start contained an ammonium content of 206 mg NH_4^+-N/l. The ammonium content decreased faster in the test replicates when compared to the control replicates. Already after 5.9 weeks of composting low ammonium levels (< 20 mg NH_4^+-N/l) were obtained for the test replicates. These low ammonium levels were maintained till the end of the test. At that moment also low ammonium levels were obtained for both control composts.

After 5.9 weeks of composting an increase of the NO_x^- -N concentration was noticed in both test replicates. Four weeks later the same had happened for both control replicates. At the end of the test an average NO_x^- -N content of 317 mg NO_x^- -N/l and 518 mg NO_x^- -N/l was found for control composts and test composts, respectively.

At the end of the test low NH_4^+-N levels were obtained for all replicates, while the NO_x^- -N content had increased. This indicates that the nitrification process had started and was proceeding well.

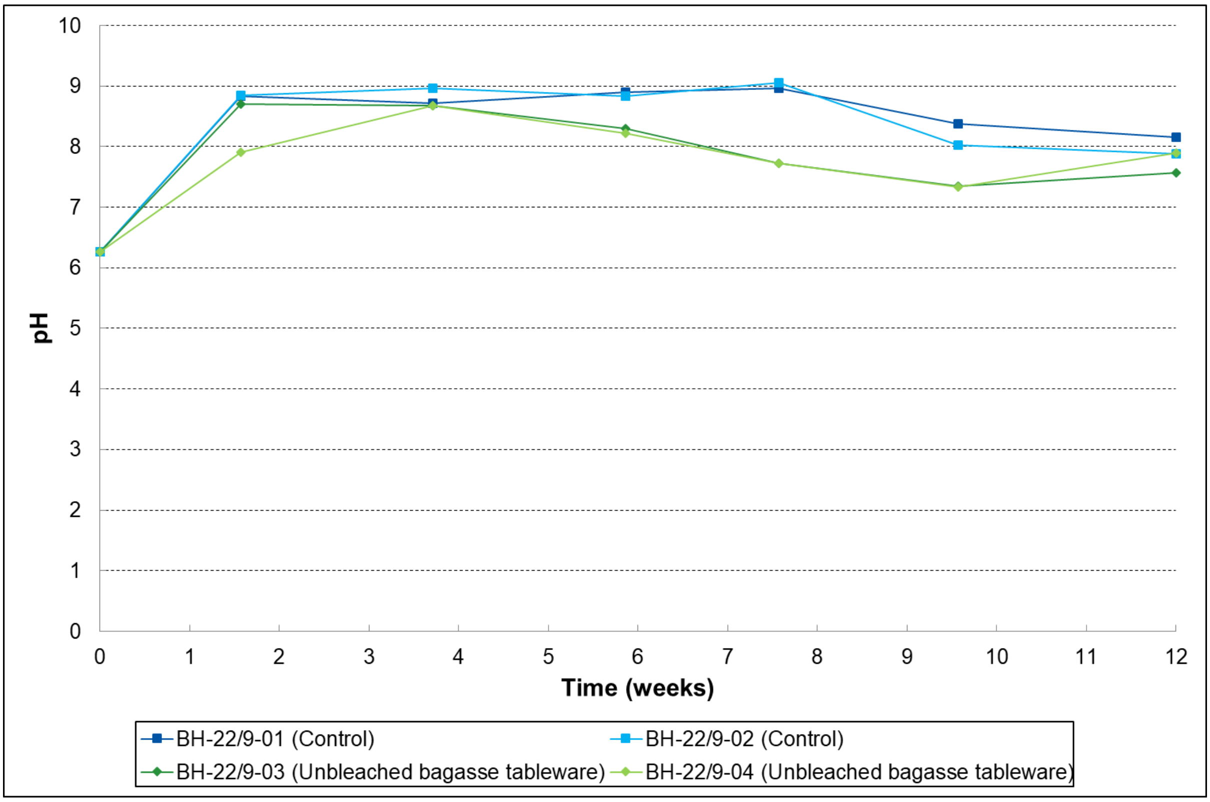

Figure 6. Evolution of pH during composting cycle

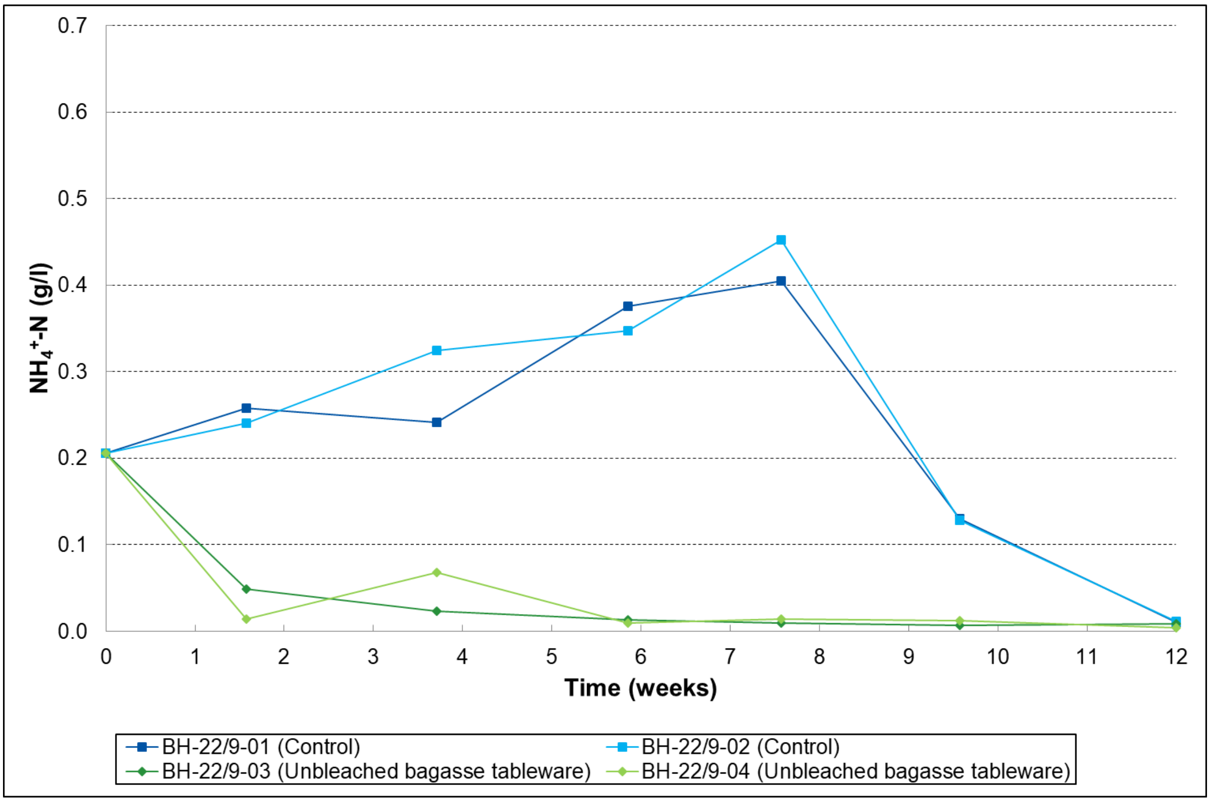

