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Ecological laboratories EMPLA
Testing laboratory No. 1110 accredited by CAI according to ČSN EN ISO/IEC 17025: 2005
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Test Report No. T 43/2019
Biodegradability

Sponsor	**TUMAX - PLUS, s.r.o.** Hornická 32 252 25 Jinočany, Praha - západ
Order No.	**2198/18** – 19.12. 2018
Requirements	**Determination of biodegradability in according to OECD Method 301 D (Closed Bottle Test)**
Sample No.	**19192**
Product description	**Bio - Akcelerator „BIUS**
Performed in period	09.01. – 05.02.2019
Performed by	Ivona Čefelínová
Date of test report issue	8.02.2019
Test report prepared by	Ivona Čefelínová
Approved by	Ing. Stanislav Eminger, CSc., Testing Laboratory Manager

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Ing. Mojmír Špaček, Ph.D.
Vedoucí analytické laboratoře
Zást. vedoucího Ekologických laboratoří EMPLA

The results relate only to the tested item.
The test report shall not be reproduced except in full, without written approval of the Ecological laboratories EMPLA.

1 Test Method

OECD 301 D

Ready biodegradability of organic substance in aqueous medium: Closed bottle test
(accredited as SOP ET 8 – equiv. Reg. (EC) No 440/2008 - C.4-E, ČSN EN ISO 10707)

Principle of the test

A medium with a mineral substratum and the test substance as the only carbon source is inoculated by a relatively low amount of microorganisms taken from a mixed culture.
Filled bottles are closed and incubated in dark space at konstant temperature.
Subsequently, the biochemical oxygen demand is determined in regular intervals over a period of 28 days, by means of test bottles prepared in parallel and withdrawn again after determination.
Biodegradation is then calculated from the ratio between the oxygen consumption that is caused by the degradation of the test item (corrected by the blank values of the inoculum), and the theoretical oxygen demand (ThOD).

A degradation extent of > 60% ThOD within 28 days and within a "10-days window" after the end of the lag-phase is defined as criterion for classifying a particular test substance as "readily" biodegradable.

Inoculum
Fresh filtered effluent from outflow of a municipal wastewater treatment plant in Hradec Králové, aerated for 24 hours.
Final concentration of inoculum in reaction mixture: 3.0 ml/l.

Bottles with tested solutions: glass bottles for BOD determination, volume 250 ml, filled without air bubble, closed

Test conditions
Incubation of BOD bottles: 20 – 25 °C in dark (fixed in termostat),
Temperature must be constant during the test (± 1 °C)

Determination of dissolved oxygen: by oxygen probe (membrane electrode)

The duration of the test: 28 days

2 Procedure

Tested item - sample No. 19192

Initial COD-Cr of tested solution:	6.5 mg/l
Concentration of base solution:	1000 mg/l
COD-Cr of base solution (1g/l):	0.051 mg/mg
Amount of tested item in test:	128 ml base solution/l

Reference substantion - Sodium benzoate

Initial COD-Cr:	6.5 mg/l
Concentration of base solution:	1000 mg/l
COD-Cr of base solution (1g/l):	1.748 mg/mg
Amount of refer. solution in test:	3.7 ml base solution /l

The number of bottles in the test

10 pcs (for tested suspension, inoculum blank and procedure control with refer. substance)

Analyzing of dissolved oxygen and pH

In day of preparation and then after 7, 14, 21, 28 days (end of testing).
The dissolved oxygen is analyzed at least two glasses for each concentration.
After that the pH is measured.

Test of inhibition

Test of inhibition was performed to determine toxicity of the test substance.

3 Calculation and expression of results

All measured values are put to the tables.
The calculation of biological degradation was made this way:
First the BOD was calculated after each time period by subtracting the oxygen depletion (mg O_2/litre) of the inoculum blank from that exhibited by the test chemical. This corrected depletion was divided by the concentration (mg/litre) of the test chemical, to obtain the specific BOD as mg oxygen per mg test chemical. The percentage biodegradability was calculated by dividing the specific BOD by the specific COD.
The average value from parallel tests was calculated (expressed in %).

