SAFETY CAR INDUSTRIES
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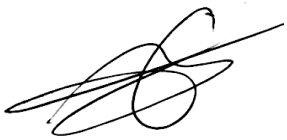
Ready biodegradability study

According to OECD 301F / EC C.4

ANALYTICAL REPORT

R/17/14635

Version #	Validation - Scientific & Technical Direction / Quality Direction	Verification – Assistant of the Scientific & Technical Direction	Edition date of the version	Date of amendment
1	J.-F. LACROIX 	G. HAZAN 	27/06/2016	/

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APPENDIX

SAMPLES IDENTIFICATION

Our References	Information provided by the customer		Date of sampling	Date of analysis
	Your references	Description		
E/17/64608	SC 5100	Gasoline additive	/	From 23/05 to 20/06/2017

Your order: Mail from 19/04/17

PICTURE OF THE ANALYSED SAMPLE

1. Mission

The firm SAFETY CAR INDUSTRIES asks to the firm Analytice to undertake study of ready biodegradability of gasoline additive.

2. Summary of results

The 28 day ready aerobic biodegradability (ultimate aerobic biodegradability) testing of Gasoline additive SC 5100, according to OECD 301F/EC C.4 – D manometric respirometry methods, was carried out at 22 °C. At the 28th day of the test the measured aerobic biodegradation of the test item attained 79.9 % (as BOD/ThOD).
The level for ready biodegradability of 60% of ThOD was reached on 6th day in a 10-day window within the 28-day period of the test. According to the OECD guidelines this result is an evidence of ready biodegradability, which means that:
Gasoline additive SC 5100 meets the criteria for ready aerobic biodegradation and it can be deemed to be readily biodegradable.

3. Materials and methods

3.1. Test item

Test item:	Gasoline additive
Name:	**SC 5100**
Appearance:	Clear, viscous liquid
Content:	Butyl glycol (CAS 111-76-2): 95.0%
	Tall oil fatty acids (CAS 61790-12-3): 5.0%

3.2. Reference item

Chemical name: sodium acetate anhydrous
Synonyms: acetic acid, sodium salt
Molecular formula: CH3COONa
CAS-No: 127-09-3
EC: 204-823-8
Purity [%]: pure p.a. min. 99.7
Form: fine, white powder
Batch number: 16/11/16
Expiry date: 11/2020

3.3. Test system – inoculum

A sample of activated sludge was taken from the aeration tank of Sewage Treatment Plant ”Czajka”, receiving predominantly domestic sewage.

3.4. Experimental part starting and completion dates

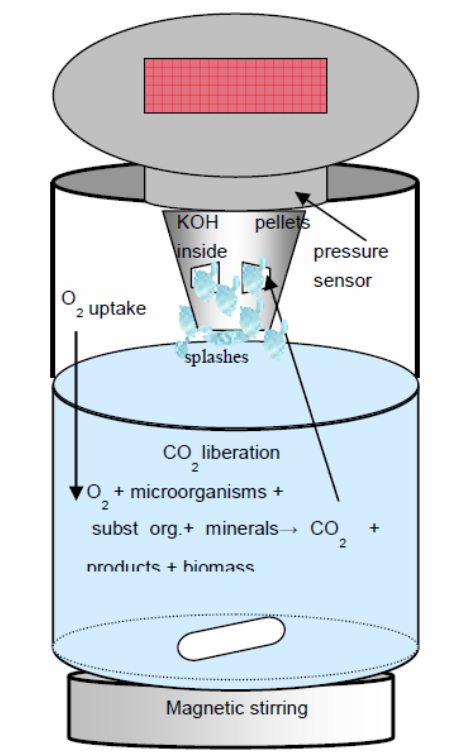
Starting date: 23.05.2017
Completion date: 20.06.2017

3.5. Principles of method

A measured volume of inoculated mineral medium, containing a known concentration of test substance (100 mg test substance/l giving at least 50-100 mg ThOD/l) as the nominal sole source of organic carbon, is stirred in a closed flask at a constant temperature (+ 1°C or closer) for up to 28 days.

A positive result obtained in a test of ready biodegradability may indicate that the chemical will undergo rapid and ultimate biodegradation in the environment. A measured, stirred volume of a mineral solution containing 100 mg/L of test item was inoculated with microorganisms and incubated under aerobic conditions (oxygen presence) in a closed respirometer flask at constant temperature of 22 ± 0.8°C for 28 days. The blank tests were run in parallel with only inoculum but without test item. A reference item (sodium acetate) was run in parallel to check the operation of the procedures. The degradation was followed by the determination of oxygen uptake and measurements were taken at sufficiently frequent intervals to allow the identification of the beginning and end of biodegradation.