Figure 7. Trend of NH_4^+-N during composting cycle

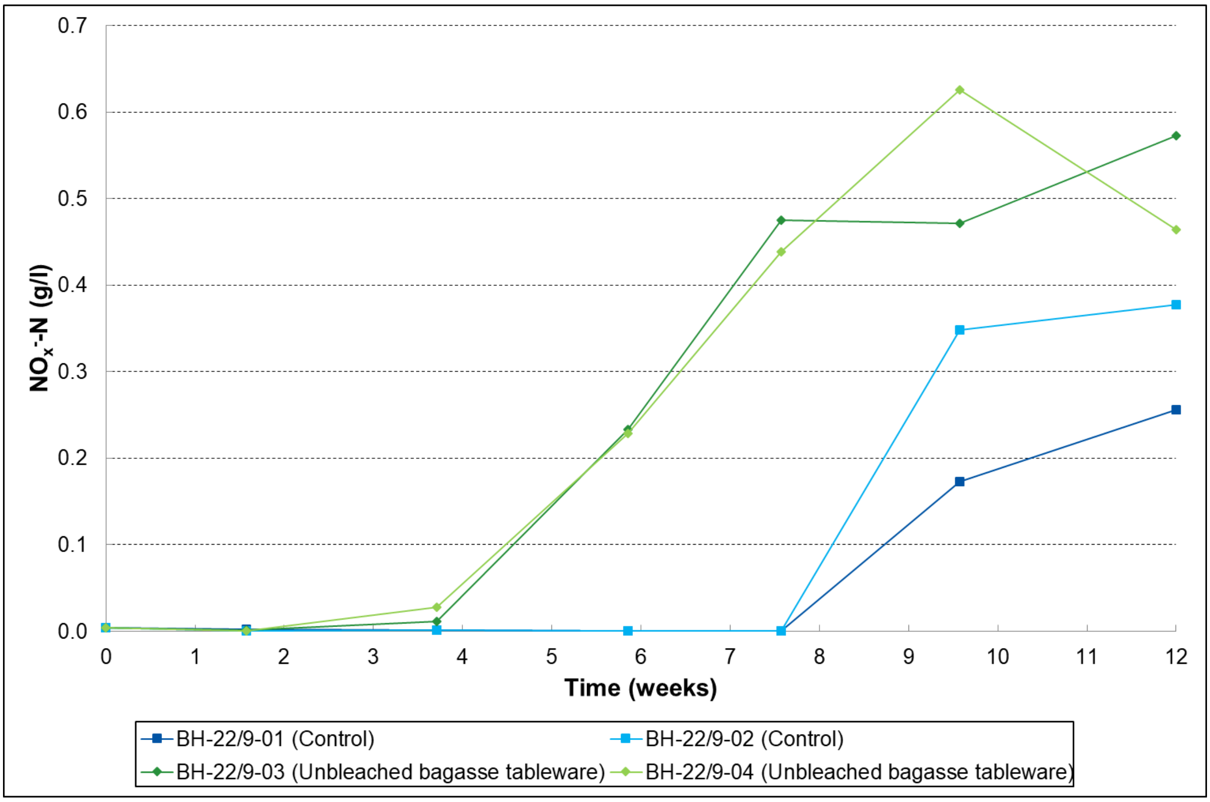

Figure 8. Trend of NO_x^- -N during composting cycle

8.5 Chemical analyses

At the end of the composting test, the whole contents of the bins were 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 4.

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 4 it can be seen that these requirements were fulfilled for all compost series.

The quality of the composts to which 10% Unbleached bagasse tableware was added at start of the composting cycle was equally good compared to the control composts. No volatile fatty acids were found in the test and control composts and they all showed a Rottegrad of V, which demonstrates that the composts were stable and mature. An average pH of 8.0 and 7.7 was measured for the control composts and the test composts, respectively. Lower average salt levels were found in the test composts (2340 µS/cm) when compared to the control composts (2670 µS/cm). A low salt content is beneficial for the compost quality. At the end of the test low NH_4^+-N levels were found in all composts, while the NO_x^--N content had increased. After 12 weeks an average NO_x^--N content of 317 mg NO_x^--N/l and 518 mg NO_x^--N/l was measured for control composts and test composts, respectively. This indicates that the nitrification process had started and was proceeding well. A rather comparable average nutrients (nitrogen (N), phosphorus (P), potassium (K) and magnesium (Mg)) content was obtained for the control composts and the test composts. An average density of 0.543 kg/l and 0.521 kg/l was found for the control composts and the test composts, respectively. The C/N ratio varied between 7 and 9.

A comparable volatile solids content was measured for the < 10 mm fraction of the test composts and the control composts. A higher average volatile solids degradation was measured for the test series when compared to the control series, indicating that the test material was degrading. Moreover, a high average volatile solids degradation was obtained for all series, demonstrating that the composting process has proceeded well (Table 5).

