Labors: Analytik, Ökotoxikologie,
Verfahrenstechnik

LAVANDAX

DEGRADATION BIOLOGIQUE INHERENTE (OECD 302 B):
Détermination de la dégradation biologique de substances organiques solubles dans l'eau dans un test statique (Test Zahn-Wellens / EMPA)

1. Informations générales

Institut de contrôle:	BMG Engineering AG, Ifangstr.11, CH-8952 Schlieren
Responsable du contrôle:	Dr. A. Häner
Echantillon d'analyse:	LAVANDAX (produit intégrale)
Numéro d'échantillon BMG:	1110077417
Numéro de rapport BMG:	1483/e-07
Donneur d'ordre:	M. O. Menegalli MENEGALLI SA, Av. de Provence 10, CH-1007 Lausanne
Date du mandat:	11 octobre 2007
Durée du test:	18.10.07 – 15.11.07 (28 jours)
Méthode de test:	OECD 302 B, adopted July 17, 1992: "Inherent Biodegradability: Zahn-Wellens / EMPA Test".
Instructions standards:	BMG-1018

Le test est effectué selon les directives ISO/IEC 17025.

Les données primaires et les copies du rapport de contrôle sont conservées dans les archives de BMG Engineering AG à Schlieren pendant 5 ans.

2. Conditions de test

Concentration:	657 mg/l (51.5 mg/l comme DOC)
Substance de référence:	49.8 mg/l diethylène glycol (comme DOC)
Température:	22.5 ± 0.5° C
Matière sèche de la boue:	0.2 g/l
Inoculum:	Boue active provenant de l'étape biologique de la centrale d'épuration de Werdhölzli (Zurich), non adaptée: 18.10.2007, 9.00.
Procédure d'analyse:	Détermination DOC (limite de décèlement: 0.05 mg/l); Shimadzu TOC-5050. Détermination du CO_2 libéré en % $ThCO_2$ (Minéralisation). Détermination de l'effet inhibiteur sur la nitrification (consommation d'ammonium).

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3. Résultat de test

Dans les conditions de la procédure d'analyse et sur la base de la détermination DOC, le produit LAVANDAX présente une élimination de **99** resp. **100 %** après **14** resp. **28 jours** de test. Une élimination presque complète de l'échantillon d'analyse après 28 jours et à un **degré de minéralisation** de **76 %** $ThCO_2$ a pu être constatée sans période d'adaptation significative.

Les résultats montrent que, dans les conditions de la procédure d'analyse (OECD 302 B), LAVANDAX présente une élimination de plus de 70 % ("**ultimately biodegradable**").

Puisque une élimination de plus de 90 % et un degré de minéralisation de plus de 70 % à été constatée, LAVANDAX peut être qualifié de **biologiquement bien dégradable**.

Pour la concentration de test, aucun effet toxique de l'échantillon d'analyse sur la population de microorganismes nitrifiants de la boue active n'a été constaté.

La substance de référence, diethylène glycol, présente une élimination de 99 resp. 100 % après 14 resp. 28 jours de test. Une minéralisation à un degré de 91 % $ThCO_2$ après 28 jours a pu être constatée. Une élimination de plus de 70 % après 14 jours démontre le choix approprié de l'inoculum et des conditions de test.

Le rapport intégrale ainsi que les données primaires peuvent être examinés sur demande auprès du donneur d'ordre ou de l'institut de contrôle sous le numéro de rapport 1483/e-07.

29 novembre 2007

Dr. A. Häner
Chef Écotoxicologie

Des indications sur les spécifications de test (valeur limite de détermination, incertitudes des mesures) peuvent être obtenues sur demande. Les résultats de test indiqués se rapportent exclusivement à l'échantillon cité ci-dessus. Des extraits du rapport ne peuvent être reproduits sans l'accord écrit du laboratoire de contrôle.

CONTENTS

PREFACE

General

Location of study	BMG Engineering Ltd., Ifangstrasse 11, CH-8952 Schlieren
Study director	Dr. A. Häner
Study title	LAVANDAX Inherent Biodegradability - Evaluation of the Aerobic Biodegradability in an Aqueous Medium: Zahn-Wellens / EMPA Test
Test substance	LAVANDAX
BMG sample no.	1110077417
BMG report no.	1483/e-07
Test system	Activated sludge
Sponsor	M. O. Menegalli MENEGALLI SA, Av. de Provence 10, CH-1007 Lausanne
Incoming orders	11 October 2007
Study timing	Start of the test: 18 October 2007; End of the test: 15 November 2007
Data storage	Raw data and a copy of the final report are stored in the archives of BMG Engineering Ltd. in Schlieren for 5 years.