The same procedure of calculation was udsed for reference substance.

The diagrams were compiled – see page 4 and 5. There is plotter value of biodagradation percentage depending on time (curve of boidegradation). The graphs reveals the biodegradability, especially lag-phase, period of biodegradation and maximum level of biodegradation.

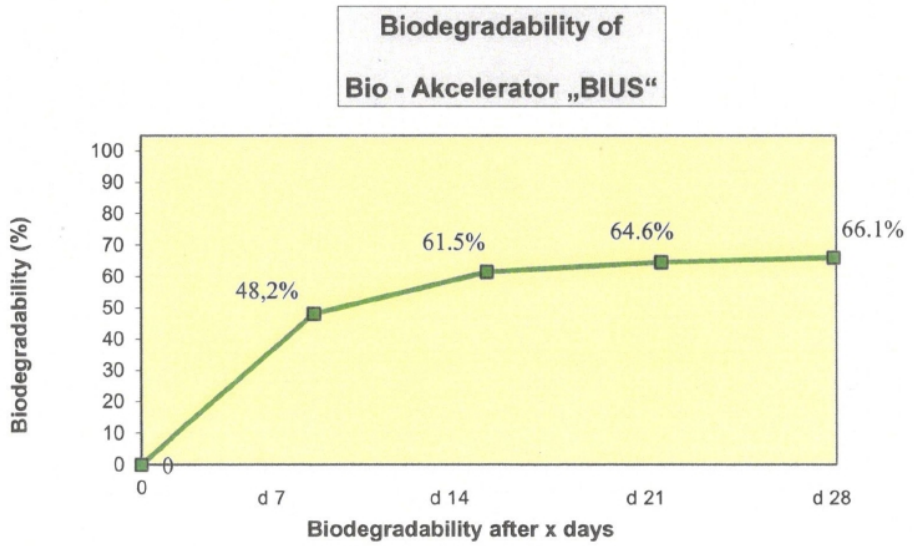
1. Bio - Akcelerator „BIUS“

Determination of dissolved oxygen

Determination type	Sample number	Concentration of dissolved oxygen in mg/l after x days				
		0 d	7 d	14 d	21 d	28 d
Tested substance with inoculum	1.	9,07	5,38	4,25	3,88	3,72
	2.	9,07	5,19	4,21	3,95	3,61
	Average	**9,07**	**5,29**	**4,23**	**3,92**	**3,67**
Blank with inoculum	1.	8,92	8,22	8,05	7,91	7,85
	2.	8,92	8,31	8,10	8,02	7,77
	Average	**8,92**	**8,27**	**8,08**	**7,97**	**7,81**

Biodegradability:

Each sample **Bio - Akcelerator „BIUS“**	Calculated biodegradability after x days				
	0 d	7 d	14 d	21 d	28 d
1.sample	0%	46,7%	61,2%	65,2%	65,2%
2.sample	0%	49,6%	61,8%	64,1%	66,9%
Average	**0%**	**48,2%**	**61,5%**	**64,6%**	**66,1%**