To check the possible inhibitory effect of the test item the toxicity test was run in parallel. The solutions containing 100 mg/L of test item and 100 mg/l of reference item, in the mineral medium, were inoculated. The consumption of oxygen was determined from the change in pressure in the apparatus. The carbon dioxide, evolved during test item degradation, was adsorbed on the flakes of potassium hydroxide. The amount of oxygen taken up by the test item (corrected for uptake by blank inoculum, run in parallel) was expressed as a percentage of calculated ThOD of the test item.



Definitions

Pass level

The pass level for ready biodegradability is 60% of ThOD for respirometric method. This pass value has to be reached in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% of ThOD and must end before day 28 of the test. This pass level of biodegradation, obtained within 28 days, may be regarded as evidence of ready biodegradability and the substance can be classed as readily biodegradable.

Lag time is the time from inoculation, until the degradation percentage has increased to at least 10%.
Degradation time is the time from the end of the lag time till the time that 90% of maximum level of degradation has been reached.
10-day window is the 10 days immediately following the attainment of 10% degradation.
Theoretical Oxygen Demand (ThOD) (mg) is the total amount of oxygen required to oxidize a chemical completely; it is calculated from the molecular formula and is expressed as mg oxygen required per mg test compound.

3.6. Method description

3.6.1. Apparatus

Usual laboratory equipment and:

- closed WTW OxiTop OC 110 respirometer for BOD determination;
- spectrophotometer Hach DR 3900 for TOC concentration measurements;
- thermo-cabinet WTW TS 606 CZ-G/3-VAR;
- electronic temperature recorder EBI 310 –T;
- pH-meter – multifunctional microcomputer meter ELMETRON CX-505;
- computer to data transfer and for calculations;
- universal laboratory aid (for mixing and averaging of inoculum).

3.6.2. Test conditions

Initial concentration of organic constituents of test item in medium, C:	100 mg/L
Volume of test solution in flask, V:	0.164 L
Calculated ThOD:	2.33 mgO_2/mg of test item
Amount of suspended inoculum in 1 l of mineral solution	30 mg/L

The temperature was recorded during the test in electronic temperature recorder EBI 310 –T.

3.6.3. Dilution water

The double-distilled water was taken from redistillation set. It must contain no more than 10% of the organic carbon content introduced by the test material. There is 100 mg/L of solid test item what gives 60.9 mg/L of organic carbon. Thus, the used water must contain less than 6.09 mg/L of organic carbon. This was checked by DOC analysis using spectrophotometer Hach DR 3900 and Hach-Lange reagents. The measured value was about 2.5 mg/L of organic carbon.

3.6.4. Basic mineral components

The solutions were prepared using the following substances:

	Substance	purity
1	Monopotassium dihydrogen orthophosphate, KH_2PO_4	pure p.a.
2	Dipotassium monohydrogen orthophosphate, K_2HPO_4	pure p.a.
3	Disodium monohydrogen orthophosphate dihydrate, $Na_2HPO_4 \cdot 2\ H_2O$	pure p.a.
4	Ammonium chloride, NH_4Cl	pure p.a.
5	Calcium chloride, anhydrous, $CaCl_2$	pure p.a.
6	Magnesium sulphate heptahydrate, $MgSO_4\ 7\ H_2O$	pure p.a.
7	Iron(III) chloride hexahydrate, $FeCl_3 \cdot 6H_2O$	pure p.a.

3.6.5. Experimental method

Selection of the appropriate method

The manometric respirometry method enables to measure the consumption of oxygen by often measuring (every 112 min) the change in pressure in the bottles. This method is suitable for substances which are poorly soluble, volatile and adsorbing.

Information

Evaluation of ready biodegradability was performed accordingly to principles of Good Laboratory Practice, on the base of the standard operating procedure SOP/BS/01/b elaborated accordingly to EEC method no. C.4 – D: Manometric respirometry which is in conformity with the OECD Guideline for Testing of Chemicals no. 301F Manometric respirometry test.

Concentrations of: test item, inoculum, elements in mineral medium

concentration of test item: mg/L	100
mg ThOD/l	50-100
concentration of inoculum	30 mg/L SS
concentration of elements in mineral medium (mg/L)	
P	116
N	1,3
Na	86
K	122
Mg	2.2
Ca	9.9
Fe	0.05–0.1
pH	7.4 ± 0.2
Temperature	22 ± 0.4 °C

