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PRELIMINARY ECOTOXICITY AND BIODEGRADABILITY ASSESSMENT OF METALWORKING FLUIDS

Kristína GERULOVÁ, Peter AMCHA, Slávka FILICKÁ

Abstract

The main aim of this study was to evaluate the potential of activated sludge from sewage treatment plant to degrade selected MWFs (ecotoxicity to bacterial consortium) and to evaluate the ecotoxicity by Lemna minor–higher plant. After evaluating the ecotoxicity, biodegradations rate with activated sludge was assessed on the basis of COD measurement. Preliminary study of measuring the ecotoxicity according to OECD 221 by Lemna minor shows effective concentration of Emulzin H at the rate of 81.6 mg l^{-1}, for Ecocool 82.9 mg l^{-1}, for BC 25 about 99.3 mg l^{-1}, and for Dasnobor about 97.3 mg l^{-1}. Preliminary study of measuringthe ecotoxicity by bacterial consortium according to OECD 209 (STN EN ISO 8192) shows effective concentration of Blasocut BC 25 at the rate 227.4 mg l^{-1}. According to OECD 302B, the biodegradations level of Emulzin H, Ecocool and BC 25 achieved 80 % in 10 days. It can be stated that these MWFs have potential to ultimate degradation, but the statement has to be confirmed by a biodegradability test with other parameters than COD, which exhibits some disadvantages in testing O/W emulsions.

Key words

biodegradability, ecotoxicity, metalworking fluids

Introduction

Metalworking fluids (MWFs) have been introduced into the cutting process with the purpose to improve the characteristics of the tribological processes which always occur on the contact surfaces between the tool and the workpiece [1]. Exposition of the working environment to fluids may cause significant contamination to the environment and health hazards for the workers [2]. Future lubricants have to be more environmentally adapted, with a higher level of performance and lower total life cycle cost (LCC) than currently used

Kristína Gerulová, PhD., Peter Amcha, MSc. Eng., Slávka Filická, MSc. Eng. – Department of Environmental Engineering, Faculty of Materials Science and Technology in Trnava, Slovak University of Technology Bratislava, Botanická 49, 917 24 Trnava, Slovak Republic, e-mail: kristina.gerulova@stuba.sk, peter.amcha@stuba.sk, slavka.filicka@stuba.sk

lubricants [3]. The use of rapidly biodegradable lubricants could significantly reduce environmental pollution. Environment-friendly alternative is available for a large variety of mineral oil-based lubricants [4]. The most interesting group for formulation of environmentally adapted lubricants is that of base fluids such as vegetable oils and synthetic fluids (polyglycols, polyalpha olefins (PAO) and synthetic ester) [1, 2, 3, 4, 5]. These oils can offer significant environmental advantages thanks to their resource renewability, biodegradability and nontoxicity [5]. In Europe, predominantly vegetable oils such as rapeseed oil and sunflower oil are used [1, 3]. Chemically, these are esters of glycerine and long-chain fatty acids (triglycerides) [3, 4, 6]. Natural triglycerides are very rapidly biodegradable and highly effective lubricants. The use of vegetable oil in metalworking applications may alleviate the problems faced by workers, such as skin cancer and inhalation of toxic mists in the work environments [7]. Biodegradable synthetic esters have much better performance than natural oils, especially in the field of low and high temperature application and oxidation stability, but they are more expensive [5]. Properties related to the environmental fate of metalworking fluids are toxicity (non toxic for human beings, fish, bacteria etc.) [6], degree of biodegradability, bioaccumulability and biomagnification, and a relative content of renewable raw materials.

Biodegradablity of MWFs

Biodegradation represents a major route for removal of oil from soil and water compartments. Hence, biodegradability studies are of significance for developing eco-friendly lubricants, devising corrective measures for cleanup in case of spillage and meeting the legislation governing the manufacture and use of lubricants in some countries. It is now a common practice in lubricant industry to assess the biodegradability of lubricants in the aquatic environment, and laboratory data are available for a wide range of products [12].

