REFERENCES

Order: Request of the 2012/05/14
Offer: DR12-2551 Revision 1
Received Rouen: 12/05/23
Requested by: M. BRUNET Eric
Client ID: CLEANER GTR PREMIUM
Description: GTR PREMIUM
Nature: DETERGENT
Comments:

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To: M. BRUNET Eric

Rouen, August 28th, 2012

REPORT
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Parameter :

Inherent aerobic biodegradability: Zahn-Wellens Test - OECD 302 B -

Principle: A mixture containing the test substance, mineral nutrients and a relatively large amount of activated sludge in aqueous medium is agitated and ventilated at 20-25°C in the dark or under diffuse light for up to 28 days. The biodegradation process is monitored by determination of DOC (or COD) in filtered samples taken at daily or other time intervals.

Inoculums: Activated sludge sampled the 12/05/24 from Rouen wastewater treatment plant. Microbial material recovered after centrifugation and re-suspension in the test media. The rate of inoculums (suspended materials) with test substance (DOC) is between 2.5/1 and 4.0/1 in final test vessel.

Sample:
- CLEANER GTR PREMIUM
  GTR PREMIUM
- Conservation: room temperature
- Preparation of tests solutions: thinning from sample

Materials::
Scale
Thermostatic room
Demineralised water production
Disoolved organic carbon analyzer
Stirrer

Products:

Dissolved organic carbon dosage
Orthophosphoric acid
Sodium persulfate
Sodium Hydrogenophtalate

Media
Potassium Dihydrogenophosphate
Dipotassium Monohydrogenophosphate
Disodium Monohydrogenophosphate
Ammonium Chloride
Calcium Chloride
Magnesium Sulfate
Iron(III) Chloride

Analysis date:

D=0: 2012/05/24
D=1: 2012/05/25
D=5: 2012/05/29
D=8: 2012/06/01
D=11: 2012/06/04
D=15: 2012/06/08
D=21: 2012/06/14
D=28: 2012/06/21

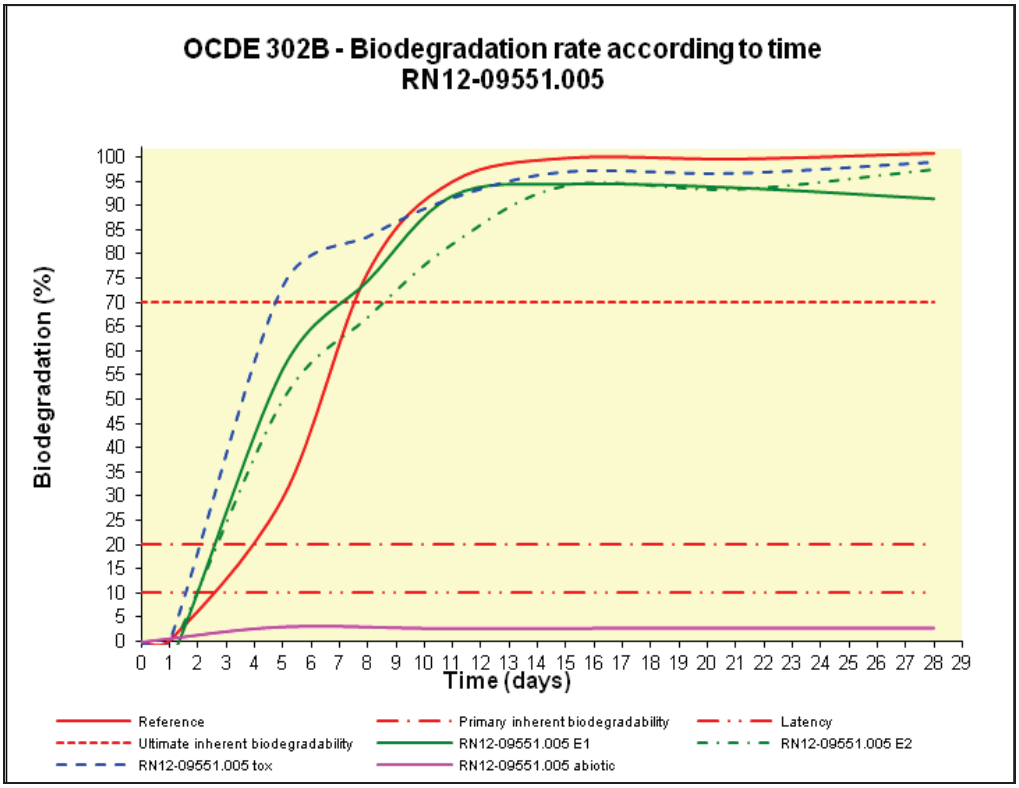
Results:

