Organic Waste Systems

FINAL REPORT

HIGH SOLIDS ANAEROBIC DIGESTION

UNDER THERMOPHILIC CONDITIONS

OF

AquaMantra Bottle

STUDY BPI-3

Biodegradable Products Institute
331 West 57th Street, Suite 415
New York, NY 10019
UNITED STATES

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1. IDENTIFICATION OF TEST

1.1. GENERAL INFORMATION

Project Number

BPI-3

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Test Item

AquaMantra bottle

Reference Item

Cellulose

Test Duration

45 days

1.2. STUDY PERSONNEL

Study Director:	Lies DEBEER
Replacement Study Director:	Johan VERMEULEN
Quality Assurance Manager:	Steven VERSTICHEL

1.3. STUDY SCHEDULE

Study initiation date:	Jan-19-2011
Experimental starting date:	Jan-19-2011
Starting date of incubation:	Jan-25-2011
Completion date of incubation	Mar-11-2011
Experimental completion date:	Mar-11-2011
Study completion date:	Mar-24-2011

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 O.W.S. These records include notebooks, study plan, study report, samples of test items and specimens. They will be stored in a file coded:

BPI-3

The training records of personnel are stored in the maps ‘Organization and Personnel’. These files are stored per person and administered by the Lab Quality Manger 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 7 years, the data and records will be destroyed.

2. CONFIDENTIALITY STATEMENT

The Testing Facility will treat strictly confidential all relevant information on the Test items 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 is performed in accordance with the OECD principles of Good Laboratory Practices (GLP).

Lies DEBEER
Study Director

4. QUALITY ASSURANCE AUDIT STATEMENT

The results reported are in accordance with the Study Plan, SOP's (standard operating procedures) and Raw Data.

A quality control is executed on...25-11...

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

Steven VERSTICHEL
QA Manager

5. SUMMARY AND CONCLUSIONS

The biodegradation at 52 °C ± 2 °C of test item AquaMantra Bottle was tested together with cellulose as reference item in a High Solids Anaerobic Digestion. The test was performed in triplicate and lasted 45 days. The biodegradation percentage is based on the net biogas production and carbon content of the test item.

The test was executed according to the Standard ASTM D.5511 at a temperature of 52 °C ± 2°C (mesophilic conditions). According to the ASTM D.5511 guideline, the test is considered valid if a) the degree of biodegradation of the reference material is >70% after 15 days, and b) the deviation of the percentage of biodegradation for the reference item in the different vessels is less than 20% at the end of the test. Both requirements were fulfilled.

The biodegradation result of the reference item cellulose, obtained after 15 days was 85.3% ± 1.1%. At the end of the test, after 45 days, biodegradation was 83.7% ± 0.4%.
During the entire test, no significant biodegradation could be measured for any of the replicates of test item AquaMantra Bottle. The test item reached a final biodegradation of 0.0% ± 1.7% (or 0.0% relative to cellulose). This means that the test item is not degradable under anaerobic conditions.

At the start of the test some cut pieces were added to each reactor to assess disintegration. At the end of the test the pieces were retrieved. No signs of disintegration could be observed.

6. INTRODUCTION

6.1. PRINCIPLE OF TEST METHOD

The biodegradability of products in a sanitary landfill or in a solid state anaerobic digestion system is determined through high-rate dry anaerobic batch fermentation. This method simulates and accelerates the biodegradation process that takes place in a landfill because it is a stationary (no mixing) and dry fermentation under optimal conditions. The incubation temperature was 52°C ± 2°C and the duration of the test was 45 days.

A small amount of test item is added to a large amount of highly active inoculum that has been stabilised prior to the start of the digestion period. Optimal conditions with regard to pH, nutrients, volatile fatty acids, etc. are provided and the mixture is left to ferment batch wise. Likewise biodegradation is not influenced by other factors than those inherent to the test item itself.

During the anaerobic biodegradation of organic materials, a mixture of gases, principally methane and carbon dioxide, are the final decomposition products while some of the organic material will be assimilated for cell growth. The volume of the biogas produced is measured and the amount of CH_4 and CO_2 produced per weight unit of test item is calculated. If the carbon content of the test item is known the percentage of biodegradation can be calculated as the percentage of solid carbon of the test item that has been converted to gaseous, mineral C.

Additionally, disintegration of the test item will be observed. At the start of test some of the test item will be added as a finished product rather than powder. In order not to affect the biodegradation results too much, only about 5 to 10% of the total amount of test item will be added as a finished product (e.g. film, pieces of bottle of bag…).

At the end of the test the pieces of film – or what has been left over – will be retrieved from the residue, washed and air-dried. The weight will be determined, weight loss calculated and pictures will be taken.

6.2. GUIDELINES USED

- ISO 15985 (2004): "*Plastics - Evaluation of the ultimate anaerobic biodegradability and disintegration under high-solids anaerobic digestion conditions - Method by analysis of released biogas*".

