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THE STUDY OF METALWORKING FLUIDS BIODEGRADABILITY BY INDIRECT MEASUREMENT OF BACTERIAL INOCULUM RESPIRATION

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Abstract

An apparatus for measuring biodegradability of metalworking fluids (MWFs) was constructed according to (1)*, based on the Zahn-Wellens test which enables a continuous determination of CO_2 production by the change in conductivity of absorption solution. Results obtained from the testing of 8 different MWFs by this modified method were compared to those obtained in standardized OECD 302 B. The comparison showed better description of bacterial inoculum activity in tested solution; lag phase was easy to indicate. Tested emulsion achieved the level of primary degradability 39.7 – 40.8 %, and semi-synthetics 19.1 – 43.5%. The samples of synthetics where the degradation level reached 43.9 - 58.6 % were identified as the most degradable metalworking fluids.*

Key words

biodegradability, Metalworking Fluids, Cutting Fluids, Carbon dioxide, CO_2

INTRODUCTION

The use of metalworking fluids in machining leads to increased tool life, improved work quality, enhanced machine tool life, effective chip management and reduced process variability (2). Selection of the best cutting fluid for a particular operation will depend on several parameters, such as cutting speed, feed rate and depth of cut, the workpiece and tool materials, required tolerances and the surface integrity of the machined-component (3), as well as on the low human health risks and environmental behavior. Biological growth and formation of metal particles and oil drops in metalworking fluids (MWFs) deteriorate manufacturing performance and ultimately necessitates disposal. Disposal of untreated MWFs can lead to significant oxygen depletion and nutrient loading in surface waters, further posing environmental risks (4). Future lubricants have to be more environmentally adapted, have a higher level of performance,

and lower total Life Cycle Cost than currently used lubricants (5). The estimation of biodegradation rates is an important source of uncertainty in chemical risk assessments mandated by new EU legislation.

The biodegradability of substances depends primarily, but not only on their molecular structure. A huge number of different enzymes located in various species of micro-organisms or encoded in their genomes and plasmids are required for the numerous catabolic pathways which lead to the final mineralization of substances (6). The **biodegradabilty of different MWFs** accordingly closely depends on their respective base stocks (7). Biodegradability of **mineral oils** is generally much lower than that of vegetable oils (8). Chemical classes of hydrocarbons (paraffins, naphtenes (cykloparaffins) and aromatics) are known to differ in their susceptibility to microbial attack. For instance, the biodegradation rates of paraffins are higher than those of naphthenic hydrocarbons. The low biodegradability of aromatic compounds is well documented (7). Out of **synthetic base fluids**, esters can have ready biodegradability essentially equivalent to those of natural oils. Polyalpha-olefins show higher biodegradability than mineral oils of equivalent viscosity because of their higher degree of linearity. Alkylbenzenes having a high degree of branching are generally of low biodegradability. The specialized non-hydrocarbon synthetic base fluid types such as silicones and perfluoroalkyl ethers are essentially non-biodegradable (8). **The following important factors** may **influence biodegradation**: **concentration of the test material**, which should be high enough for the analytical methods chosen but sufficiently low in the case of toxic substances or when real environmental concentrations are to be simulated; **physico-chemical properties** of the test substances, such as volatility or water solubility, which determine their bioavailability and their abiotic elimination from water; **composition and concentration of inorganic nutrients** in the test medium, especially nitrogen and phosphorus, but also trace elements, and a sufficient buffer capacity of the medium; **presence or absence of other degradable substances** in the same medium for co-metabolic processes; **conditions and properties of the test systems** such as volume and shape of the test vessels, open or closed bottles, temperature, mode of mixing or shaking and oxygen supply; **test duration** (6)**.** Methods for measuring biodegradability of organic substances can be divided into two principal groups: direct measurement of parent compound concentrations and indirect measurement of parent compound bioconversion (9) as shown in Table 1.

