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Report N° 1241338A01 v1

BIODEGRADABILITE

26 July 2021

Quotation 2021/66055 (DSP 844512)
Reference: Devis 2021 - Essais de biodégradabilité 301 F

Tested product

Designation: SAVON DE MARSEILLE TRADITION 74% 850745/850740 F007006
Reference: -
Batch N°: -
Brand: -
ATS reference: 819924

RAPPORT D'ANALYSE

N° de rapport d'analyse : AR-21-IY-010004-01 Version du : 26/07/2021
Dossier N° : 21G002556 Date de réception : 21/04/2021
Référence bon de commande : pocos210528

N° Ech	Matrice	Référence échantillon	Observations
003	Autres Matrices	819924 / SAVON DE MARSEILLE TRADITION 74% 850745/850740 f00	

Nouzové telefonní číslo: +420224919293 , +420224915402 (telefon 24hod/ denně) Toxikologické informační středisko, Na Bojišti 1, 128 08 Praha2)

Prélèvement effectué par (1)	Prélevé par vos soins	**Date de réception**	21/04/2021 10:34
Date de prélèvement	Non communiquée	**Début d'analyse**	26/07/2021

Biodégradabilité

	Résultat	Unité			
IY0M2 **:** Biodégradabilité facile : Essai de respirométrie manométrique Prestation réalisée par nos soins Technique [Biodégradabilité facile : Essai de respirométrie manométrique] - OCDE 301 F	see linked report				

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THE EASY BIODEGRADABILITY TEST FOR THE SAMPLE 819924- SAVON DE MARSEILLE TRADITION 74% FOLLOWING THE OECD 301 F GUIDELINE

Test report n°21TC7V-1176– 2021/07/26

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I. I. REPORT OBJECT

Ekokoza s.r.o., IČ:07508247
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This report gives results obtained on a sample received the 2021/04/21 for the realization of biodegradability assay.

II. SAMPLE PRESENTATION

Order reference: POCOS210528

Designation: SAVON DE MARSEILLE TRADITION 74%

ATS reference : 819924

Batch No.: 850745 / 850740 F007006

Expiration date: not communicate.

Conservation conditions: ambient temperature

III. DESCRIPTION THE “EASY” BIODEGRADABILITY TEST (OECD 301 F)

III.1 Principle

Biodegradability of organic compounds by micro-organisms in an aquatic medium is determined using a static test system. The test blend contains an inorganic medium, the organic compound as the only nominal source of carbon and energy with a theoretical demand in oxygen of 100 mg/L, and a mixed inoculation from an urban waste water treatment plant (amount of suspended matter inferior to 30 mg/L in the final blend).

O_2 created during the microbial degradation is trapped in measured by the system OXYTOP. Released O_2 is compared to the theoretical released quantity (THOD) and given in percentage.

The degradation rate at a given time is determined using the following equation:

$$\%\ \text{deg}\ radation\ = \frac{BOD\ (mgO\ 2\ /\ mg\ )}{ThOD\ (mgO\ 2\ /\ mg\ )} x100$$

The test standardly lasts 28 days, but can be extended if the biodegradation curve does not reach a plateau on the 28th day.

III.2 Definitions

BOD: Biochemical Oxygen Demand (mg) is the amount of oxygen consumed by micro-organisms when metabolising a test compound; also expressed as mg oxygen uptake per mg test compound.

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 latency period and ends when 90% of the degradation maximal rate is reached.

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

Easily biodegradable: a product is considered as easily 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.

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

III.3 Standard tests configuration

An analytic session has:

- Negative control (mineral medium and inoculum): 2 replicas
- Positive control (mineral medium, reference compound and inoculum): 1 replica
- Test substance (mineral medium, test substance and inoculum): 2 replicas
- Toxicity control*: 1 replica

*A test product's toxicity control: mineral medium + mix of the reference substance and the test product (tested concentrations) + inoculum

III.4 Standardtive references

- OECD 301 F Guidelines « Manometric Respirometry Test ».
- Test meTHOD C.4D: « Manometric Respirometry Test » in annex of the Commission Regulation (CE) n° 440/2008 dated May, 30th 2008 laying down test meTHODs pursuant to Regulation (CE) n° 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH).
- Water quality - Determination of the chemical oxygen demand index (ST-THOD) - Small-scale sealed-tube meTHOD following the ISO 15705 standard – subcontracting analysis in another Laboratory.

