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ECOTOXICITY AND BIODEGRADABILITY ASSESSMENT OF METALWORKING FLUIDS BY ACTIVATED SLUDGE BACTERIA

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Abstract

The main aim of this study was to evaluate toxicity of metalworking fluids to bacterial consortium of activated sludge according to OECD 209 (STN EN ISO 8192) and a potential of the same sludge to degrade a part of the fluids according to OECD 302B.

Toxic impact can affect different responses, particularly the inhibition of respiration measured from the oxygen consumption in a closed bottle. The degradation rate was calculated from COD according to the authors such as van der Gast and Ian Thompson (1, 2) who tested the degradability of some MWFs in bioreactors by measuring the COD. The lowest toxic MWF´s were Cimstar 597 and Emulzin H (the highest tested concentration was below EC_{50}), then Zubora TXS (EC_{50} - 11 349 mg l^{-1}), Aquamet LAK-E (EC_{50} - 5 228 mg l^{-1}), Adrana D 407 (EC_{50} - 4 351 mg l^{-1}) followed, and finally, Hocut 3380 (EC_{50} - 2 339 mg l^{-1}) was assessed as the most toxic.

Important in this test (OECD 302B) is that the starting concentration of the tested substance must not decrease below 20% after 3 hours of cultivating. After that, it is impossible to distinguish biological degradation of organic matter from abiotic elimination from the suspension through adsorption. Tested were 8 MWFs of similar concentration and different addition of activated sludge – 0.25 g l^{-1}, 0.50 g l^{-1} and 1.00 g l^{-1}. The test showed that, after the first 3 hours of cultivating, adsorption grew with the increasing amount of inoculums, except of Akvol B (the decrease of the starting concentration after the first 3 hours of cultivating was the lowest of all and below 20%). It can be stated that, according to the test basic conditions, all the tested MWFs have a potential to ultimate degradation.

Key words

biodegradability, ecotoxicity, metalworking fluids

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Introduction

Future lubricants or coolants, which are used in cutting process, have to be more environmentally adapted, have a higher level of performance, and lower total life cycle cost (LCC) than presently used lubricants (3). The most interesting group for formulation of environmentally adapted lubricants is base fluids such as vegetable oils, synthetic fluids (polyglycols, polyalpha olefins (PAO), synthetic ester) (3-7). Properties related to environmental fate of metalworking fluids are toxicity (non toxic to human beings, fishes, bacteria etc.) (8), degree of biodegradability, bioaccumulability and biomagnification, and relative content of renewable raw material.

Biodegradablity of MWFs

Biodegradation represents a major route for removal of oil from soil and water compartments. Although the biodegradability of organic substances can be evaluated using a wide variety of testing procedures, the greatest significance is put to the standard tests specified in the OECD. Guidelines for the testing of chemicals and in the ISO standardized methods. The biodegradation process can be analyzed under aerobic or anaerobic conditions, in fresh water or seawater, or in soil (9).

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 such compounds are assumed to biodegrade rapidly and completely 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 (10-13). ***Inherent biodegradability*** demonstrates the potential degradability of a compound. Unlike in ready biodegradability tests, the conditions for biodegradation are optimum. Also, these methods have a screening function in the sense that "hard" chemicals are selected - a negative result indicates that a chemical is too persistent and that, tentatively, no further research on biodegradation has to be performed (14).

The choice of an appropriate test is an essential prerequisite for the determination of the biodegradability of lubricants. The CEC L-33-A-93 test, for example, enables the evaluation of primary biodegradability while the OECD test permits the ultimate biodegradability to be assessed. The OECD tests for ready biodegradability (OECD 301 series and OECD 310) play an important role in the environmental classification of chemicals. A substance is considered as readily biodegradable if it has reached a sufficient level of degradation in one of these tests, 70% in the case of DOC removal or 60% for CO_2 or BOD. The pass-levels have to be reached within the 28-day test period by the end of a 10-day window, which begins when biodegradation reaches 10%. When the substance fails to meet the ready biodegradable criterion, it can be made subject to inherent biodegradability tests, which are used to assess whether a substance has any potential for biodegradation. Biodegradation rates above 20% (measured as $ThCO_2$, ThOD, DOC or COD) may be regarded as evidence of inherent,

primary biodegradability, whereas biodegradation rates above 60% $ThCO_2$, ThOD or 70% DOC or COD may be regarded as evidence of inherent, ultimate biodegradability (9).

