

**THE STUDY INTO POTENTIAL ENHANCEMENT
OF METALWORKING FLUIDS BIODEGRADABILITY
BY THE APPLICATION OF O₃/UV**

Kristína GERULOVÁ, Ondrej TATARKA, Tomáš ŠTEFKO,
Tomáš ŠKULAVÍK

Ing. Kristína Gerulová, PhD., Ing. Ondrej Tatarka, Ing. Tomáš Štefko, Ing. Tomáš Škulavík, PhD.
Slovak University of Technology in Bratislava, Faculty of Materials Science and Technology in
Trnava, Institute of Safety, Environment and Quality, Paulínska 16, 917 24 Trnava, Slovak Republic
e-mail: kristina.gerulova@stuba.sk, ondrej.tatarka@stuba.sk, tomas.stefko@stuba.sk,
tomas.skulavik@stuba.sk

Abstract

The increase in mineralization and biodegradability of MWFs by ozone/ultraviolet in comparison with ozone were investigated. Studied were two similar synthetic fluids pre-treated by the combination of the O₃/UV advanced oxidative method. Expectations that the pre-treatment could enhance biodegradability of the metalworking fluid were not confirmed. The combined oxidation process at the defined conditions resulted in 1-35 % decrease of the achieved primary degradation level. Samples were prepared from real concentrates and diluted to approximately 350 mg/L of TOC.

Key words

biodegradability, Metalworking Fluids, AOP's, Advanced oxidation processes, Ozone, UV-irradiation

INTRODUCTION

Treatment of Spent Metalworking Fluid

To conform to the European legislation (EURAL), all spent metalworking fluids (MWFs) are to be disposed of as hazardous waste. The internal disposal of used emulsions via the waste water is not allowed (1). A wide variety of recycling equipment is available for the contaminant removal, and most of the recycling equipment is generally easy to operate and maintain. A cutting fluid recycling equipment includes **filters** (particulates), **centrifuges** (tramp oil, particulates, and bacteria), **skimmers** (tramp oil), **flotation** and **magnetic separators** (particulates) (2). Spent cutting-oil emulsions are one of the largest volumes of oily wastewaters in metalworking industries. They must be treated before disposal, which is due to their

detrimental effects on aquatic life and their interference with conventional wastewater treatment processes (3). In the early 1990s, the dominant disposal methods were **chemical and physical processes**; *e.g.*, **adding chemicals** (lime, alum, sodium aluminate, etc.) **or polymers**, and using **ultrafiltration** and **evaporation** (4). Almost all techniques used (except incineration and biologic treatment) do not ultimately solve the problem of safe removal of MWFs constituents (5). Technologies to reduce the effluent volume and to improve the quality of the effluent significantly include **microfiltration/ultrafiltration**, **settling**, **dissolved air flotation**, **centrifugation**, **sorption processes**, **hybrid ultrafiltration/ membrane distillation**, **membrane distillation reverse osmosis** and **membrane bioreactors** (3-5). Possible is also **electrochemical oxidation** (6). A combination of ≥ 2 methods generally gives more promising results than using only one of them alone (5). Many of the current disposal methods (**evaporation**, **landfill** or **incineration**) are increasingly non-viable options because they are uneconomical, inefficient or environmentally unacceptable. Many of these methods for the MWFs waste management according to the EU directives (2000/60/EC and 200/76/EC) will no longer be viable. Because of these limitations, there is an increasing interest in the biological treatment systems for processing industrial wastes, such as MWFs (7). Nowadays, the **biological methods** are extensively recognized as being able to offer the most cost-effective option for the organic matter removal, and therefore are used for the MWFs wastewater treatment (8).