Biodegradation rate in percentage according to time:

Time (days)	0	1	5	8	11	15	21	28
Control	0	0,2	29,5	76,1	95,0	99,8	99,7	100,8
RN12-09551.005 E1	0	-4,4	56,2	74,4	92,0	94,4	93,7	91,4
RN12-09551.005 E2	0	-2,1	49,8	66,9	82,0	94,1	93,3	97,4
RN12-09551.005 tox	0	0,1	73,3	83,6	91,6	97,0	96,7	99,0
RN12-09551.005 abiotic	0	-	3,1	-	2,8	-	2,9	2,9

E1 and E2 correspond to two different test concentrations at D=0 according to the criteria of the test beginning. The tests were performed in double with differences of results lower than 20 % according to the validity criteria fixed by the OECD guidelines.

The toxicity control shows that the sample has no inhibitory effect on the inoculum.
The abiotic control shows that the sample does not deteriorate significantly from abiotically.



Remarks: Not adsorption on activated sludge
. Latency time (a) is less than 5 days.
Biodegradation time (b) is approximately of 11 days.
Biodegradation maximal rate (c) is upper 100%

a) Duration from the beginning of inoculation to the moment when the rate of degradation has reached 10%
b) Interval between the end of latency time and the moment when approximately 90% of the maximum rate of biodegradation is reached.
c) Approximate value from which there are more biodegradation during the test

Comments:

Reference control is a known readily biodegradable compound and permits to validate the assay. In this essay, reference control reach 90% of biodegradability in 11 days, therefore this essay is performed in suitable conditions.

The OECD guidelines have set a threshold of 20% biodegradation beyond which the substance demonstrates a primary inherent biodegradability and a 70% threshold beyond which the substance demonstrates an ultimate inherent biodegradability. For this analysis, there is a biodegradation of the test substance from 90% to 100% in 28 days.

The sample « CLEANER GTR PREMIUM – ***GTR PREMIUM*** » is « **inherent biodegradable without pre-adaptation** » according to OECD criteria extrapolated to a finish product.
Biodegradation kinetics (the level is reached in less than 7 days and latency in less than 3 days) leads to the conclusion of rapid biodegradation in a waste water treatment plant and extrapolate a biodegradation rate constant (k = 0.1 (h-1) in the case of a pure substance).

➢ *Comments:* OECD 302B is a standard to evaluate ready biodegradability with pure substance and not for complex compounds. Conclusion of this report is reserved and can be discussed for the different organics constituents of this sample.

OECD guidelines extract (March, 23rd 2006) (pure chemicals):

When the results indicate that inherent, ultimate biodegradability does occur, it indicates that the substance has a potential for degradation under favourable conditions, e.g. in well-operated STPs.

Inherent biodegradability tests are used to assess whether a chemical has any potential for biodegradation. The European Commission Technical Guidance Document (5) proposes that results of the Zahn-Wellens/EMPA Test (TG 302 B) and the Modified MITI Test (II) (TG 302 C) may be used for extrapolation to a rate constant in models for estimation of the elimination of chemicals in STPs (5).
However, this extrapolation is only allowed, if the inherent biodegradability tests fulfil specific criteria.

-The pass level of 70% degradation in the Zahn-Wellens/EMPA Test must be reached within 7 days, including the lag-phase and the log-phase.
-The lag-phase should be no longer than 3 days.
-The percentage removal in the test before biodegradation occurs should be below 15%.

When a negative result is obtained in a test of inherent biodegradability, it may lead to a preliminary conclusion of environmental persistence and to an evaluation of potential adverse effects of transformation products.

Results approved by **Aline JOURDAN** Tel : +33 2 35 07 91 15
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