Table 4. Chemical analysis of the compost fractions after 12 weeks of composting

Parameter	Control composts		Test composts	
	BH-22/9-01	BH-22/9-02	BH-22/9-03	BH-22/9-04
> 10 mm fraction				
Total solids (TS, %)	69.2	49.2	59.1	51.1
Volatile solids (VS, % on TS)	31.1	37.6	30.1	40.8
Ash (% on TS)	68.9	62.4	69.9	59.2
< 10 mm fraction				
Total solids (TS, %)	65.6	51.9	56.1	51.3
Volatile solids (VS, % on TS)	32.9	35.5	33.0	35.5
Ash (% on TS)	67.1	64.5	67.0	64.5
pH	8.1	7.8	7.5	7.8
Volatile fatty acids (VFA, g/l)	b.r.	b.r.	b.r.	b.r.
Total N (g/kg TS)	20.4	19.0	23.0	20.8
Total P (g/kg TS)	4.42	3.99	3.62	3.75
Total K (g/kg TS)	16.0	14.3	14.3	14.6
Total Mg (g/kg TS)	3.94	3.85	4.57	4.37
NH_4^+-N (mg/l)	10.5	11.2	b.r.	b.r.
NO_x^--N (mg/l)	256	377	573	464
Electrical conductivity (µS/cm)	2850	2450	2630	2050
Rottegrad	V	V	V	V
Density (kg/l)	0.545	0.540	0.524	0.518
C/N	8	9	7	9

b.r. = below reporting limit
Reporting limit: VFA = 0.3 g/l
NH_4^+-N = 10 mg/l

Table 5. Volatile solids degradation for the different test series

Test series	Volatile solids degradation	
	Average %	%
Control composts	58.1	
BH-22/9-01		58.9
BH-22/9-02		57.2
Test composts	67.1	
BH-22/9-03		66.9
BH-22/9-04		67.2

Ecotoxicity tests

Earthworm acute toxicity test on compost residuals of Unbleached bagasse tableware

Report R-BH-22/10

FINAL REPORT
BH-22/10

Earthworm acute toxicity test
on compost residuals of
Unbleached bagasse tableware

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1 Identification of the test

1.1 General information

Project number

BH-22/10

Sponsor

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Test item

Unbleached bagasse tableware compost

Reference item

Blank compost without any addition

1.2 Study personnel

Study Director:	Sieglinde Debruyne
Replacement Study Director:	Steven Verstichel
Study Director QA:	Nike Mortier

1.3 Study schedule

Starting date study:	July 31st, 2020
Experimental starting date:	August 10th, 2020
Incubation starting date:	August 12th, 2020
Incubation completion date:	August 26th, 2020
Duration of incubation:	14 days
Experimental completion date:	August 26th, 2020
Completion date study:	September 4th, 2020

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:

BH-22/10

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).

Sieglinde Debruyne
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 Sep-08-2020

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

Nike Mortier
Study Director QA

5 Summary and conclusions

An earthworm, acute toxicity test was performed on Unbleached bagasse tableware compost obtained at the end of a pilot-scale composting test, in which Unbleached bagasse tableware was added in a 10% concentration to biowaste at start of the composting. The pilot-scale composting test is reported in report R-BH-22/9.

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

The test method is based on ASTM E1676 *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation tests with the Lumbricid Earthworm Eisenia Fetida and the Enchytraeid Potworm Enchytraeus albidus* (2012) with the modifications as prescribed in the Australian standard AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006).

100% survival was measured for the artificial soil, which means that the pass level of 90% survival is easily reached and that the test is valid.

According to the Australian standard AS 4736 *Biodegradable plastics - Biodegradable plastics suitable for composting and other microbial treatment* (2006) the difference in the morbidity or mean weight of the surviving worms between the test compost and the control (blank) compost should not be greater than 10%.

Complete (100%) or almost complete (98%) survival was observed in the 25% mixture of the blank compost and in the 25% mixture of the test compost, respectively, while the survival in the 50% mixture of the test compost (85%) was higher when compared to the survival in the 50% mixture of the blank compost (25%). Moreover, the mean weight (as % of start) of the surviving earthworms in both mixtures of the test compost was higher when compared to the mean weight (as % of start) of the surviving earthworms in the corresponding mixture of the blank compost. Therefore, it can be concluded that the requirements of AS 4736 (2006) on earthworm toxicity are fulfilled.

