To:

AVEBE

EVALUATION OF READY BIODEGRADABILITY: DISSOLVED ORGANIC CARBON DIE-AWAY FOLLOWING THE OECD 301 A GUIDELINE

Sample:
"SOLVITOSE GREENMELT"

Test report n° 20FER6-0853 - 2020/09/25

This report has 10 pages.

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TABLE

I. REPORT OBJECT

Client details :
Name: AVEBE
Address: Avebe Innovation Center – Zernikelaan 8 – 9747 AA Groningen – The Netherlands

This report gives results obtained on a sample received the 2020/07/28, for the realization of biodegradability assay, following quotation reference B5QVFR200108-02.

II. SAMPLE PRESENTATION

Client sample reference: SOLVITOSE GREENMELT

Reception date: 2020/07/28.

Expiration date: unknown

Conservation temperature: Ambient temperature.

EUROFINS Ecotoxicologie France reference: 20G007026-002.

Dissolved Organic Carbon (DOC) threshold of a 100 mg/L solution: 15 mg/L.
The biodegradability test has been performed on the sample at a theoretical quantity of DOC of 15.0 mg/L which equals 100 mg/L of sample.

This report is only about products tested.

III. PHYSIC-CHEMICALS ANALYSE

Organic Carbon dosage following the NF EN 1484 (September 1997) standard “Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC) - Persulfate oxidation method”.

IV. DESCRIPTION OF THE EVALUATION OF "READY" BIODEGRADABILITY TEST FOLLOWING THE OECD301 A GUIDELINE

IV.1 Principle

Bacterial seeding: from a sample of an urban biological Wastewater treatment plant sample of activated sludge (Suspension Matter concentration in the final mix to test inferior or equal to 30 mg/L).

The assay is conducted by including these following preparations:

- The tested sample with a concentration of 15 mg/L of DOC (2 flasks),
- A control assay of the seeding (blank) (2 flasks),
- An assay with reference substance with a biodegradability rate superior to 90% (sodium acetate at 15 mg/L of DOC),
- An assay with the control of inhibition containing the tested sample and the reference substance at tested concentrations.

Several assays are under aeration stirring at 22 +/- 2°C.

The DOC is measured at the beginning and at the end of the assay (generally 28 days), with complementary measures at Days 1, 4, 7, 11, 14, 18, 21 and 25.

IV.2 Definitions

Lag phase: period between the sowing moment and the moment when the percentage of degradation has reached around 10 %

Degradation period: period which begins at the end of the lag phase and ends when 90% of the degradation maximal rate is reached.

10-days window: 10 days which directly follow the moment when the biodegradation rate has reached 10 %.

Readily biodegradable: a product is considered as readily biodegradable if the biodegradation rate has reached at least 60% in the 10-day interval which has to fall within the 28 (firsts) days of the test.

Readily biodegradable without respecting the 10-day interval: a product is considered as readily biodegradable without respecting the 10-day interval if the biodegradation rate has at least reached 60% within the 28 (first) days of the test without having reached that limit in the 10-day interval.

IV.3 Mineral medium

Each solution is prepared in ultra-pure water.

Stock solution composition:

Stock solution A:

- Potassium dihydrogen phosphate, KH_2PO_4: 8.50 g
- Potassium hydrogen phosphate, K_2HPO_4: 21.75 g
- Sodium hydrogen phosphate dihydrate, Na_2HPO_4, $2H_2O$: 33.40 g
- Ammonium chloride, NH_4Cl: 0.50 g

Dissolve in water and make up to 1 litre; pH equals to 7.4.

Stock solution B:

- Anhydrous calcium chloride, $CaCl_2$: 27.50 g

or calcium chloride dihydrate, $CaCl_2$, $2H_2O$: 36.40 g

Dissolve in water and make up to 1 litre.

Stock solution C:

- Magnesium sulfate heptahydrate, MgSO4, $7H_2O$: 22.50 g

Dissolve in water and make up to 1 litre.

Stock solution D (Must be prepared extemporaneously):

- Ferric chloride hexahydrate, $FeCl_3$, $6H_2O$: 0.25 g

Dissolve in water and make up to 1 litre.

Preparation of the mineral medium:

Stir 10 mL of solution A with 800 mL of ultra-pure water, then add 1 mL of solutions B, C and D and complete to 1 Litre.

IV.4 Reference substance

Sodium Acetate.
Weighed mass for a DOC grade of 15.0 mg/L: 51.2 mg.

IV.5 Bacterial inoculum

Origin: activated sludge from Maxeville's water treatment plant (France) dealing primarily with domestic waste water (98%)
Sampling date: 2020/08/10.

On the sampling day : sludge is washing by a series of three centrifugations (1100 *g* during 10 minutes) after resuspending the pellet in the mineral medium and filtration on a stainless filter-sieve of 100 µm.
After the treatment, a measure of the solids in suspension is realized.
The solids concentration in the test vessels containing the inoculum must be less than or equal to 30 mg/L.

