

Study on the Biodegradability of Organometallic Modifier Used in Liquid Fuel Combustion

Joanna Guziałowska-Tic*, Wilhelm Jan Tic

Department of Environmental Engineering, Opole University of Technology,
5 Mikołajczyka, 45-271 Opole, Poland

Received: 11 August 2015

Accepted: 7 March 2016

Abstract

Our objective in this study was to assess the biodegradability and to calculate the percentage degradation for the modifier used in liquid fuel combustion processes and for sodium acetate (the reference material). We performed an assessment study evaluating high biodegradability in an aqueous environment using the manometric respirometry method (according to the OECD 301F guideline). Based on the results, it was found that the biodegradation of the test material reached 62.18% at the end of testing (after 28 days). On the other hand, the biodegradation of the reference material was at 85.94% at the end of our tests.

Keywords: ecotoxicological studies, biodegradability, iron-based modifier, combustion of liquid fuels

Introduction

The amount of organic compounds emitted to the environment has significantly increased in recent years. As a consequence, we have seen the development of research methods and monitoring of biodegradation processes, while providing the essential knowledge on undertaking decisions related to environmental protection. Substantially, there has been a focus on the biodegradability of organic compounds in an aqueous environment [1].

Biodegradation is the susceptibility of the compound to biochemical degradation by micro-organisms. In most cases, the compounds are completely destroyed and ultimately they are converted from relatively complex compounds into carbon dioxide, water and inorganic salts. Although the biodegradation is considered as a degradation to simple inorganic compounds, there can

also arise more complex or more dangerous chemicals [2]. Natural chemical substances that occur in nature are often easily degradable; on the other hand, man-made organic substances are in many cases sparingly degradable. In an aqueous medium such substances can stay a long time, even for many years.

The primary objective of the biodegradation test is to assess the susceptibility of individual organic compounds and their mixtures on microbial decomposition.

Pursuant to the regulation by the minister of health on the criteria and mode of classification of chemical substances and mixtures [3], substances and mixtures are considered to be easily degradable if the following criteria are satisfied. Firstly, after biodegradation testing lasting for 28 days, the level of degradation reaches 70% for tests based on designations on dissolved organic carbon and degradation at the level of 60% for tests based on oxygen loss or carbon dioxide generation. Secondly, in the case of data given explicitly on the size of COD and BOD₅, a substance or mixture shall be considered as easily degradable if the ratio of BOD₅ to COD is greater

*e-mail: j.guzialowska@po.opole.pl

than or equal to 0.5. Furthermore, a substance or mixture is considered easily degradable when other convincing scientific evidence confirms that it can be biotically or abiotically degraded in an aquatic environment within 28 days by exceeding the level of 70%.

Low biodegradability in water does not necessarily mean that the test product is not biodegradable. Application of selected strains of micro-organisms allows for obtaining a high degree of distribution even of these substances, which are in fact very difficult for biodegradation. Second, the medium in which the research tests are carried out is a very important factor affecting biodegradability, and the conditions under which the sample is located [4].

Test Material

In order to reduce emissions of harmful products of liquid fuel combustion processes into the atmosphere, it is required to use specific modifiers. One of the types is organic salts of metals being soluble in fuel, which form oxides of metals to improve the oxidizing properties of fuels in conditions of combustion processes. The product discussed in research is the reaction mass of soaps and salts of iron (III) generated in the production process, creating stable emulsion in the organic solvent – fuel oil [5].

The metal catalyst was obtained from salt of Fe^{3+} soluble in water. At the stage of the first synthesis, a mixture of iron hydroxide (III) and ammonium hydroxide was prepared, which then reacts with fatty acids dissolved in diesel oil. After the reaction, the organic phase was separated from the aqueous phase. The organic phase containing ammonium soaps and metal hydroxides in fuel oil is considered a catalyst.

In the case of high concentrations of iron in fuel oil (100 g Fe/dm^3), it is required that approximately 25% of iron occurs in the form of ammonium soaps, while the remaining part of the metal occurring in the form of hydroxide is suspended in the solvent as a colloid.