From these results it can be concluded that after composting Unbleached bagasse tableware, added in a 10% concentration to the biowaste at start of the composting process, no residuals were left such as metabolites, undegraded components and inorganic components that exert a negative influence on the survival and mean weight of earthworms.

6 Introduction

6.1 Purpose and principle of test method

The earthworm toxicity 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 earthworm toxicity test is to evaluate any toxic effect of the test compost containing the test item residuals in comparison to blank compost to which no test item was added at the start of the preceding composting.

In the test earthworms are kept in mixtures of a precisely defined artificial soil and compost in different ratios. Survival of the worms is assessed 14 days after application.

The toxicity of the test compost is evaluated by comparing the percentage survival of the earthworms and the mean weight of the earthworms (expressed as a percentage of the weight at start) in the test compost/artificial soil mixtures with the corresponding blank compost/artificial soil mixtures.

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

6.2 Standards followed

The test method is based on ASTM E1676 *Standard Guide for Conducting Laboratory Soil Toxicity or Bioaccumulation tests with the Lumbricid Earthworm Eisenia Fetida and the Enchytraeid Potworm Enchytraeus albidus* (2012) with the modifications as prescribed in the Australian standard AS 4736 *Biodegradable plastics – Biodegradable plastics suitable for composting and other microbial treatment* (2006).

7 Materials and methods

7.1 Test items

At the end of the pilot-scale composting test (see R-BH-22/9) the content of the bins was sieved over a mesh size of 10 mm. The compost fraction < 10 mm is used to perform the ecotoxicity tests.

Following types of compost were tested for their ecotoxicological effect:

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 BH-22/9-01 and BH-22/9-02.

Test compost: Homogenous 50:50 mixture on weight basis of the < 10 mm fraction of the compost from bins BH-22/9-03 and BH-22/9-04 to which 10% Unbleached bagasse tableware (milled < 4 mm) was added at start of the composting.

7.2 General procedure

The composts are thoroughly mixed before they are used for the earthworm test.

The earthworm, acute toxicity test is done in glass jars of 1000 ml, containing a mixture of compost and artificial soil. Each compost is tested in 2 mixing ratios of compost and reference substrate: (1) 75% artificial soil & 25% compost and (2) 50% artificial soil & 50% compost on weight basis. Each mixture is tested in 4 replicates. Also the pure artificial soil is tested in 4 replicates.

One day before start-up of the test, the worms are conditioned in the artificial soil. At the start of the test, each glass jar is filled with 600 g of compost/artificial soil mixture or artificial soil. Subsequently, 10 viable earthworms are put on top of the mixture. The weight of the worms is determined at start.

After all glass jars are filled, they are closed and put at room temperature (20°C ± 2°C) and with continuous lighting. During the incubation, extra water is added if needed.

The test is finished after 14 days. At the end of the test the survival and the live weight of the earthworms is determined for each jar separately. Any behavioral or pathological symptoms are also noted. The toxicity of possible residuals of the test item is evaluated by comparing the survival and the mean weight in the test compost to that in the blank compost.

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

pH

The pH is measured with a pH meter after calibration with standard buffer solutions (pH = 4.00, pH = 7.00 and pH = 10.00), as described in 'M_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 'M_057 Extraction of water and potassium chloride soluble nutrients and elements'.

Weight determination

During the test 2 types of balances are used. A Sartorius ACCULAB ATILON with internal calibration (max. 220 g, d = 0.1 mg) for the determination of the weight of the earthworms and the test item. A Sartorius AX6202 (max. 6200 g, d = 0.01 g) is used for weighing of the artificial soil.

8 Results

8.1 Test conditions and set-up

The composts obtained after the preceding composting test were thoroughly mixed, prior to use. In total, 20 glass jars were used. Each jar is filled with 600 g. The test set-up is given in Table 1.

