



Certificate of analysis

Inherent aerobic biodegradability: Zahn-Wellens test – OECD 302 B

Date: Almere Octoberr 9, 2012

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

Inoculum: Activated sludge from Almere (the Netherlands) wastewater treatment plant for domestic wastewater treatment. Microbiological material is washed with mineral medium. Concentration in the test vessel is about 1 g/l.

Sample:

- Reference (Monoethyleneglycol)
- Rustconverter ARP662 (A300D026)
- Rustconverter ARP662 (Cat II)

Materials:

- Scale
- Incubator
- Aeration pump
- pH-meter
- COD analyzer

Reagents:

mineral nutrients:

10 ml Stock A + 1.0 ml Stock B, 1.0 ml Stock C en 1.0 ml Stock D.

Stock A: potassium dihydrogen orthophosphate KH_2PO_4 8.5 g
Potassium hydrogen orthophosphate K_2HPO_4 21.75 g
Disodium hydrogen orthophosphate dihydrate $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 33.4 g
Ammoniumchloride 0.5 g

Solution 1 liter with demineralised water in een volumetric flask.

Stock B: Calcium chloride dihydrate $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ 36.4 g

Solution 1 liter with demineralised water in a volumetric flask.

Stock C: Magnesium sulfate heptahydrate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 22.5 g

Solution 1 liter with demineralised water in a volumetric flask.

Stock D: Iron (III) Chloride hexahydrate $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 0.25 g

Solution 1 liter with demineralised water in a volumetric flask.

COD analysis:

Test tubes from Hanna Instruments containing:

Sulphuric Acid

Mercury (II) Sulphate

Potassiumdichromate

Results:

Biodegradation rate in percent according to time:

Concentration 0.05 g/l reference / rustconverter (Graph 1)

| | D = 0 | D = 1 | D = 3 | D = 6 | D = 8 | D = 13 | D = 15 | D = 20 | D = 23 | D = 28 |
|-----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Reference | 0 | 18 | 63 | 99 | 100 | 100 | 100 | 100 | 100 | 100 |
| A300D026 | 0 | 48 | 95 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| CAT II | 0 | 65 | 96 | 94 | 100 | 100 | 100 | 100 | 100 | 100 |

Duplo with 0.1 g/l reference / rustconverter (Graph 2)

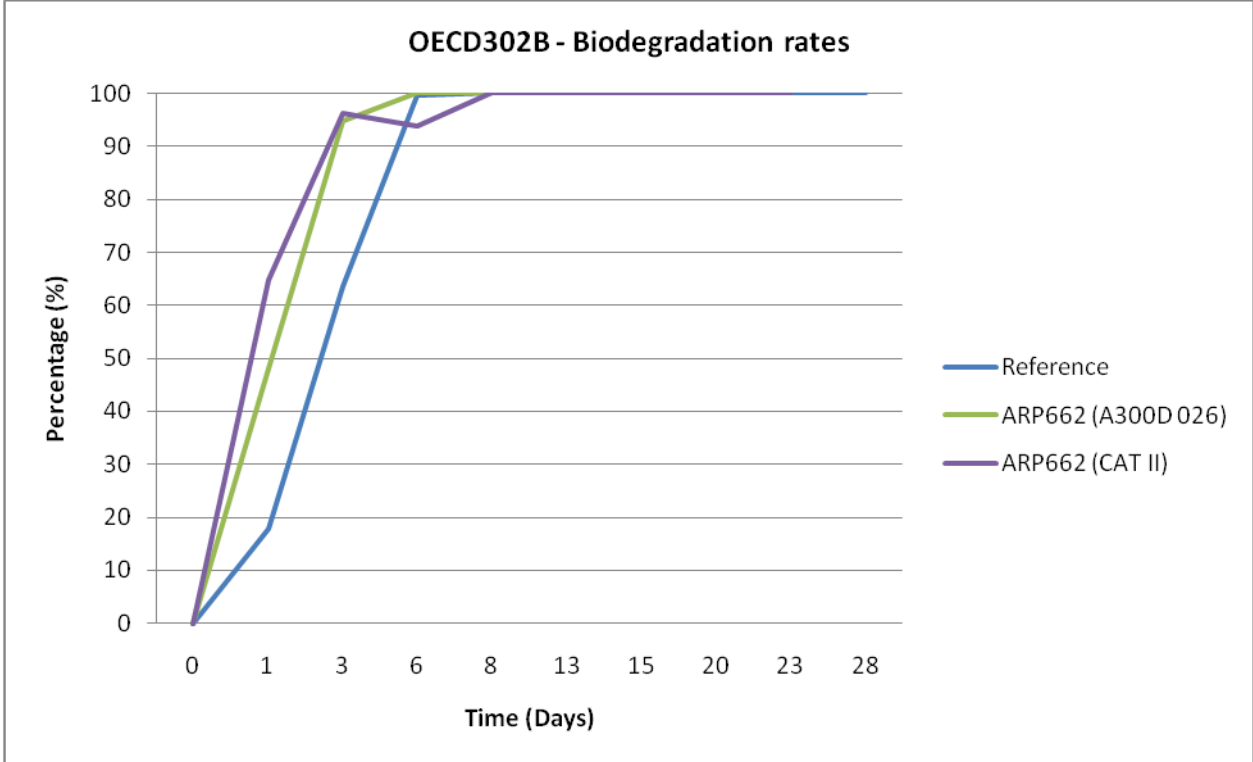
| | D = 0 | D = 1 | D = 3 | D = 7 | D = 9 | D = 14 | D = 16 | D = 21 | D = 23 | D = 28 |
|-----------|-------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Reference | 0 | 11 | 43 | 90 | 98 | 96 | 82 | 94 | 100 | 100 |
| A300D026 | 0 | 9 | 43 | 74 | 74 | 88 | 92 | 97 | 100 | 100 |
| CAT II | 0 | 40 | 75 | 98 | 100 | 100 | 100 | 100 | 100 | 100 |