The threat from fuel additives is related to the presence of organic iron salts in their composition and the petroleum-derived solvents they are dissolved in. The risk of soil and water contamination with iron modifier occurs at the stage of its manufacture, storage, and addition to fuel.

In terms of water contamination with iron compounds, it was ascertained that the toxicity of this element is particularly distinct in higher concentrations. In this case, the contaminants are quickly absorbed by the gastrointestinal tract, which may be harmful for the organisms living in a particular aquatic environment [6, 7].

Moreover, uncontrolled leakage of a petroleum-derived substance may substantially interfere with a soil and water environment. Hydrocarbons hinder gas exchange, limit light, reduce the concentration of oxygen, degrade groundwaters, contaminate soil and ground, and above all are toxic, mutagenic, and carcinogenic to all organisms [8].

In the future, it is planned to launch iron modifier production of more than 100 Mg per year; therefore, it is required, among others, to perform tests on toxicological and ecotoxicological properties required for product registration in the REACH system [9, 10]. Research tests necessary to assess the risks to human health and hazards to environment were performed in accordance with the principles of good laboratory practice in the Institute of Organic Industry in Pszczyna [11, 12]. We conducted eight toxicological tests and 10 ecotoxicological tests on the basis of production volumes and the data contained in annexes VII-IX of the REACH regulation [13].

In turn, physicochemical research tests on the iron-based modifier of combustion in liquid fuels were conducted in accordance with the methodology entered in commission regulation (EC) No 440/2008 laying down test methods complying with the REACH regulation [14]. Table 1 summarizes results of basic physicochemical properties of iron-based modifier during combustion in liquid fuels.

Our paper presents the results of biodegradability according to the OECD 301 guideline for the discussed iron-based modifier of combustion processes in liquid fuels.

Experimental Procedures

We performed an assessment study on high susceptibility of the test material (iron-based modifier) on biodegradation in the aquatic environment using the manometric respirometry method in accordance with the OECD 301 guideline [19]. This method is suitable for a wide range of substances, and due to continuous mixing process, testing the substance was kept at a constant level throughout the entire period of the studies [4]. The study was conducted in BOD bottles, in which appropriate volumes of mineral nutrient solution, microbial inoculum,

Table 1. Results of physicochemical tests.

Type of test	Result	Unit
Physical state at 20°C and pressure of 101.3 kPa	liquid	-
Boiling point according to [14]	277±22	°C
Relative density by [15]	0.9071±0.001	g/cm ³
Solubility in water by [16] at a temperature of 20°C±0.5	base oil: 132.3 iron salt: 47.6	mg/dm ³
Distribution coefficient of n- octanol / water according to [14]	no marking	
Flash point according to [16]	94±2	°C
Auto-ignition temperature according to [17]	250 (±6) p = 990.5 hPa	°C
Kinematic viscosity by [18] at 20°C	11.89	mm ² /s

and sodium acetate (or a proper test portion of the test material) were provided. The concentration of the test material and sodium acetate used in the experiment equaled 100 mg/dm³. The results were read out using the Sensomat system, after the period of 1, 3, 5, 7, 14, 21, and 28 days from the beginning of the experimental tests.

Testing Procedure and Conditions

Activated sludge derived from the biological wastewater treatment plant was used as a microbial inoculum. The activated sludge was downloaded from the aeration tank into plastic containers and transported to the laboratory. Immediately after it was transported, the activated sludge was poured through a sieve having a mesh size of 1 mm; it was decanted and washed three times with tap water and mineral medium. After these treatments, the sludge was intensively aerated. At the same time, two sediment samples (of 50 cm³ each) were downloaded in order to determine the dry matter in accordance with the BS EN 12880: 2004 [20]. We used 20 mg/dm³ dry mass of sludge.

Table 2 shows composition of the medium used in the experimental tests.

Each of the solutions from (a) to (d) were dissolved in water and made up to 1 dm³. All the above solutions were mixed in the following proportions: 10 cm³ of the solution (a) was mixed with 800 cm³ of deionized water, and then 1 cm³ of solutions (b), (c), and (d) were each added. All of this was supplemented with water to the level of 1 dm³.