A key performance characteristic when assessing environmental acceptability is the likely fate of the released product (i.e. biodegradation or persistence). Biodegradability is usually determined by measuring either the loss of extractable hydrocarbon (primary biodegradation) in the Coordinating European Council (CEC) L-33-A-93 test or ultimate biodegradation to CO_2, H_2O, inorganic salts and new microbial biomass using tests for ready biodegradability. The CEC test has seen more widespread use as it requires only standard laboratory glassware and an infra-red (IR) spectrophotometer, the latter being available in most oil product laboratories. Although the CEC test is only recommended for use with two-stroke outboard engine lubricants, it has been shown to be applicable to other lubricants. Because the CEC extraction solvent (1,1,2-trichlorotrifluorethane) is an ozone-depleting chemical, its supply and use are restricted by the Montreal Protocol it can be replaced by CCl_4 as extraction solvent [12]. ASTM test method D 5864 determines lubricant biodegradation. This test determines the rate and extent of aerobic aquatic biodegradation of lubricants when exposed to an inoculum under laboratory conditions. The inoculum may be the activated sewage-sludge from a domestic sewage-treatment plant, or it may be derived from soil or natural surface waters, or any combination of the three sources. The degree of biodegradability is measured by calculating the rate of conversion of the lubricant to CO_2. A lubricant, hydraulic fluid or grease is classified as readily biodegradable when 60 % or more of the test material carbon is converted to CO_2 in 28 days, as determined using this test method [13]. In [14] was used as the indicator of biodegradation the COD – chemical oxygen demand and this was used also in this study instead of its disadvantages, according OECD 302 B.

Although microbial deterioration of MWFs during their usage is a problem, such deterioration can be used to advantage for the disposal of operationally exhausted fluids. There is growing interest in exploiting the biocatalytic potential of micro-organisms to biodegrade MWFs in bioreactor-based processes. This can best be achieved by improving our understanding of microbial diversity within metalworking fluids [15]. Recently, genetic engineering has also been employed to specify certain species that can enhance overall treatment performance [16, 17]. The most common extracted bacterial isolates are defined in [15]. In [17] for established bioreactors to be effective for treating chemically mixed wastes such as metal working fluids (MWF), it is essential that they harbour microbial populations that can maintain sufficient active biomass and degrade each of the chemical constituents present. The reduction in the COD by the consortium (*Clavibacter michiganensis, Methylobacterium mesophilicum*, *Rhodococcus erythropolis* and *Pseudomonas putida*) was approximately 85 % of the total pollution load, and 30 – 40 % more effectively than any other treatment (indigenous MWF community alone or activated sludge). Many chemical components of the MWF proved to be recalcitrant in the other treatments. The results of this study confirm that assemblage of an inoculum, based on a comprehensive knowledge of the indigenous microbial community, in the target habitat, is a highly effective way of selecting microbial populations for bioaugmentation of bioreactors [17].

Toxicity of metalworking fluids

Toxicity to aquatic organisms is generally used to reveal potentially adverse environmental effects of a compound or product [8]. Experience has shown that the toxicological properties of fully formulated lubricants are related to those of the base fluid and additive components. The measured toxicity of mixtures is found to be close to the sum of component toxicities [3]. It is widely recognized that the ecotoxicological effects of the main MWFs components (biocides, corrosion inhibitors, extreme pressure and anti wear agents, emulsifiers, and surfactants) cause a major problem regarding the disposal of MWFs and their environmental impact. Much scientific research indicates the need for wider toxicologic monitoring of industrial effluents and receiving waters [9].

Toxicity of a substance is generally evaluated by conducting an acute toxicity test. The most common test methods used by the lubricant industry for evaluating the acute toxicity and European ecolabelling board of their products are EPA 560/6-82-002 (Sections EG-9 and ES-6); and OECD 201 (Algae growth inhibition test), OECD 202 (Daphnia acute immobilization test) and OECD 203 (Fish acute toxicity test) [1, 2, 8, 9]. The tests are used to determine acute toxicity, and do not evaluate adverse effects after long time exposure. Even so, problems arise from poor water solubility of the test substance. If a hydrophobic compound that is poorly soluble in water is discharged into the natural environment, it will probably end up in sediment and soil rather than remain in the water phase. Development of assays for sediment and soil are underway. The following scales of toxicity have been used: EC_{50}<1 mg l^{-1} highly toxic, 1 − 10 mg l^{-1} toxic, 10 − 100 mg l^{-1} hazardous, and >100 mg l^{-1} without acute toxicity [8].

