

# **Biodegradability of Universol**

Client: Grobe Nederland

Mr. R. van Bergen

PO Box 780

9400 AT Assen-Netherlands

Ordernumber: HR207037

Projectleader: dr. ir. A.E. Duisterwinkel

performed by: C.M. Görtz, dr.ir. A.E. Duisterwinkel

final responsibility: ir. H. Smits

#### Resume

#### **GENERAL**

With the two-phase closed bottle test (NEN 6515, OECD301D), the biodegradability of the cleaning agent Universol has been determined.

#### **RESULT**

After 28 days a degradation percentage of 80% is found on the basis of oxygen uptake. In the OECD guidelines for testing for chemicals, a substance is assessed as readily biodegradable if a degradation percentage of at least 60% is achieved for 28 days. From this it can be concluded that cleaning agent Universol is biodegradable.

The percentage DOC removal corresponds to the percentage of oxygen uptake. This confirms biodegradability.

No toxicity was observed in toxicity testing tests at high (400 milligrams COD / liter) and low dose (100 milligrams COD / l) cleansers.

Rapportnummer: HR207037 Instituut voor Reinigingstechnieken TNO

#### **INDEX**

#### 1. PREFACE

#### 2. METHOD

- 2.1 Two phase closed bottle test
- 2.2 Analytical methods
- 2.3 Samples

## 3. RESULTS AND DISCUSSION

- 3.1 Toxicity check
- 3.2 Oxidation percentages
- 3.3 Discussion
- 3.4 Conclusion

### **ATTACHMENTS**

Appendix 1: Toxicity check at high and low concentration of test substance

Appendix 2: Graphic representation of oxidation percentages

Appendix 3: Results of DOC analyses

Rapportnummer: HR207037 Instituut voor
Reinigingstechnieken TN0

#### 1. INTRODUCTION

Commissioned by Grobe Nederland in Assen, the institute for cleaning techniques TNO has determined the biodegradability of a cleaning agent called Universol. The degradability is determined using the two-phase (air-water) closed bottle test according to applicable standards (NEN 6515, OECD 301D)

#### 2. METHOD

### 2.1 two phase closed bottle test

Degradability is determined using the two-phase air-water closed bottle test (NEN 6515, OECD 301D). In this test, test substances are added as the only carbon and energy source to a light graft of active slip. The samples are incubated for 4 weeks and sampled weekly. The degradation of the test substance is calculated by determining the oxygen intake and comparing it with the maximum theoretical oxygen consumption. The theoretical oxygen consumption is determined prior to the test by analyzing the chemical oxygen demand. To check the found degradability percentages, additional analyzes are performed to determine the dissolved organic carbon (DOC) content.

The following samples have been used per test substance:

- test substance, in triplicate;
- toxicity check to check toxic effects of the test substance (in triplicate) The toxicity was evaluated one week after the start of the test;

In addition, the following references and blanks were used per trial:

- reference sample (sodium acetate) to test the quality of the graft used (in triplicate);
- blank without test substance, but with graft to check an autonomic oxygen uptake (in triplicate).

#### 2.2 analytical methods

For the execution of the study, the following methods were used:

- Biodegradability of organic substances according to NEN 6515 (analogue OECD 301D);
- Chemical oxygen demand according to a method derived from NEN 6633;
- Dissolved organic carbon (DOC) determined with a TOC device according to NPR 6522.

Rapportnummer: HR207037

## 2.3 Samples

A sample of the Universol detergent has been received from the client. A sample was analyzed for COD and NH4 + \_N prior to the degradability test. The results are included in table 1.

Table 1: Data samples

parameter		Universol		
CZV	mg/l	69.000		
NH4+_N	mg/l	0,8		

#### 3. RESULTS AND DISCUSSION

#### 3.1 Toxicity control

Derivation of toxicity check concluded that the addition of sample Universol does not have an inhibiting effect on the activity of the sludge (see Appendix 1). Both at high  $(400 \text{mg COD} \ / \ l)$  and at low concentrations  $(100 \text{mg COD} \ / \ l)$  of the cleaning agent an oxygen uptake (oxidation) takes place that is higher than or equal to the oxygen uptake in the reference sample.

#### 3. 2 Oxidation percentages

The oxidation percentages found are shown in Table 2. The oxidation percentage is calculated on the basis of the weekly oxygen intake over a period of 4 weeks. A graphical representation of the oxidation percentages can be seen in Appendix 2. Already after 14 days more than 60% of the test substance is broken down is calculated from the oxygen uptake. An appointment rate of 80% is found after 28 days on the basis of oxygen uptake.