Determining Chemical Oxygen Demand

Before performing the experiment test to assess the high biodegradability of the test material and reference material method in the aquatic environment using manometric respirometry, chemical oxygen demand

Table 2. The composition of the medium used in the experimental tests.

Solution	Substance	Concentration [g/dm ³]
a	Monobasic potassium dihydrogen phosphate - KH_2PO_4	8.50
	Dipotassium monohydrogen orthophosphate - K_2HPO_4	21.75
	Disodium monohydrogen phosphate dihydrate - $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	33.40
	Ammonium chloride - NH_4Cl	0.50
b	Calcium chloride - CaCl_2	27.50
c	Magnesium sulfate heptahydrate - $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	22.50
d	Iron (III) chloride xehahydrate - $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$	0.25

(COD) was designated. Designation of COD was made using dichromate based on the ISO 6060: 2006 standard [21]. This value is needed to calculate the percentage distribution of the test and reference materials.

Designation of (COD) by dichromate method consists of determining the number of milligrams of potassium dichromate, calculated on the amount of oxygen being consumed in the oxidation process of the test compound dissolved in water. The oxidation is carried out in a sulfuric acid medium in the presence of silver sulfate as a catalyst. For designation purposes, relevant test portions of the test material (two samples at concentrations of 100 mg/dm³), the reference material solution (100 mg/dm³), and a blank sample were used. Each sample was prepared twice. COD was determined immediately after the samples were prepared.

Experimental Testing

In the research test, six BOD bottles were used. Bottles 1 and 2 contained the test material and inoculum of micro-organisms. In turn, bottles 3 and 4 explicitly contained inoculum – a blank sample (BS). Bottle No. 5 contained a reference material (sodium acetate) and inoculum of micro-organisms, whereas No. 6 contained the test material, the reference material (sodium acetate), and inoculum of micro-organisms for toxicity control (Table 3).

Before proceeding to the experiment, the pH of each solution and mineral nutrient solution were measured, which amounted to 7.30. Adequate volumes of the basic solutions of test material (bottles 1, 2, and 6), of the reference material (bottles 5 and 6), and of the mineral medium (all bottles) were introduced into the BOD bottle and placed in a thermostatic cabinet. Once the solutions reach the desired temperature (22°C), their contents were inoculated with activated sludge in such a manner that the concentration of solid particulates in mixtures being tested was 20 mg per dm³. Each bottle was placed on a magnetic stirrer, and 45% of potassium hydroxide solution was applied as CO₂ absorber.

The following criteria should be fulfilled for the test to be recognized as credible:

- Oxygen uptake by a blank sample should not be higher than 60 mg per dm³ within the testing period of 28 days, whereas oxygen uptake by the test material should exceed the amount of 60 mg per dm³ within the testing period of 28 days.
- The distribution of toxicity covering both the test substance and the reference substance within 14 days should be higher than 25%.
- The pH at the end of the experiment should be in the range of 6-8.5.

However, if the pH value at the end of testing is not within the limits stated above, and simultaneously the oxygen consumption by the test substance is less than 60%, the test should be repeated using a lower concentration of test substance [17].

Table 3. The pH value in the tested bottles.

BOD bottle	pH value (on day 0)	pH value (on 28. day)
B1 (blank)	7.55	7.48
B2 (blank)	7.57	7.45
RM (reference material)	7.28	7.18
TC (toxicity control)	7.61	7.55
TM1 (test material)	7.58	7.55
TM1 (test material)	7.63	7.58

Test Results

Biochemical oxygen demand (BOD) – the amount of oxygen consumed by micro-organisms for metabolic processes of chemical compounds – was computed on the basis of the obtained test results, according to the following formula:

$$BOD = \frac{mg\ O_2\ uptake\ by\ test\ material - mg\ O_2\ uptake\ by\ blank}{mg\ test\ item\ in\ vessel} = mg\ O_2 / mg\ test\ material \quad (1)$$

In turn, the percentage degradation of the test material and of the reference material were calculated based on the following formula:

$$\% degradation = \frac{BOD(mg\ O_2 / mg\ test\ material)}{ThOD\ or\ COD\ (mg\ O_2 / mg\ test\ material)} \times 100 \quad (2)$$

... where ThOD is theoretical oxygen demand, calculated based on the molecular formula of the substance, meaning the total amount of oxygen required for oxidation of a chemical compound; and COD is chemical demand on oxygen – the amount of oxygen being consumed during oxidation of the test compound using a hot acidified solution of potassium dichromate.