Metalworking fluids fundamental composition and potential of biological oxidation

High organic content of most MWFs makes them good candidates for degradation by bacteria for waste treatment. However, the high oil content of these fluids typically **eliminates using biological treatment as the primary treatment method** (9). The **biodegradability of lubricants** is accordingly closely dependent on that of their respective **base stocks**. The most commonly used base stocks are mineral oils (10). Their biodegradability is generally much lower than that of vegetable oils (11). Chemical classes of hydrocarbons (paraffins, naphthenes (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 (10). Among synthetic base fluids, esters can have ready biodegradabilities 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 (11). A chemical or physical treatment process precedes the use of biological treatment for MWFs, which could be an excellent "secondary or tertiary" treatment. However, this process is most economical for large users, especially where there is not an option for sewer discharge (9). It should also be pointed out that certain types of additives are not biodegradable (12). Although microbial deterioration of MWFs during their use 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 MWFs (13). Recently, genetic engineering has also been employed to specify certain species that can enhance overall treatment performance. Van der Gast and colleagues have also carried out a series of waste MWF treatment studies (7,14).

Advanced Oxidation Processes (AOPs)

Advanced oxidation processes are remediation technologies that generate highly reactive, short-lived and non-selective hydroxyl radicals which oxidize and mineralize most organic compounds (15). AOP's can cause the entire mineralization or the partial oxidation of organic substances (16, 17). The hydroxyl (the second most powerful oxidizing agent generated in situ), some strong oxidants such as ozone and other radicals produced in these technologies, oxidize recalcitrant, toxic and non-biodegradable compounds into different by-products and eventually into inert end-products (17). This suggests that AOP's are worth considered as pre-treatment methods of biological processes for the treatment and disposal (17). Hence, AOP's may not be economically and technically feasible options as stand-alone treatment techniques and need to be used in combination with other remediation processes (16, 17). The costs associated with a solely chemical process can often be prohibitive (17). Furthermore, they all consume energy (radiation, ozone, etc.) and chemical reagents (catalysts and oxidizers) which increase with treatment time (18). A potentially feasible approach is the integration of chemical and biological process for treating recalcitrant chemicals in wastewater. This hybrid approach employs a pre-treatment step which targets the toxic and recalcitrant components resulting in production of biodegradable intermediates, which can then be more readily treated in subsequent biological treatment steps (15). The combination of the partial oxidation and consequent biological processes belong to the cheaper processes of the resistant and toxic organic substances treatments.

Ozone is a very powerful oxidizing agent ($E^\circ = 2.07 \text{ V}$) that can react with most species containing multiple bonds (such as $\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{N}=\text{N}$, etc.), but not with singly bonded functionality such as $\text{C}-\text{C}$, $\text{C}-\text{O}$, $\text{O}-\text{H}$ at high rates. This is mainly due to the fact that there is no easy chemical pathway for the oxidation to take place. Usually higher values of pH are recommended. It has been reported that at high pH values, ozone reacts almost indiscriminately with all organic and inorganic compounds present in the reacting medium. (19). Oxidation of organic species may occur due to a combination of reactions with molecular ozone and reactions with $\bullet\text{OH}$ radicals (20,21). While disinfection occurs dominantly through ozone, oxidation processes may occur through both oxidants, ozone and $\bullet\text{OH}$ radicals. Ozone is a very selective oxidant; $\bullet\text{OH}$ radicals react fast with many dissolved compounds and the water matrix (21). The rate of the attack by $\bullet\text{OH}$ radicals is typically 106 to 109 times faster than the corresponding reaction rate for molecular ozone. The major operating cost for the ozone oxidation process is the cost of electricity for ozone generation. The energy requirement for ozone synthesis using air as a feed gas ranges from 22 to 33 kWh/kg O_3 including air handling and ozone contacting with water. The energy requirement for ozone production from pure oxygen is in the range from 12 to 18 kWh/kg O_3 to which the cost of oxygen should be added (20). The growing toxicological concern about degradation products resulting from the oxidation of micro pollutants calls for thorough product studies. The reaction products from the reaction of ozone with organic compounds in aqueous solutions are often difficult to predict and establish. The information is scattered throughout the available literature and no comprehensive collection is available (21).

The **photo-activated chemical reactions** are characterized by a free radical mechanism initiated by the interaction of photons of a proper energy level with the molecules of chemical species present in the solution, with or without the presence of the catalyst. The radicals can be easily produced using UV radiation by the homogenous photochemical degradation of oxidizing compounds like hydrogen peroxide and ozone. An alternative way to obtain free radicals is the photocatalytic mechanism occurring at the surface of semiconductors (such as TiO_2). Various chalcogenides (oxides such as TiO_2 , ZnO , ZrO_2 , CeO_2 etc. or sulphides such as

CdS, ZnS etc.) have been used as photo-catalysts. For highly concentrated effluents, absolutely no destruction may be observed and dilution is essential in this case (19).