Table 2: amount of degraded test substance in percent after n days on the basis of the oxygen uptake.

sample	n = 6	n = 14	n = 21	n = 28
reference	69	86	95	100
Universol	47	67	75	80

Rapportnummer: HR207037

At the end of the test, the DOC content of the samples from day 21 and 28 was determined. The DOC removal percentages are shown in table 3. In appendix 3 the results of the DOC analyzes are included.

Table 3: DOC removal (in %) after n days

sample	n = 21	n = 28
Universol	85	81

The DOC measurements confirm the conclusion about biodegradability.

An oxidation percentage of 75 to 85% corresponds in general to 100% degradation of the test substance. As a result of cell growth, a deviation between the DOC removal and the oxidation percentage is possible. A difference between the percentage DOC removal and the oxidation percentage greater than 25% may be due to defects in the test. Such a difference is not observed.

#### 3. 3 Discussion

The NH4 + \_N content of the sample has been determined. If the sample contains a high concentration of NH4 + \_N (> 0.1 milligrams per liter after dilution) then a correction should be made on the oxygen uptake for the conversion of NH4 + \_N to NO3\_N. The extra oxygen uptake should be deducted. The concentration found (see table 2) is so low that no correction is needed. The inaccuracy of the oxygen uptake, including the deviation from the blank tests, is, if the test is performed in triplicate, about 5% at high (120 milligrams per liter) concentrations and 15% at low (9 milligrams per liter) concentrations of test substance.

The dissolved organic carbon (DOC) of the sample is determined for the control of the two oxygen uptake found oxidation percentages. This assumes that the substances tested are completely soluble in water. The lower DOC removal after 28 days is due to the inaccuracy of the DOC determination and the low DOC level at which the test is used. The limit of determination of the DSC analysis is 1 milligram DOC / l. The accuracy is in the same order of magnitude with which a difference in DOC removal can be explained.

#### 3. 4 Conclusion

In the OECD guidelines for testing of chemicals, a substance is assessed as readily biodegradable if a degradation percentage of at least 60% is reached in 28 days. Measured is a degradation percentage of 80% with an inaccuracy of about 5%. From this

Rapportnummer: HR207037 Instituut voor Reinigingstechnieken TNO

it can be concluded that the cleaning agent Universol is biodegradable. This conclusion is confirmed by the DOC measurement.

Rapportnummer: HR207037

Appendix 1. Toxicity control at high and low concentration of test substance.

Actual oxygen concentration of the samples

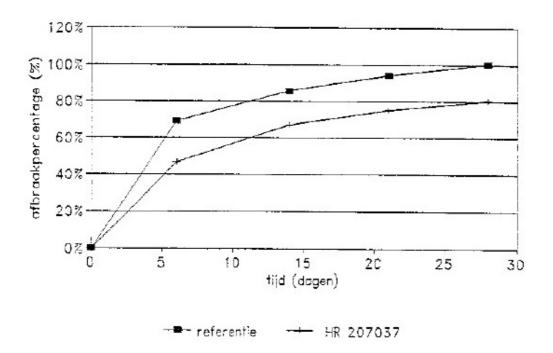
sample	reference	Universol (mg O2/I)		
	mg O2/I)	400 mg CZV/I	100 mg CZV/l	
1	5	0,5	0,1	
2	3,3	1,4	0,0	
3	4,1	1,0	0,3	
average	4,2	1,0	0,1	

The toxicity control was performed at a high and low concentration of the test substance. An amount of reference substance has been added to this. The oxygen uptake of the toxicity check should be higher or equal to the oxygen uptake in the reference sample.

The current oxygen concentration of the samples is shown in the table above. The oxygen concentration of the sample is lower than that of the reference, so the oxygen uptake is higher. There is therefore no question of toxicity.

Appendix 2: graphical representation of oxidation percentages

## degradability of cleaning agent based on oxygen uptake



Rapportnummer: HR207037 Instituut voor Reinigingstechnieken TNO

## Appendix III: results DOC analyses

sample	stock dilution mg O2/I)	volume stock dilution	added amount	day 21	day 28
blanco				8	18
Universol	3600	1,5	27	12	23

The added amount of DOC is calculated from the concentration of the stock solution, the volume used and the liquid in the flask. (see appendix 4 rough data).