ThOD – Theoretical Oxygen Demand, SS – Suspended Solids

Preparation of mineral medium

Stock solutions of mineral components. To make up the test solution, stock solutions of appropriate concentrations of mineral components were made up.
Stock solutions:
Prepare the following stock solutions, using analytical grade reagents.
(a) Monopotassium dihydrogen orthophosphate, KH_2PO_4 8.50 g
Dipotassium monohydrogen orthophosphate, K_2HPO_4 21.75 g
Disodium monohydrogen orthophosphate dihydrate $Na_2HPO_4 \cdot 2\ H_2O$ 33.40 g
Ammonium chloride, NH_4Cl 0.50 g
Dissolve in water and make up to 1 litre, the pH of the solution should be 7.4.
(b) Calcium chloride, anhydrous, $CaCl_2$ 27.50 g
Dissolve in water and make up to 1 litre
(c) Magnesium sulphate heptahydrate, $MgSO_4 \cdot 7\ H_2O$ 22.50 g
Dissolve in water and make up to 1 litre.
(d) Iron(III) chloride hexahydrate, $FeC_{13} \cdot 6H_2O$ 0.25 g
Dissolve in water and make up to 1 litre.
Mix 10 ml of solution (a) with 800 ml dilution water, add 1 ml of solutions (b) to (d) and make up to 11 with dilution water.

Preparation of inoculum

A sample of activated sludge was taken from the aeration tank of Sewage Treatment Plant ”Czajka”. The coarse particles were removed by settling and the supernatant was discarded. The sludge was washed in the mineral medium. The concentrated sludge was suspended in mineral medium to yield a concentration of 3-5 g suspended solids/l and it was aerated for 5 days, at the test temperature of 22 °C, up to application. A sample was withdrawn just before use for the determination of the dry weight of the suspended solids.

Preparation of flasks

The test was carried out according to the following methods and procedures:

- Evaluation of ultimate aerobic biodegradability was performed on the base of the standard operating procedure SOP/BS/01/b. The test conditions and the raw data are recorded in appropriate sheet.
- The run of oxygen demand evaluation in the respirometer was conducted accordingly to Appendix no. 1 and 2 of SOP/BS/01/b and PN-EN ISO 9408:2005.

The calculations and the graphs were performed using SigmaPlot 9.0 software of SYSTAT Software, Inc., USA purchased from GAMBIT CoiS Ltd, Poland.

Solution of the test item and reference item:
The solutions of the test and reference items in test flasks were prepared, in separate batches, in mineral medium 100 mg/L of test item and 100 mg/l of reference item. 0.2500 g of reference item was weighed into 250 ml measuring flask and made up to the mark giving a concentration of 1 g of reference item in 1 liter.
Test item was added directly to the mineral medium at this stage on a weight basis. The amount of test item as much as 16.4 mg was weighed into each of triplicate test flasks when needed. 16.4 ml of stock solution of the reference item was introduced into each of the duplicate right flasks. Mineral medium only was added to further flasks (for inoculum controls). Three flakes of potassium hydroxide were added to each of the CO_2-absorber compartments. The determined content of inoculum at the end of preconditioning was equal to 4.21 g suspended solids/l. To give a concentration of suspended solids equal to 30 mg/L in each flask, 1.17 ml of preconditioned suspension of inoculum was added into each flask.
The flasks were made up to 164 ml volume with prepared mineral medium.

Number of flasks (in triplicate):

Flasks # 13, 14, 15: Test suspension containing test item (100 mg substance/L) and inoculum 30 mg/L SS.
Flasks # 1, 2 and 3: Inoculum blank containing only inoculum 30 mg/L SS.
Flask # 4, 5 and 6: Procedure control containing reference item (sodium acetate 100 mg/L) and inoculum 30 mg/SS.
Flask 16, 17 and 18: Toxicity control containing test item, reference item at the same concentrations as in the individual solutions and inoculum 30 mg/L SS.

Performance of test

The vessels were placed into a thermo-cabinet and allowed to reach the desired temperature and appropriate vessels were inoculated with prepared activated sludge to give a concentration of suspended solids equal to 30 mg/L. After assembling the equipment, the stirrers were started and the measurement of oxygen uptake was started. The necessary readings were taken and daily checks were made to see that the correct temperature and adequate stirring were maintained.
The oxygen uptake from the readings taken at regular and frequent intervals was calculated, using the methods given by the manufacturer of the equipment. The data were read out every 112 min during the 28 day test (40 320 min that is 360 readings) and were recoded and stored in the measuring heads of the sample bottles. These collected data, using the controller, were infrared read out from the heads and stored in the controller. Using software Achat OC version 2.03 of Aqualytic the data were transferred from to controller to a computer.
The calculations and the graphs were performed using SigmaP lot 9.0 software of SYSTAT Software, Inc., USA purchased from GAMBIT CoiS Ltd, Poland.
The temperature in thermo-cabinet was recorded with temperature recorder EBI 310 –T from Ebro.
At the end of incubation, normally 28 days, the pH of the contents of the flasks was measured.

