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Certificate

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

Date: Almere June 10, 2011

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)
- HM-604
- WB-100
- WB-200
- SG-1

Materials:

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

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
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Solution 1 liter with demineralised water in een volumetric flask.

Stock C:	Magnesium sulfate heptahydrate $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	22.5 g
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Solution 1 liter with demineralised water in een volumetric flask.

Stock D:	Iron (III) Chloride hexahydrate $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.25 g
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Solution 1 liter with demineralised water in een volumetric flask.

*COD analysis:**Test tubes from Hanna Instruments containing:*

Sulphuric Acid

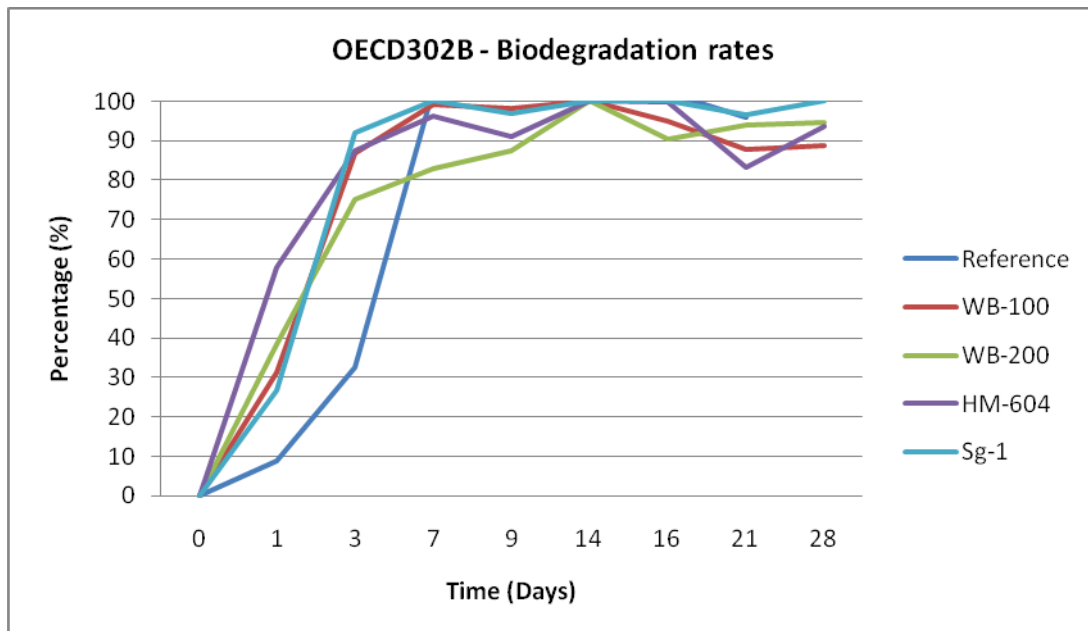
Mercury (II) Sulphate

Potassiumdichromate

Results:

Biodegradation rate in percent according to time:

	D = 0	D = 1	D = 3	D = 7	D = 9	D = 14	D = 16	D = 21	D = 28
Reference	0	9	33	100	100	100	100	96	96
HM-604	0	58	88	96	91	100	100	83	93
WB-100	0	31	87	99	98	100	95	88	89
WB-200	0	39	75	83	87	100	90	94	94
SG-1	0	27	92	100	97	100	100	97	100



- 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. (it gets 95% of degradation in less than 5 days, the experience was realized within favourable 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 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.

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