Before starting the OECD 302B test, it exist a presumption with appropriate methods that no inhibition of sludge occurs at the chosen concentration of test substance if this is not already known [22]. If an inhibitory effect is found, reduce the concentration of test substance to a level which is unlikely to be inhibitory. The test of activated sludge inhibition of respiration is recommended also when OECD 301A-F for primary biodegradation is realized. Compounds with an EC_{50} value greater than 300 mg l^{-1} are not likely to have toxic effects in ready biodegradability testing [18].

Materials and methods

In the first test, *Lemna minor* was used as the organism for ecotoxicity testing and this was obtained from the ECOTOX s. r. o. Bratislava in 2008. It was cultivated in Hoagland E medium by [10] in axenic condition before test started according OECD 221 [11]. For evaluating EC_{50} value was applied linear regression. Inhibition was calculated by the area under the growth curve to control groups as it is described in [10]. In the first test were used four types of MWF´s such as Emulzin H, Ecocool, Blasocut BC 25 and Dasnobor.

As inoculums for the second test (toxicity and degradability by activated sludge), fresh activated sludge from sewage treatment plant from Jaslovské Bohunice, collected on the same day as the experiment started, was used. In each test, 0.1 g of dry matter per liter in final volume for toxicity and 0.5 g of dry matter per liter in final volume for degradability evaluation was used. In this test, Blasocut BC 25 and Adrana D407 were preliminary tested. The toxic effect of tested substance signified decrease of oxygen consumption according OECD 209 [19]. The degradability evaluation was carried out according to OECD 302B.

Results and discussion

Preliminary study of selected MWFs ecotoxicity on *Lemna minor* is shown in Figs. 1a-d. For the test to be valid, the doubling time of frond number in the control must be less than 2.5 days (60 h), corresponding to approximately a seven-fold increase in seven days and an average specific growth rate of 0.275 d^{-1}. Average specific growth rate were evaluated for all tests in the range 0.23 – 0.24 d^{-1} and pass the criteria for the test. Figs. 3 a-d show dependences of growth inhibition from logarithms of concentration of three tested metalworking fluids such as Emulzin H, Ecocool, BC 25 and Dasnobor.

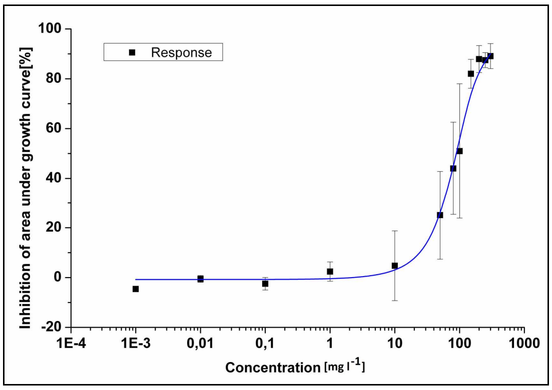

***Fig. 1a** – Dose-response (concentration-response) curve of Emulzin H for all tested concentrations*

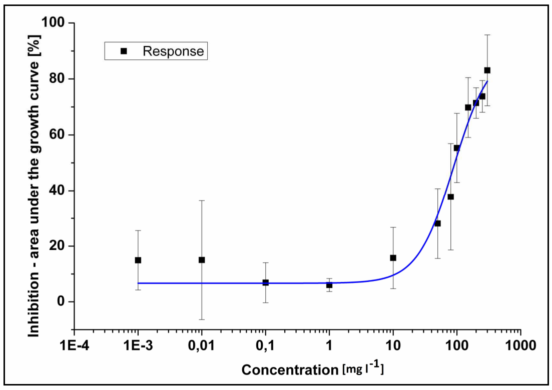

***Fig. 2b** – Dose-response (concentration-response) curve of Ecocool for all tested concentrations*

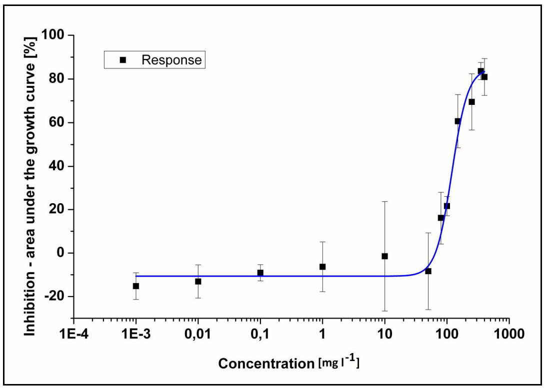

Fig. 1c – *Dose-response (concentration-response) curve of BC 25 for all tested concentrations*