III.5 Inoculum

Origin: activated sludge from Maxeville's water treatment plant (France) dealing primarily with domestic waste water (98 %).
Activated sludge is used within 24 hours following sampling.

The solid concentration in tests vessels containing inoculum must be lower than or equal to 30 mg/L.

III.6 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
Dihydrate Sodium hydrogen phosphate, 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 dihydrate Dihydrate, $CaCl_2$, $2H_2O$: 36,40 g

Dissolve in water and make up to 1 litre.

Stock solution C:
Magnesium sulfate heptahydrate, $MgSO_4$, $7H_2O$: 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 liter.

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 liter.

III.7 Reference substance

Sodium Acetate.

III.8 Test criterion validity

According to the OECD 301F guideline:

- The median of the reference substance's (sodium acetate) degradation percentage has reached at least 60% on day 14.
- The consumption of oxygen in the blank control (negative control) containing the inoculum standardly is between 20 mg/L and 30 mg/L of medium at the end of the test and can't exceed 60 mg/L. If the value is above 60 mg/L, results and experimental meTHODs will have to be examined with a critical mind.
- If, during the toxicity test of the product to be tested and the reference substance, the degradation is below 25 % on day 14, it can be considered that the test substance has an inhibitory effect. The session must be repeated, using a lesser concentration of product and/or a higher inoculum concentration (without being over than 30 mg/L of suspended matters).
- At the end of the test, if the value of the pH is not between 6 and 8.5 and if the consumption of oxygen by the test substance is less than 60%, the test should be redone with a lesser concentration of the product.

III.9 Calculation rules

Correction of consumed Oxygen:
Biodegradation calculations are performed after subtracting the average value of the cumulated oxygen results of the negative controls (representing the inoculum endogenous activity).

Biodegradation result for the positive controls or products to test:
For a given product, the result of biodegradation at the different times of measurement corresponds to the average of the biodegradation values achieved.

The 10 days interval choice:
Per default, selecting the closer biodegradability's results day to 10%. When the gap between to consecutives values is the same from either side to 10%, choose the corresponding day to the value inferior to 10% like the starting 10 days window.
If there is litigation, a graphic evaluation can be made.

III.10 Preparation of the bacterial inoculum

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 lower than or equals to 30 mg/L.

IV. PERIOD OF INVESTIGATION

Ready biodegradability test, OECD 301 F : June, the 08th of 2021.

V. INOCULUM

Origin: activated sludge from Maxeville's water treatment plant (France) dealing primarily with domestic waste water (98%)
Sampling date: June, the 07th of 2021.

VI. RESULTS

VI.1 Theoretical Oxygen Demand (ThOD)

Result of the ThOD for the product: 2.71 mg O_2/mg.
Biodegradability tests on the product and the test substance being realized with a 37.19 mg for 1000 mL.

Test product initial concentration in medium: 6.1 mg.
Volume in test flasks: 164 mL.
ThOD are respectively equal to:
- 100 mg for the vessels " tests products " and "Positive control"
- 200 mg for the test vessel "toxicity control ".

VI.2.1 Follow-up of the consumed O_2 cumulated: negative control

Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Negative control 1	5,6	8,4	11,3	14,1	19,7	19,7	22,5	22,5	25,3	25,3	28,1	28,1	28,1	30,9	30,9	30,9	33,8	33,8	33,8	36,6	36,6	36,6	36,6	36,6	36,6	36,6	36,6	39,4
Negative control 2	2,8	5,6	8,4	11,3	14,1	14,1	19,7	19,7	19,7	22,5	22,5	25,3	25,3	25,3	28,1	28,1	28,1	28,1	28,1	30,9	30,9	30,9	30,9	30,9	30,9	30,9	30,9	30,9
Average	***4,2***	***7,0***	***9,9***	***12,7***	***16,9***	***16,9***	***21,1***	***21,1***	***22,5***	***23,9***	***25,3***	***26,7***	***26,7***	***28,1***	***29,5***	***29,5***	***31,0***	***31,0***	***31,0***	***33,8***	***33,8***	***33,8***	***33,8***	***33,8***	***33,8***	***33,8***	***33,8***	***35,2***
Standard deviation	***3,5***	***2,0***	***2,1***	***2,0***	***4,0***	***4,0***	***2,0***	***2,0***	***4,0***	***2,0***	***4,0***	***2,0***	***2,0***	***4,0***	***2,0***	***2,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***4,0***	***6,0***
Coefficient of variation	***3,9***	***28%***	***21%***	***16%***	***23%***	***23%***	***9%***	***9%***	***18%***	***8%***	***16%***	***7%***	***7%***	***14%***	***7%***	***7%***	***13%***	***13%***	***13%***	***12%***	***12%***	***12%***	***12%***	***12%***	***12%***	***12%***	***12%***	***17%***