Determination of dissolved organic carbon

Dissolved Organic Carbon in water was determined using Hach-Lange reagents for TOC measurements:
LCK 380: Photometer with Barcode-System.
Parameter meas. range
TOC 2 – 65 mg/L, TC 12 – 75 mg/L, TIC 10 – 73 mg/L
Program filter 435 nm

3.6.6. Calculations

In order to calculate the amount of oxygen up-taken by microorganisms for organic mass degradation the oxygen uptake (mg) of the test item after a given time (corrected for that by the blank inoculum control after the same time) was divided by the weight of the test item used. This yields the BOD expressed as mg oxygen/mg test item:

$$\%\ \text{biodegradation} = \%\text{COD} = \frac{\text{BOD (mg O}_2\text{/mg test item)}}{\text{ThOD (mg O}_2\text{/mg test item)}} \times 100 \qquad (3.5.6.2.)$$

The biodegradation level (%) can be calculated from:

$$BOD = \frac{\text{mg } O_2 \text{ uptake by test item - mg } O_2 \text{ uptake by blank sample}}{\text{mg test item in flask}} \qquad (3.5.6.1)$$

$$= \text{mg } O_2 \text{ per mg test item.}$$

The chemical compound may be expressed as CcHhClclNnNanaOoPpSs.
The theoretical oxygen demand of it (ThOD), as the elemental composition is known, can be calculated using the following equation:

$$ThOD = \frac{16\left(2C + \frac{1}{2}(H - Cl - 3N) + 3S + \frac{5}{2}P + \frac{1}{2}Na - O\right)}{\text{test item molecular weight}} \text{ mg}O_2/\text{mg test item} \qquad (3.5.6.3.)$$

Content of the test item, according to the Sponsor information, is as below:

- butyl glycol (CAS 111-76-2) 95.0%
- tall oil fatty acids (CAS 61790-12-3) 5%, which is a mixture of various compounds

It was assumed that it consists mostly of oleic acid, 5% of all test item.
The ThOD values of the test item constituents, using the above equation, were calculated to be:

- butyl glycol $C_6H_{14}O_2$ - 2.30 mgO_2/mg,
- tall oil fatty acids (as oleic acid) $C_{18}H_{34}O_2$ – 2.89 mgO_2/mg,

The value of the ThOD of test item was finally calculated on a weighted-average basis using the weight fractions of the above calculated ThOD values of individual components.
The obtained value equals 2.33 mgO_2/mg.
In this test the test item, in fact, as a nitrogen-free substance, has no affect the oxygen uptake because of nitrification reaction.
The corrected (for that by the blank inoculum control) BOD values for oxygen consumption due to C oxidation could then be compared with the calculated ThOD equals 2.33 mgO_2/mg test item.
During the performed test the values of BOD directly obtained, in apparatus, are expressed in mg O_2/l of test solution. This way the biodegradation level of test item (%) can be calculated using this equation:

$$\frac{\text{mg/L } O_2 \text{ uptake by test item - mg/L } O_2 \text{ uptake by blank sample}}{\text{ThOD (mg } O_2\text{/mg test item)} \times \text{concentration of test item (mg test item/L)}} \times 100\% \qquad (3.5.6.4)$$

3.6.7. Validity of method

1. A test is considered valid if the difference of extremes of replicate values of the removal of test item at the plateau, at the end of the test is less than 20% and if the percentage, degradation of the reference substance has reached the level for ready biodegradability by 14 days. If either of these conditions is not met, the test should be repeated.
2. If in a toxicity test, containing both the test item and a reference chemical, less than 25% (based on ThOD) occurred in 14 days, the test item can be assumed to be inhibitory.
   The test series should be repeated, if possible using a lower concentration of test item and/or a higher concentration of inoculum, but not greater than 30 mg solids/l for manometric respirometry.
3. For manometric respirometry the oxygen uptake of the inoculum blank is normally 20-30mg O_2/l and should not be greater than 60 mg/L in 28 days. Values higher than 60 mg/L require critical examination of the data and experimental techniques.
4. If the pH value is outside the range 6-8.5 and the oxygen consumption by the test item is less than 60%, the test should be repeated with a lower concentration of the test item.

4. Results

The mineral medium solution pH was equal to 7.44.
The preconditioned suspension of inoculum pH was equal to 7.32.
The initial concentration of test item, as organic substances, in test flasks was equal to 100 mg/L.

Table 4.1. The pH values of test flasks

flask #	13	14	15	1	2	3	4	5	6	16	17	18
	Test item			Inoculum blank			Reference item			Toxicity test		
initial	7.43	7.45	7.46	7.38	7.44	7.44	7.42	7.43	7.45	7.47	7.47	7.48
final	7.84	7.64	7.85	7.43	7.44	7.44	8.71	8.78	8.69	7.85	7.66	8.13

No adjustment of pH was conducted.