Toxicity of metalworking fluids

Toxicity to aquatic organisms is generally used to reveal potentially adverse environmental effects of a compound or product (15).

According to OECD 302B test, there is a presumption with appropriate methods that no inhibition of sludge occurs at the chosen concentration of the test substance if this is not already known. If an inhibitory effect is found, it is needed to reduce the concentration of the 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.

MATERIALS AND METHODS

Fresh activated sludge from sewage treatment of the Jaslovské Bohunice plant was used as inoculums for the both tests (toxicity and degradability by activated sludge) the same day as the experiment started. In each of degradability tests, used were 0.25, 0.50 and 1.00 g of dry matter per liter in the final volume and 0.10 g of dry matter per liter in the final volume for toxicity evaluation. The degradability of MWFs was evaluated by OECD 302 B, while the toxic effect caused by inhibition of respiration was evaluated by OECD 209.

Adrana D407 – Shell Adrana D 407 is used for general and medium severe machining on materials as steel, aluminum and cast iron. Shell Adrana D 407 is specially recommended for all kinds of grinding operations in the bearing industry are good stability when tramp oil is present, it has very good stability in water with high hardness, good anti-foam characteristics in soft water, extremely good anti-corrosion protection, even with higher chloride content and high detergency properties, due to balance of boron, amine and biocide (17). Per oral acute toxicity LD50 > 2000 mg kg^{-1} (rat), dermal toxicity – information are out-of-reach, eye irritation – information are out-of-reach, dermal irritation – information are out-of-reach. The producer of the fluid wrote in the MSDS that ecotoxicity was not especially assessed. No information on degradability was found in the sheet. Water class dangerous (German class WGK) is by the MSDS No. 2 (18, 19).

Aquamet LAK-E – There is no information regarding acute or chronic toxicity, mutagenity, carcinogenity or teratogenity in MSDS. Also, there is no information on ecotoxity or biodegradability. Water Class Dangerous (German class WGK) was established as No. 2 (20).

Hocut 3380 – Hocut 3380 is a low foaming milky emulsion which gives superior surface finish and tooling performance. It is ideal for machining automotive aluminum, titanium, inconel and alloy steels due to its non-staining characteristics. It is particularly designed for high-pressure coolant systems associated with modern CNC machine tools with ultra-long life and freedom from biocide additions, making it suitable for both centralized systems and single-sump machines (21). The information on toxicity and ecological information are not available according to the material safety data sheet supplies by producer. By the MSDS, the main components defined as hazardous ingredients are Amine (Cas No. 101-83-7) 1-5%,

Ethoxylated Alcohol (Cas No. 68213-23-0) 1-10%, Mineral Oil (Cas No. 64742-52-5) 30-60%, and Monoisopropanolamine (Cas No. 78-96-6) 1-5% (22).

Cimstar 597 **–**MSDS was not available due to the poor communication with the producer.

Akvol B **–** By the MSDS, the main components defined as hazardous ingredients are 1, 3, 5-hexahydrotriazine (Cas No. 4719-04-4) 1-3%. Toxicological and ecological information is not defined in MSDS (23).

Ecocool MK3 – **Fuchs Ecocool MK 3** is specially compounded emulsifying cutting oil composed of petroleum oil and special emulsifiers. The information on toxicity and ecological information is not available according to material safety data sheet supplied by producer. By MSDS, Water Class Dangerous (German class WGK) was established as No. 1 (24).

Emulzin H **–** exotoxicity test was carried out according to fishes LC50, 96 hours exp.: 30.1 mg/l, and daphnia EC50, 48 hours: 9.8 mg/l. Ecological information states that the product has unfavorable effect on environment. According to CEC L-33-A-93, products and water form biodegradable emulsion and the level of biodegradation were evaluated to 67%. By MSDS, the main components defined as hazardous ingredients are sulfonated acids (Cas No. 271-871-5) > 6%, ethoxylated alcohol C13 (Cas No. 500-027-2) 2.4%, alkylalcohol (Cas No. 248-469-2) < 0.9%, ethoxylated alcohol C12-C15 (Cas No. 500-195-7) 0.8%. (25).