Ozonation followed by biological filtration has been shown to effectively remove NOM from water. Ozone and/or hydroxyl radicals ($\bullet\text{OH}$) generated from the decomposition of ozone degrade recalcitrant organic compounds to smaller molecules, which are more hydrophilic and biodegradable. Bacteria in the biological filter utilize the oxidized organic compounds as the substrate (22). In combined chemical and biological wastewater treatment, it is very important to keep in mind how the characteristics of each individual treatment, such as the chemical oxidant to be used (photo-Fenton or Fenton reagent, $\text{O}_3/\text{H}_2\text{O}_2$, O_3/UV , $\text{H}_2\text{O}_2/\text{UV}$, TiO_2/UV , etc.) can improve the destruction of a persistent contaminant. Measurement of the combined process efficiency depends on the purpose of the treatment, but normally requires the independent optimization of each chemical and biological step. Calculation of the individual biological and chemical oxidation efficiencies is important for finding the optimal operating conditions for the combined process. Selection of the best treatment option for remediation of a specific industrial wastewater is a highly complex task. The choice of one or more processes to be combined in a certain situation depends on the quality standards to be met and the most effective treatment with the lowest reasonable cost (18).

In (23), explored was the feasibility of employing a hybrid treatment combining ozonation with a post bio-degradation step for the treatment of a recalcitrant semi-synthetic MWFs wastewater. The final reduction in COD was over 70% when combined processes were applied sequentially. The study is the first investigation of the feasibility of employing ozone to reduce the toxicity and enhance biodegradability of waste MWF and specific recalcitrant constituents (23).

In (24), authors try to investigate the applicability of UV-based AOPs ($\text{UV}/\text{H}_2\text{O}_2$, photo-Fenton and UV/TiO_2) in the treatment of MWF wastewater. The potential of using AOPs to enhance the biodegradation of spent MWFs was also addressed. A semi-synthetic MWF was firstly pre-treated using ultrafiltration to remove the emulsified oil. The potential of direct photolysis, $\text{UV}/\text{H}_2\text{O}_2$, $\text{UV}/\text{Fe}^{2+}/\text{H}_2\text{O}_2$ and, particularly, UV/TiO_2 processes to treat the UF permeate was investigated with the possibility of combining biodegradation and AOPs to enhance the final effluent quality. They observed an increase in the biodegradability of the MWF wastewater during the photo-Fenton process with the BOD_5/COD ratio increasing by 44% at $20.7 \text{ J}/\text{cm}^2$ and by 59% at $34.5 \text{ J}/\text{cm}^2$.

MATERIAL AND METHODS

All **chemicals** were analytical grade and employed without any further purification. List of chemicals used for the preparation of mineral medium for the biodegradability test is defined in (25). Selected were two similar synthetic metalworking fluids for the experiment, which were obtained from the CASTROL Company. Volume of treated samples was estimated to 2.5 L, and the starting concentration was set up to 1 % v/v. Both samples were identified as synthetic coolants. **TOC** (total organic carbon) **measurements** were determined by direct injection of the diluted sample (1:25, 1:2) into a Shimadzu TOC- V_{CPN} analyser, calibrated with standard solutions of potassium phthalate. **Ozone** was produced from air using OZONFILT® OZV a type 2 ozone generators (Prominent), which are capable of producing up to 15 g of O_3 per hour. Compressed air was supplied as feed gas for the production of ozone at a rate of 20 L/min. **UV reactor** was composed from three main compartments: 400 Watt medium pressure irradiation lamp, quartz immersion well reactor RQ 400 and the 400 ml flow-through reaction vessel. The reaction vessel was made of borosilicate glass isolated from the environment by the aluminium foil. The 400 W medium pressure mercury lamp radiate predominantly 365-366 nm radiation

with smaller amounts in the ultraviolet at 265, 297, 303, 313 and 334 nm as well as significant amounts in the visible region at 404-408, 436, 546 and 577-579 nm. Schema of the **ozone/UV apparatus** consist 400 ml UV reactor and 5L Ozone reactor (Figure 1) while these reactors are connected by two inert pumps (peristaltic pump) to circulate reactant from the O₃ reactor to UV reactor. Flow of the liquid portion from O₃ to UV vessel was set up to 80 mL/min. Samples were taken from the UV vessel in time interval 30, 60 and 90 min. Total liquid volume of the sample was 2.5 L.