- ASTM method D.5511-02: "Standard *Test Method for Determining Anaerobic Biodegradation of Plastic Materials under High-Solids Anaerobic-Digestion Conditions*".

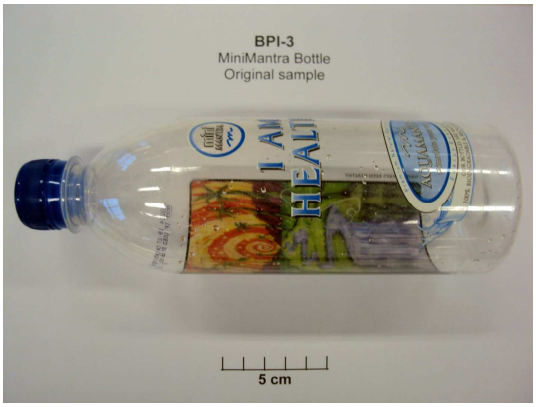
7. MATERIALS AND METHODS

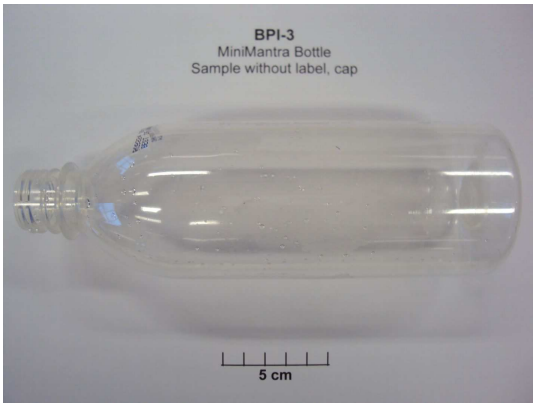
7.1. TEST ITEM AND REFERENCE ITEM

TEST ITEM 1

Name:	AquaMantra bottle
Type:	MiniMantra bottle
Batch N°:	n/a*
Production date:	n/a*
Colour:	Transparent
Sample preparation:	Cap and label removed (see photo's below) Cryogenically milled (< 800 µm) Cut into pieces (app. 2 by 4 cm) for disintegration testing
Carbon content:	62.2% (on fresh weight)
Storage conditions:	Room temperature in the dark

*n/a not applicable; sample was store bought





REFERENCE ITEM

Name:	Cellulose
Purity:	Native cellulose powder for thin layer chromatography (Avicel)
Physical form:	Powder
Colour:	White
Batch number:	K39658431930
Expiration date:	October 2014
Storage conditions:	Room temperature in the dark
Carbon content:	42.5% (on fresh weight)
Brand:	Merck Art. Nr. 2331

7.2. GENERAL PROCEDURE

7.2.1. Preparation of the inoculum

The inoculum is derived from a properly operating anaerobic digester functioning with pre-treated household waste as a sole substrate. The digester is operated for a period of at least 4 months on household waste with a retention time of a maximum of 30 days and under dry (>20% solids) and thermophilic conditions (52°C). Gas production is at least 15 Nml of biogas per gram of dry matter in the digester and per day on the average for at least 30 days. The normalised biogas volume (Nl) is the volume of biogas recalculated and transformed to volumes at standard conditions of temperature (273°K) and pressure (1 ATM) (=STP).

The prepared inoculum is stabilised during a short post-fermentation of several days until the biogas production rate is decreased to a level below 1.5 Nl biogas/kg.d. This means that the concentrated inoculum is not fed but allowed to post ferment the remains of the previously added organics. This is to ensure that large easily biodegradable particles are degraded during this period and to reduce the background level of biogas from the inoculum itself.

At the start of the digestion period a sufficient amount of inoculum for all test vessels is removed from the post fermentation digester and carefully mixed. The most important biochemical characteristics of the inoculum should be as follows: pH between 7.5 and 8.5, volatile fatty acids (VFA) below 1 g/kg wet weight and NH_4^+-N between 0.5 and 2 g/kg wet weight.

7.2.2. Preparation of Blank, Reference and Test Reactors

At the start of the experiment, each reactor is filled with the same amount of inoculum.
For the reference and test reactors a well known amount of reference/test item is added, thoroughly mixed and closed.

7.3. INCUBATION

After all test reactors are filled, they are put in the (cold) incubator and closed. The gas columns are filled with water till level 0. Before start-up, each column is rinsed several times so that the headspace gas in Test reactor and columns consists of air only. Consequently the heating system is switched on and the digesters are incubated in the dark at 52 °C ± 2°C for 45 days. At the end of the test the final biogas production is measured.

7.4. ANALYTICAL METHODS

Weight Determination

During the test 2 balances are used. A Sartorius AC 210 S with internal calibration (max. 200 g; d = 0.1 mg) for the determination of dry and volatile matter and for the weight of test and reference items. . An Acculab ATL-4202 (max. 4200 g; d = 0.01 g) is used for the weight of inoculum and mineral medium.