Table 1. Test set-up

Treatment	Artificial soil (g/pot)	Compost (g/pot)
4 x Artificial soil	600	0
4 x Blank compost (25%)	450	150
4 x Blank compost (50%)	300	300
4 x Test compost (25%)	450	150
4 x Test compost (50%)	300	300

The artificial soil consists of a mixture of 10% peat, 20% kaolin clay and 70% industrial sand (on dry weight basis). The artificial mixture should have a pH of 7.0 ± 0.5 and a moisture content of about 35% of the dry weight. A pH of 7.1 was measured, while a moisture content of 35.7% on dry weight was obtained. The used worm species is *Eisenia foetida foetida*. The worms are reared at OWS nv, Dok-Noord 5, 9000 Gent, Belgium.

8.2 Survival and weight

The test was stopped after 14 days. Table 2 shows the average percentage of survival and mortality at the end of the test. Also the live weight per worm and the average preservation of the weight at start of the surviving worms are given.

Figure 1 gives a visual presentation of the average survival of the earthworms, while Figure 2 gives a visual presentation of the average weight of the surviving earthworms (expressed as a percentage of the weight at start). Figure 3 gives an overview of the earthworms at the end of the test.

Based on the results represented in Table 2 and Figure 1 it can be concluded that 100% survival was measured for the artificial soil, which means that the pass level of 90% survival is reached and that the test is valid.

Table 2. Average and standard deviation of percentage survival, percentage mortality and live weight yield for each test series

Test series	Survival (%)		Mortality (%)	Live Weight Yield (g per worm)		Live Weight Yield (% of start)	
	AVG	SD	AVG	AVG	SD	AVG	SD
Artificial soil	100	0	0	0.32	0.01	88	2
Blank compost 25%	100	0	0	0.30	0.02	82	4
Blank compost 50%	25	10	75	0.10	0.02	28	5
Test compost 25%	98	5	2	0.33	0.02	88	3
Test compost 50%	85	19	15	0.19	0.02	50	3