COMPARISON OF DIRECT AND INDIRECT MEASUREMENTS OF DEGRADABILITY TESTS ACCORDING TO MEASURED PARAMETER Table 1

	Direct measurement	**Indirect measurement**	**Indirect measurement**
Measured parameters	• Concentration of parent compound concentration • Concentration of rising metabolites	• Measurement of organic content via summary parameters TOC / DOC / COD	• Indirect measurement of microbial activity such as evolved CO_2 or consumed O_2
Disadvantages	• Time consuming • Analytically complicated • Low number mixture - mostly water soluble	• Impossible to distinguish the biological degradation from the abiotic elimination of the substance from the water phase. • Only for water soluble and non-adsorbing compounds	Strongly temperature dependent if oxygen consumption is measured • Impossible to evaluate the rate of biodegradation
Advantages	• Information about the rising metabolites	• Easy measured parameters	• Available for poorly soluble substances

For biodegradability of lubricants (or poorly soluble or adsorbing substances), the industry recognized mostly the following tests such as **OECD 301 B** (**C.4-C Reach method**, **ISO 9439**), **ASTM 6139-11** and **ASTM 5864-11.** All these tests focus on the indirect measurement of bacterial activity during the degradation process.

In the Ready biodegradability test **OECD 301 B** (10) exactly the same as in C.4-C Reach method (11), a defined volume of inoculated mineral medium, containing a known concentration of the test substance (10-20 mg DOC or TOC per liter) as the nominal sole source of organic carbon is aerated by the passage of carbon dioxide-free air at a controlled rate (30 – 100 mL/min.) in the dark or in diffuse light. CO_2 produced by the bacterial inoculum (approximately 30 mg of SS per liter) is trapped in barium or sodium hydroxide and is measured by titration of the residual hydroxide or as inorganic carbon (corrected for that derived from the blank inoculum). If barium hydroxide is used, there are three absorption bottles, each containing 100 ml of 0.0125 M $Ba(OH)_2$ in series to each cultivation bottle. If sodium hydroxide is used, two connected traps containing 200 ml of 0.05 M NaOH, the second acting as a control one, to demonstrate that all the carbon dioxide was absorbed in the first one. The apparatus for preparing carbon dioxide free air is not defined in the OECD 301 B/C.4-C, but it is recommended to use the oxygen and nitrogen mixtures (20% O_2 : 80% N_2) (10). ISO 9439 describes the apparatus consisting of two adjacent bottles, one containing sodium hydroxide and the other one barium hydroxide (12). ASTM 6139-11 uses the CO2 scrubbing apparatus; the system consists of 8 Erlenmeyer flasks for each reaction bottle. Schematic view of the test is shown in the Figure 1.

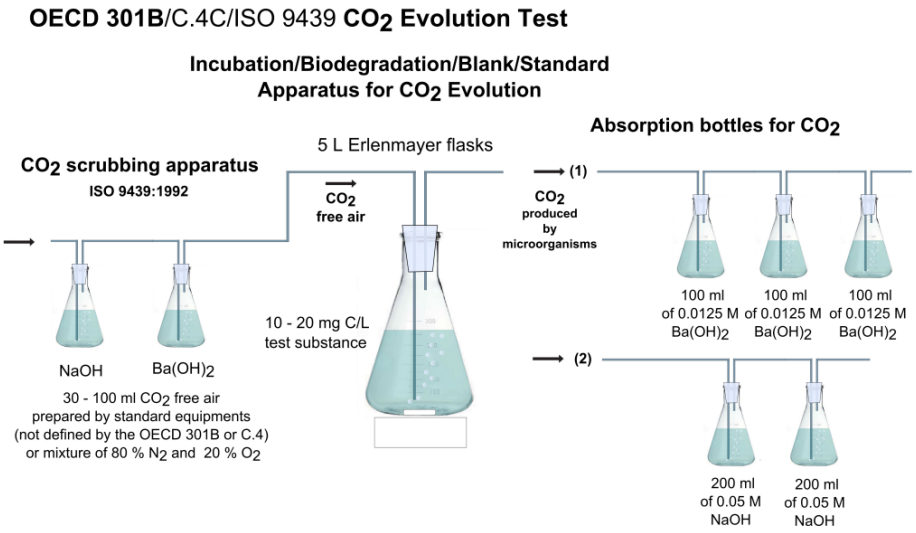

Fig. 1 *Schematic view of OECD 301 B /C.4-C test for Ready Biodegradability Test - CO_2 Evolution (Modified Sturm Test)*