Due to the molecular structure of the test material, the content of nitrate and nitrite in the samples containing the test material were not analyzed at the beginning and at the end of the experiment. In the final calculation, the correction for nitrification was not considered in this case.

Results

All of the above-mentioned reliability criteria have been fulfilled in the conducted study on biodegradability. Table 3 summarizes the results of pH measurements for all samples tested on day 0 and all through until the 28th day of the experiment. At the beginning of the research test, the level of pH varied between 7.28 and 7.63; and at the end of the trail, it was in the range from 7.18 to 7.58. In turn, the average oxygen consumption and biochemical oxygen demand (BOD) on days 0, 3, 5, 7, 14, 21, and 28 of the analysis for the test material, the reference material and the control of toxicity are presented in Tables 4-6. For the test material, BOD ranged from 0.006 mg O₂ per dm³ in the third day of the experiment to O₂ value of 0.36 mg per dm³ in the final day of the test study (Table 4-6). Whereas for the reference material, BOD O₂ value ranged from 0.157 mg per dm³ on the first day of the experiment, and on the final day of the study O₂ was of 0.660 mg per dm³ (Table. 5). As for the control of toxicity, the biochemical demand on oxygen in the first day of the experiment amounted to 0.157 mg of O₂ per dm³; and the highest value was set out on the seventh day of the

Table 4. The results obtained for the test material.

Day	Sample	The average uptake of O ₂ [mg O ₂ /dm ³]	Sample	Uptake of O ₂ [mg O ₂ /dm ³]	O ₂ uptake by test material, corrected for average power consumption of O ₂ by blank sample [mg O ₂ /dm ³]	BOD [mg O ₂ /dm ³ mg of the test material]
1	B1	11.3	TM1	12.0	0.7	0.007
	B2		TM2	13.5	2.2	0.022
3	B1	19.0	TM1	25.0	6.0	0.060
	B2		TM2	25.0	6.0	0.060
5	B1	26.5	TM1	41.0	14.5	0.145
	B2		TM2	41.0	14.5	0.145
7	B1	32.0	TM1	52.0	20.0	0.200
	B2		TM2	47.0	15.5	0.150
14	B1	46.0	TM1	76.0	30.0	0.300
	B2		TM2	67.0	21.0	0.210
21	B1	47.5	TM1	82.0	34.5	0.345
	B2		TM2	79.0	31.5	0.315
28	B1	46.0	TM1	82.0	36.0	0.360
	B2		TM2	81.0	35.0	0.350

Table 5. The results obtained for the reference material.

Day	Sample	The average uptake of O ₂ [mg O ₂ /dm ³]	Sample	Uptake of O ₂ [mg O ₂ /dm ³]	O ₂ uptake by test material, corrected for average power consumption of O ₂ by blank sample [mg O ₂ /dm ³]	BOD [mg O ₂ /dm ³ mg of the test material]
1	B1 B2	11.3	RM	27.0	15.7	0.157
3	B1 B2	19.0	RM	50.5	31.5	0.315
5	B1 B2	26.5	RM	87.0	60.5	0.605
7	B1 B2	32.0	RM	97.0	65.0	0.650
14	B1 B2	46.0	RM	110.0	64.0	0.640
21	B1 B2	47.5	RM	110.0	62.5	0.625
28	B1 B2	46.0	RM	112.0	66.0	0.660

test, and it reached the O₂ value of 0.470 mg per dm³ (Table 6).

Prior to the experiment being performed, the values of COD were also determined for the test material and the reference material. COD value for the test material was 0.571 mg of O₂ per dm³, while the COD value for the reference material was 0.768 mg O₂/dm³ (the ThOD value for sodium acetate, based on the structural formula, amounted to 0.78 mg O₂/dm³).