With AVG = average and SD = standard deviation

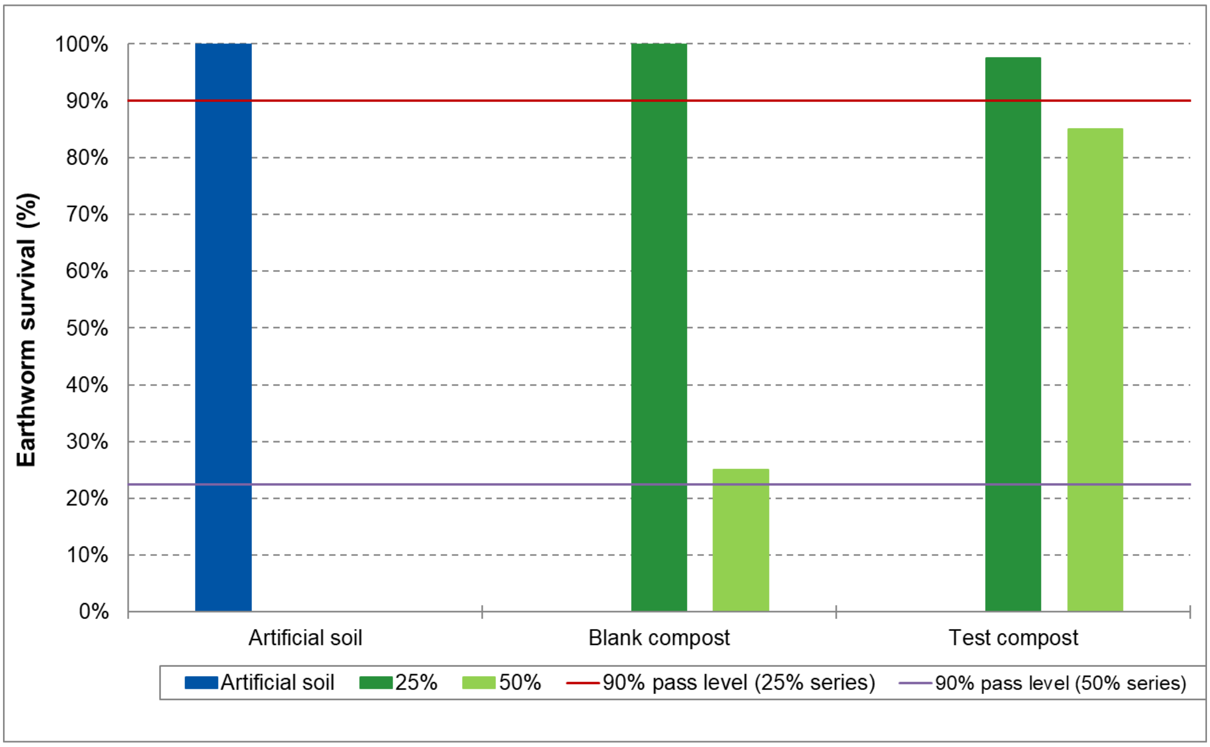

Figure 1. Average survival of earthworms

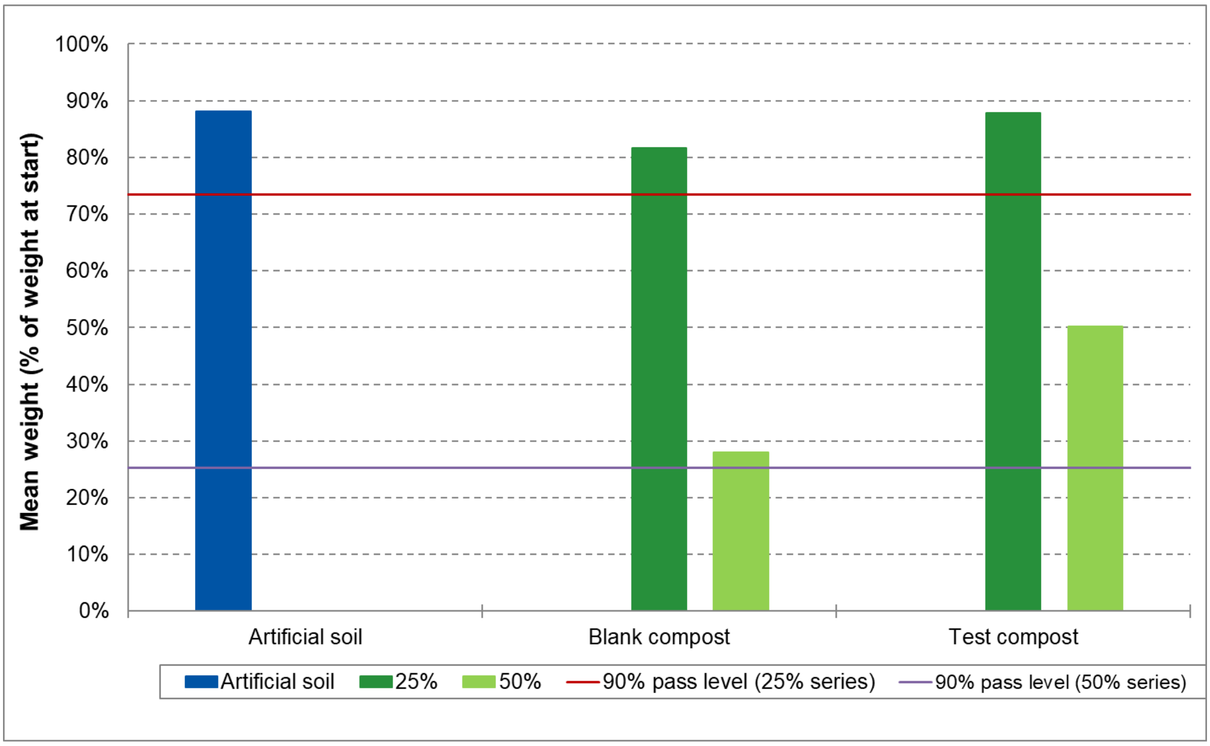

Figure 2. Average weight of earthworms (as % of weight at start)