The test usually runs with two flasks containing test substance and inoculum, two flasks containing only inoculum (blank), one flask containing reference compound (procedure control). If necessary, one flask containing test substance and sterilizing agent (abiotic sterile control) and another flask containing test substance, reference compound and inoculum (toxicity control) are added to the test (10). The degree of biodegradation may also be calculated from supplemental DOC analysis made at the beginning and end of incubation. Frequency of titrations is as necessary, for example, when substantial precipitation is seen in the first trap, or at least weekly (10,11). The pass level for ready biodegradability is 70 % removal of DOC and 60 % of $ThCO_2$ production and it has to be reached during the 10-day window within the 28-

day period of the test. The 10-day window begins when the degree of biodegradation has reached 10 % DOC of ThCO_2 and must end before the 28th day of the test. The pass level for the degradability test for the respirometric method is lower since, as some of the carbon from the test chemical is incorporated into new cells (the percentage of CO_2 produced is lower than the percentage of carbon being used). It is important to note that total CO_2 evolution in the blank must not exceed 40 mg/l medium. For measuring the amount of carbon dioxide produced by the bacterial inoculum during the degradation process, both methods (10, 11) allow the use of a device inorganic carbon analyzer, but do not define them exactly. ISO 9439 (12) allows the use of a carbon dioxide analyzer.

ASTM D 6139-11 Standard Test Method for Determining the Aerobic Biodegradation of Lubricants or their Components using Gledhill Shake Flask (14). This relatively simple shake-flask system for determining CO_2 evolution was developed to assess the ultimate biodegradability by soil and sewage microorganisms of chemicals which enter the environment previously for linear alkylbenzene sulfonates as it was described in (15). A standard test method for determining the aerobic biodegradation of lubricants is quite similar. This test method is an ultimate biodegradation test that measures CO_2 evolution. Biodegradation of a lubricant or the components of lubricant is estimated by collecting and measuring the CO_2 produced when the lubricant or component are exposed to microorganisms under controlled aerobic conditions. This value is then compared to the theoretical amount of CO_2 which could be generated if all of the carbon in the test material were converted to CO_2. The evolved CO_2 is trapped in a $Ba(OH)_2$ or other alkaline solution, and the amount of CO_2 absorbed is determined discontinuously by titrating the remaining hydroxide in solution (14). Inoculum from activated sludge freshly sampled from a well operated predominantly domestic sewage treatment plant or inoculum from the soil or from the surface waters should be used. These three inoculum sources may be combined in any proportion. Also the pre-adaptation of the inoculum is allowed. The suggested reference compound for water insoluble test materials LEAR – low eruic acid rapeseed oil such as canola oil may be used. Sodium benzoate or aniline may be used in case the test material is water soluble. Schematic view of the test is shown in Figure 2.

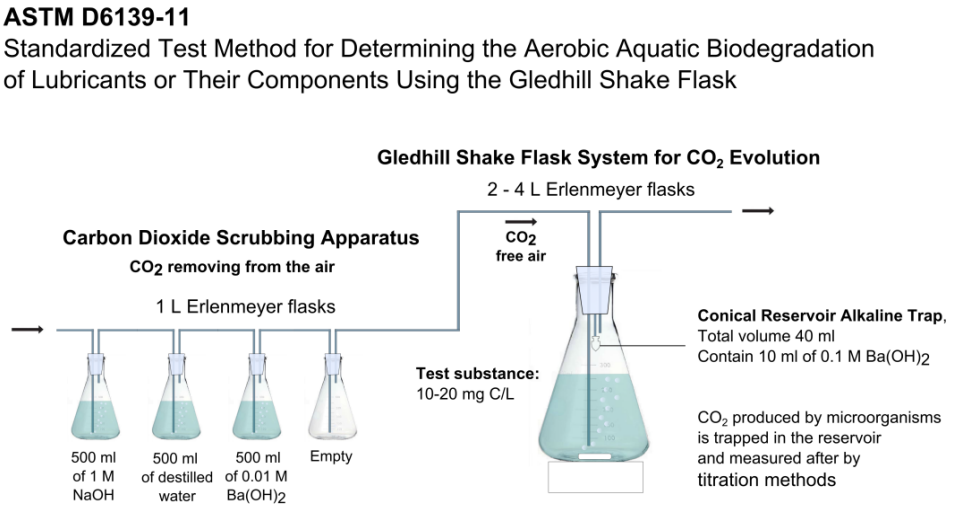

Fig. 2 *Schematic view of ASTM D 6139-11 Standard Test Method for Determining the Aerobic Biodegradation of Lubricants or Their Components Using Gledhill Shake Flask*

ASTM D 5864-11 Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components (13). This test method is quite similar to the OECD 301

B/C.4-C/ISO 9439 with the differences in construction of the carbon dioxide scrubbing apparatus. The same source of inoculum as the same reference compounds as in ASTM D 6139-11 may be used. Schematic view of the test is shown in Figure 3.