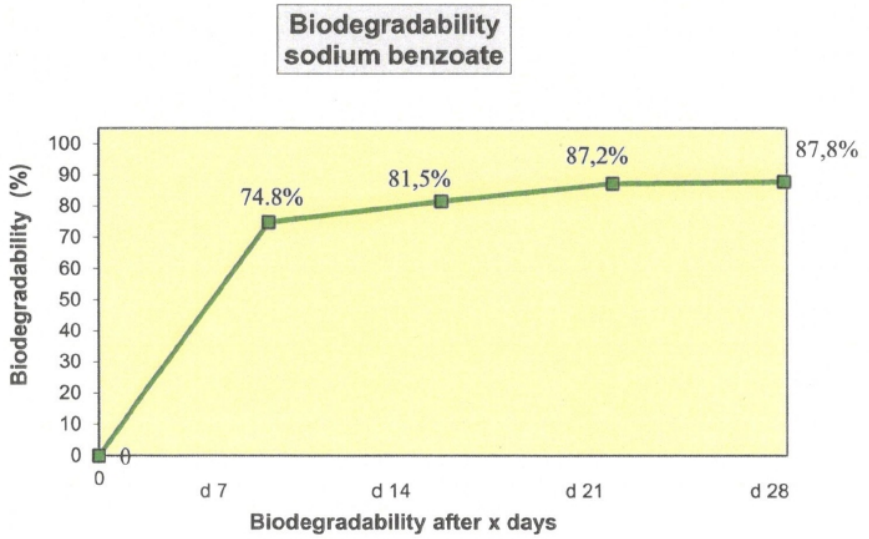
2. Test results - Reference substance

Determination of dissolved oxygen

Determination type	Sample number	Concentration of dissolved oxygen in mg/l after x days				
		0 d	7 d	14 d	21 d	28 d
Tested substance with inoculum	1.	8,86	3,37	2,77	2,25	2,03
	2.	8,86	3,31	2,66	2,22	2,06
	Average	**8,86**	**3,34**	**2,72**	**2,24**	**2,05**
Blank with inoculum	1.	8,92	8,22	8,05	7,91	7,85
	2.	8,92	8,31	8,10	8,02	7,77
	Average	**8,92**	**8,27**	**8,08**	**7,97**	**7,81**

Biodegradability

Each sample **Sodium benzoate**	Calculated biodegradability after x days				
	0 d	7 d	14 d	21 d	28 d
1.sample	0%	74,4%	80,7%	87,0%	88,0%
2.sample	0%	75,3%	82,4%	87,5%	87,5%
Average	**0%**	**74,8%**	**81,5%**	**87,2%**	**87,8%**



4 Summary of results

Tested sample – No. 19192

Bio - Akcelerator „BIUS“

Biodegradability
determined in accordance with OECD 301 D = 66.1 % after 28 days

Biodegradability of tested sample was 66.1 % after 28 days.

Reference sample – Sodium benzoate

Biodegradability
determined in accordance with OECD 301 D = 87.8 % after 28 days

Reference substance is well biodegradated. After 28 days the ready biodegradability percentage was 87.8 %. After 14 days the biodegradability percentage was 81.5 %. The validity of biodegradability of reference substance is according to criteria quality.

Validity of results

- Oxygen depletion in the inoculum blank should not exceed 1.5 mg dissolved oxygen/l after 28 days.
  In this test – max. oxygen demand in blank after 28 days: 1,11 mg/l

- Dissolved oxygen concentration in bottles should not be lower then 0.5 mg/l. In this test – the lowest concentration of dissolved oxygen was 2.05 mg/l (in reference solution).

- Difference of end values of the parallel degradation testing substance at the end of test stall be lower than 20 %.
  In this test – difference of end values in parallel determinations were lower then 20%.

- The biodegration percentage of the reference substance must be 60 % after 14 day of incubation.
  In this test – biodegradation of reference substance has reached the value 81.5 % after 14 days of incubation.

End of document

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Test report no. T 89/2019

Customer:	**TUMAX - PLUS, Ltd**
	Hornická 32
	252 25 Jinočany, Praha - západ
	Czech Republic
Order No.:	258/19
Performed in period:	18. 2. 2019 – 8. 3. 2019
Test report prepared by:	Ivona Čefelínová
Head of Ecol. lab. EMPLA:	Ing. Stanislav Eminger, CSc.