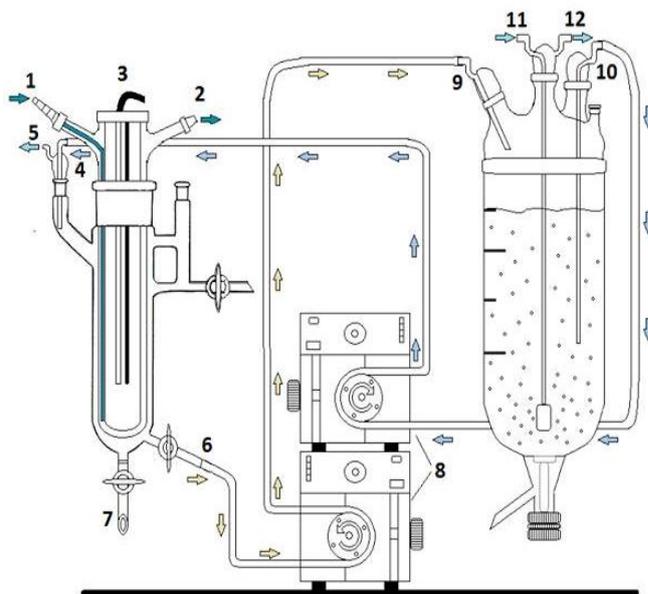


Fig. 1 UV/O₃ apparatus schema:

UV reactor:

- 1 – cooling water inlet;
- 2 – cooling water outlet;
- 3 – UV lamp;
- 4 – input of ozonized sample;
- 5 – safety outlet for gases;
- 6 – UV treated sample outlet;
- 7 – sampling valve;
- 8 – peristaltic pumps;

O₃ reactor:

- 9 – UV treated sample inlet for ozonation;
- 10 – ozonized sample outlet for UV;
- 11 – gas ozone inlet;
- 12 – gas outlet.

Degradability test

The principle of the biodegradability measuring is described in (25). The aerobic degradation was carried out in a closed apparatus consisting of two vessels where the air part was pumped by the peristaltic pump from culture bottle to absorption bottle for better reaction of evolved CO₂ by the absorption solution. Prepared were 10 apparatus for the blank, control and tested substances. In culture vessel, there was 750 mL of culture medium with 0.1 g/L of SS prepared from activated sludge the same day as the test began. The concentration of the test substance was in the range 192 – 281 mg of TOC per vessel. During the biodegradability test in the cultivation bottle, pH was set up to 7.5. Aeration intensity was 50-60 mL/min. and the air: liquid volume was settled to 1:1. The amount of carbon dioxide produced by the microbial inoculum during the degradation was measured by the change in conductivity in absorption bottle which contain 1L of 0.0175 mol/l Ba(OH)₂ by calibrated conductivity probe continuously during the whole test. When the conductivity decreased under 1.5 mS/cm, the absorption solution was replaced by a fresh one. While the apparatus was opened, the system was filled with fresh air. The performance of the test is more similar to OECD 302 B in the parameters such as the amount of inoculum and concentration of the test substance, but it resembles to OECD 301 B by the degradability measured parameter – CO₂. It eliminates misinterpretation in the degradability evaluation when adsorption occurred in poorly soluble substances and the COD or TOC from water phase is used for calculation. The amount of microorganisms used was 3-times higher and concentration of tested substance was 6-times higher than in OECD 301 B.