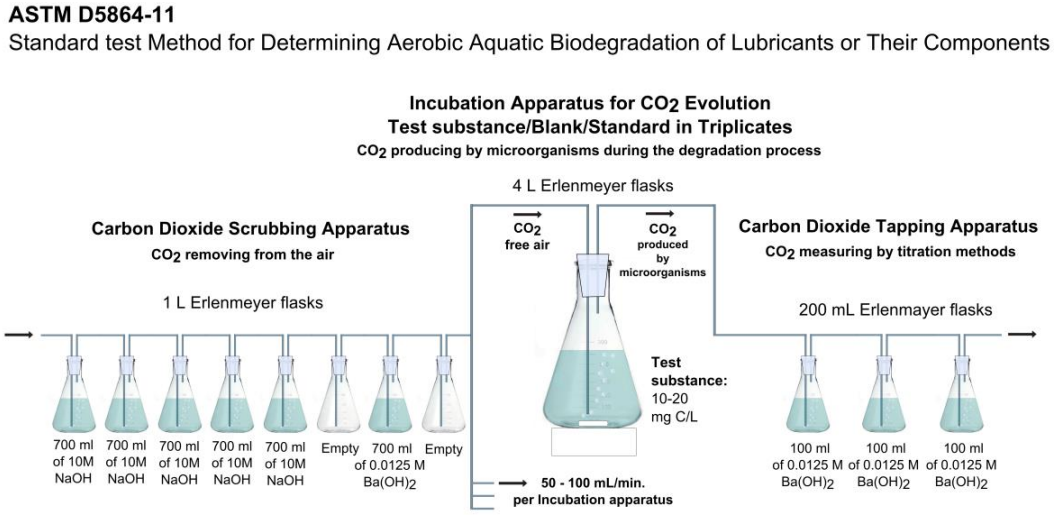

Fig. 3 *Schematic view of ASTM D 5864-11 Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components*

The amount of used activated sludge as an inoculum is about 30 mg/l of suspended solids in all above described tests. The concentration of tested substance is different in the EN ISO 9439, where up to 40 mg TOC per litre of tested media is allowed. Monitoring the degradability of poorly soluble substances is very difficult because of the measuring parameter. In the inherent degradability test (high concentration of inoculum), TOC or COD parameters are possible. In the case where the poor soluble substance is adsorbing on to the walls of the reactor or floccules of the activated sludge, evaluated biodegradability values could be misleading. In the case of primary degradability (these tests are more stringent, use less amount of inoculum) possible parameters evolved are carbon dioxide or consumed oxygen. When measuring oxygen, it is very important to maintain constant temperature, otherwise it allows testing only low concentrations of test substance. The tests, where decomposition of carbon dioxide is the main parameter, use CO_2-free air passing through the bioreactor in order to analyse microbiologically produced carbon dioxide after reaction in absorption solution by mostly titration methods. This measurement is complicated and discontinued. Some standardized method allow to measure evolved carbon dioxide by gaseous sensors.

Modified methods for degradability measurement

Norr et al., 2001 developed and defined a modified test system in (1) based on the Zahn-Wellens test which enables a continuous and parallel determination of O_2 consumption (pressure measurement) and CO_2 production (conductivity measurement). It is a closed test system consisting of a culture flask, a CO_2 absorption flask, as well as integrated measuring and control instruments. The air circulating within the test system causes the CO_2 present in the test solution to be stripped out completely and directly absorbed by the absorption solution. Authors in (1) state, that the testing method facilitates to test poorly soluble, adsorbing and volatile substances for inherent biodegradability, and constitute an appropriate complement to the standardized Zahn-Wellens test. Three years later, Strotmann et al., 2004 in (9) released their study on a multicomponent biodegradation test system. This method used the direct linear relationship between the CO_2 production and the change of conductivity in a well-specified,

calibrated system. Together with the ions of an aquatic *KOH* solution, the biogenously evolved CO_2 produced K_2CO_3. The carbonate was less dissociated, and therefore showed less conductivity. The linear correlation between the amount of CO_2 liberated and the change in conductivity could be used to determine the formed CO_2 very accurately. The main purpose of this study was the establishment of a conductivity-based online CO_2 evolution test. Authors in (9) state that this test fulfilled the requirements of standardized biodegradation tests and may serve as a basis for further development of biodegradation tests in different areas. In such a case, information obtained from three independent analytical parameters may lead to a better and more reliable prediction of biodegradability. Both modified system used closed bottles, and therefore measured also the pressure of the system while calculating the O_2 consumption. The linear correlation between conductivity and evolved CO_2 may be useful to exclude discontinued titration in the test.