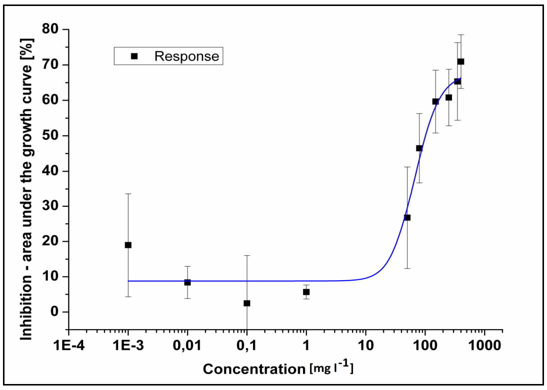

Fig. 1d – *Dose-response (concentration-response) curve of Dasnobor for all tested concentrations*

Hormesis effect was shown in the presence of small concentration of Blasocut BC 25. In the concentration 0.001 mg l^{-1}; 0.01 mg l^{-1}; and 0.1 mg l^{-1} of all tested MWFs were observed hormesis – stimulatory effect on growth. Effective concentration of tested MWFs that effect 50 % of growth inhibition was evaluated from the area under the growth curves and for Emulzin H was about 99.19 mg l^{-1}, for Ecocool 99.66 mg l^{-1}. BC 25 has affected inhibition about 99.3 mg l^{-1} and Dasnobor a little bit lower than the others – 97.3 mg l^{-1}. Tab. 1 compares evaluated effective concentrations and Tab. 2 lists effective concentrations that cause 20, 50 and 80 % of growth inhibition.

EC_{50} OF EMULZIN H, ECOCOOL, BC 25 AND DASNOBOR ACCORDING TO OECD 221, TESTING ORGANISM WAS *LEMNA MINOR*

Table1

MWF	EC_{50} [mg l^{-1}]	R^2	95% confidence interval [mg l^{-1}]
Emulzin H	81.6	99.19	75.6 - 86.9
Ecocool	82.9	99.66	73.5 – 85.5
BC 25	133.6	99.3	112.0 – 201.3
Dasnobor	108.4	97.3	75.5 – 193.6

CALCULATED VALUES OF EC_{20}, EC_{50} AND EC_{80} FOR THE MWFs

Table 2

MWF	EC_{20} [mg l^{-1}]	EC_{50} [mg l^{-1}]	EC_{80} [mg l^{-1}]
Emulzin H	28.2	81.6	223.9
Ecocool	22,4	82.9	281.8
BC 25	89.1	99.3	316.2

The test suggets that all tested MWFs have comparable toxic effect on higher plant – *Lemna minor* in the range 97.3 − 99.66 mg l^{-1}. The test was carried out in static conditions but it seems to be advantageous to study the effect on *Lemna minor* in semi-static or flow-through conditions with renewal of testing solution due to the observed little precipitation during the test.

Preliminary study of selected MWFs ecotoxicity with bacterial consortium is shown in the Figs. 2 − 3. The purpose of the method is to provide the quick screening test to identify the substances with unfavorable influence on sewage treatment plant and to identify noninhibition concentration of the tested substances, applicable in biodegradability test according to OECD 301 A-D. Respiration rate wass calculated from the oxygen decrease curves for all of tested substances concentrations approximately between 6.5 – 2.5 mg l^{-1} O_2. A part of the respiration curve above which the respiration rate is calculated has to be linear.