Dry Matter or Total Solids

The dry matter is determined by drying at 105°C during at least 16 hours and weighing, as described in "METH L.009 Compost - Determination of moisture content". The dry matter is given in percent on wet weight.

Volatile Solids - Ash

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

pH

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

Volatile Fatty Acids (VFA)

The volatile fatty acids are determined as described in "METH L.203 Determination of volatile fatty acids". The sample is diluted with water and centrifuged to remove the suspended solids. Afterwards ether is added and the acids are extracted by centrifugation. The actual analysis is done by gas chromatography. The gas chromatograph is a Clarus 480. The column used is a Stabilwax of 30 m. The carrier gas is He. 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 kg wet weight.

Ammonium - Nitrogen (NH_4^+-N)

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

Gas Composition

The gas analyses are performed on a gas chromatograph. The gas composition is measured using two channels. The first channel contains a Unibeat column followed by a molecular sieve (MolSieve 13X). This channel allows to measure concentrations of N_2, O_2 and H_2. The second channel consists of a Porapak-Q-collumn and allows determining concentrations of H_2O, CO_2, CH_4 and H_2S. The gas chromatograph is calibrated with a standard gas mixture consisting of 0.504% O_2; 44.01% CO_2; 2.03% N_2; 53.4% CH_4; 101.7 ppm H2 and 502.2 ppm H_2S. Every day gas analyses were executed the gas chromatograph was validated.

Total organic carbon (TOC)

The TOC, total organic carbon, is determined in another laboratory. The sample is pretreated with acid to remove the inorganic carbon. The used method is an element CHN analysis. The sample is burned in a small excess of oxygen at 900°C. Due to the exothermic combustion of the tin capsule the temperature raises to about 1800°C. The sample is at that temperature decomposed to NO_x, N_2, CO, CO_2, H_2O, SO_2, SO_3, salts and metals. The gas mixture is led to oxidators and catalysts and converted to NO_x, N_2, CO_2 and H_2O. After reduction, the end products CO_2 and H_2O are separated on different absorption columns while the N_2 gas is directly measured by means of a katharometer. Thereafter CO_2 and H_2O are measured. The carbon content is given in percent on wet weight.
The apparatus used is an Elementar Vario EL III. Reagents used are oxygen gas (as burning gas), helium gas (carrier stream), and standards for calibration. The analysis is executed according to EN 13137 procedure B, Direct method.

8. RESULTS

8.1. TEST CONDITIONS AND SET-UP

A set of 9 equal vessels with a total volume of 2 l each was used. Each reactor was filled with 1 kg of inoculum and 15 g of reference of test item (except for the blank reactors). The test item was added as powder, but also about 10% test item was added under the form of cut pieces (app. 2 by 4 cm) to assess disintegration. In total 15 g of test item was added. The reactors were kept at 52°C ± 2°C in an incubator. The test set-up is given in Table 1.

Table 1. Test set-up.

RN	Test series	Inoculum (g/reactor)	Test/Reference item (g/reactor)			% TOC	C added (g/reactor)
			powder	piece	total		
1	Blank	1000.4	-	-	-	-	-
2	Blank	999.9	-	-	-	-	-
3	Blank	999.4	-	-	-	-	-
4	Cellulose	998.9	15.00	-	15.0	42.5	6.37
5	Cellulose	999.7	15.07	-	15.1	42.5	6.40
6	Cellulose	998.6	15.42	-	15.4	42.5	6.54
7	AquaMantra Bottle	998.3	13.76	1.21	15.0	62.2	9.31
8	AquaMantra Bottle	998.1	13.83	1.14	15.0	62.2	9.31
9	AquaMantra Bottle	997.8	13.85	1.15	15.0	62.2	9.33

8.2. ANALYSES INOCULUM AND TEST ITEMS

The inoculum was taken from a digester that has been operated during several months on the organic fraction of household waste. Before use, the inoculum was left to stabilize during 6 days. This post-fermentation was needed to reduce the biogas production rate. The characteristics of the inoculum are given in Table 2. It is recommended that the pH is between 7.5 and 8.5, the NH_4^+-N content between 0.5 and 2.0 g/kg and the volatile fatty acids content < 1 g/kg. In Table 2, it can be seen that pH is somewhat higher, but still acceptable. The overall quality of the inoculum was good.

Table 2. The characteristics of the inoculum.