MATERIALS AND METHODS

All **chemicals** were analytical graded and employed without any further purification. List of chemicals used for the preparation of mineral medium for the biodegradability test is defined in (1). For the experiment, selected were eight different MWFs from the semisynthetic to synthetics emulsion types. All **concentrates** were obtained from the CASTROL Company. **TOC (total organic carbon) measurements** were determined by direct injection of the diluted sample (1:25) into a Shimadzu TOC-V_{CPN} analyser, calibrated with standard solutions of potassium phthalate. The starting concentration of the TOC for samples was set up to 350 mg/L, while the total volume of the solution was 750 mL. The **conductivity measurements** were executed by Vernier CON-BTA conductivity probes connected to self-developed interface. The developed interface is a 12 channel microprocessor controlled data acquisition and processing device with RS-485 communication interface. As a HMI, a standard PC with a USB port connected to the communication network through an RS-485/USB converter is used. Communication takes place between the data acquisition devices and HMI and Master-Slave, wherein the HMI is the Master node. A GUI application was also implemented to the HMI which allows the online monitoring of the measured values and historical data export to xls. The developed interface is equipped with SRAM and a supercapacitor which together allows the internal measured data storing. This prevents the data loses during the communication failure or the HMI crash.

Modified Biodegradability apparatus for indirect measuring of evolved CO_2

Ten closed apparatuses were constructed according to (1); the air part was pumped by the peristaltic pump for better reaction of evolved CO_2 by the absorption solution at constant rate. Signal from the conductivity probes was transmitted through the conversion unit to the PC and measured continuously during the whole test. The principle of the measurement is described in (1). The CO_2 evolved by microbial consortium during the degradation process of different types of MWFs was continuously measured by the change in the conductivity in the absorption bottle where the carbon dioxide reacts with the barium hydroxide solution. The amount of absorbed CO_2 as $\mathrm{mg\,C}$ in a defined interval for the test/control/blank apparatus was calculated as a difference between the CO_2 concentration in the absorption solution at the time t and the CO_2 concentration in the absorption solution at the start of the test $\mathrm{t_0}$. In the test, we used the air that was not cleaned from the presence of CO_2 as it is accomplished in a standardized test, but the same level occurred also in blank. During the test, the absorption solution was replaced when it reached the level of conductivity below 1.5 mS/cm. The apparatus was opened, so that

inoculum was supported also by fresh oxygen. The percentage degradation of the test substance was calculated according to Equation 6:

$$D = 100.\ (m_{CO_2\ test} - m_{CO_2\ blank}) / ThOC\ , \qquad [\%] \qquad [6]$$

where, ThOC is the carbon input by the application of the test substance in mg C.

RESULTS AND DISCUSSION

Preliminary tested were **18 samples** of different MWFs according to OECD 302 B with different addition of activated sludge as an inoculum (0.01; 0.1; 0.25; 0.5 and 1.0 g/L of SS). Mostly synthetic fluids (4 of all samples) and only one emulsion were in the limit significantly under 20 % (0.1 – 5 %) of adsorption after first 3 hours of cultivating in all tested additions of inoculum (Figure 4a). Seven emulsions types and three semisynthetic fluids were classified in to the 2nd group where all samples were in the limits of 20 % of adsorption but, as the inoculum increased in the test, the adsorption level increased simultaneously (Figure 4b). In 9 of 10 cases, the highest additions of inoculum exceeded a bit the level of 20 %. Last four of all tested samples (three semi-synthetics and one emulsion) exceeded the level of 20 % of adsorption in more than 3 additions of inoculum (Figure 4c). These experiments showed that inherent degradability (potential to be degradable according OECD 302 B) depends on the addition of inoculum (0.1 – 1.0 g/L of SS) which may be critical in passing the fundamental criteria for the test. In all of 18 samples, the lag phase was not observed or only very slightly, which may be considered as the misinterpretation that the compounds of tested MWFs are very rapidly degradable instead of being eliminated by the other mechanisms.