Analytical chemistry of the test media:

DOC determinations: daily during the first phase of the test, directly after sampling; then at least once a week and on the 27th and 28th days, or, if the plateau is attained in less than 28 days, on the last two days of the test.

CO_2 production: determined as inorganic carbon (IC) 4 times during the test.

NH_4^+ consumption: at the same time of measurement as DOC until complete consumption was reached.

Guidelines

The study procedure described in this report was based on the recommendations of the following guideline:

Organisation for Economic Cooperation and Development (OECD):

OECD Guidelines for the Testing of Chemicals, "Inherent Biodegradability: Zahn-Wellens / EMPA Test", Procedure 302B, adopted on 17 July 1992.

The test procedure was conducted as described in detail in BMG's Standard Operating Procedure (Standardarbeitsanweisung BMG-1018).

Quality assurance statement

The study described in this report was conducted in accordance with ISO/IEC 17025.

1. SUMMARY

The biodegradability of LAVANDAX exposed to activated sludge of a municipal sewage treatment plant was investigated under aerobic static conditions.

Based on the data of the individual DOC determinations LAVANDAX reached a biodegradation of 100 % after 28 days.

The main degradation process occurred during the first 4 days, when more than 95 % of the initial DOC was removed. Maximum biodegradation was attained after 11 days of incubation. No significant adaptation period (lag phase) was observed.

No significant elimination of the test substance due to adsorption to the activated sludge, on the glass surface or other physico-chemical processes was found as determined by means of DOC measurements 3 h after the start of the test.

The positive control, diethyleneglycol, showed 99 % biodegradation after 14 days of incubation thus confirming suitability of inoculum and test conditions. The test was considered valid, since the degradation of the reference compound reached more than 70 % within 14 days of incubation.

The biodegradation of LAVANDAX based on CO_2 evolution and calculated as % $ThCO_2$ reached 76 % after 28 days of incubation, showing that the main part of the organic carbon of the test material was transformed into CO_2. The extent of mineralization of the procedure control with diethyleneglycol was within the same range showing a value of 91 % based on $ThCO_2$.

The test substance showed no significant toxic effects to the activated sludge.

Based on these results LAVANDAX can be termed as ultimately biodegradable under the conditions of the OECD guideline no. 302, since more than 70 % degradation was attained after 28 days of contact time.

2. PURPOSE

The objective of this study was to determine the biodegradability of LAVANDAX under aerobic static conditions using the Zahn-Wellens / EMPA Test.

In this method, activated sludge, mineral nutrients and the test material as the sole carbon source were incubated together in a glass vessel placed on an orbital shaker under controlled conditions. The degradation of the test material was monitored by the determination of the dissolved organic carbon (DOC) at regular time intervals. The ratio of eliminated DOC after each interval to the value 3 hours after the start of the test was expressed as percentage biodegradation. The percentage biodegradation was plotted against time to give the biodegradation curve.

The produced CO_2 was determined at least 3 times during the test as an additional measure of the biodegradation expressed as the percentage of the theoretical value (% $ThCO_2$).

A potential toxicity of the test material was assessed by the determination of NH_4^+ consumption.

3. MATERIAL AND METHODS

3.1. Test substance

Substance name	LAVANDAX
Batch number	-
Concentration in the test	657 mg/l
Concentration as DOC	51.5 mg/l
Procedure control	Diethyleneglycol, 49.8 mg/l (as DOC)

3.2. Test system and test conditions

Inoculum	Activated sludge from the aeration tank of a municipal biological waste water treatment plant, not adapted, not pre-conditioned. 0.2 g/l dry matter in the final mixture ARA Werdhölzli, CH-8048 Zürich, 18 October 2007; 9.90 a.m.
Test unit	1200 ml closed glass bottle containing a total volume of test solution of 800 ml; aerated with CO_2-free air and fitted to gas-absorption bottles containing 125 ml of 0.13 M KOH
Test medium	Aerobic mineral salts medium (Table 5) prepared with double distilled water (conductivity: <1.5 μS/cm; DOC: <0.3 mg/l)
Feed	None, test substance or procedure control as sole organic carbon sources
Test duration	28 days
Incubation	Temperature-controlled dark room (22 ± 0.5 °C)

3.3. Test procedure

The activated sludge was used immediately after sampling from the treatment plant without adaptation. Prior to the test the sludge was washed twice with tap water. The test material was diluted with mineral salts medium to give a final DOC concentration of about 50 mg/l.

