SGS INSTITUT FRESENIUS GmbH · Im Maisel 14 · D-65232 Taunusstein

APP All Protect
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Taunusstein, January 29, 2021

1 General Information

Titel:	**Test for Ready Biodegradability of Products**
Sponsor:	APP All Protect
Sponsor's contact person:	Mr. Erik Amesz
Study director:	Sebastian Vogel
Sample designation:	"Anti vandaal coating PSS-20"
SGS Sample-No.:	201197098
Date of order:	November 26, 2020
Sample receipt:	November 26, 2020
Test method:	OECD-Guideline 301 B (CO_2 Evolution Test) 1992-07; accredited to DIN EN ISO 17025
Testing period:	December 10, 2020 to January 8, 2021
Pages:	7

2 Test Information

Inoculum:	Filtrate of homogenised activated sludge from the waste-water treatment plant at Taunusstein-Bleidenstadt; Lot-No.: December 9, 2020
Incubation time:	28 days
Detection method:	CO_2-measurement
Control:	Sodium benzoate, ≈20 mg/L TOC
Application method:	A stock solution in DCM was prepared and a defined amount of the stock solution was applied on a glass filter. After drying at room temperature, the glass filter was torn and put into the incubation vessel.

Definitions:

TOC: total organic carbon

TCO2: theoretical amount of CO_2 which may be developed from the test item (expressed as mg CO_2 / g test item). This value is calculated from the carbon content of the test item and the relation of molar masses of CO_2 (= 44.01) and carbon (= 12.01).

ThCO2: theoretical amount of CO_2, which may be developed from the test item within the whole test solution (= 3.5 L).

3 Method description

The test item and the polyvalent inoculum from an activated sewage plant dealing predominantly with domestic sewage are incubated together in a mineral nutrient medium at a temperature range of 19 to 25 °C. The test item is the sole carbon and energy source. The test solutions are aerated with CO_2-free compressed air and are stirred on a magnetic stirrer. When the test material is mineralised it is converted to CO_2 which is trapped in a system of gas-washing bottles into barium hydroxide. The CO_2 is quantified by titration of the remaining barium hydroxide with HCl. Comparing the amount of CO_2 produced upon degradation of the test item with the theoretical amount of CO_2 ($ThCO_2$) the percentage of degradation is calculated. Two Blank controls with inoculum but without any test item are run in parallel in order to determine the amount of CO_2 derived from the inoculum. The mean of these values have to be subtracted from those values determined for the test solutions with the test item. At the end of the test the reaction in the test solutions is stopped by addition of 1 mL HCl conc. to each of the test solutions, and inorganic carbonates are made volatile. Aeration with CO_2 -free compressed air is continued for another 1 day in order to purge the remaining CO_2 off the test solutions. Two test solutions with the test item are tested in parallel at ≈10 mg/L TOC. The test duration normally is 28 days (+ 1 day purging off the dissolved CO_2 from the test solutions after acidification of the test solutions).

4 Evaluation

$$\%\ \mathrm{TCO_2} = \frac{\text{mg } \mathrm{CO_2} \text{ produced x } 100}{(\text{mg Test item in test solution}) \text{ x } (\mathrm{TCO_2})} = \%\ \text{Degradation}$$

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The test solutions were prepared according to the OECD guideline 301 B.

Carbon content of the test item (calculated from TOC-measurement): **14.4 mg C / g Test item**[1)]

Relation molar masses of CO_2 : C = 44.01 : 12.01 = 3.667

Resulting: TCO_2 = 53 mg CO_2 / g Test item

Volume of test solutions: 3500 mL

Amount of test item within:

Test solution 1: 2432.0 mg / 3500 mL

$ThCO_2$ = 128.3 mg CO_2 / 3500 mL Test solution

Test Solution 2: 2441.3 mg / 3500 mL

$ThCO_2$ = 128.8 mg CO_2 / 3500 mL Test solution

Amount of control item sodium benzoate within:

Test Solution 3: 127.4 mg / 3500 mL Test solution

$ThCO_2$ = 271.7 mg CO_2 / 3500 mL Test solution

Amount of test and control items in the toxicity control in:

Test solution 4: 2453.3 mg test item / 3500 mL test solution;
+ 126.0 mg sodium benzoate / 3500 mL test solution

$ThCO_2$ = 398.2 mg CO_2 / 3500 mL test solution

[1)] This measurement was performed according to DIN EN 15407:2011-05 at SGS INSTITUT FRESENIUS GmbH Berlin being also accredited according DIN EN ISO 17025

6 Validity criteria

Total-CO_2-Evolution of the Blanks:

Blank solution A = 91.63 mg CO_2 / 3.5 L [26.18 mg CO_2 / L] in 28 d
Blank solution B = 104.93 mg CO_2 / 3.5 L [29.98 mg CO_2 / L] in 28 d
Mean blank solutions = 98.28 mg CO_2 / 3.5 L [28.08 mg CO_2 / L] in 28 d

Positive Control:
The control item sodium benzoate was degraded 87 % within 28 days. The threshold of "ready biodegradability" of ≥ 60 % was passed within 7 days (66 %).

The validity criteria defined by guideline OECD 301 B were met / complied with:

- The inorganic carbon content of the test item in the test solution is < 5 % of the total carbon content.
- The CO_2 formation of the negative controls / blanks is < 40 mg CO_2 / L in each case.
- The positive control meets the requirements with regard to the minimum degree of degradation and a 10-day window.
- The result deviation in the parallel batches with test substance (Run 1 and 2) is < 20 %.