Zubora TXS – By MSDS, the main components defined as hazardous ingredients are oxazolidine derivate (Cas No. 66204-44-2) 1-3% and fatty alcohol polyglycolether 3-5%. There is no information on biodegradability of the product in MSDS (26).

Quakercool 7030 **–** Information on ecotoxicity is not available. MSDS listed dangerous components such as polyol polyether in addition 1-5%, Alky alcohol in addition 1-5% and Poly(oxy-1,2-ethanediyl), a-tridecyl-w-hydroxy-, branched (Cas No. 69011-36-5) in addition 1-5% (27).

RESULTS AND DISCUSSION 1

Biodegradability testing

The test is considered valid if the procedural control shows the removal of the reference compound by at least 70 % within 14 days. If the rate of biodegratation reaches a constant level (more than 80%) before the 28th test day, the test is considered completed. If the degradation starts at the end just before the 28th day, the test has to be prolonged until the biodegradation is finished. The displayed data of biodegradation curve suggest that: ***Lag phase*** t_1 – is defined as the time from inoculation until the biodegradations rate increases up to 10 % from the initial COD (or DOC) value. Lag phase is variable and the repeatability is low. It is measured in days. ***Ultimate biodegradation degree*** – it is the degree of biodegradation, over which no other biodegradation occurs. ***Biodegradation time*** t_2 – is defined as the degradation time from the end of the *Lag phase* until it reaches 90 % of biodegradation's rate.

Figures 1-8 show the degradation/elimination curves from testing eight selected metalworking fluids: Adrana D407, Hocut 3380, Aquamet LAK-E, Quakercool 7030, Cimstar 597, Akvol B, Ecocool MK3 and Emulzin H, which are commonly procurable in the Slovak market. If more than 20% of adsorption occurs after the first 3 hours of cultivating, the curve can be considered an elimination one, since it is impossible to distinguish between biotic and abiotic elimination of substance from the solution. Figure 9 shows the adsorption rates after the first 3 hours of cultivating in different additions of activated sludge – 0.25 g l^{-1}, 0.50 g l^{-1}, and 1.00 g l^{-1}.