Characteristics	**Inoculum**
Total solids (TS %)	19.3
Volatile solids (VS % on TS)	56.3
Ash (% on TS)	43.7
pH	8.6
NH_4^+-N (g/kg)	1.56
Volatile fatty acids (g/kg)	b.r.*

* Below reporting limit: VFA= 0.15 g/kg

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

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

Test item	**TS (%)**	**VS (% on TS)**	**TOC (%)**
Cellulose powder	96.4	100.0	42.5
AquaMantra Bottle	99.6	100.0	62.2

8.3. BIOGAS PRODUCTION

The averages and standard deviations of the final gas readings in Nl (Normalized litre. i.e. litre converted to standard conditions of temperature and pressure) are summarized in Table 4. The background activity of the inoculum was clearly lower compared to the reference reactors, which shows that the inoculum was stabilized sufficiently during the post-fermentation period. The low background activity improved the accuracy of the test.

Table 4. Average and standard deviation of the final gas readings (Nl) after 45 *days.*

Test series	**Biogas Production (Nl)**	
	Average	**Standard Deviation**
Blank	7.2	0.2
Cellulose	17.2	0.1
AquaMantra Bottle	7.1	0.3

Table 5 shows the biogas composition and the pH after 45 days of testing. The pH's and gas compositions were within a normal range for all reactors. The composition of the biogas has no influence on the biodegradation percentage, but gives an idea on the fermentation process. A high CO_2 concentration and a low CH_4 content could indicate a bad fermentation. As can be seen from Table 5 this was certainly not the case for the test item.

Table 5. Average biogas composition (%) and pH at end of test.

Test series	**CO_2 content (%)**	**CH_4 content (%)**	**pH**
Blank	42.4	57.6	7.6
Cellulose	45.1	54.9	7.7
AquaMantra Bottle	43.4	56.6	7.6

Figures 1 to 3 show the evolution of the total cumulative biogas production (in normalized liter of biogas) for the 3 replicates of the different test series.

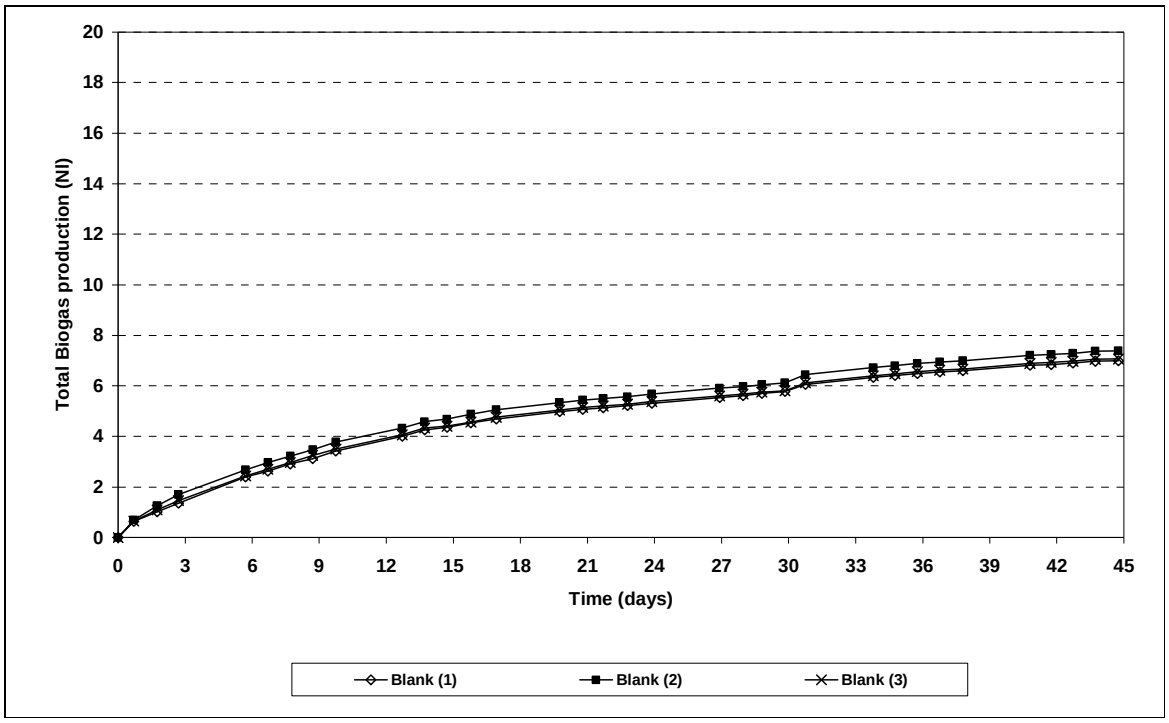


Figure 1. Total biogas production of the blank reactors.