Validity criteria as determined by the OECD 301 F guideline:

The average of the total consumption of O_2 in the blank control is not between 20 mg/L and 30 mg/L, but doesn't exceed 60 mg/L at the end of the test: 35.2 mg.

The test is considered valid.

VI.2.2 Follow-up of the consumed O_2 cumulated: positive control

Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Positive control	39,4	47,8	56,3	64,7	70,3	78,8	81,6	87,2	90,0	92,8	95,7	101,0	101,0	104,0	107,0	107,0	110,0	113,0	113,0	113,0	113,0	113,0	115,0	115,0	115,0	115,0	115,0	115,0

Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Savon de Marseille tradition 74%-1	14.1	22.5	30.9	39.4	45.0	53.5	61.9	67.5	70.3	76.0	78.8	81.6	84.4	84.4	87.2	87.2	90.0	90.0	90.0	92.8	90.0	92.8	92.8	92.8	95.7	95.7	95.7	98.5
Savon de Marseille tradition 74%-2	14.1	28.1	36.6	45.0	53.5	61.9	67.5	73.2	76.0	78.8	84.4	87.2	87.2	92.8	90.0	95.7	95.7	95.7	95.7	98.5	101.0	101.0	101.0	101.0	104.0	104.0	104.0	104.0
Average	***14.1***	***25.3***	***33.8***	***42.2***	***49.3***	***57.7***	***64.7***	***70.4***	***73.2***	***77.4***	***81.6***	***84.4***	***85.8***	***88.6***	***88.6***	***91.5***	***92.9***	***92.9***	***92.9***	***95.7***	***95.5***	***96.9***	***96.9***	***96.9***	***99.9***	***99.9***	***99.9***	***101.3***
Standard deviation	***0.0***	***4.0***	***4.0***	***4.0***	***6.0***	***5.9***	***4.0***	***4.0***	***4.0***	***2.0***	***4.0***	***4.0***	***2.0***	***5.9***	***2.0***	***6.0***	***4.0***	***4.0***	***4.0***	***4.0***	***7.8***	***5.8***	***5.8***	***5.8***	***5.9***	***5.9***	***5.9***	***3.9***
Coefficient of variation	***0%***	***16%***	***12%***	***9%***	***12%***	***10%***	***6%***	***6%***	***6%***	***3%***	***5%***	***5%***	***2%***	***7%***	***2%***	***7%***	***4%***	***4%***	***4%***	***4%***	***8%***	***6%***	***6%***	***6%***	***6%***	***6%***	***6%***	***4%***
Difference between the two values of cumulated oxygen consumption	***0%***	***-25%***	***-18%***	***-14%***	***-19%***	***-16%***	***-9%***	***-8%***	***-8%***	***-4%***	***-7%***	***-7%***	***-3%***	***-10%***	***-3%***	***-10%***	***-6%***	***-6%***	***-6%***	***-6%***	***-12%***	***-9%***	***-9%***	***-9%***	***-9%***	***-9%***	***-9%***	***-6%***

Validity criteria as determined by the OECD 301 F guideline:

The gap between consumption values cumulated of oxygen is inferior or equal to 20% every measurement days.

VI.2.4 Follow-up of the O_2 consumption cumulated: Toxicity control of SAVON DE MARSEILLE TRADITION 74% products.

Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Toxicity control	30.9	42.2	53.5	84.4	104.0	113.0	124.0	129.0	138.0	146.0	152.0	158.0	166.0	172.0	177.0	180.0	183.0	186.0	189.0	191.0	191.0	194.0	194.0	194.0	197.0	197.0	197.0	200.0

VI.2.5 Follow-up of the degradation compared to THOD: positive control

Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Positive control	35%	41%	46%	52%	53%	62%	61%	66%	68%	69%	70%	74%	74%	76%	78%	78%	79%	82%	82%	79%	79%	79%	81%	81%	81%	81%	81%	80%

Validity criteria as determined by the OECD 301 F guideline:
The degradation percentage of the reference substance has at least reached 60% after 14 days: 76%.
The test is considered valid.

VI.2.6 Follow-up of the degradation compared to the THOD: SAVON DE MARSEILLE TRADITION 74% products

Number of days	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***23***	***24***	***25***	***26***	***27***	***28***
Savon de Marseille tradition 74%-1	10%	17%	21%	27%	28%	37%	41%	46%	48%	52%	54%	55%	58%	56%	58%	58%	59%	59%	59%	59%	56%	59%	59%	59%	62%	62%	62%	63%
Savon de Marseille tradition 74%-2	10%	23%	27%	32%	37%	45%	46%	52%	54%	55%	59%	61%	61%	65%	61%	66%	65%	65%	65%	65%	67%	67%	67%	67%	70%	70%	70%	69%
Average	***10%***	***20%***	***24%***	***30%***	***32%***	***41%***	***44%***	***49%***	***51%***	***54%***	***56%***	***58%***	***59%***	***61%***	***59%***	***62%***	***62%***	***62%***	***62%***	***62%***	***62%***	***63%***	***63%***	***63%***	***66%***	***66%***	***66%***	***66%***
Standard deviation	***0%***	***4%***	***4%***	***4%***	***6%***	***6%***	***4%***	***4%***	***4%***	***2%***	***4%***	***4%***	***2%***	***6%***	***2%***	***6%***	***4%***	***4%***	***4%***	***4%***	***8%***	***6%***	***6%***	***6%***	***6%***	***6%***	***6%***	***4%***
Coefficient of variation	***0%***	***20%***	***17%***	***13%***	***19%***	***15%***	***9%***	***8%***	***8%***	***4%***	***7%***	***7%***	***3%***	***10%***	***3%***	***10%***	***7%***	***7%***	***7%***	***7%***	***13%***	***9%***	***9%***	***9%***	***9%***	***9%***	***9%***	***6%***

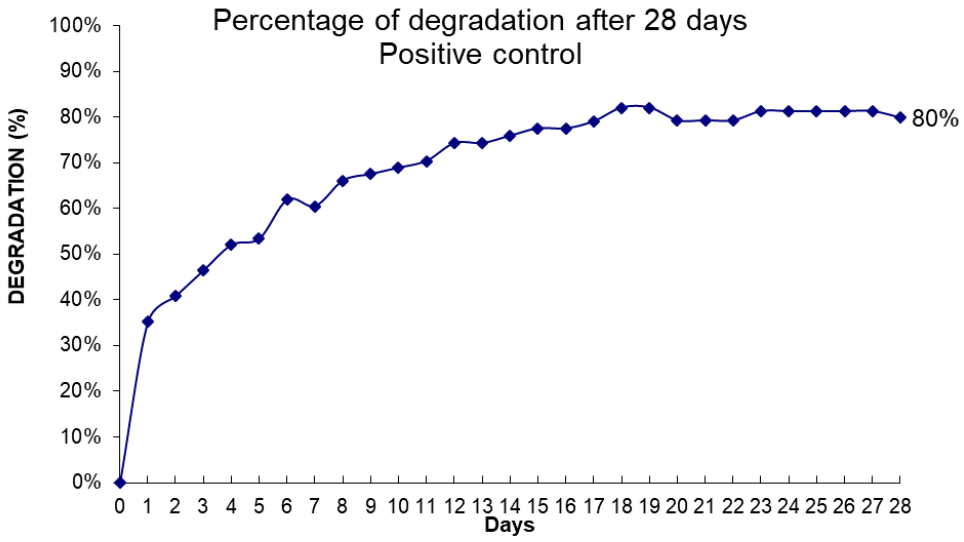
VI.2.7 Follow-up of the degradation compared to the THOD : toxicity control of the SAVON DE MARSEILLE TRADITION 74% products