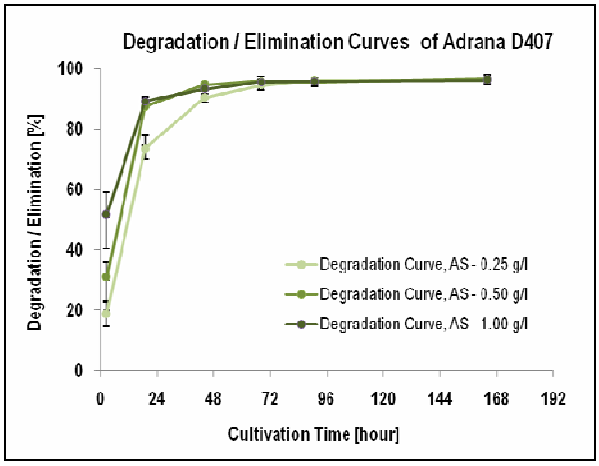

Fig. 1 *Degradation/Elimination curves of Adrana D 407*

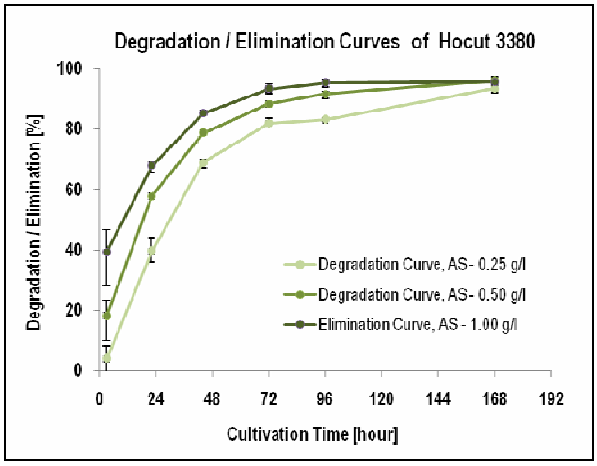

Fig. 2 *Degradation/Elimination curves of Hocut 3380*

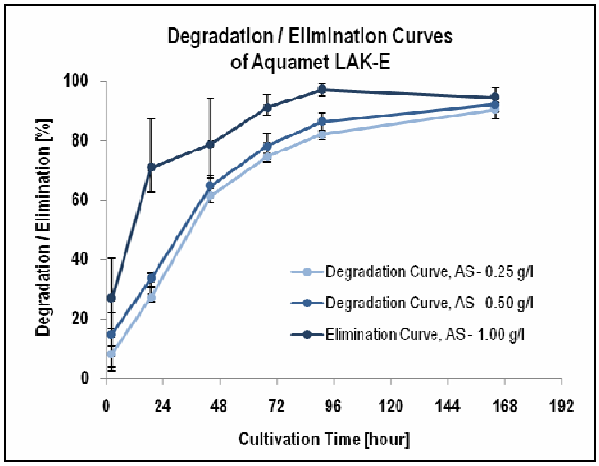

Fig. 3 *Degradation/Elimination curves of Aquamet LAK-E*

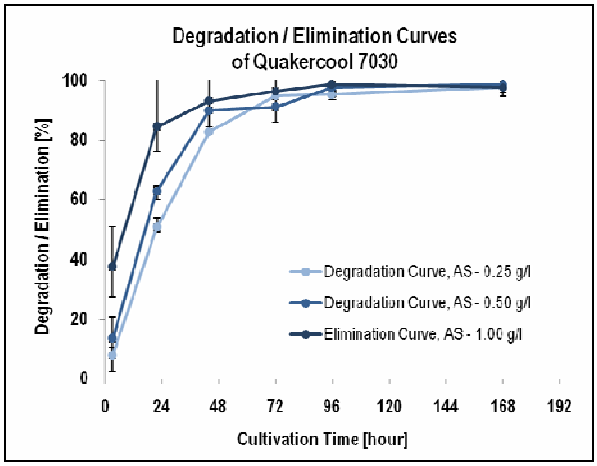

Fig. 4 *Degradation/Elimination curves of Quakercool 7030*

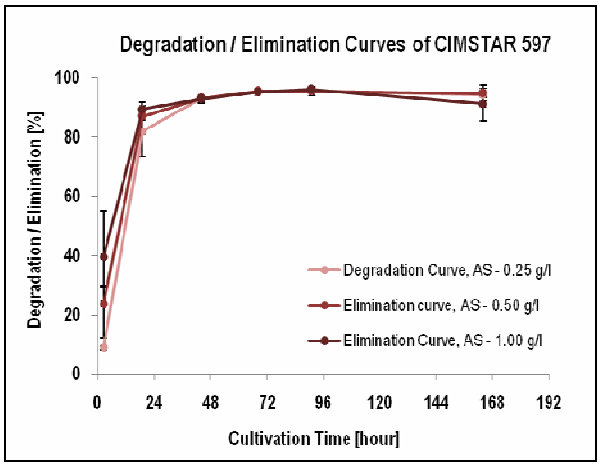

Fig. 5 *Degradation/Elimination curves of CIMSTAR 597*

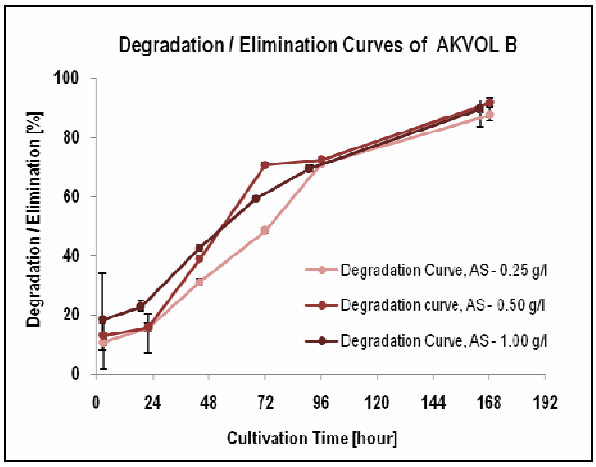

Fig. 6 *Degradation/Elimination curves of Akvol B*

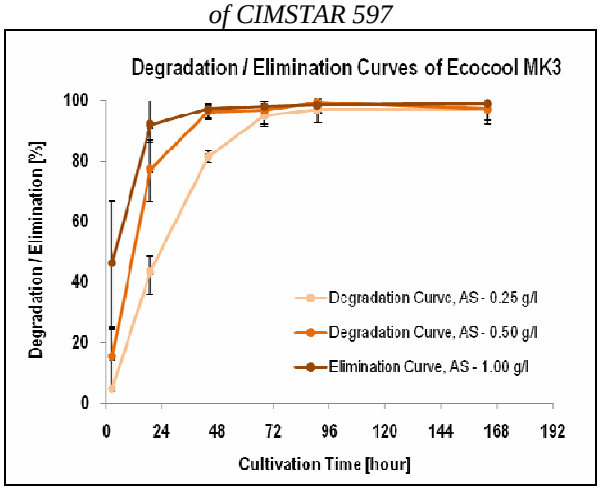

Fig. 7 *Degradation/Elimination curves of Ecocool MK3*

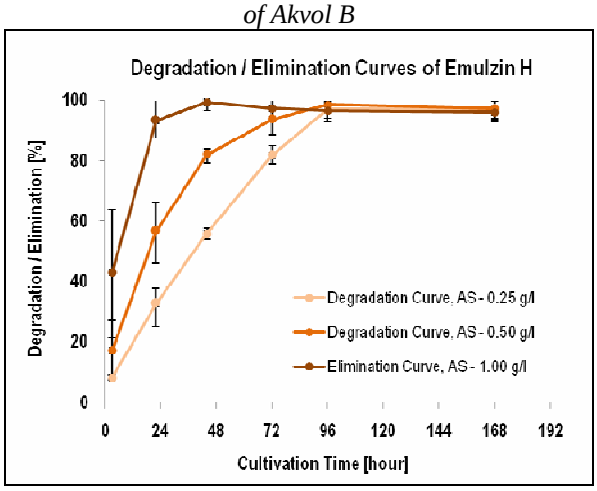

Fig. 8 *Degradation/Elimination curves of Emulzin H*

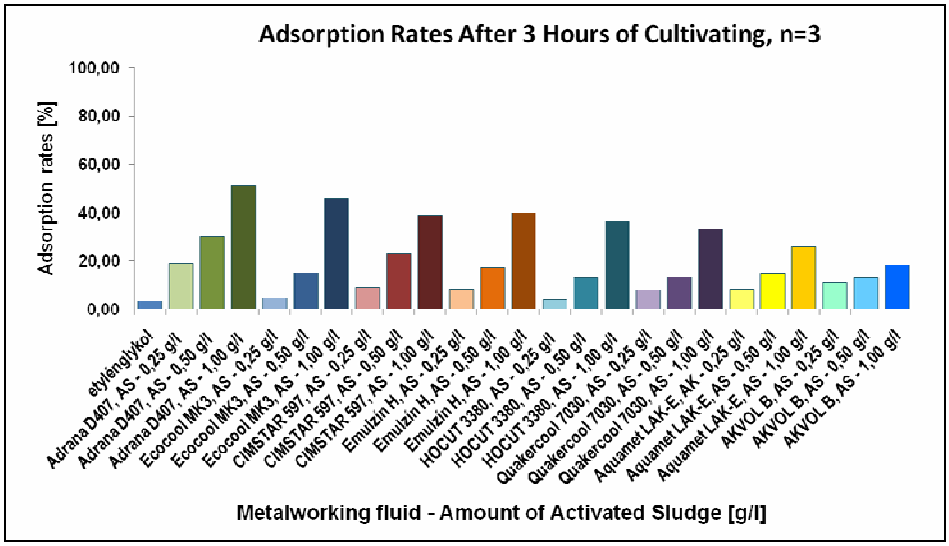

Fig. 9 *Adsorption rate of tested substances after the first 3 hours of cultivating*
AS – addition of activated sludge

As all graphs in the Figs. 1-9 show that, with the increasing addition of activated sludge (AS) inoculums, the adsorption rate also increases after the first 3 hours of cultivating. The abovementioned suggests that adsorption on the floccules of AS plays an important role in elimination of the organic matter from the substance. To distinguish the main route of elimination (adsorption or degradation), it is recommended to use other analytical methods, such as the absorbance of Methyl (C-H) bond stretch in 2926 cm^{-1} and Methylene ($C-H_2$) bond stretch in 2956 cm^{-1} by FTIR analyses according to the CEC L-33-A-93 test which was released mainly to measure degradability of automotive lubricants. The lowest adsorption rates were recorded for Akvol B and Aquamet LAK-E. None of the tested metalworking fluids passed 20% of adsorption after the first 3 hours of cultivating with addition of activated sludge 0.25 g l^{-1}. In the case of Akvol B, the *Lag*-phase was also registered, which means probably the adaptation of the bacteria to the source of organic carbon in the solution. Results of the degradability testing are summarized in Table 1.