Table 4.2. Sample oxygen uptake: biodegradability

		time, days												
		1	3	5	7	9	12	14	16	18	21	23	25	28
Test item O_2 uptake, mg/L	a_1	11.2	55.9	148.0	175.4	193.4	206.6	211.6	213.8	216.2	219.3	219.6	224.7	226.5
	a_2	14.6	42.5	146.9	172.3	189.5	203.1	208.7	213.0	215.6	219.0	222.1	225.3	229.3
	a_3	13.0	37.0	142.1	165.6	180.3	194.5	199.9	203.8	207.6	211.4	212.1	216.9	219.8
	a_m. avg	13.0	45.1	145.7	171.1	187.7	201.4	206.7	210.2	213.1	216.6	217.9	222.3	225.2
Blank test O_2 uptake. mg/L	b_1	7.4	14.7	19.1	24.1	26.8	29.8	31.6	33.4	33.7	36.8	35.1	37.9	40.3
	b_2	10.8	16.1	18.4	19.1	22.3	23.7	24.9	25.9	27.2	28.8	31.2	32.1	34.5
	b_3	16.8	21.1	23.8	26.2	27.2	31.2	34.4	34.0	34.7	38.9	37.3	39.6	42.0
	b_m. avg	11.7	17.3	20.4	23.2	25.4	28.2	30.3	31.1	31.9	34.8	34.5	36.5	38.9
Reference item O_2 uptake. mg/L	w_1	27.8	53.7	68.8	75.8	78.1	83.4	85.4	87.7	88.2	92.6	92.5	93.1	95.5
	w_2	29.3	56.5	70.7	77.5	83.0	88.4	90.9	94.3	96.8	99.1	101.2	104.3	106.0
	w_3	24.5	54.0	68.1	76.9	80.7	87.1	90.5	94.0	96.0	100.6	103.1	105.2	110.9
	w_m. avg	27.2	54.7	69.2	76.8	80.6	86.3	88.9	92.0	93.7	97.5	98.9	100.9	104.1
Toxicity control O_2 uptake. mg/L	tox_1	32.8	97.6	138.6	174.2	200.5	233.3	246.1	255.8	261.7	270.5	275.3	279.4	284.7
	tox_2	31.6	98.1	142.3	172.6	199.9	231.9	241.9	249.8	253.9	262.9	267.4	269.6	277.3
	tox_3	31.7	99.9	139.6	174.2	200.9	234.7	246.0	252.1	257.9	263.8	268.9	273.5	280.2
	tox_m avg	32.0	98.6	140.2	173.7	200.4	233.3	244.7	252.6	257.8	265.7	270.5	274.2	280.7
Corrected test item O_2 uptake, mg/L	(a_1-b_m)	-0.5	38.6	127.6	152.2	168.0	178.4	181.3	182.7	184.4	184.4	185.0	188.1	187.6
	(a_2-b_m)	3.0	25.2	126.5	149.1	164.1	174.9	178.4	181.9	183.7	184.2	187.6	188.7	190.3
	(a_3-b_m)	1.4	19.7	121.7	142.4	154.9	166.3	169.6	172.7	175.7	176.6	177.6	180.4	180.9
Reference item % degradation $\frac{BOD}{ThOD \times C} \times 100$ ThOD = 0.78 mgO_2/mg C = 100 mg/L	R_1(w1)	20.6	46.7	62.1	67.5	67.6	70.8	70.7	72.6	72.2	74.1	74.4	72.5	72.5
	R_2 (w2)	22.6	50.2	64.4	69.7	73.9	77.1	77.7	81.0	83.2	82.4	85.4	86.9	86.0
	R_3 (w3)	16.5	47.0	61.1	68.9	70.8	75.5	77.1	80.6	82.2	84.3	87.9	88.1	92.2
	$R_w$avg	19.9	48.0	62.6	68.7	70.8	74.5	75.2	78.1	79.2	80.3	82.6	82.5	83.6
Test item % degradation $\frac{BOD}{COD \times C} \times 100$ ThOD = 2.56 mgO_2/mg C = 30 mg/L	R_1(a1)	0.0	16.6	54.8	65.3	72.1	76.6	77.8	78.4	79.1	79.2	79.4	80.7	80.5
	R_2(a2)	1.3	10.8	54.3	64.0	70.4	75.1	76.6	78.1	78.9	79.0	80.5	81.0	81.7
	R_3(a3)	0.6	8.5	52.2	61.1	66.5	71.4	72.8	74.1	75.4	75.8	76.2	77.4	77.6
	Raavg	0.6	12.0	53.8	63.5	69.7	74.3	75.7	76.9	77.8	78.0	78.7	79.7	79.9
Toxicity control % degradation	R_1 (tox)	6.8	25.8	38.0	48.6	56.3	65.9	69.4	72.3	73.9	75.8	77.4	78.1	79.0
	R_2 (tox)	6.4	26.0	39.2	48.0	56.1	65.5	68.0	70.3	71.4	73.3	74.9	75.0	76.6
	R_3 (tox)	6.4	26.6	38.3	48.6	56.4	66.4	69.4	71.1	72.7	73.6	75.4	76.2	77.6
	$R_a$avg	6.5	26.1	38.5	48.4	56.3	66.0	68.9	71.2	72.7	74.2	75.9	76.4	77.7