Suspended matter concentration after pre-treatment: 10.5 g/L
Volume added in tested flasks to obtain a suspended matter concentration inferior or equal to 30 mg/L in the final mix: 2.9 mL.

Enumeration of the revivable aerobic flora at 22°C in the mix medium + inoculum: $5.7x10^7$ UFC/L (Theorical value following the OECD 301B: concentration between 10^7 and 10^8 cells/L).

IV.6 Preparation of trial mixtures

The quantity of product is added directly in assay bottles with mineral medium and activated sludge.
After the addition of 900 mL of mineral medium, the pH is measured in flasks of trial batch of the tested product, and then adjusted to 7.4 +/- 0.2 if necessary with HCL (1N) or NaOH (1N)
Optionally, the flask pH « Toxicity control of the product » is adjusted.
The inoculum is added and the volume adjusted to 1 Liter.

V. RESULTS

V.1 pH

Mineral medium pH: 7.5 at 22.2 °C.

Batch trial "SOLVITOSE GREENMELT-1" pH before adding inoculum: 7.5 at 22.1°C.

Adjustment of pH: No.

V.2 Test of ready biodegradability (OECD 301 A)

Beginning date of assay: 2020/08/12.

Following of the DOC (mg/L)

Number of days	0	1	4	7	11	14	18	21	25	28
SOLVITOSE GREENMELT - 1	16.0	15.0	4.7	3.4	3.0	2.2	2.4	2.4	2.5	3.0
SOLVITOSE GREENMELT - 2	18.0	18.0	4.3	3.7	2.8	2.5	2.4	2.0	2.6	3.0
Average - SOLVITOSE GREENMELT	*17.0*	*16.5*	*4.5*	*3.6*	*2.9*	*2.4*	*2.4*	*2.2*	*2.6*	*3.0*
Gap between values	*-11.8%*	*-18.2%*	*8.9%*	*-8.5%*	*6.9%*	*-12.8%*	*0.0%*	*18.2%*	*-3.9%*	*0.0%*
Positive control	17.0	6.3	1.7	2.1	2.2	2.1	1.7	1.8	1.9	3.0
Inhibition assay	33.0	23.0	10.0	6.3	5.4	2.7	4.0	4.3	1.9	4.2
Negative control 1	1.6	1.8	1.6	2.2	1.8	1.6	1.6	1.4	1.5	2.4
Negative control 2	1.7	1.9	1.8	2.0	2.4	1.7	1.5	1.6	1.6	2.7
Average Negative control	*1.7*	*1.9*	*1.7*	*2.1*	*2.1*	*1.7*	*1.6*	*1.5*	*1.6*	*2.6*

Following of DOC in mg/L after substraction of “Control”

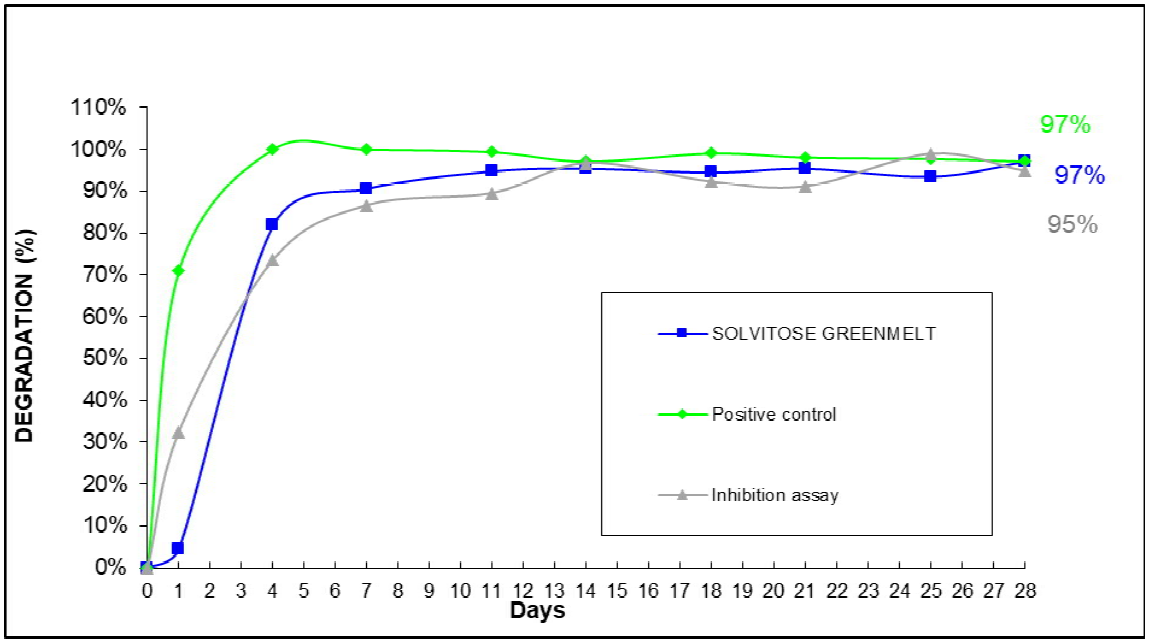
Number of days	0	1	4	7	11	14	18	21	25	28
SOLVITOSE GREENMELT - 1	14.4	13.2	3.0	1.3	0.9	0.6	0.9	0.9	1.0	0.5
SOLVITOSE GREENMELT - 2	16.4	16.2	2.6	1.6	0.7	0.9	0.9	0.5	1.1	0.5
Average - SOLVITOSE GREENMELT	*15.4*	*14.7*	*2.8*	*1.5*	*0.8*	*0.7*	*0.9*	*0.7*	*1.0*	*0.5*
Positive control	*15.4*	*4.5*	*0.0*	*0.0*	*0.1*	*0.5*	*0.2*	*0.3*	*0.4*	*0.5*
Inhibition assay	31	21.2	8.3	4.2	3.3	1.1	2.5	2.8	0.4	1.7