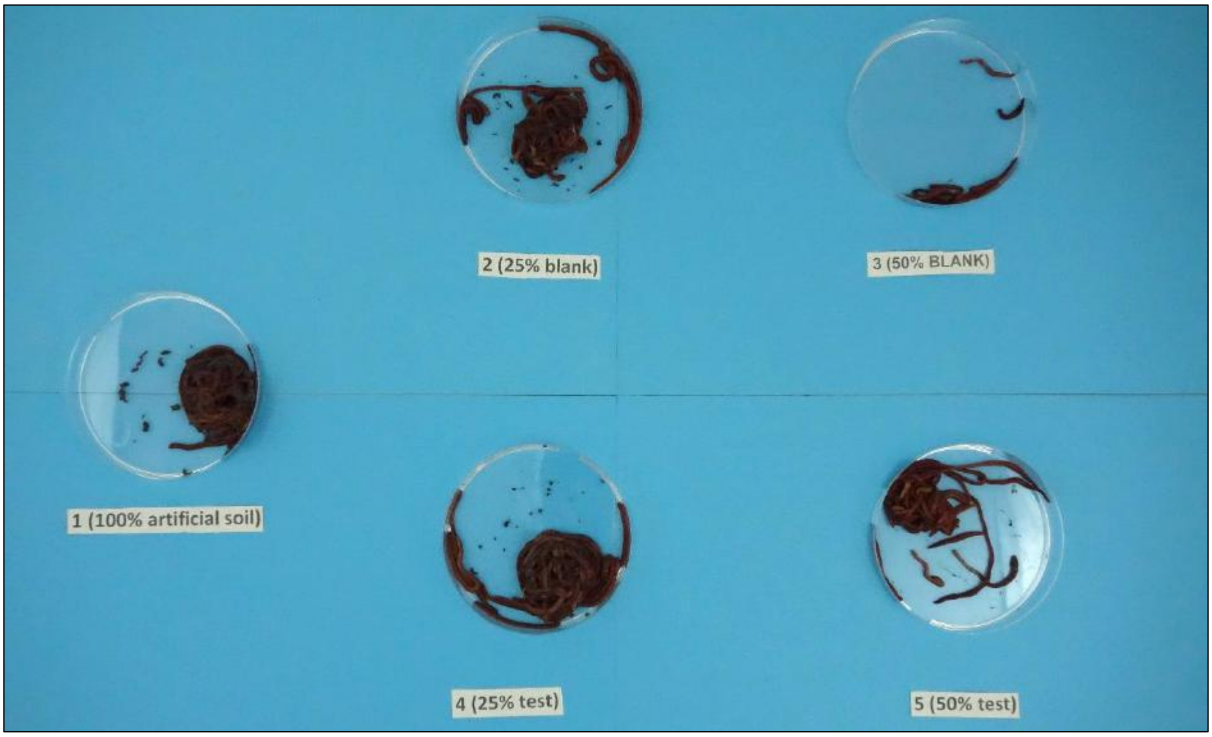

Figure 3. Overview of the earthworms retrieved at the end of the test

According to the Australian standard AS 4736 *Biodegradable plastics - Biodegradable plastics suitable for composting and other microbial treatment* (2006) the difference in the morbidity or mean weight of the surviving worms between the test compost and the control (blank) compost should not be greater than 10%.

Complete (100%) or almost complete (98%) survival was observed in the 25% mixture of the blank compost and in the 25% mixture of the test compost, respectively, while the survival in the 50% mixture of the test compost (85%) was higher when compared to the survival in the 50% mixture of the blank compost (25%). Moreover, the mean weight (as % of start) of the surviving earthworms in both mixtures of the test compost was higher when compared to the mean weight (as % of start) of the surviving earthworms in the corresponding mixture of the blank compost. Therefore, it can be concluded that the requirements of AS 4736 (2006) on earthworm toxicity are fulfilled.

Table 3. Survival of earthworms in the test compost (as % of start) and mean weight of earthworms in the test compost (as % of start) as a percentage of the corresponding mixture of blank compost

Test series	Survival (%)	Mean Weight (%)
Test compost 25%	98	107
Test compost 50%	340	179

On basis of these results it can be concluded that after composting Unbleached bagasse tableware, added in a 10% concentration to the biowaste at start of the composting process, no residuals were left such as metabolites, undegraded components and inorganic components that exert a negative influence on the survival and mean weight of earthworms.