The test item biodegradation time course was fitted with the three parameters, logistic function of the form:

$$\text{biodegradation, \%} = \frac{77.99}{1+\left(\frac{t}{4.177}\right)^{-3.308}}$$

The reference item biodegradation time course was fitted with the three parameters, logistic function of the form:

$$\text{biodegradation, \%} = 79.78(1-e^{-0.3066t})$$

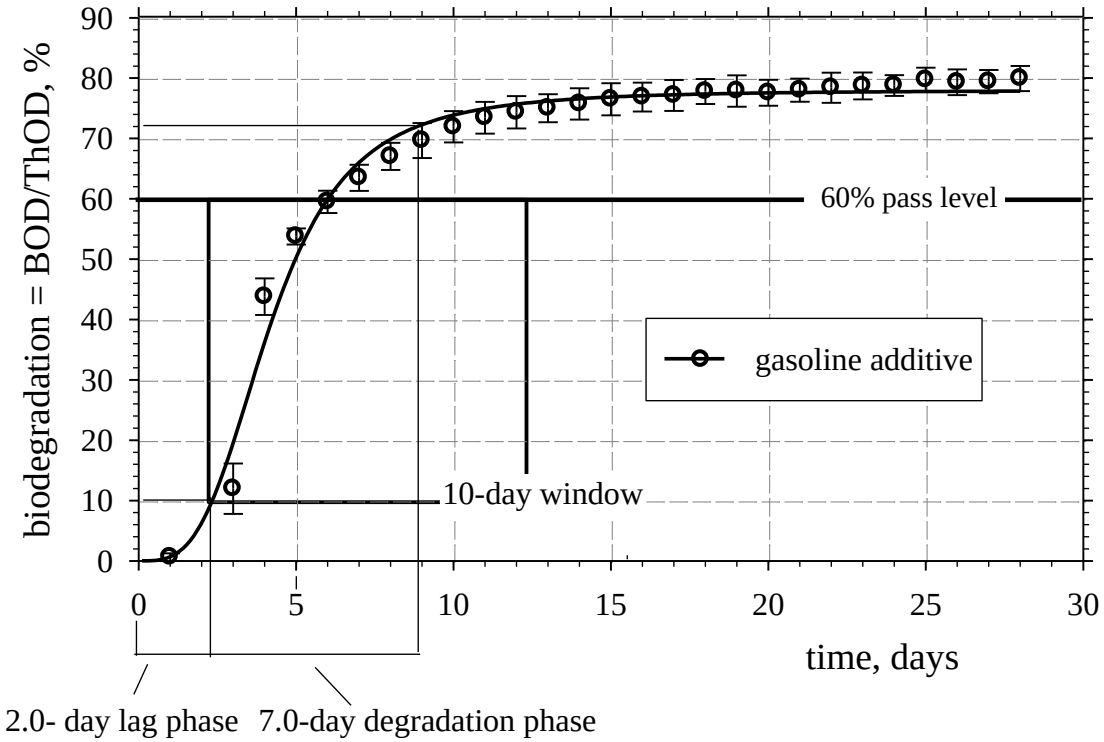


Fig. 4.1. The biodegradability curve for manometric respirometry test.
initial concentration of test item 100 mg/L