For each test series the following number of test flasks was set up:

Test suspension (T) 2 replicates:	containing activated sludge + test medium + test substance
Inoculum blank (B), 2 replicas:	containing activated sludge + test medium
Procedure control (R), 1 replicate:	containing activated sludge + test medium + diethyleneglycol as ready biodegradable reference compound

The test vessels were stirred (100 r.p.m.) and aerated with synthetic CO_2-free air for a maximum test period of 28 days. The air leaving the individual vessels was passed through gas-absorption bottles filled with NaOH. It was assured that during the test the oxygen concentration was >6 mg/l. The pH-value was checked periodically and adjusted to pH 6.5-8.0 with NaOH or HCl, if necessary.

The elimination of the test material was followed by DOC determinations at regular intervals. First samples were analyzed at the beginning of and 3 h after starting the test. The trapped CO_2 was determined as inorganic carbon (IC). NH_4^+ decrease was followed during the first days of incubation until total consumption was attained.

3.4. Chemical analyses

Dissolved organic carbon (DOC) was determined in duplicate with a Shimadzu 5050 TOC-Analyzer using the NPOC-mode. For each determination at least 3 single injections were performed. In Table 1 the mean values of the individual measurements are given.

Aliquots from the individual test vessels were centrifuged (15 min at 4500 g) and the supernatant acidified to pH <2. Prior to analysis the samples were sparged with CO_2-free high purity air for 5 min to remove inorganic carbon. Inorganic carbon (IC) was determined in the same way as DOC without sparging the samples before analysis.

NH_4^+ was determined according to EDI guidelines.

3.5. Data handling

The degree of biodegradation/elimination attained at the end of the test after 28 days or, if degradation is attained in less than 28 days, at an earlier time, is reported as "ultimate biodegradability in the static test (after x days)" if more than 70 % biodegradation was reached. Evidence of "inherent biodegradability" is given, if an elimination of the test substance between 20 and 70 % was reached.

If the result of analysis of the sample 3 h after the start of the test is significantly different from the initial value, the amount of deficient DOC is reported as "elimination due to adsorption by the activated sludge or other physico-chemical processes".

Additionally, the degree of biodegradation was calculated as % of $ThCO_2$. The $ThCO_2$ (Theoretical carbon dioxide evolution in mg) is the total quantity of carbon dioxide calculated to be produced from the known or measured carbon content of the test material when fully mineralized.

Calculation of the percentage degradation

The percentage removal of DOC was calculated using the following equation:

$$D_T = \left[1 - \frac{C_T - C_B}{C_A - C_{BA}} \right] \times 100$$

D_T is the biodegradation/elimination at time t in %
C_T is the mean DOC value of the test suspension at time t in mg/l
C_B is the mean DOC value of the blanks at time t in mg/l
C_A is the mean DOC value of the test suspension after 3 h in mg/l
C_{BA} is the mean DOC value of the blanks after 3 h in mg/l

The percentage biodegradation/elimination rounded to the nearest full percent was calculated from the DOC values of the last measurement.

The percentage biodegradation from the produced CO_2 was calculated from:

$$\% ThCO_2 = \frac{\text{mg IC from test unit - mg IC from inoculum blank}}{\text{mg DOC added as test material}} \times 100$$

% $ThCO_2$ is the biodegradation at time t in % of the theoretical CO_2
IC is the mean inorganic carbon value of the individual absorption bottles at time t in mg/l
DOC is the organic carbon content of the added test material at the beginning of the test

4. RESULTS AND DISCUSSION

4.1. Determination of biodegradation / elimination

Based on the data of the individual DOC determinations (Table 1, Figure 1) LAVANDAX reached a biodegradation of 100 % after 28 days.

The main degradation process occurred during the first 4 days, when more than 95 % of the initial DOC was removed. Maximum biodegradation was attained after 11 days of incubation. No significant adaptation period (lag phase) was observed.

No significant elimination of the test substance due to adsorption to the activated sludge, on the glass surface or other physico-chemical processes was found as determined by means of DOC measurements 3 h after the start of the test.

The positive control, diethyleneglycol, showed 99 % biodegradation after 14 days of incubation thus confirming suitability of inoculum and test conditions. The test was considered valid, since the degradation of the reference compound reached more than 70 % within 14 days of incubation.

