

Test for ready biodegradability according to OECD 301 B

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Test for Ready Biodegradability of Products

1 Sample designation: „Detergent Sheets”

- 1.1 Sample receipt: 16-Jul-2018
- 1.2 Storage conditions: Room temperature

2 Sponsor:

- 2.1 Address:

3 Test facility:

- 3.1 Address: SGS INSTITUT FRESENIUS GmbH
Im Maisel 14, D-65232 Taunusstein-Neuhof; Germany
- 3.2 Study director: Dr. H. Lebertz

4 Test method:

- 4.1 Inoculum: OECD-Guideline 301 B (CO₂ Evolution Test)
Filtrate of homogenised activated sludge from the waste-water treatment plant at Taunusstein-Bleidenstadt; Lot-No.: 23-July-2018
- 4.2 Application: 28 days
- 4.3 Detection method: CO₂-measurement

- 5 Control: Sodiumbenzoate, ≈20 mg/L TOC

6 Definitions:

TOC: total organic carbon

TCO₂: theoretical amount of CO₂ which may be developed from the test item (expressed as mg CO₂ / g test item). This value is calculated from the carbon content of the test item and the relation of molar masses of CO₂ (= 44.01) and carbon (= 12.01).

ThCO₂: theoretical amount of CO₂, which may be developed from the test item within the whole test solution (= 3.5 L).

7 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 CO₂-free compressed air and are stirred on a magnetic stirrer. When the test material is mineralised it is converted to CO₂ which is trapped in a system of gas-washing bottles into bariumhydroxide. The CO₂ is quantified by titration of the remaining bariumhydroxide with HCl. Comparing the amount of CO₂ produced upon degradation of the test item with the theoretical amount of CO₂ (ThCO₂) 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 CO₂ derived from the inoculum. The mean of these values

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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 CO₂-free compressed air is continued for another 1 day in order to purge the remaining CO₂ 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 CO₂ from the test solutions after acidification of the test solutions).

8 Evaluation

$$\% \text{ TCO}_2 = \frac{\text{mg CO}_2 \text{ produced} \times 100}{(\text{mg Test item in the test solution}) \times (\text{TCO}_2)} = \% \text{ Degradation}$$

9 Test report

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

Carbon content of the test
item (calculated from
TOC-measurement):

446 mg C / g Test item¹⁾

Relation molar masses of CO₂ : C = 44.01 : 12.01 = 3.667

Resulting: TCO₂ = 1634.34 mg CO₂ / g Test item

Volume of test solutions: 3500 mL

Amount of test item within:

Test solution 1: 78.9 mg / 3500 mL

ThCO₂ = 128.95 mg CO₂ / 3500 mL Test solution

Test Solution 2: 79.6 mg / 3500 mL

ThCO₂ = 130.09 mg CO₂ / 3500 mL Test solution

¹⁾ This measurement was performed at SGS INSTITUT FRESENIUS GmbH Berlin being also accredited according DIN EN ISO 17025

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Amount of control item sodium benzoate within:

Test Solution 3: 120.2 mg / 3500 mL Test solution

ThCO₂ = 255.43 mg CO₂ / 3500 mL Test solution

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

Test solution 4: 82.7 mg test item/ 3500 mL test solution;
+ 124.5 mg sodium benzoate/ 3500 mL test solution
ThCO₂ = 399.73 mg CO₂ / 3500 mL test solution

10 Results

The results are given in table 1 and 2, and a plot of the degradation curve is included in the report.

10.1 Total-CO₂-Evolution

of the Blank: 84.56 mg CO₂ / 3.5 L in 28 d (+ 1d purging off the dissolved CO₂ from the test solutions after acidification of the test solutions)

10.2 Control: The Control was degraded 81% within 28 days. The threshold of "ready biodegradability" of $\geq 60\%$ was passed within 9 days (67%) and thus within the "10-days-window" as requested by the OECD test guideline 301B.

10.3 Test item: The threshold of "ready biodegradability" of $\geq 60\%$ was met within both test solutions. The mean value of 90% is higher than the pass value of the OECD Guideline 301B. Thus the test item „**Detergent Sheets**“ 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.

SGS Institut Fresenius GmbH

- BioServices -

D-65232 Taunusstein, date: 27-August-2018

i.V.