The results marked in red are discarded because of false high or false low results and are not in the graphics.

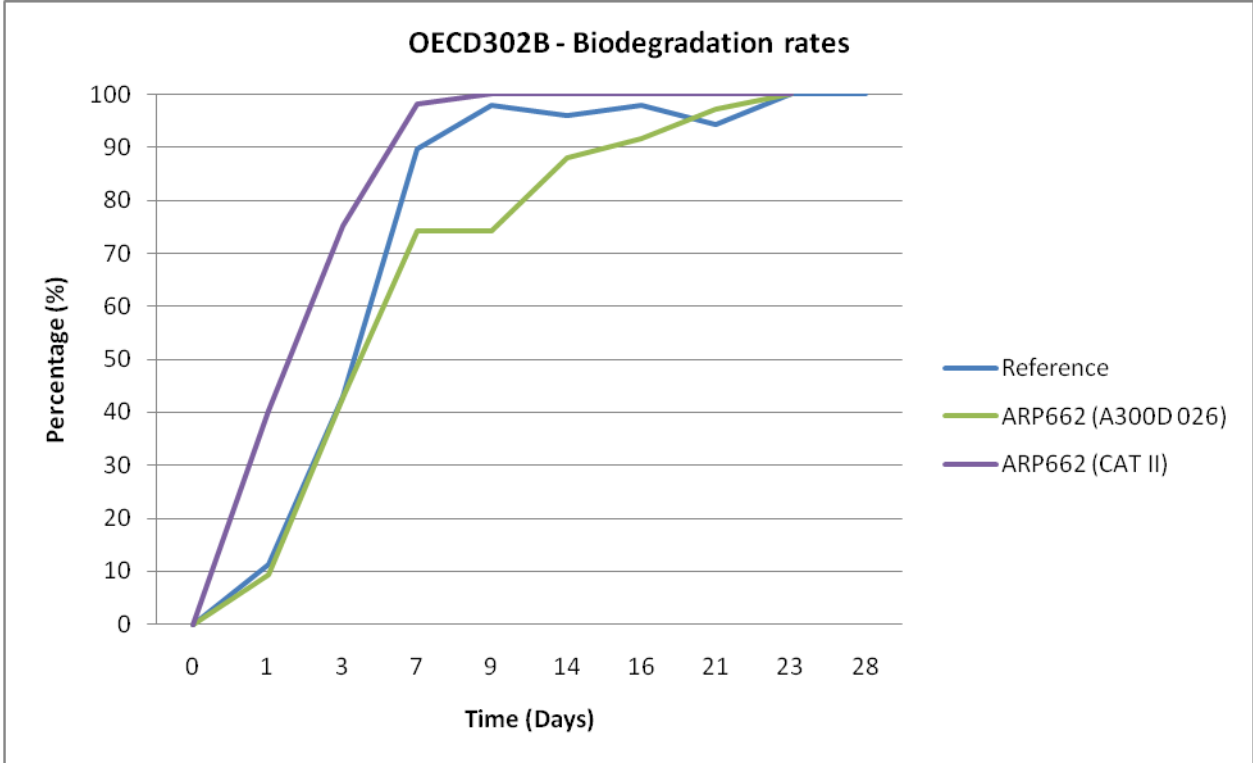
The test with 0.05 g/l test substance was ended after 8 days because of full degradation of the water soluble part of the test substance.

The test with 0.1 g/l test substance was ended after 21 days because of full degradation of the water soluble part of the test substance.

Graph 1:



Graph 2:



- Primary inherent biodegradability is at 20%
- Ultimate inherent biodegradability is at 70%

Remark: No adsorption on activated sludge.

Comments:

The reference is known for its ability to be biodegraded and will validate the whole experience. (the reference must reach at least 70% within 14 days).

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 biodegradability. For this analysis, there is a biodegradation of all test substances of at least 70% in 28 days.

All substances tested in this experience show ultimate inherent biodegradability according to OECD criteria.

NOTE: The substances tested conform this method are fully dissolved in water. Substances that do not dissolve in water will not be taken into account for biodegradation since these will be filtered from the solution (=residue). The watery filtrate is than tested for COD content.

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