The biodegradation of LAVANDAX based on CO_2 evolution and calculated as % $ThCO_2$ reached 76 % after 28 days, showing that the main part of the organic carbon of the test material was transformed into CO_2. The extent of mineralization of the reference compound diethyleneglycol was within the same range showing a value of 91 % based on $ThCO_2$ (Table 3).

At the applied initial test substance concentration the test substance showed no significant toxic effects to the activated sludge, since the consumption of NH_4^+ in the test unit proceeded as fast as in the inoculum blank (Table 2) indicating no inhibition of the nitrifying microorganisms.

Based on these results LAVANDAX can be termed as ultimately biodegradable under the conditions of the OECD guideline no. 302, since more than 70 % degradation was attained after 28 days of contact time.

4.2. Remarks and alterations of the standard procedure

None.

5. REFERENCES

Organisation for Economic Cooperation and Development (OECD):

OECD Guidelines for the Testing of Chemicals, "Inherent Biodegradability: Zahn-Wellens / EMPA Test", Procedure 302B, adopted on 17 July 1992.

Eidgenössisches Departement des Inneren (EDI):

Richtlinien für die Untersuchung von Abwasser und Oberflächenwasser, 1. Teil: Abwasser, 30 Ammoniak/Ammonium, September 1983.

Table 1: DOC concentrations of test suspension, inoculum blank and procedure control and calculation of degradation data.

	Inoculum blank (B) *	Procedure control with diethylene glycol (R) *			Test suspension with test material (T) *		
Time (days)	DOC (mg/l)	DOC (mg/l)	DOC net. (mg/l)	Degradation (%)	DOC (mg/l)	DOC net. (mg/l)	Degradation (%)
0	0.6	51.4	50.8	-	49.8	49.1	-
0.125	1.5	51.7	50.1	0	51.4	49.9	0
1	1.7	52.4	50.7	-1	42.9	41.2	17
4	3.5	42.8	39.3	22	5.1	1.6	97
8	2.1	5.0	2.9	94	4.1	2.0	96
11	2.5	2.9	0.4	99	2.7	0.2	100
14	2.0	2.4	0.4	99	2.3	0.4	99
18	2.0	2.3	0.3	99	2.4	0.4	99
21	2.2	2.8	0.5	99	2.4	0.2	100
25	2.1	2.5	0.4	99	2.4	0.3	99
27	2.0	2.2	0.1	100	2.3	0.3	99
28	2.0	2.2	0.1	100	2.2	0.2	100