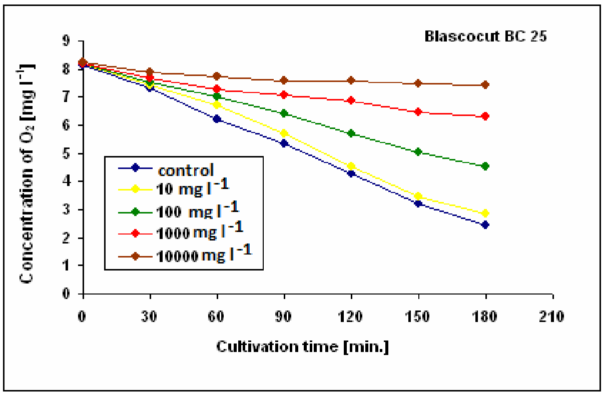

Fig. 2 Oxygen decreased curves in the range finding test of BC 25, part of the curve between 6.5 and 2.5 mg O_2 per litre has to be linear

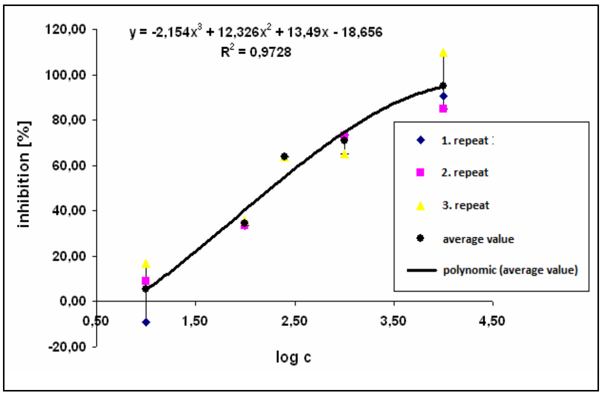

***Fig. 3** EC_{50} evaluation of BC 25with bacterial consortium become from sewage treatment plant activated sludge*

Tab. 3 shows the calculated values of EC_{20}, EC_{50}, EC_{80} for the MWFs Blasocut BC 25. EC_{20} of BC 25 was calculated to 30.3 mg l^{-1}; EC_{50} was calculated to 227.4 mg l^{-1} and EC_{80} for the MWFs Blasocut BC 25 was calculated to 3 293.1 mg l^{-1}.

CALCULATED VALUES OF EC_{20}, EC_{50} AND EC_{80}
FOR THE MWFs BLASOCUT BC 25

Table 3

	EC_{20} [mg l^{-1}]	**EC_{50} [mg l^{-1}]**	**EC_{80} [mg l^{-1}]**
Average value	30.3	227.4	3293.1

According to the OECD 209 regulation, it is sufficient to range the tested substance into one of four classes (<1 mg l^{-1}, 1 − 10 mg l^{-1}, 10 – 100 mg l^{-1} and <100 mg l^{-1}). BC 25 comes under the 4th category and it is possible to observe that BC 25 is relatively low toxic to the activated sludge bacteria from sewage treatment plant. In the case of using the concentration like this it is presumable that it will not be toxic for the biodegradability test according to OECD 301 A-F. It is recommended to carry out also test to the reference compound such as 3.5-dichlorphenol to determine the right activity of the bacteria in the sludge and also the physical-chemical test for the inorganic elimination of the oxygen by the testing substance.

The same test was repeated also for Adrana D407. Though EC_{50} of Adrana is much higher than Blascocut BC 25 − 3 288.5 mg l^{-1}, it belongs to the group > 100 mg l^{-1}.

The preliminary ecotoxicity study was followed by the biodegradability evaluation according to OECD 302 B. Validity and interpretation of the test is considered valid if the procedural control shows the removal of the reference compound by at least 70 % within 14 day and if the removal of DOC (or COD) in the test suspension took place relatively gradually over days or weeks, since this indicates biodegradation. Ultimate degradation degree of a reference compound was 95 % in 10 days window (started concentration of COD was in the required range up to 1000 mg l^{-1} and was 772 mg $l^{-1}$of COD). Starting concentration of MWFs samples passed COD 1101 – 1225 mg l^{-1}. As it is presented in the Fig. 4a-4d all tested cutting fluids satisfy 80% of degradation in 10 days. *Lag phase* were shown in the cases of Emulzin H and Ecocool and extend 2 days (Tab. 4).