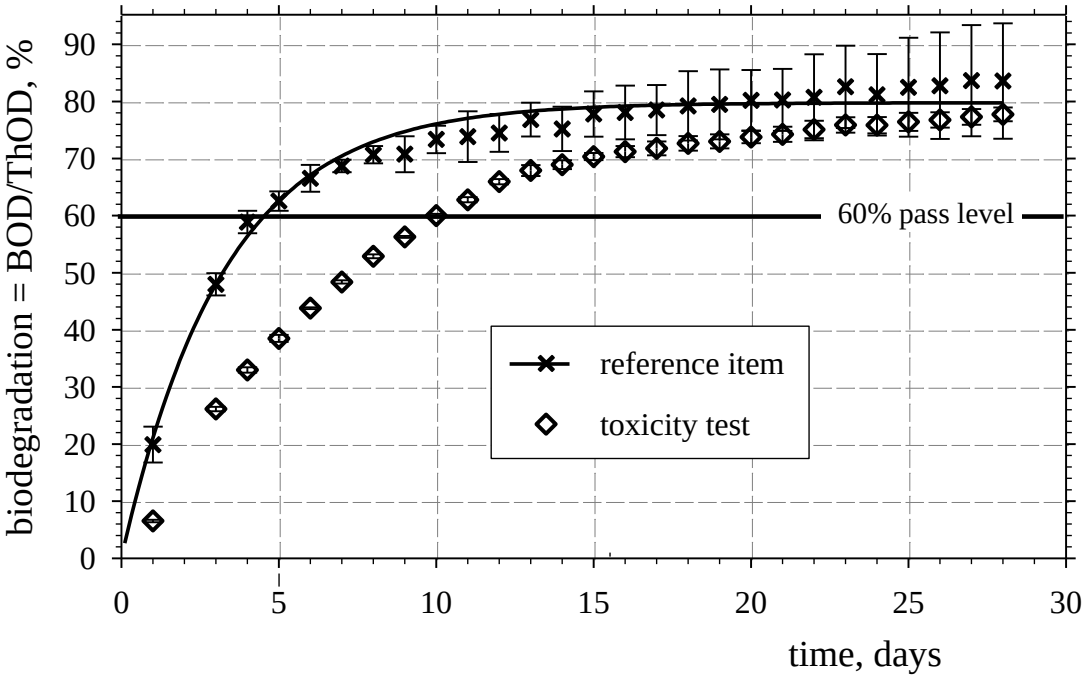


Fig. 4.2. The biodegradability curve for manometric respirometry test.
Initial concentration of sodium acetate 100.0 mg/L
Toxicity test: test item 100 mg/L + sodium acetate 100 mg/L

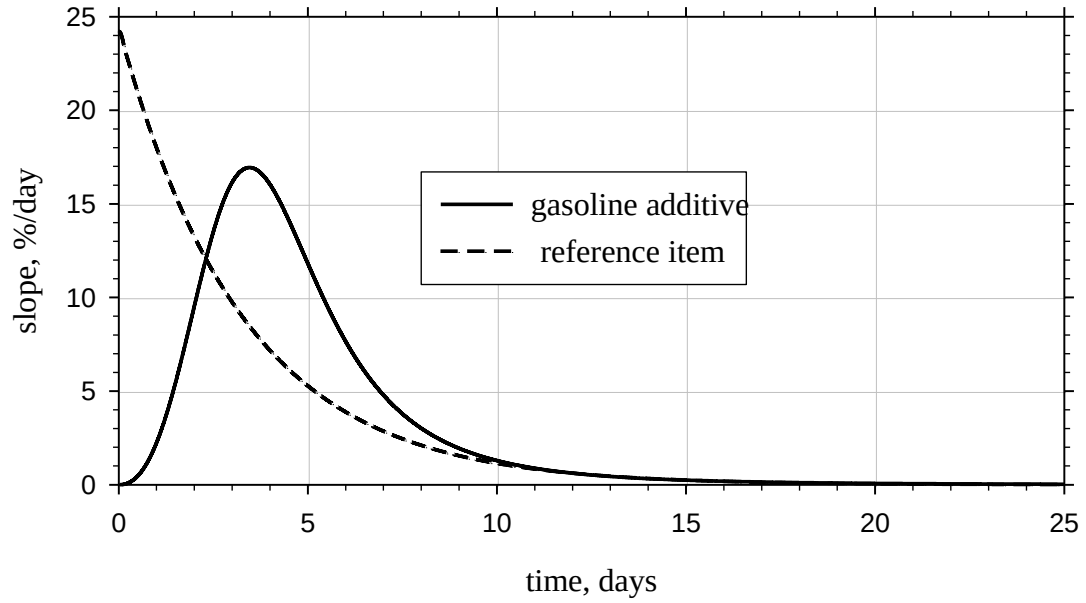


Fig. 4.3. The slope of biodegradability curves during manometric respirometry test.

The recorded measurement temperature didn't exceed - by 0.40 °C - the expected 22 °C.
At the 28th day of the test the aerobic biodegradation of the testing:
o **The test item attained 79.9% of biodegradation,**
o **The reference item reached 83.6% of biodegradation and the level for ready biodegradability by 5 days,**
o In the toxicity test the biodegradation was equal to 77.7% and 68.9% at 14th day,
o The oxygen uptake of the inoculum blank was equal to 38.9 mg/L in 28 days,
o The pH values of all flasks were inside the range 7.43-8.85 (see table 4.1.)

In a toxicity test, containing both the test item and a reference item more (68.9%) than 25% of biodegradation (based on COD) occurred in 14 days and the test item cannot be assumed to be inhibitory.

According to the OECD guidelines requirement of pass level:

- The pass level for ready biodegradability - 60% of ThOD - was reached on 6th day in a 10-day window within the 28-day period of the test. The 10-day window begins when the degree of biodegradation has reached 10% of ThOD and must end before day 28 of the test. This pass level of biodegradation, obtained within 28 days, is an evidence of ready biodegradability and the test item can be classed as readily biodegradable.