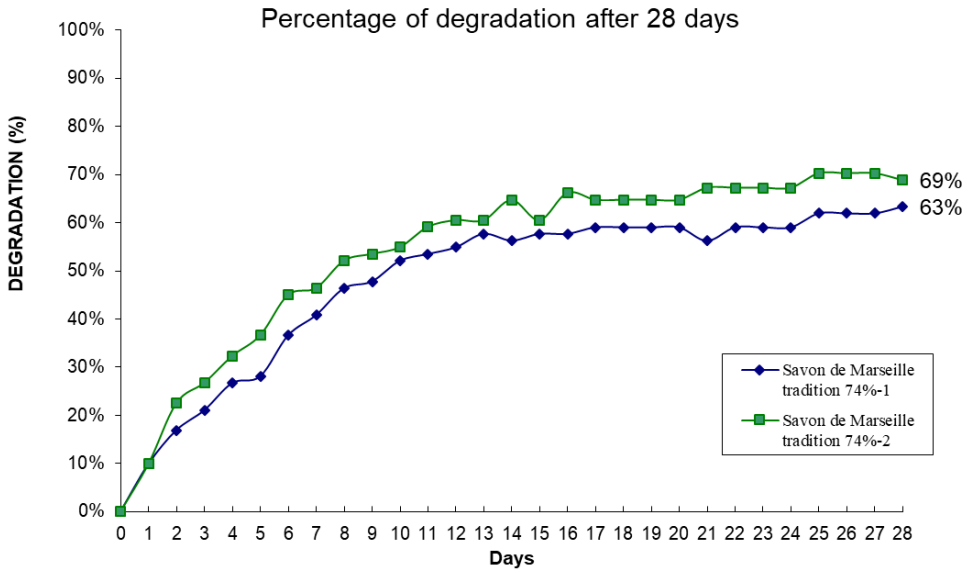
Number of days	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*	*27*	*28*
Toxicity control	13%	18%	22%	36%	44%	48%	51%	54%	58%	61%	63%	66%	70%	72%	74%	75%	76%	78%	79%	79%	79%	80%	80%	80%	82%	82%	82%	82%

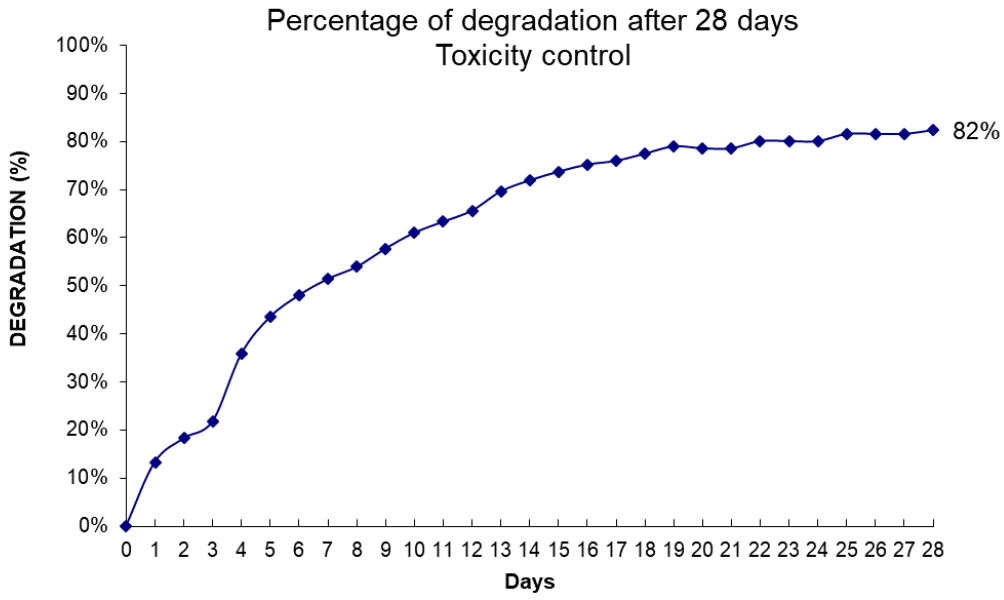
Validity criteria as determined by the OECD 301 F guideline:

The degradation of the flask containing the test substance and the reference compound, after 14 days is better than 25% (72%): the test substance can be assumed to be not inhibitory towards bacterium inoculum.