On the basis of the calculated values of COD and BOD (Tables 4-5), on the basis of formula (2), the biodegradability of the test material and a reference material, that is sodium acetate, was determined (Fig. 1).

The biodegradability for the control of toxicity is presented in Table 7, which is considered to be reliable due to the fact that the toxicity degradation covering both the test substance and the reference substance performed over 14 days exceeded 25%.

Discussion of the Results

Table 4 shows consumption of O₂ after days 1, 3, 5, 7, 14, 21, and 28 of the experiment measured for the control samples and the test material. The table also shows the calculated values of BOD (mg O₂ per mg of the test material) on different days; and then the biodegradation of

Table 6. The results obtained for the control of toxicity.

Day	Sample	The average uptake of O ₂ [mg O ₂ /dm ³]	Sam- ple	Uptake of O ₂ [mg O ₂ /dm ³]	O ₂ uptake by toxicity control, corrected for average power consumption of O ₂ by blank sample [mg O ₂ /dm ³]	BOD [mg O ₂ /dm ³ mg of the reference material / test material]
1	B1 B2	11.3	TC	27.0	15.7	RM 0.157 TM 0.157
3	B1 B2	19.0	TC	49.5	30.5	RM 0.305 TM 0.305
5	B1 B2	26.5	TC	52.0	25.5	RM 0.255 TM 0.255
7	B1 B2	32.0	TC	79.0	47.0	RM 0.470 TM 0.470
14	B1 B2	46.0	TC	87.0	41.0	RM 0.410 TM 0.410
21	B1 B2	47.5	TC	87.0	39.5	RM 0.395 TM 0.395
28	B1 B2	46.0	TC	92.0	46.0	RM 0.460 TM 0.460

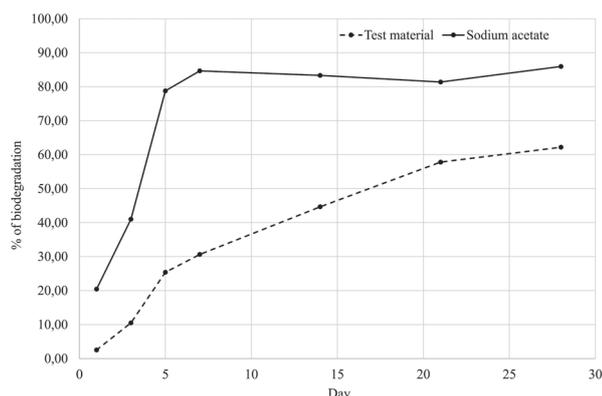


Fig. 1. Percentage of the distribution of reference material and test material in subsequent days of the experiment.

Table 7. The percentage of distribution for the control of toxicity.

Day	% of RM degradation	% of TM degradation
1	20.44	27.50
3	39.71	53.42
5	33.20	44.66
7	61.20	82.31
14	53.39	71.80
21	51.43	69.18
28	59.90	80.56

iron-based modifier was calculated (Fig. 1). Based on the obtained results for the test material, it was concluded that its biodegradation on day 7 amounted to 30.65%, on day 14 to 44.66%, on day 21 to 57.80%, and to 62.18% on the last day of the experiment. Pursuant to the regulation of the minister of health on the criteria and classification of chemical substances and mixtures [3], it can be concluded that iron-based modifier is easily degradable in an aquatic environment because a level of 60% degradation was exceeded in tests based on oxygen loss.

For comparison, sodium acetate was used as the reference substance, which satisfies the criteria for high biodegradability. Table 5 shows the measured uptake of O_2 after days 1, 3, 5, 7, 14, 21, and 28 of the experiment for the control samples and the reference material. In addition, the table shows calculated values of BOD (mg O_2 /mg of reference material) on particular days. Based on the results obtained for the reference material, it was stated that its biodegradation in day 7 was 84.64%, on day 14 83.33%, on day 21 81.38%, and 85.94% on the last day of the experiment.

Conclusions

Based on analysis of the test results on biodegradability carried out using the manometric respirometry test

method, it can be concluded that iron modifier is easily degradable in water environment due to the fact that after 28 days of experimental studies its biodegradability exceeded 60%. This means that, in accordance with the regulation of the minister of health on criteria and mode of classification of chemical substances and mixtures [3], iron modifier is safe for aquatic environments.