Dr. H. Lebertz
Study director

i.V.

for 
S. Vogel
Group leader

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Results of the Study on the Biodegradability according to CO₂ Evolution-Test, OECD 301 B

Test item: „Detergent Sheets“

Table 1: Test Solution 1 with the test item

Time [d]	Concentration: 78.9 mg / 3.5 L ThCO ₂ : 128.95 mg CO ₂ / 3.5 L	
	mg CO ₂ produced in the Test solution. cumulative	% TCO ₂ (= % Degradation)
3	15.72	12
6	42.59	33
9	64.62	50
14	82.79	64
17	90.08	70
24	104.03	81
28	110.83	86 ¹⁾
29	117.44	91

Table 1b: Test Solution 2

Time [d]	Concentration: 79.6 mg / 3.5 L ThCO ₂ : 130.09 mg CO ₂ / 3.5 L	
	mg CO ₂ produced in the Test solution. cumulative	% TCO ₂ (= % Degradation)
3	11.34	9
6	35.02	27
9	53.50	41
14	66.80	51
17	75.86	58
24	91.97	71
28	104.09	80 ¹⁾
29	114.99	88

- 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: 124.5 mg Sodium Benzoate + 82.7 mg Test Item	
	ThCO ₂ : 399.73 mg CO ₂ / 3.5 L	
	mg CO ₂ produced in the Test solution. cumulative	% TCO ₂ (= % Degradation)
3	126.04	32
6	176.45	44
9	225.84	56
14	261.44	65
17	271.63	68
24	292.22	73
28	303.87	76 ¹⁾
29	318.61	80

Table 4: Positive Control Sodium Benzoate

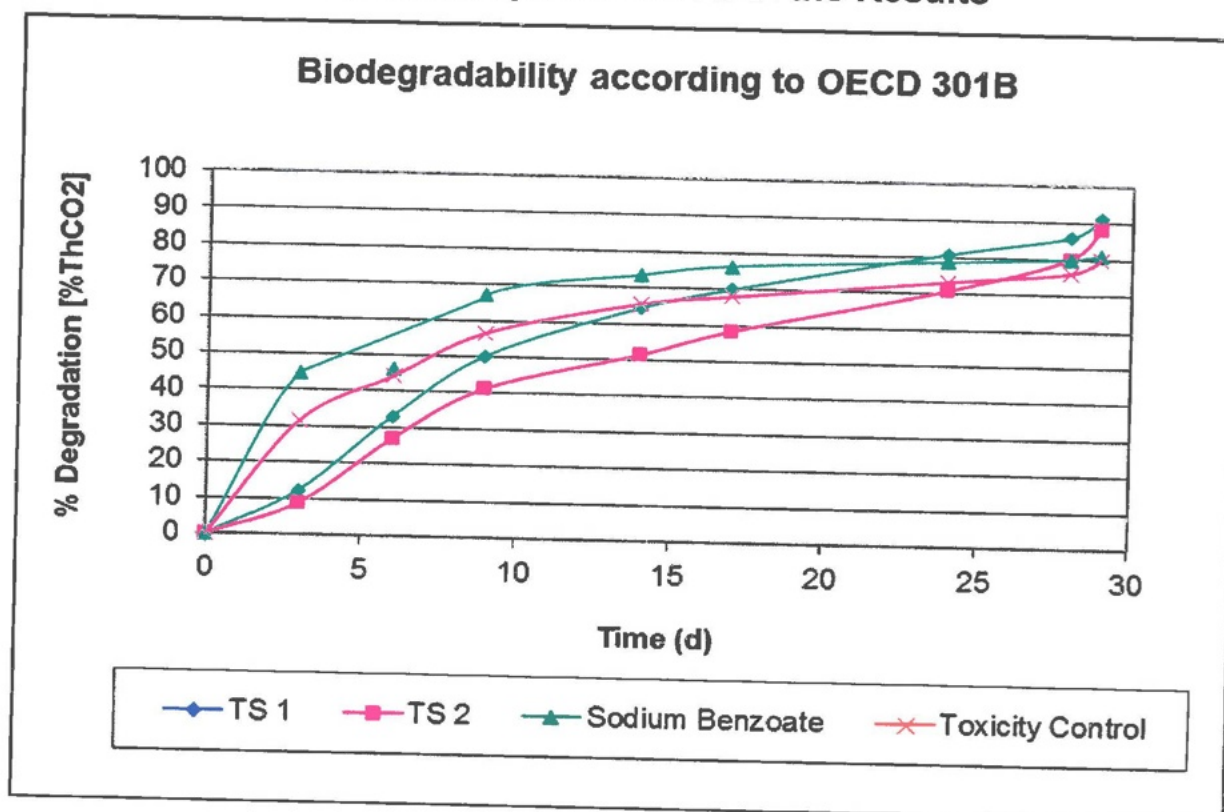
Time [d]	Concentration: 120.2 mg / 3.5 L ThCO ₂ : 255.43 mg CO ₂ / 3.5 L	
	mg CO ₂ produced in the Test solution. cumulative	% TCO ₂ (= % Degradation)
3	113.93	45
6	117.53	46
9	171.35	67
14	187.39	73
17	194.79	76
24	200.74	79
28	204.05	80 ¹⁾
29	207.38	81

- 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}.

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Graphical Representation of the Results



TS 1 = 1st Test solution with the test item
 TS 2 = 2nd Test solution with the test item

-End of Report-

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