VI.2.8 Degradation curves







VI.2.9 Validity criteria as determined by the OECD 301 F guideline

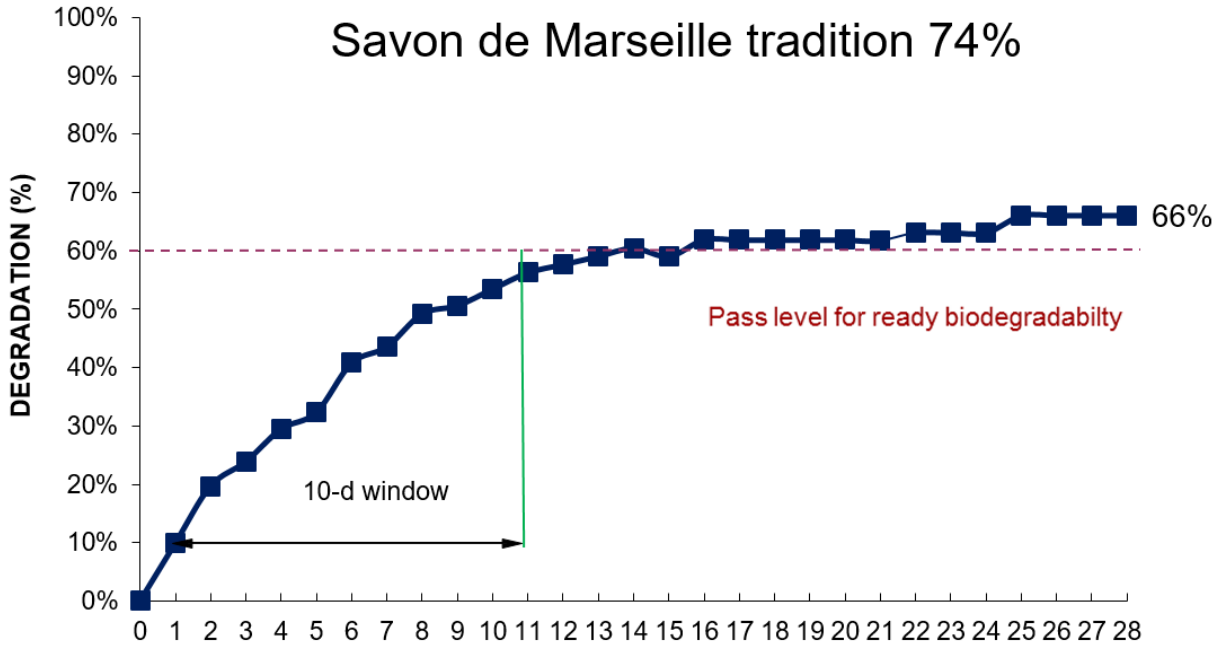
- The total median O_2 consumption in the blank test (with inoculum, so the negative control) is not between 20 mg/L and 30 mg/L, but doesn't exceed 60 mg/L at the end of the test: 35.2 mg.
- The degradation percentage of the reference substance has reached at least 60% in 14 days: 77%.
- For the toxicity test (test product and reference substance), the degradation is superior to 25% (72%) after 14 days: the SAVON DE MARSEILLE TRADITION 74% product hasn't an inhibitor effect towards the bacterial inoculum.
- At the end of the test, the pH value is in the interval which is between 6 and 8.5 (7.7).

VII. CONCLUSION

Under the experimental conditions of the test:

- The test product « SAVON DE MARSEILLE TRADITION 74% » is biodegradable without fulfilling the 10 days window criterion.
- The biodegradability of the product « SAVON DE MARSEILLE TRADITION 74% » is 66% after 28 days.

NB: the "easily biodegradable" mention is applicable on pure substances.



Maxéville, 2021/07/26,
Ludivine Spoor, Biodegradability Group Leader