7 Results

Table 1: Test Solution 1 with the test item

Time [d]	Concentration: 2432.00 mg / 3.5 L ThCO$_2$:	128.33 mg CO_2/ 3.5 L
	mg CO_2 produced in the Test solution. cumulative	% TCO_2 (= % Degradation)
4	0.00	0
7	26.11	20
11	52.63	41
13	67.80	53
19	74.44	58
25	74.49	58
28	75.22	59 [1)]
29	81.69	64

[1)] The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

Table 2: Test Solution 2 with the test item

Time [d]	Concentration: 2441.30 mg / 3.5 L ThCO$_2$: 128.82 mg CO$_2$/ 3.5 L	
	mg CO$_2$ produced in the Test solution. cumulative	% TCO$_2$ (= % Degradation)
4	0.00	0
7	24.30	19
11	50.43	39
13	68.53	53
19	76.96	60
25	78.27	61
28	80.14	62 [1)]
29	81.66	63

[1)] The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

Table 3: Toxicity Control

Time [d]	Concentration: 126.00 mg Sodium Benzoate + 2453.30 mg Test Item ThCO$_2$: 398.16 mg CO$_2$/ 3.5 L	
	mg CO$_2$ produced in the Test solution. cumulative	% TCO$_2$ (= % Degradation)
4	15.79	4
7	170.26	43
11	244.12	61
13	268.44	67
19	285.90	72
25	297.60	75
28	313.48	79 [1)]
29	324.22	81

[1)] The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

Table 4: Positive Control Sodium Benzoate

Time [d]	Concentration: 127.40 mg / 3.5 L $ThCO_2$: 271.69 mg CO_2/ 3.5 L	
	mg CO_2 produced in the Test solution. cumulative	% TCO_2 (= % Degradation)
4	103.04	38
7	179.38	66
11	199.41	73
13	212.99	78
19	220.86	81
25	226.76	83
28	229.88	85 [1)]
29	236.58	87

[1)] The test solution was stopped at time 28d by the addition of 1 mL conc. HCl. The final titration was performed at time t_{29d}.

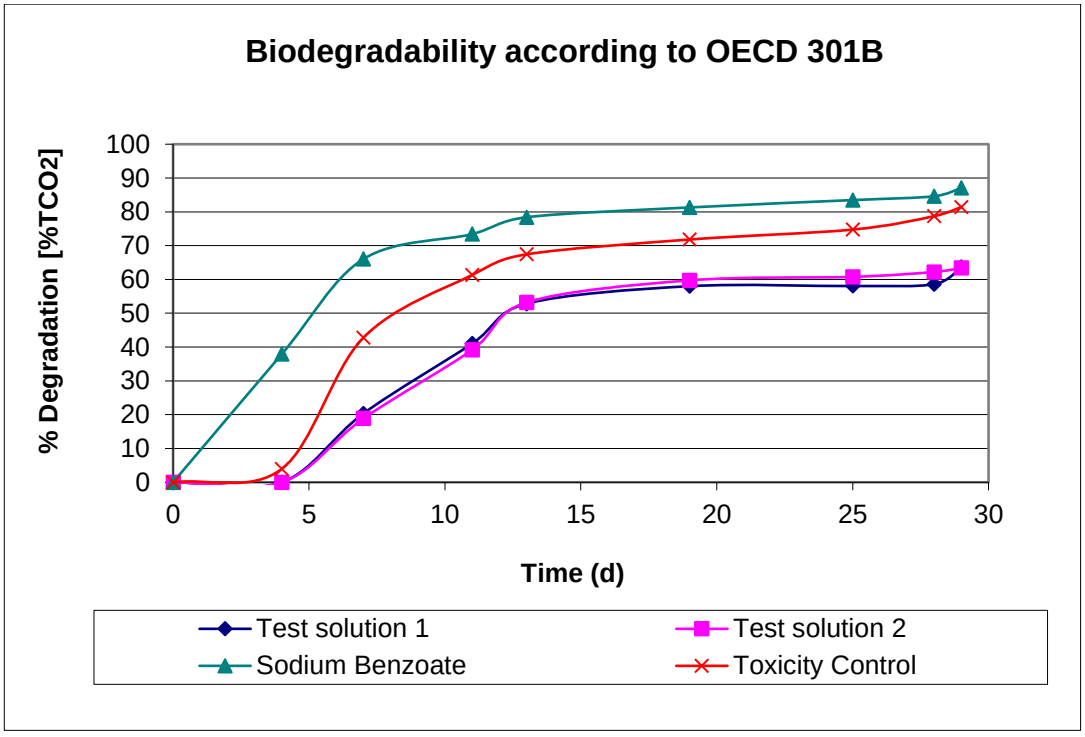

Figure 1: Graphical Presentation of the Results

8 Conclusion

The threshold of "ready biodegradability" of ≥ 60 % was met within both test solutions. The mean value of 64 % is higher than the pass value of the OECD Guideline 301B. Thus the test item **"Anti vandaal coating PSS-20"** can be termed "readily biodegradable". According to the results of the toxicity control, a toxic effect towards microorganisms at the concentration tested can be excluded.

Annotation: It should be noted that the bio degradation test method according to OECD 301B was designed to test pure substances, but not for multi-substance mixtures. Therefore, it cannot be excluded that individual product components or degradation (intermediate) products / metabolites are ready degradable, inherent degradable and / or non-degradable, as this guideline only detects the sum of the mineralized organic carbon compounds.

SGS Institut Fresenius GmbH
D-65232 Taunusstein, January 29, 2021

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The test results refer exclusively to the examined test items and the date of the test under the test specifications

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