SUMMARIZED RESULTS FROM ZAHN-WELLENS TEST Table 1

	Average value n=3 AS addition	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 [%]	Biodegradation time t_2 [day]	ultimate biodegradability (pass 80 %)
Adrana D407	0.25 g l^{-1}	915	no	19	-	96	2	yes (2 days)
	0.50 g l^{-1}	871	no	30	96	-	-	yes (2 days)
	1.00 g l^{-1}	746	no	51	96	-	-	yes (2 days)
Aquamet LAK - E	0.25 g l^{-1}	957	no	8	-	90	6	yes (7 days)
	0.50 g l^{-1}	880	no	15	-	92	11	yes (7 days)
	1.00 g l^{-1}	818	no	26	97	-	-	yes (4 days)
CIMSTAR 597	0.25 g l^{-1}	852	no	9	-	95	2	yes (2 days)
	0.50 g l^{-1}	848	no	23	95	-	-	yes (2 days)
	1.00 g l^{-1}	782	no	39	96	-	-	yes (2 days)
Ecocool MK3	0.25 g l^{-1}	1038	no	4	-	98	3	yes (3 days)
	0.50 g l^{-1}	1012	no	15	-	99	2	yes (2 days)
	1.00 g l^{-1}	867	no	45	99	-	-	yes (2 days)
Hocut 3380	0.25 g l-1	1153	no	4	-	93	7	yes (4 days)
	0.50 g l-1	981	no	13	-	95	-	yes (3 days)
	1.00 g l-1	1046	no	36	96	-	-	yes (2 days)

Quakercool 7030	0.25 g l-1	1019	no	8	-	98	4	yes (2 days)
	0.50 g l-1	1066	no	13	-	99	3	yes (2 days)
	1.00 g l-1	937	no	33	98	-	-	yes (1 day)
Akvol B	0.25 g l-1	879	yes (1 day)	11	-	87	-	yes (6 days)
	0.50 g l-1	808	yes (1 day)	13	-	92	7	yes (6 days)
	1.00 g l-1	790	yes (1 day)	18	-	90	7	yes (6 days)
Emulzin H	0.25 g l-1	1128	no	8	-	98	5	yes (3 days)
	0.50 g l-1	1109	no	17	-	98	4	yes (2 days)
	1.00 g l-1	987	no	40	96	-	-	yes (1 day)

Ecotoxicity testing on bacteria of activated sludge – inhibition of the respiration according to OECD 209 (STN EN ISO 8192)

Preliminary study of six selected MWFs' ecotoxicity with bacterial consortium is shown in Figures 13-18. The purpose of the method is to provide the quick screening test to identify substances that have unfavourable influence on sewage treatment plant and identify noninhibition concentration of tested substances applicable in biodegradability test according OECD 301 A-D. Respiration rate is calculated from the oxygen decrease curves for all of tested concentrations of substances 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.