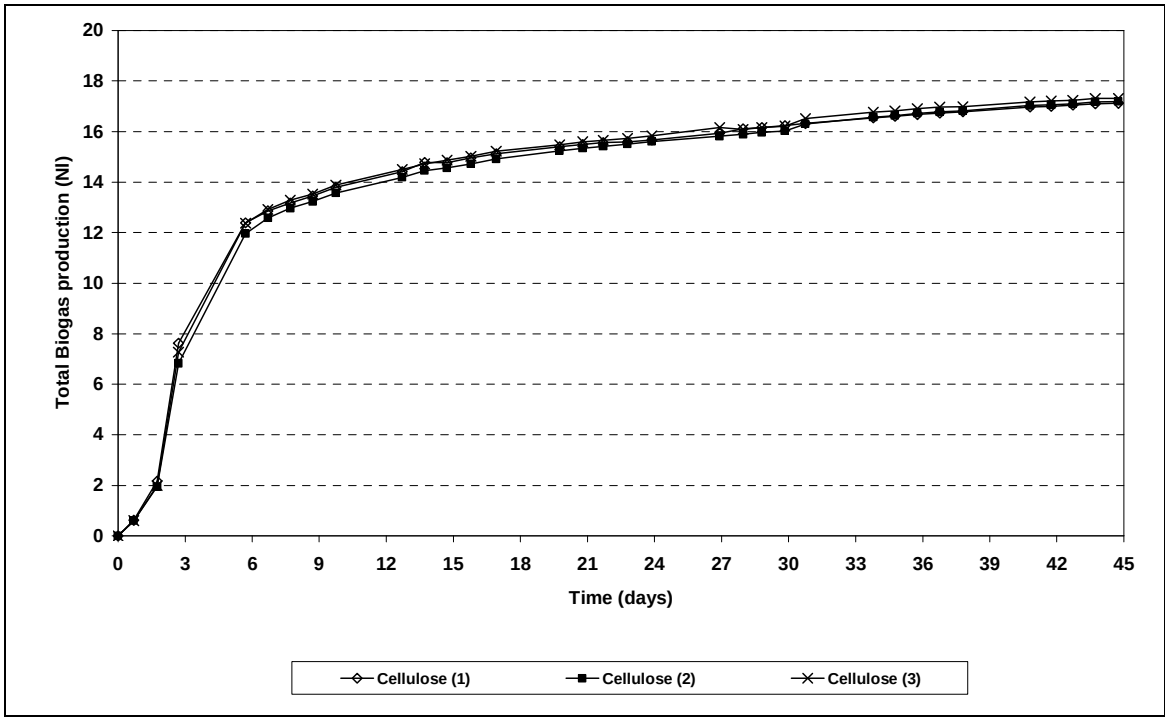


Figure 2. Total biogas production of the Cellulose reactors.

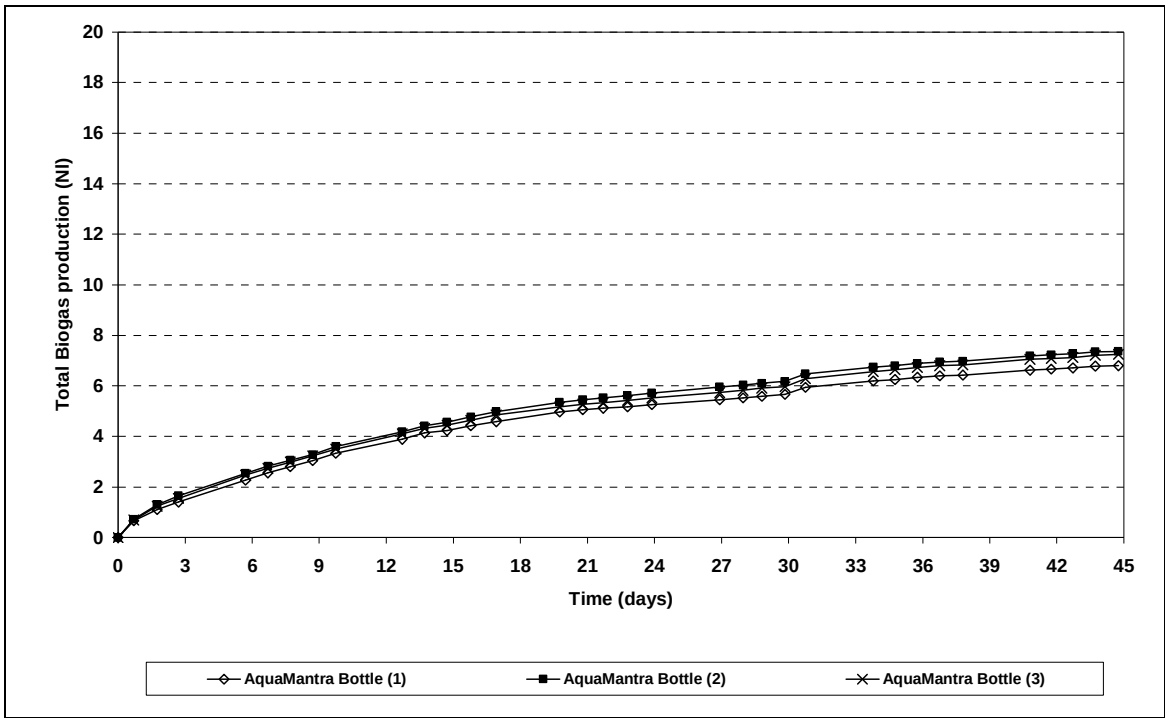


Figure 3. Total biogas production of test item AquaMantra Bottle.