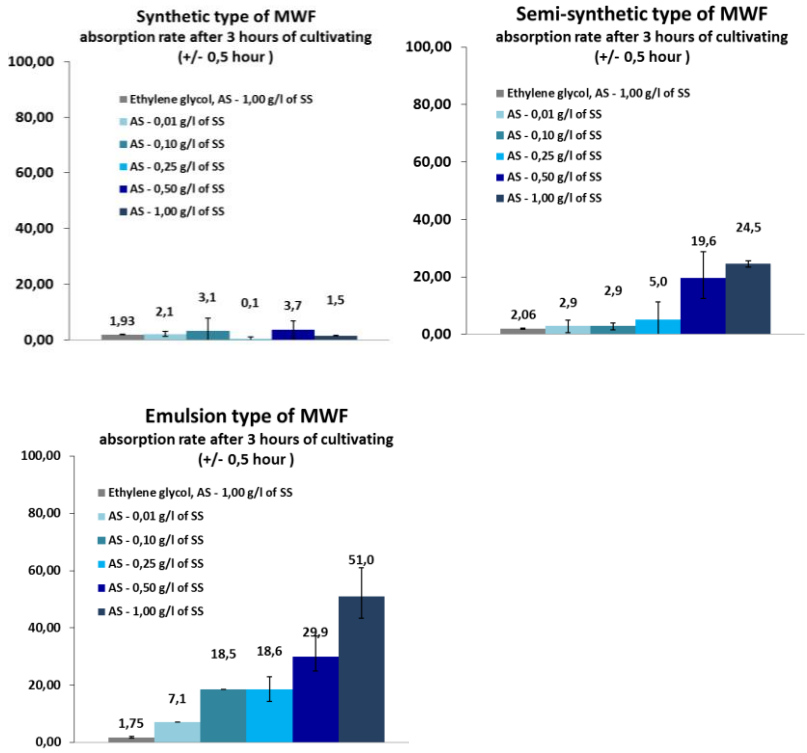

Fig. 4 a, b, c *– Adsorption rates for different kinds of MWFs.*

Below (Figure 5-12) are displayed the results from the biodegradability test obtained by the measurement of CO_2 evolved biologically in the constructed apparatus. We used 3-times higher concentration of inoculum than normally occurred in OECD 301 B, and also 5-6 higher concentrations of tested samples. Activity of the inoculum was controlled by measuring ethylene glycol. Endogenous respiration in blank, even when the concentration of the inoculum was 3-times higher, was in the allowed range of OECD 301 B.