SUMMARIZED RESULTS FROM ZAHN-WELLENS TEST OF EMULZIN H STANDARD

Table 4

	Culti-vation	Starting concentration of COD [mg l^{-1}]	lag phase t_l [day]	adsorption [%]	total decrease D_t' (if >20 % of adsorption)	ultimate degradation degree D_t [%]	Biodegra-dation time t_2 [day]	ultimate biodegradability (pass 80 %)
Emulzin H	1	1 203	no	13	-	95	3	**yes (3 days)**
	2	1 333	no	8	-	85	no	**yes (8 days)**
	3	959	no	15	-	76	no	**no**
	4	1 101	2	1	-	96	11	**yes (10 days)**
Ecocool	1	1 220	no	16	-	94	6	**yes (5 days)**
	2	1 358	1	9	-	90	11	**yes (8 days)**
	3	1 552	no	33	91	88	no	**yes (7 days)**
	4	1 125	2	3	-	85	no	**yes (10 days)**
BC 25	1	1 252	no	44	90	83	no	**yes (5 days)**
	2	1 497	no	36	90	85	no	**yes (8 days)**
	3	776	no	7	-	72	no	**no**
	4	1 043	no	28	86	82	no	**yes (10 days)**
Dasnobor	1	1 120	No	18	-	78	**no**	**n**o
	2	872	no	24	93	91	13	yes (4 day)

Lag phase t_l – time from inoculation until the biodegradation percentage increase up 10 % from initial COD (or DOC). Lag phase is variable and repeat with difficulties. It is measured in days. *Ultimate biodegradation degree* – it is the degree of biodegradation, above no other biodegradation occurs. *Biodegradation time t_2* – degradation time from the end of lag phase until 90 % of biodegradation occurs.