8.4. BIODEGRADATION PERCENTAGES

Table 6 shows the biodegradation percentages of reference and test item after 45 days. They are calculated as the amount of carbon in the sample that was converted to carbon in the biogas (methane and carbon dioxide).

Table 6. Average biodegradation percentages after 45 days of HSAD.

Test item	Average C_{input} (g)	Average $C_{gaseous}$ (g)	Biodegradation (%)			95% CL
			AVG	STD	Relative to Cellulose	
Cellulose	6.44	5.39	83.7	0.4	100.0	3.5
AquaMantra Bottle	9.32	0.00	0.0	1.7	0.0	3.8

The values in Table 6 do not include the amount of carbon which was originally present in the test or reference item and which in the course of the digestion has been converted to biomass carbon. Some of the carbon that is biodegraded is indeed used for the building of new bacterial biomass. For anaerobic digestion the biomass yield factor is between 10% and 30%. This means that for 1 g of carbon consumed, between 10% and 30% is used for new cell biomass while 70% to 90% is converted to gaseous, mineral carbon under the form of CH_4 or CO_2.

Figure 4 shows the evolution of the average biodegradation percentages of the reference and test item. While Figures 5 and 6 show the evolution of the biodegradation percentage of the 3 replicates of Cellulose and the test item.

The test is considered valid if after 15 days the biodegradation percentage of the reference item is more than 70% and if the standard deviation of the biodegradation percentage of the reference item is less than 20% at the end of the test. After a lag phase of about one day, biodegradation of Cellulose started at a high rate. After 6 days already a biodegradation percentage of 81% was reached. From then on the activity slowed down to reach a value of 85.3% ± 1.1% after 15 days. The final degradation was 83.7% ± 0.4% after 45 days. This means that all requirements are fulfilled.

During the entire test, no significant biodegradation could be measured for any of the replicates of test item AquaMantra Bottle. The test item reached a final biodegradation of 0.0% ± 1.7% (or 0.0% relative to cellulose). This means that the test item is not degradable under anaerobic conditions.

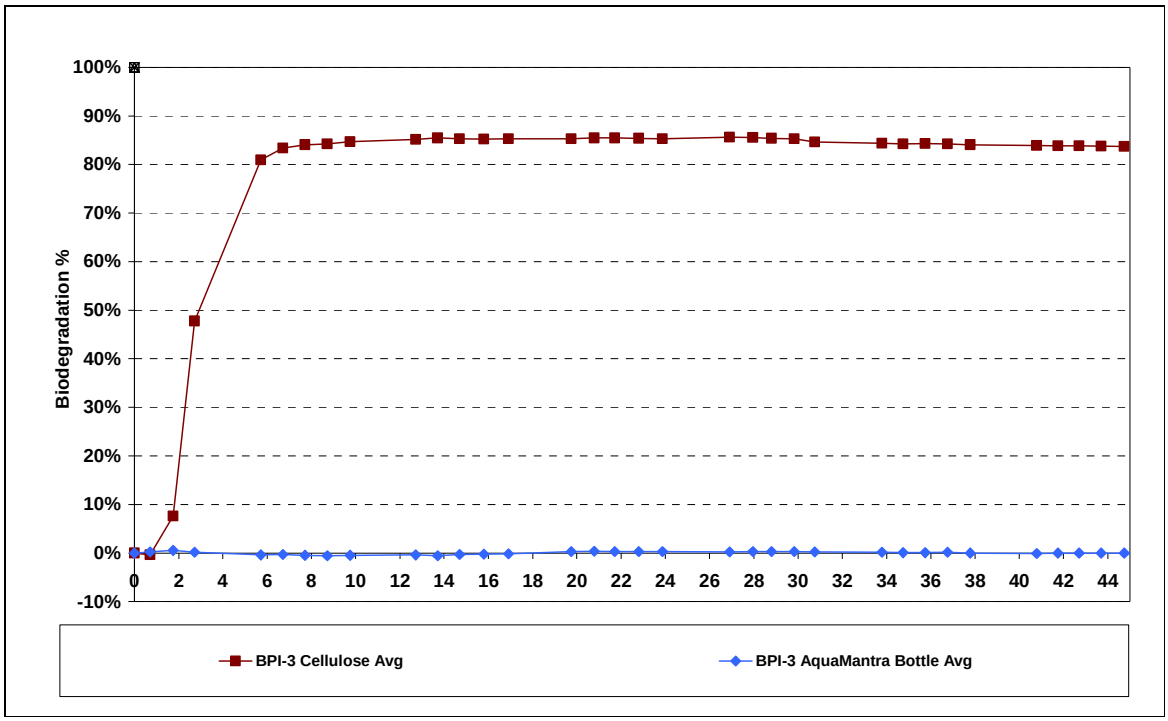


Figure 4. Evolution of the average biodegradation percentage of Cellulose and test item.