Validity of test

1. It can be seen in fig.4.1 that the difference of extremes of replicate values of the BOD of the test item at the plateau and at the end of the test is less than the limit of 20%.
2. The reference item has reached the pass level (60%) on day 4 (the limit is by day 14).
3. In a toxicity test, containing both the test item and a reference item, on the 14th test day the biodegradation (based on COD) attained 68.9%.
4. The oxygen uptake of the inoculum blank is 38.9 mg O_2/l in 28 days (should not be greater than 60 mg/L).
5. The pH values of almost all flasks were inside the range 6-8.5 and the oxygen consumption by the test item is not greater than 60%.

5. Conclusions

At the 28th day of the test the measured aerobic biodegradation of the test item attained **79.9 %** (as BOD/ThOD).
The level for ready biodegradability of 60% of ThOD was reached on 6th day in a 10-day window within the 28-day period of the test. According to the OECD guidelines this result is an evidence of ready biodegradability, which means that:
Gasoline additive SC 5100 meets the criteria for ready aerobic biodegradation and it can be deemed to be readily biodegradable.
The lag phase lasted 2.0 days, and degradation phase of 7.0 days.

6. Archiving

The following items will be retained in the archive of the Institute of Industrial Organic Chemistry for the 10 year retention period (till 2027):

- Raw data (test sheet and results reading chart)
- Final report

The samples of test item will be retained in the samples archive for the 2 year retention period or if samples activity is maintained.

7. Literature

1. COUNCIL REGULATION (EC) No 440/2008 of 30 May 2008 laying down test methods pursuant to Regulation (EC) No 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH).

2. OECD Guidelines for the Testing of Chemicals, Section 3 Degradation and Accumulation, Ready Biodegradability, 301 F Manometric Respirometry test, Adopted: 17.07.92.

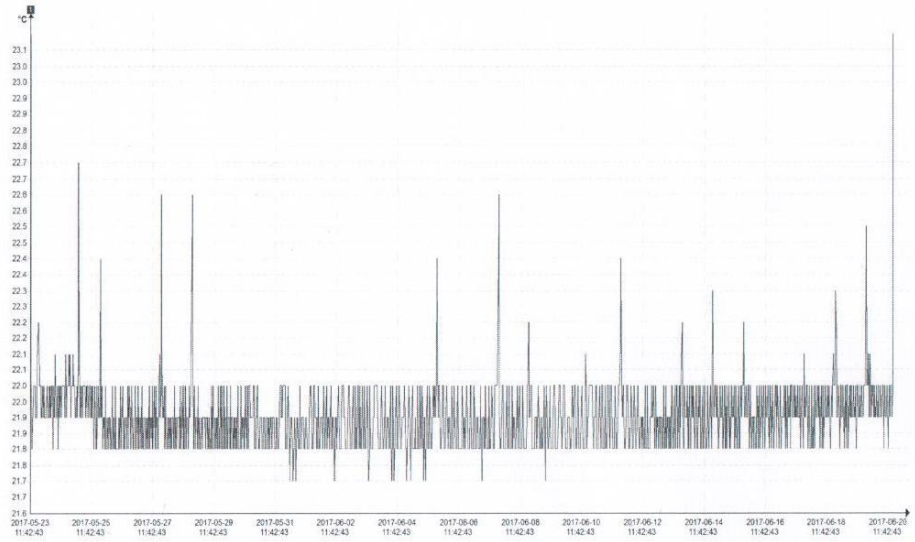
3. OECD GUIDELINES FOR THE TESTING OF CHEMICALS REVISED INTRODUCTION TO THE OECD GUIDELINES FOR TESTING OF CHEMICALS, SECTION 3 PART 1: PRINCIPLES AND STRATEGIES RELATED TO THE TESTING OF DEGRADATION OF ORGANIC CHEMICALS DEGRADATION OF ORGANIC CHEMICALS, 2.3 Ready biodegradability tests, 2.7 Interpretation of results Ready biodegradability tests, Adopted : 23 March 2006

APPENDIX

APPENDIX no. 1

Temperature run

Compact report



Logger	EBI 310
Serial	73103697
E/17/64608	
Location	
User	Admin
Measurement	
Start time	2017-05-23 11:45:24
Interval	30 Min.
Measurements	1351
User information	
JZ	
IPO	
WARSAW	

Curve	Unit	Name	Min. limit	Max. limit	Serial	Last calibration
1	°C	Channel 1	20,00	24,00	73103697	2015-06-15 08:21:08
	Statistic	Min	Max	Duration		
		21,7	23,1	28:03:00:00		

Untitled 2017-06-20 14:57:50 1/1