* Mean of two replicates

Figure 1:
Degradation curve for LAVANDAX

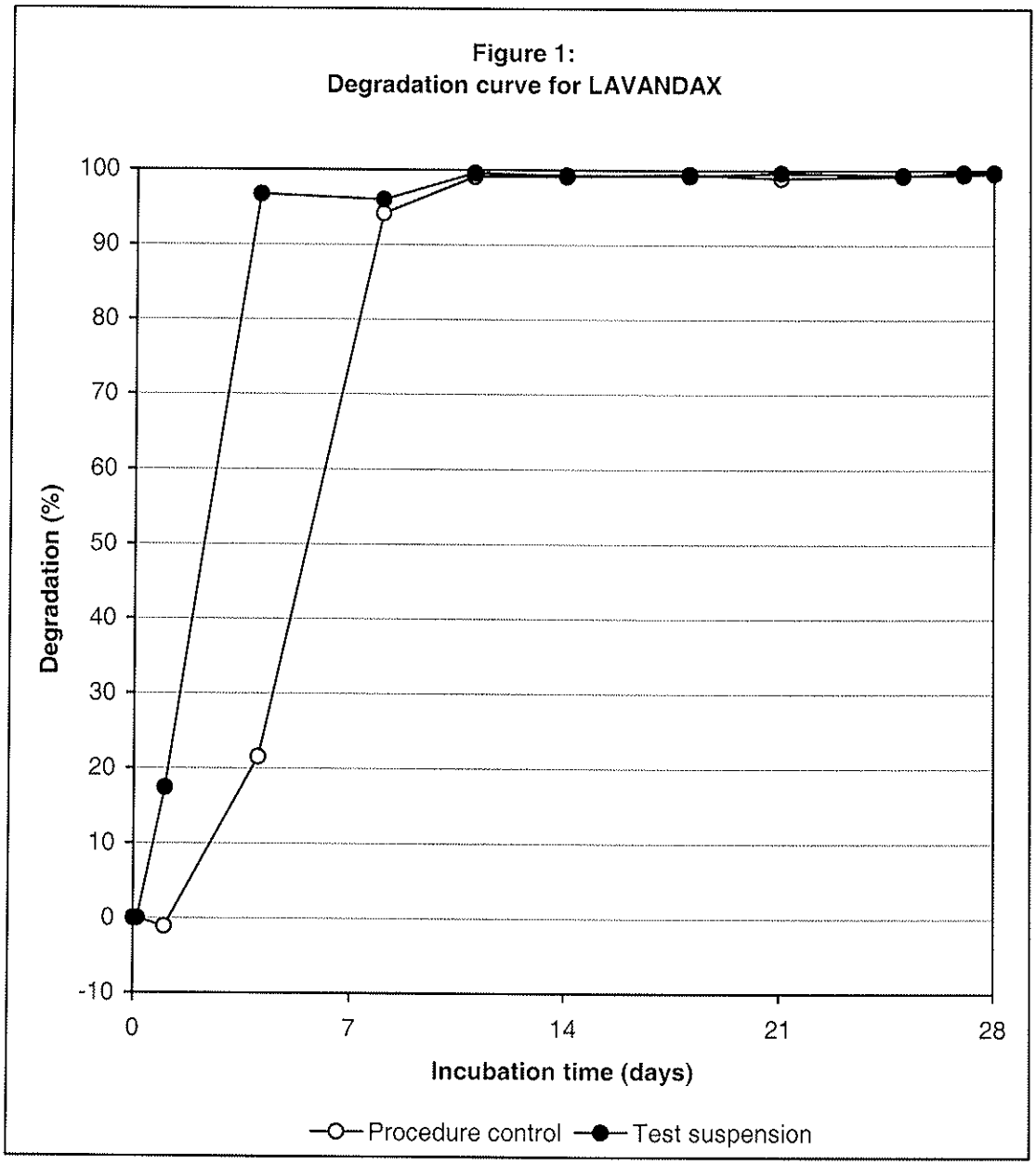

Table 2: NH_4^+ concentrations of test suspension, inoculum blank and procedure control.

Time (days)	Test suspension NH_4^+ (mg/l)	Procedure control NH_4^+ (mg/l)	Inoculum blank NH_4^+ (mg/l)
0	1.70 [a)]	1.70 [a)]	1.70 [a)]
1	<0.05	<0.05	<0.05
8	<0.05	<0.05	<0.05
14	<0.05	<0.05	<0.05

a) Nominal concentration

Table 3: IC concentrations, calculated from the concentrations in the gas absorption bottles, of test suspension, inoculum blank and procedure control and corresponding degradation data.

Time (days)	Test suspension IC (mg/l)	Procedure control IC (mg/l)	Inoculum blank IC (mg/l)	Biodegradation Test suspension (% $ThCO_2$)	Biodegradation Procedure control (% $ThCO_2$)
0	nd	nd	nd	-	-
8	37.2	35.4	12.8	48	46
14	49.4	60.6	20.1	57	81
21	59.1	64.4	22.1	72	85
28	65.8	72.4	26.9	76	91

nd not determined

Table 4: Composition of the mineral salts medium.

Stock solution I (pH 7.4)	10 ml/l	Ammonium chloride, NH_4Cl Di-sodium hydrogenphosphate, $Na_2HPO_4 \times 2\ H_2O$ Potassium dihydrogenphosphate, KH_2PO_4 Di-potassium monohydrogenphosphate, K_2HPO_4	0.50 g 33.40 g 8.50 g 21.75 g
Stock solution II	1 ml/l	Calcium chloride, anhydrous, $CaCl_2$	27.50 g
Stock solution III	1 ml/l	Magnesium sulfate, $MgSO_4 \times 7\ H_2O$	22.50 g
Stock solution IV	1 ml/l	Iron (III) chloride, $FeCl_3 \times 6\ H_2O$	0.25 g

6. SIGNATURE

It is certified that the test for inherent biodegradability of LAVANDAX was carried out under the study director's supervision using the guidelines and standard operating procedures described in this report and that this report forms a true and accurate record of the procedures performed and of the results obtained.

The study described in this report was conducted in accordance with ISO/IEC 17025.

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Dr. A. Häner (Study Director)

29 November 2007
Date