Biodegradability percentage of DOC

Number of days	1	4	7	11	14	18	21	25	28
SOLVITOSE GREENMELT	5%	82%	91%	95%	95%	94%	95%	93%	97%
Positive control	71%	100%	100%	99%	97%	99%	98%	98%	97%
Inhibition assay	33%	74%	87%	89%	97%	92%	91%	99%	95%

V.3 Degradation curves

Degradation percentages after 28 days



V.4 Test validity

Considering:

1. The difference between DOC measurement values of the studied product is inferior to 20% each sample.
2. The degradation percentage of reference substance (sodium acetate) is superior to 70% (97%) day 14.
3. The elimination degree of DOC in inhibitor assay, containing the tested substance and the reference substance, being superior to 35% at day 14 (97%), the sample is not considered as toxic for the seeding.

The test is thus valid.

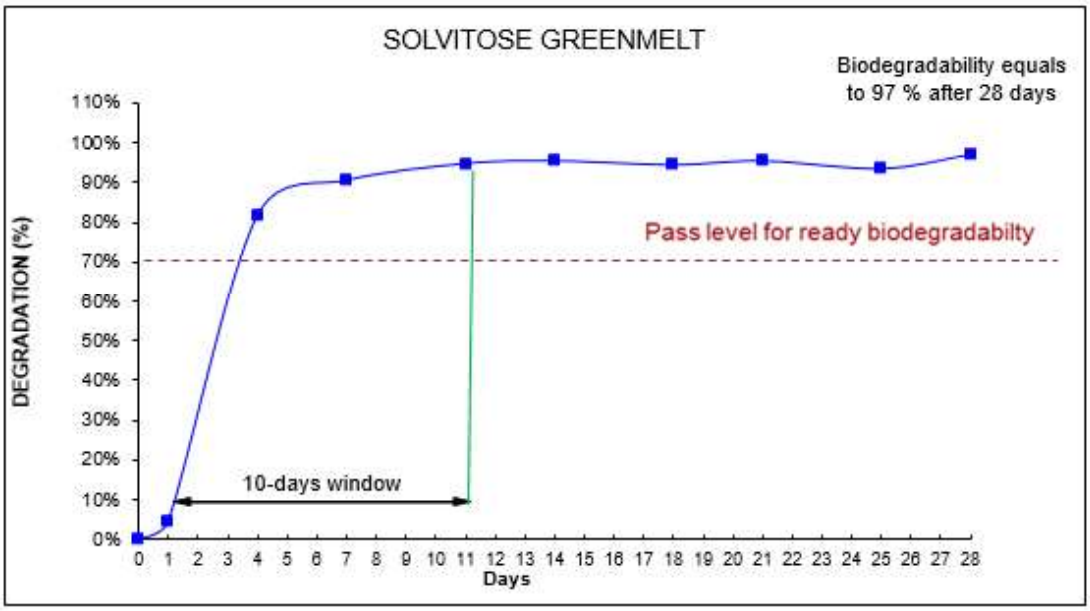
VI. CONCLUSION

Under the experimental conditions of the test:

- The product **«SOLVITOSE GREENMELT» is considered as readily biodegradable**:
  - The diminution threshold of DOC of 70% is reached in the 10 days window interval.

- The product **«SOLVITOSE GREENMELT» is biodegradable at 97% after 28 days of test.**

NB: The « readily biodegradable » mention is only applicable to pure substances.



In Maxéville, 2020/09/25.
Eloïse Renouf, Project Engineer