Acknowledgements

Our work was performed within the framework of the Innovative Economy Operational Program 2007-13 (No. POIG.01.04.00-16-159/12).

References

- BALLESTEROS M.M., GARCIA B.E, ORTEGA-GOMEZ E., SÁNCHEZ-PÉREZ J.A. A new bioseed for determination of wastewater biodegradability: analysis of the experimental procedure. *Environ. Sci. Pollut. Res.* **21**, 9522, **2014**.
- HOFFMAN D.J., RATTNER B.A., BURTON G.A. Jr., CAIRNS J. Jr. *Handbook of Ecotoxicology*, 2nd Ed.; Lewis Publishers, Boca Ranton, **2002**.
- Regulation of the Minister of Health of 10 August 2012 on the criteria and methods for the classification of chemical substances and mixtures. *Poland's Journal of Laws* of **2012**, Item 1018 [In Polish].
- GĘBALA P., MICKIEWICZ A. Spinosad – ready biodegradability test in water. *J. Ecol. Health* **16** (2), 55, **2012** [In Polish].
- TIC W.J. Metal Salts as the Homogeneous Catalysts for an Effective Combustion of Liquid Fuels. *Pol. J. Environ. Stud.* **17** (3), 439, **2008**.
- BABY J., RAJ J.S., BIBY E.T, SANKARGANESH P., JEEVITHA M.V., AJISHA S.U., RAJAN S.S. Toxic effect of heavy metals on aquatic environment. *Int. J. Biol. Chem. Sci.* **4** (4), 939, **2010**.
- ZEITOUN M.M., MEHANA E.E. Impact of Water Pollution with Heavy Metals on Fish Health: Overview and Updates. *Global Veterinaria.* **12** (2), 219, **2014**.
- PIEKUTIN J. Organic pollution in ground water. *Inż. ekol.* **26**, 95, **2011** [In Polish].
- Regulation (EC) of the European Parliament and of the Council, on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH); No 1907/2006 of 18 December **2006**.
- GUZIAŁOWSKA-TIC J., TIC W. J. Current requirements for the registration of products under the REACH system using an example of ecological solvents. *Chemik* **65** (11), 1204, **2011**.
- Directive 2004/10/EC of the European Parliament and of the Council of February 11, **2004** on the harmonization of laws, regulations, and administrative provisions relating to the application of the principles of Good Laboratory Practice and the verification of their applications for tests on chemical substances (codified version).
- Polish regulation: Regulation of the Minister of Health of May 22, **2013** on Good Laboratory Practice and conducting studies in compliance with the principles of Good Laboratory Practice (Poland's Journal of Laws No 108, Item 665) [In Polish].
- GUZIAŁOWSKA-TIC J. Selected toxicological and

- ecotoxicological properties of additive for combustion of liquid fuels. *Chemik* **68** (10), 831, **2014**.
14. 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, Authorization, and Restriction of Chemicals (REACH). Part V. Manometric respirometry test (Method C.4 – D).
 15. OECD Guidelines for the Testing of Chemicals: NF T 20-053:**1985**. „Chemical products for industrial use -Determination of density of solids in powder and liquids. Pycnometric method”.
 16. PN-EN ISO 2592: **2008**. „Petroleum products – Determination of flash and fire points – Cleveland open cup method” [In Polish].
 17. DIN 51794:05:**2003**. „Determining the ignition temperature of petroleum products”.
 18. PN-EN ISO 3104:**2004**. “Petroleum products. Transparent and opaque liquids. Determination of kinematic viscosity and calculation of dynamic viscosity EQP”. OECD Method No 114 [In Polish].
 19. OECD Guideline for the Testing of Chemicals No. 301F (1992): “Ready biodegradability, manometric respirometry test”.
 20. PN – ISO 6060: **2006** Water quality. Determination of chemical oxygen demand [In Polish].
 21. PN – EN 12880: **2004** Activated sludge – Determination of dry weight and water content [In Polish].