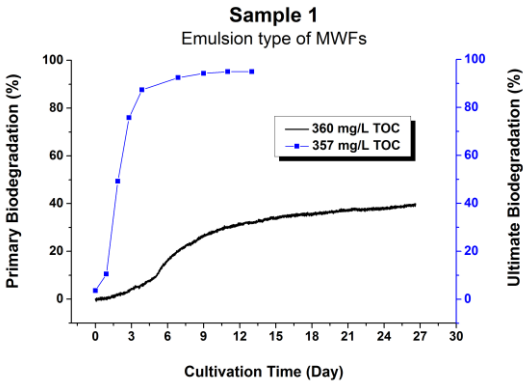

Fig. 5 *Degradation curves for Sample 1*

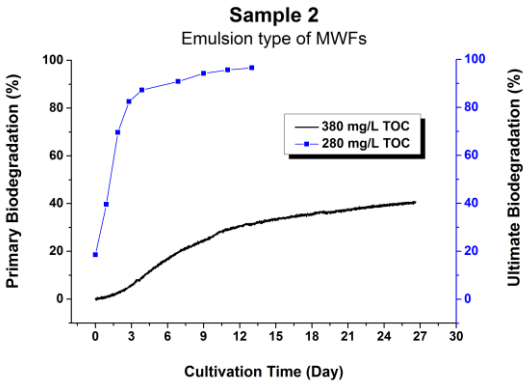

Fig. 6 *Degradation curves for Sample 2*

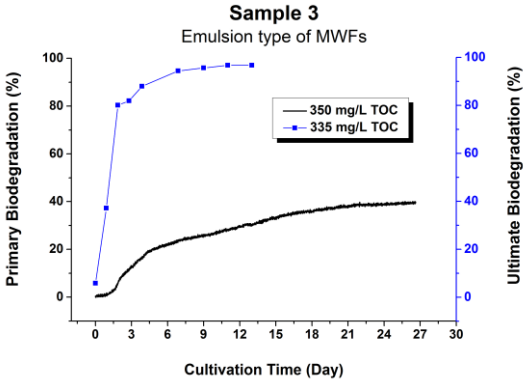

Fig. 7 *Degradation curves for Sample 3*

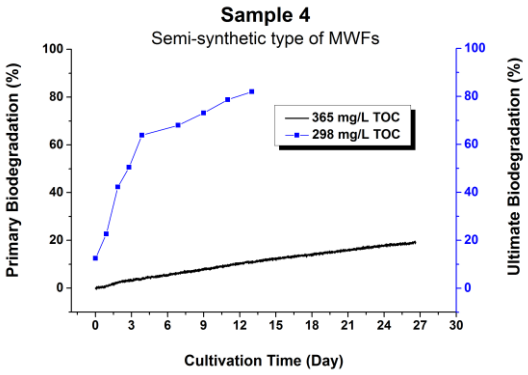

Fig. 8 *Degradation curves for Sample 4*

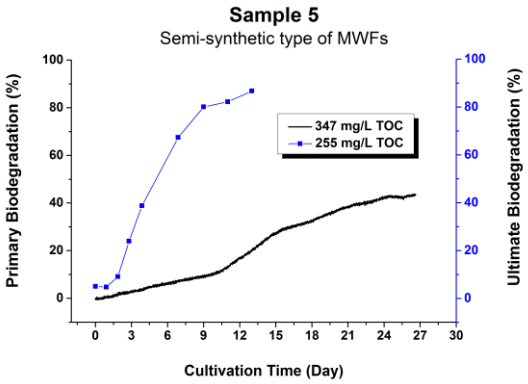

Fig. 9 *Degradation curves for Sample 5*

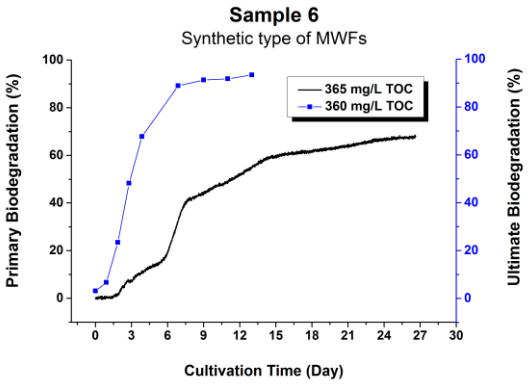

Fig. 10 *Degradation curves for Sample 1*

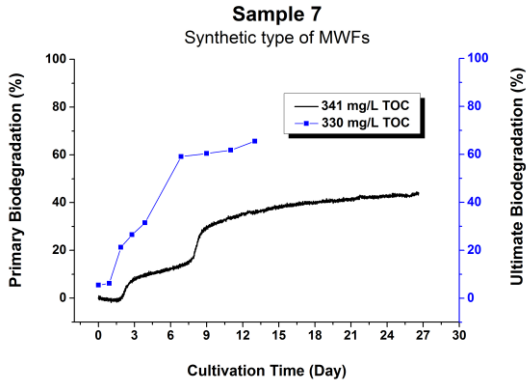

Fig. 11 *Degradation curves for Sample 7*

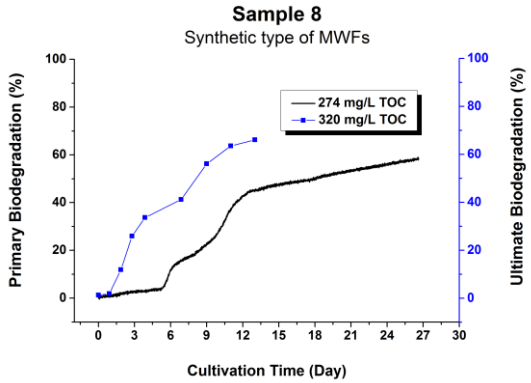

Fig. 12 *Degradation curves for Sample 8*

Note: OECD 301 A-F ***primary biodegradation*** *is the alteration in the chemical structure of a substance, brought by biological action, resulting in the loss of a specific property of that substance.* ***Ultimate biodegradation*** *(aerobic) is the level of degradation achieved when the test compound is totally utilized by microorganisms resulting in the production of CO_2, H_2O, mineral salts and new microbial cellular constituents (biomass).* ***Readily biodegradable*** *is an arbitrary classification of chemicals which have passed certain specified screening tests for ultimate biodegradability; these tests are so stringent that it is assumed that such compounds will rapidly and completely biodegrade in aquatic environments under aerobic conditions.* ***Inherently biodegradable*** *is classification of chemicals for which there is unequivocal evidence of biodegradation (primary or ultimate) in any test of biodegradability.*

Blue line in the graphs (Figure 5-12) indicates that in the presence of the *Lag phase*, ultimate degradability (OECD 302 B) is very short and the duration is only 24 hours (Samples 1, 5-8). In addition, samples 2 and 4 have relatively high level of adsorption (blue line in the graphs). Entirely all tested samples (except for samples 7-8) reached 80 % of the ultimate degradation according OECD 302 B before the 28th day of the test, which is one of the critical parameters to state that the substance has potential to be degradable. According to these results, we may state that tested samples except for samples 7-8, are very easily degradable. In comparison to the primary biodegradation (black line in the graphs Figures 5-12), the *Lag phase* has longer duration (mostly 3-6 days), that means that the inoculum is adapted to the organic carbon source provided; while this is not obvious from OECD 302 B. In the OECD 301 B regime, a compound is regarded as readily biodegradable if it reaches 