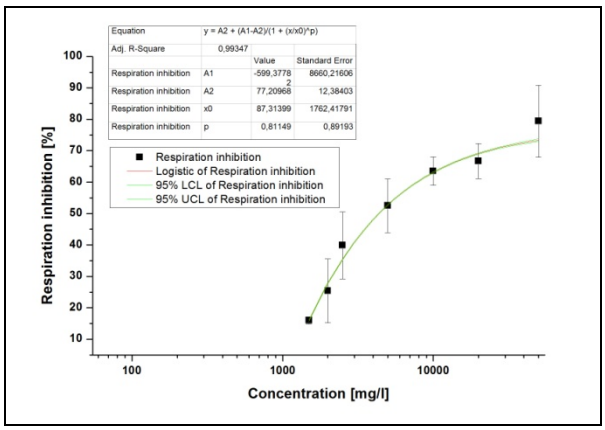

Fig. 13 *EC_{50} evaluation of Adrana D407 (Origin Pro8)*

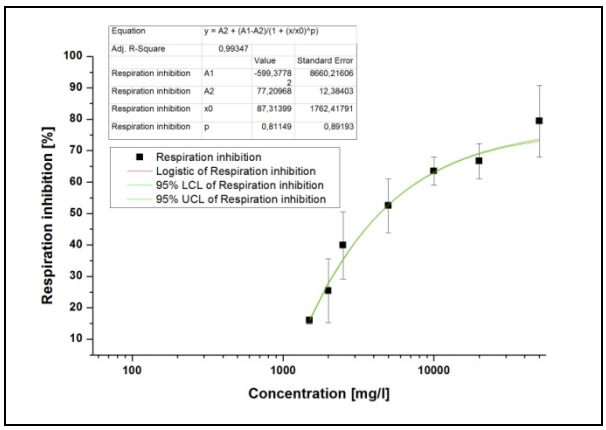

Fig. 14 *EC_{50} evaluation of Aquamet Lak-E (Origin Pro8)*

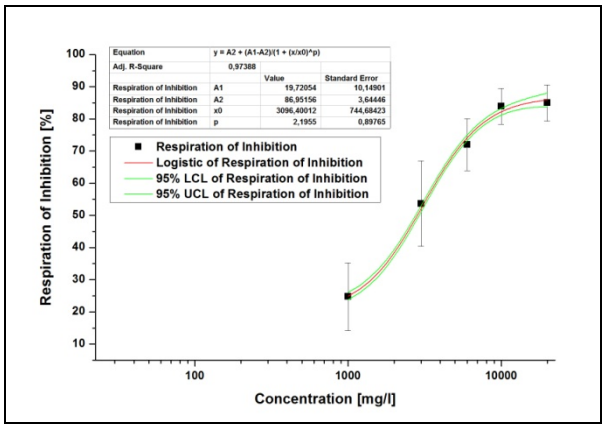

Fig. 15 *EC_{50} evaluation of Hocut 3380 (Origin Pro8)*

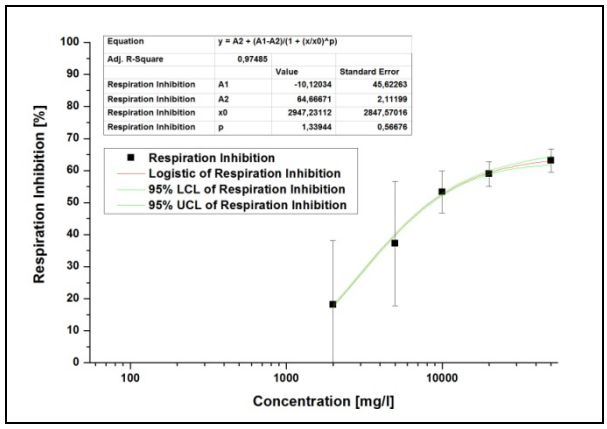

Fig. 16 *EC_{50} evaluation of Zubora TXS (Origin Pro8)*

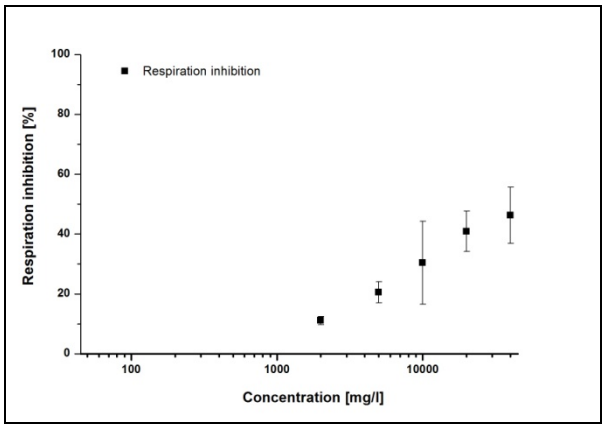

Fig. 17 *EC_{50} evaluation of Cimstar 597 (Origin Pro8)*

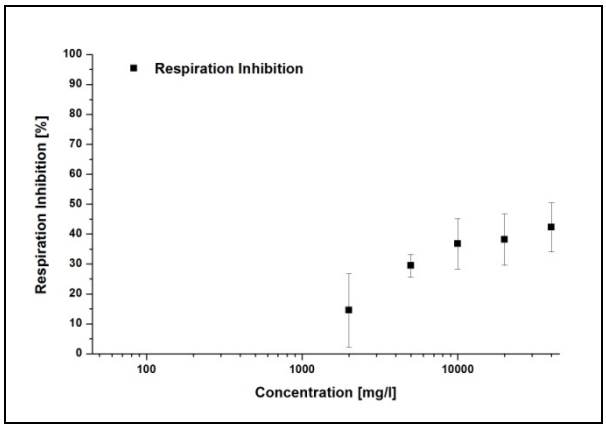

Fig. 18 *EC_{50} evaluation of Emulzin H (Origin Pro8)*

Table 2 are summarised results of testing. Shown are the calculated values for EC_{20}, EC_{50}, EC_{80} for the MWFs such as Cimstar 597, Emulzin H and Hocut 3380. Adrana D407, Aquamet LAK-E and Zubora TXS by the two methods – polynomial function of Excel application and Logistic function by OriginPro8. Just selected data (those which were in replicates, with the error bars) was used for the OriginPro8 program which allows calculating also 95% confidence interval to calculate the inhibition of the respiration.

CALCULATED VALUES OF EC_{20}, EC_{50} AND EC_{80} FOR THE MWFs Table 2

Type of Metalworking Fluid Emulsion	Statistical Function	EC_{20} [mg l^{-1}] 95 % confidence interval	EC_{50} [mg l^{-1}] 95 % confidence interval	EC_{80} [mg l^{-1}] 95 % confidence interval
1. CIMSTAR 597	Polynomial	4 943	-	-
	Logistic	4 808 4 726 – 4 890	- -	- -
2. EMULZÍN H	Polynomial	2 425	-	-
	Logistic	2 625 2 539 – 2 719	- -	- -
3. ZUBORA TXS	Polynomial	2 264	11 349	-
	Logistic	2 196 2 166 – 2 228	8 449 8 309 – 8 587	- -
4. Aquamet LAK-E	Polynomial	1 010	5 228	-
	Logistic	- -	5 600 5 567 – 5 658	- -
5. Adrana D 407	Polynomial	1 688	4 351	62 252
	Logistic	1 643 1 643 – 1 654	4 354 4 354 – 4 405	- -
6. HOCUT 3380	Polynomial	894	2 339	9 931
	Logistic	- -	2 827 2 740 – 2 911	8 282 7 758 – 9 060