TESTING LABORATORY ACCREDITED by CAI
EMPLA
Chemical, microbiological and ecotoxicological examinations
No. 1110

in Hradec Králové 8. 3. 2019

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Ing. Stanislav Eminger, CSc.
Vedoucí Ekologických laboratoří
EMPLA

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Approved by

1. Sample information

Description:	**Bio – Akcelerator BIUS**
Producer:	Russian federation, Company „BioMix“
	PSC-603037, Nizhny Novgorod, Ul. Fedosenko d. 57A
Sample No.:	**1570**
Sample protocol No.:	sampled by customer
Performed by:	Ivona Čefelínová

2. Purpose od the test

Determination of ecotoxicological properties of the product **Bio – Akcelerator BIUS** on aquatic organisms.

The control solutions and measurements were included in each test.
Potassium dichromate used as a reference substance (controled twice a year).

3. Metters

All metters used during testing were calibrated.

4. Test methods

5. Results

Fish acute toxicity test

OECD 203 – Fish, Acute Toxicity Test
Accredited test No. 301, SOP ET 1

Principle of the test

This test is used for determination of accute lethal toxicity of water-soluble substances under specific conditions.
The fish are exposed to various concentrations of the test substance for a period of 96 hours without feeding and aeration. Mortality is recordered at 24, 48, 72 and 96 hours period and the concentration which killed 50% of the fish (LC_{50}) is determined where possible.

Test conditions

Test system:	freshwater fish *Poecilia reticulata* *(Teleostei, Poeciliidae)*
Temperature during the test:	23°C ± 1°C

Exposition:	96 hours
Light:	16 hours photoperiod daily
Medium:	150 ml / 1 pcs of test organism
Numer of fish:	7 fish in limit test 3 fish / test concentration in preliminary test, 7 fish / test concentration in basic test
Concentration at limit test:	1 (100 mg/l)
Concentrations at preliminary test:	5
Concentrations at basic test:	5

	Control	**1570**
Test item concentration (mg/l)	0	6000
Fish mortality in 96 h (pcs) / number of fish in the test (pcs)	0/7	15/35
LC_{50} in 96 h (mg/l)	0	***1364,1***

Declaration to protocol of test no. T89/2019

Tests of acute toxicity are performed on the solid sample after leaching to water according to ČSN EN 12457-4 (in case the tested solid sample is subject of leaching).

In case the substance or mixture is not subject of leaching but is subject of dilution then the solution is tested directly in required dilution according to the appropriate standards.

In case of sample No. 1570 (Bio-Akcelerator BIUS) test was carried out according to ČSN EN ISO 7346, Czech versions of European standards used for example for evaluation of dangerous quality HP14.

Result of ecotoxicity test do not exceed limit value stated in regulation No. 94/2016 Journal of law in up-to-date version, table 1.1 and Commission regulation (EU) No. 1357/2014 EC. Above-mentioned legislation is valid in EU.

Accomplished by:

Ivona Čefelínová

Date of report: 8. 3. 2019

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EMPLA
Chemical, microbiological and ecotoxicological examinations
No. 1110

Approved by: Ing. Stanislav Eminger, CSc.

EMPLA AG L.t.d.

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Test report no. T 461/2018

Customer:	**TUMAX - PLUS, Ltd**
	Hornická 32
	252 25 Jinočany, Praha - západ
	Czech Republic
Order No.:	1533/18
Performed in period:	10. 9. 2018 – 11. 10. 2018
Test report prepared by:	Ivona Čefelínová
Head of Ecol. lab. EMPLA:	Ing. Stanislav Eminger, CSc.

in Hradec Králové 24. 10. 2018

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TESTING LABORATORY ACCREDITED by CAI
Chemical, microbiological and ecotoxicological examinations
No. 1110

Ing. Mojmír Špaček, Ph.D.
Vedoucí analytické laboratoře
Zást. vedoucího Ekologických laboratoří EMPLA

Approved by

1. Sample information

Description:	**Bio – Akcelerator BIUS**
Producer:	Russian federation, Company „BioMix“
	PSC-603037, Nizhny Novgorod, Ul. Fedosenko d. 57A
Sample No.:	**12889**
Sample protocol No.:	sampled by customer
Performed by:	Ivona Čefelínová, Zuzana Smetanová

2. Purpose od the test

Determination of ecotoxicological properties of the product **Bio – Akcelerator BIUS** on aquatic organisms.