60% degradation during the 28 day period when the parameter is $ThCO_2$. Even if we do not evaluate the degradability level (we used higher concentration of inoculum and also tested substance), it is obvious that it better describes how the tested substance mineralizes to CO_2 during the test. Only two samples reached the level of 60 % according OECD 301 B (sample 6 and 8). Very low biodegradability was observed in sample 4 (in comparison to OECD 302 B where the sample was evaluated as a very rapidly degradable). The reached level of degradation at the 26 day of the test was 18 % of $ThCO_2$. Table 2 summarizes all results. Tested emulsions had similar degradability (39.7 – 40.8 %), semi-synthetics had very different degradability (samples 4 – 19.1 %; sample 5– 43.5 %). The most degradable metalworking fluids were the samples of synthetics where the degradation level reached 43.9 % (sample 6), 58.6 % (sample 7) and 68.1 % (sample 8).

Table 2

			OECD 302 B				OECD 301 B modified
sample	**TOC [mg/L]**	***Lag phase***	**Adsorption [%]**	**Degradability [%]**	**TOC [mg/L]**	***Lag phase***	**Degradability [%]**
1 Emulsion	357	No	3.6	94.9	360	5 days	39.8
2 Emulsion	280	No	18.5	96.5	380	4 days	40.8

3 Emulsion	335	No	5.8	96.7	350	3 days	39.7
4 Semi-synthetic	298	No	12.5	82.0	365	15 days	19.1
5 Semi-synthetic	255	2 days	5.0	86.7	347	11 days	43.5
6 Synthetic	360	1 days	5.5	65.5	365	4 days	43.9
7 Synthetic	330	1 days	1.3	66.1	341	5 days	58.6
8 Synthetic	320	2 days	3.1	93.4	274	6 days	68.1

CONCLUSIONS

There is a relatively high potential in misinterpretation of the biodegradability results (according OECD 302 B) when the parameter such as TOC is measured in testing substances such as metalworking fluids, even when no adsorption (less than 5%) after the first 3 hours of cultivating is observed. Rapid decrease in the measured parameter may indicate that the chemical substance biologically degrades rapidly based on physical-chemical elimination. When a different parameter is chosen (e.g. CO_2), the behaviour of the process is more accurate. Typically in measuring degradability of soluble substances, primary degradation is still lower than ultimate biodegradability. According to modified OECD 301 B test, emulsions had similar degradability (39.7 – 40.8 %), semi-synthetics had very different degradability (sample 4 – 19.1 %; and sample 5 – 43.5 %). The investigated samples of synthetics where the degradation level reached 43.9 % (sample 6), 58.6 % (sample 7) and 68.1 % (sample 8) were identified as the most degradable metalworking fluids were.

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