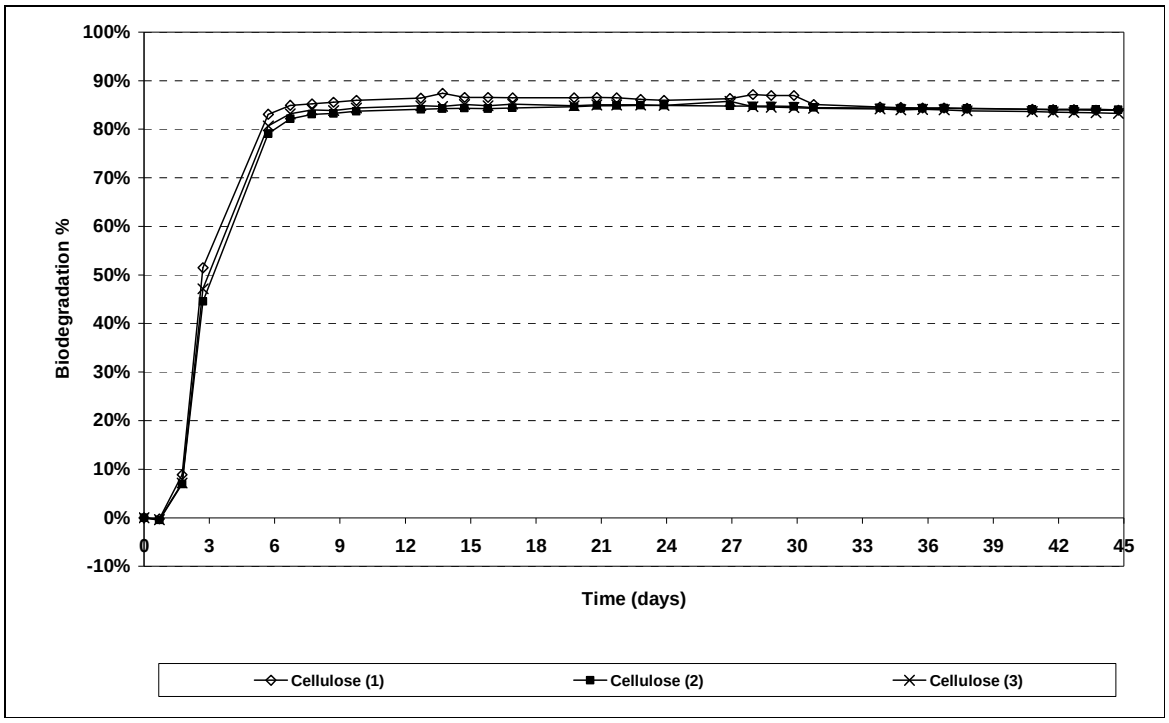


Figure 5. Evolution of the biodegradation percentage of the 3 replicates of Cellulose.