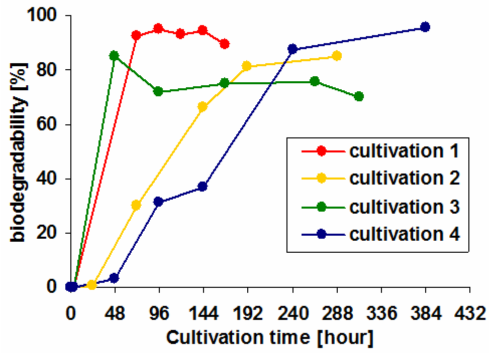

***Fig. 4a** – Biodegradability curves of Emulzin H*

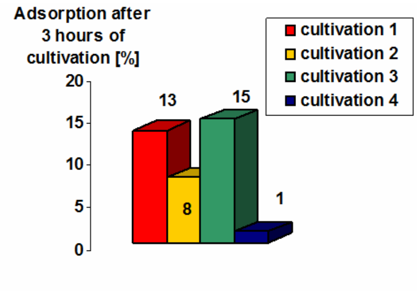

***Fig. 5a** – Adsorption after 3 hours of cultivating of Emulzin H*

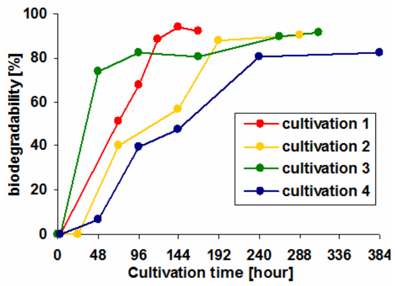

***Fig. 4b** – Biodegradability curves of Ecocool*

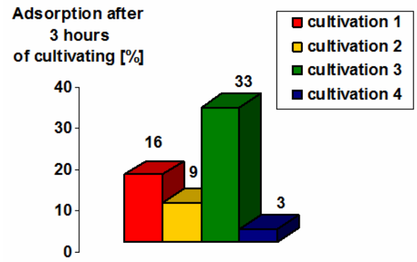

***Fig. 5b** – Adsorption after 3 hours of cultivating of Ecocool*

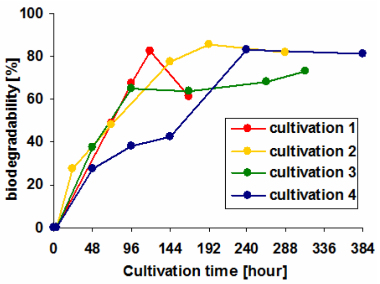

***Fig. 4c** – Biodegradability curves of BC 25*

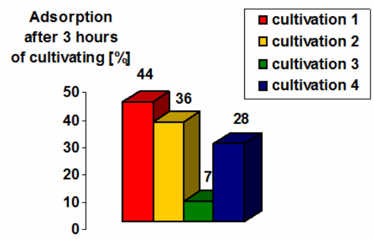

***Fig. 5c** – Adsorption after 3 hours of cultivating of BC 25*

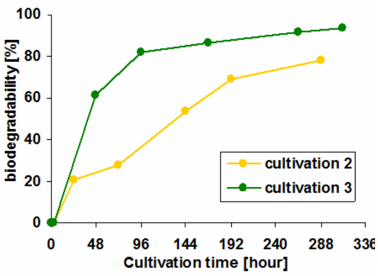

***Fig. 4d** – Biodegradability curves of Dasnobor*

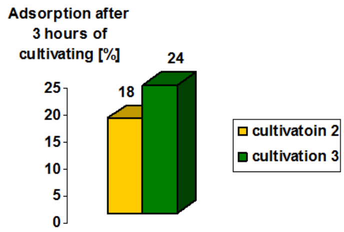

***Fig. 5d** – Adsorption after 3 hours of cultivating of Dasnobor*

All tested MWFs standards exhibited ultimate degradability by the Zahn-Wellens test, even when the concentration of COD was higher by 43 – 125 mg l^{-1} than this test requires. However, physical-chemical adsorption can, in some cases, play a role and this is indicated when there is complete or substantial removal in the first 3h and the difference between blanks and test solutions remains at an unexpected low value. In such cases, additional information is obtained from the comparison between the 3h value and initial value of test measured before the inoculum is added. If a more precise distinction between biodegradation (and partial degradation) and adsorption is to be drawn, carry out further tests, preferably a respirometric test for ready biodegradation, using the supernatant of the acclimatized sludge as inoculums.

Tab. 4 shows the adsorption of tested emulsions after 3 hours of the test (+/- 30 min.). Decrease of COD of tested MWFs in testing vessel were higher than 20 % in Ecocool, where presented 33 % of COD decrease and in the case of BC 25 it was 28 %, 36 % and 44 % of COD decrease, so there was no possibility to assess if the decrease was the result of the microbial activity, or other adsorption kinetics (physico-chemical). It is necessary to evaluate the level of biodegradation by other biodegradation method for BC 25 e.g. OECD 301 B or by CEC-L-33-A-93. As foor Emulzin H and Ecocool, COD decrease was within the limits of Zahn-Wellens test validation (< 20 %).

Conclusion

Preliminary study into the measuring of ecotoxicity by *Lemna minor* shows effective concentration of Emulzin H at the rate of 93.1 mg l^{-1}, for Ecocool 99.7 mg l^{-1}, for BC 25 about 99.3 mg l^{-1} and for Dasnobor 97.3 mg l^{-1}. The toxicity effect to *Lemna minor* of tested MWFs was comparable. Small concentrations of testing fluids indicate hormetic effect. It is required to test Emulzin H, Ecocool, BC 25 and Dasnobor by semi-static or flow-through condition. Preliminary study into the measuring of ecotoxicity by the consortium of activated sludge bacteria shows effective concentration of BC 25 is 227.4 mg l^{-1} and Adrana D 407 is 3 288.5 mg l^{-1}. By the results Adrana is less toxic than BC 25, both of belong to the fourth category > 100 mg l^{-1} so the concentrations about EC_{50} values did not cause problems in tests of biodegradability according OECD 301 A-F. The determined level of tested MWFs (Emulzin H, Ecocool, BC 25 and Dasnobor) biodegradation by Zahn-Wellens test achieved 80 % in 10 days, so, according to this test, they have a potential to ultimate degradation. Disadvantage of Zahn-Wellens test is that DOC measurement does noe enable evaluating all presented organics; even when they are in a insoluble form (in the case of COD) it is possible to identify only oxidable forms and some inorganics, which deforms the result. At high rate of adsorption, it is impossible to differentiate between biotic degradation and other form of abiotic elimination, which was shown in the case of BC 25 (28 and 38 % of COD decrease after 3 hours of cultivating). This method is also discontinuous. The future intended study will use the modified test method of Zahn-Wellens test by measuring CO_2 production and O_2 consumption continuously, or replacing it by the modifications of CEC test.

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Reviewers:

Mária Linkešová, Assoc. Professor, PhD. - Department of Chemistry, Faculty of Education, University in Trnava

Maroš Soldán, Assoc. Professor, PhD. - Institute of Safety and Environmental Engineering, Faculty of Materials Science and Technology in Trnava, Slovak University of Technology Bratislava.