Two of the six metalworking fluids – Cimstar 597 and Emulzin H were evaluated as the less toxic. In these two emulsions, the highest used concentration caused around 50%response, so that it was impossible to calculate the EC_{50} value with required accuracy. Than the list continues with Zubora TXS (EC_{50} - 11 349 mg l^{-1}), Aquamet LAK-E (EC_{50} - 5 228 mg l^{-1}), Adrana D 407 (EC_{50} at 4 351 mg l^{-1}) and finally Hocut 3380 which proves to be the most toxic of all the tested substances (EC_{50} - 2 339 mg l^{-1}). Unfortunately, all tested emulsions passed 100 mg l^{-1}. According to [16], it is satisfactory to arrange the tested substances into 4 classes < 1 mg l^{-1}, 1-10 mg l^{-1}, 10-100 mg l^{-1}, > 100 mg l^{-1}. We suppose that the tested metalworking fluid will not cause inhibitory problems in degradability test when lower concentrations are used, as listed in Tab. 2.

Conclusion

The study of degradability of 8 MWFs selected according to OECD 302B shows the increasing adsorption after the first 3 hours of cultivating with increasing the addition of activated sludge. This may play an important role in elimination of the organic matter from the solution. To distinguish biotic and abiotic eliminations, it is recommended to use other appropriate analytical methods.

The study of ecotoxicity of 6 selected MWFs according to OECD 209 shows very high effective concentration which can mean that the tested MWFs are low in toxicity to the

inoculums. As the less toxic substance were evaluated two from six metalworking fluids – Cimstar 597 and Emulzin H. In these two emulsions, the highest used concentration caused the response around 50%, so that it was impossible to calculate the EC_{50} value with required accuracy. The following on the list are Zubora TXS with EC_{50} at 11 349 mg l^{-1}, Aquamet LAK-E with EC_{50} at 5 228 mg l^{-1}, Adrana D 407 with EC_{50} at 4 351 mg l^{-1} and finally Hocut 3380, which proved to be the most toxic of the tested substances.

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