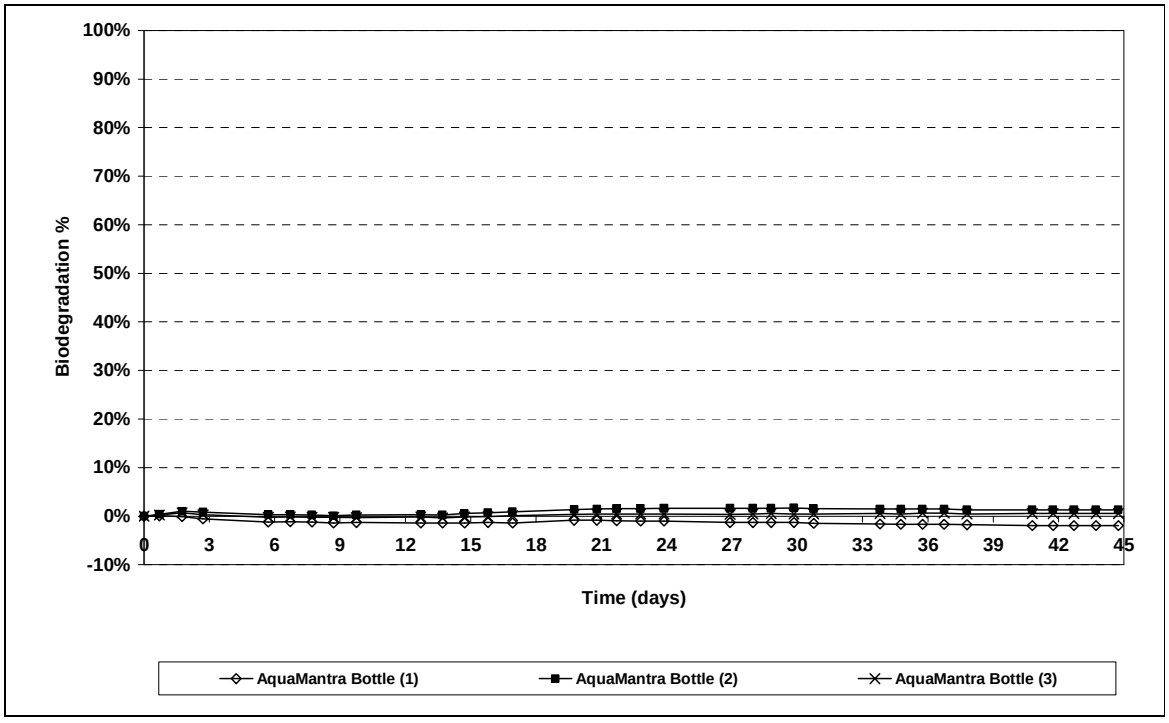


Figure 6. Evolution of the biodegradation percentage of the 3 replicates test item AquaMantra Bottle.

8.5. DISINTEGRATION RESULTS

At the start of the test, 4 pieces of test item were added. These were retrieved at the end of the test. No visual disintegration or weight loss could be observed.
Photo 1 shows the sample before and after the anaerobic disintegration / biodegradation test. On the photo some discoloration but no signs of disintegration can be seen.

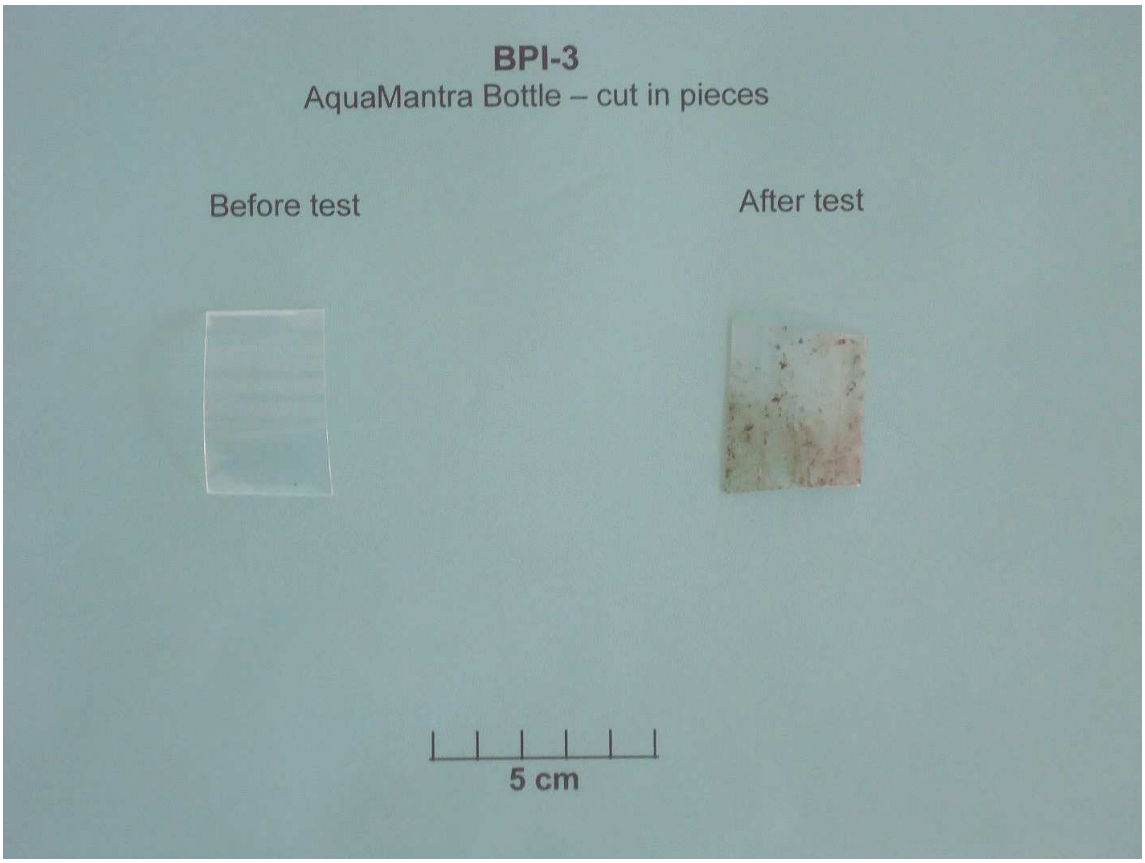


Photo 1. Test item AquaMantra Bottle before and after the degradation/disintegration test.