The control solutions and measurements were included in each test.
Potassium dichromate used as a reference substance (controled twice a year).

3. Metters

All metters used during testing were calibrated.

4. Test methods

Aquatic crustacean acute immobilisation test

OECD 202 – *Daphnia sp.*, Acute Immobilisation Test
Accredited test No. 302, SOP ET 2

Principle of the test

Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations for a period of 48 hours without feeding and aeration. Immobilisation is reccordered at 48 hours and compared with control values. The concentration which immobilised 50 % of the daphnids (EC_{50}) is determined.

Test conditions

Test system:	aquatic crustacean *Daphnia Magna Straus (Cladocera, Crustacea)*
Temperature during the test:	20 °C ± 1 °C
Exposition:	48 hours
Light:	none

Medium:	10 ml / 1 pcs of test organism
Number of daphnids:	10 pcs / test concentration in preliminary test, 10 pcs / test concentration in basic test
Concentrations at preliminary test:	5
Concentrations at basic test:	5 (two replicates)

OECD 201 – Freshwater Alga, Growth Inhibition Test (accredited test No. 303 – SOP ET 3)

Test conditions

Test system: freshwater alga *Desmodesmus subspicatus (Chlorococcales, Chlorophyta, Chlorophyceae),*
Temperature during the test: 23 °C ± 1 °C (fixed in thermoluminostat)
Exposition: 72 hours
Light: 24 h daily, intensity 6000-10000 lx
Stirring: 4 times a day
Starting cell density of algal: approximately 10^4 cells in 1 ml
Medium: 3 x 25 ml
Concentrations in limit test: 1 (100 mg/l)
Aeration: none

The cell density was determined at least at 72 hours after the start of the test in Bürker chamber with microscope.

5. Results

Description of the sample

Prepared volume of the sample:	3000 ml
Appearance of the sample:	clear, brownish

Variance from test procedure: **none**

OECD 202 – *Daphnia sp.*, Acute Immobilisation Test (accredited test No. 302, SOP ET 2)

	Control	**Sample no. 12889**
Test item concentration (mg/l)	0	1000
Daphnids immobilization in 48 h (pcs) / number of daphnids in the test (pcs)	0/20	67/120
EC_{50} in 48 h (mg/l)	0	***693,2***

OECD 201 – Freshwater Alga, Growth Inhibition Test (accredited test No. 303 – SOP ET 3)

Results: **72h IC50 = 887,3 mg/l**

	Control	**Sample no.12889**
Concentration of test item (mg/l)	0	1000
Replicates	3	3
IC50 in 72 h (mg/l)	0	887,3

Declaration to protocol of test no. T461/2018

Tests of acute toxicity are performed on the solid sample after leaching to water according to ČSN EN 12457-4 (in case the tested solid sample is subject of leaching).

In case the substance or mixture is not subject of leaching but is subject of dilution then the solution is tested directly in required dilution according to the appropriate standards.

In case of sample No. 12889 (Bio-Akcelerator BIUS) tests were carried out according to ČSN EN ISO 6341 and ČSN EN ISO 8692, Czech versions of European standards used for example for evaluation of dangerous quality HP14.

Results of two ecotoxicity tests do not exceed limit value stated in regulation No. 94/2016 Journal of law in up-to-date version, table 1.1 and Commission regulation (EU) No. 1357/2014 EC. Above-mentioned legislation is valid in EU.

Determination of biodegradability was performed according to OECD 301 D method (see protocol No. T 43/2019). Biodegradability of tested sample was 66,1 % in 28 days. Requirement of biodegradability is 60 % in 28 days.

Accomplished by:

Ivona Čefelínová

Date of report: 8. 2. 2019

Approved by: Ing. Stanislav Eminger, CSc.

EMPLA AG Ltd.

Hradec Králové

Czech Republic