RESULTS AND DISCUSSION

These preliminary experiments investigated O_3/UV pre-treatment to increase/decrease the MWFs biodegradability. Though in previous experiments evaluated were also the reaction of tested synthetic MWFs to the presence of high concentration of ozone solely and also in the combination to O_3/UV without presence of any kind of catalyst. In the case of Sample 1 (Figure 2), we observed 18.6 % decrease of organic matter after 90 min. of ozonation (from 3 286 to 2 674 mg/L of TOC) and 8.3 % decrease in the case of O_3/UV combination method. In the case of Sample 2 (Figure 3), we observed 3.8 % decrease of organic matter after ozonation (from 3 895 to 3 746 mg/L of TOC) and 0.8 % decrease of TOC after O_3/UV (from 3 818 to 3 788 mg/L). It has to be pointed out that ozonation was performed in one reactor where were placed 1 L of 1 % v/v solution prepared from concentrate, while in the combination with method O_3/UV , it was treated by 2.5 L of 1 % v/v solution which was continuously pumped from the ozonation reactor to UV reactor and then back under 80 ml/min. flow. The nature of the organic matter decrease was not studied in these experiments. We observed no separation of oil phase on the surface. Another study will be needed to investigate whether oxidation or destabilization of the solution primarily takes place in the system. For the degradability measurement, we calculated the water phase volume corresponding to the same concentration of TOC for each treating time. The calculated concentration was set up to 350 mg/L of TOC (final volume in the cultivation vessel was 750 mL). After treatment with ozone and UV radiation, the samples were mixed for 12 hours for complete removal of $\bullet OH$ radicals and ozone molecules before adding the inoculum.

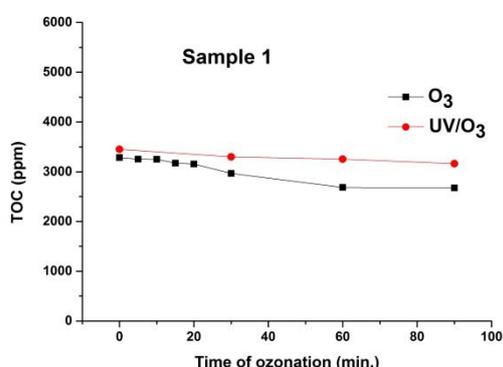


Fig. 2 Kinetics of TOC in Sample 1

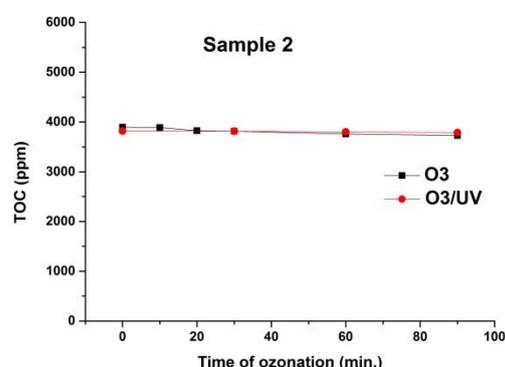


Fig. 3 Kinetics of TOC in Sample 2

The graphs in Figures 4 and 6 there describe the calculated **carbon dioxide evolution** during the test, and Figures 5 and 7 show the **primary degradation curves** for the control and tested samples of MWFs. In this preliminary biodegradability tests, it was not important to study the achieved level of the primary biodegradation, but to observe the increase/decrease in the comparison to the untreated sample. Therefore, we did not evaluate all typical biodegradability parameters according to OECD 301 B. In addition, we utilized higher concentration of inoculum and concentrations of tested samples that normally occurred in the standard test.

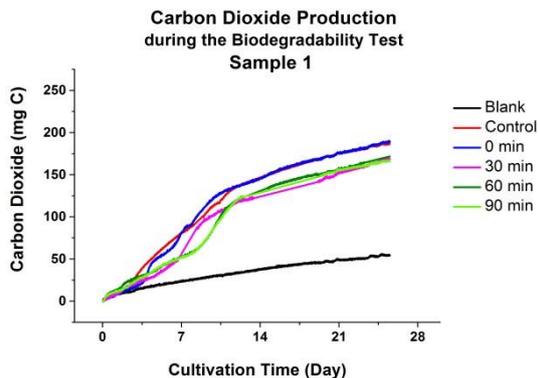


Fig. 4 Carbon dioxide production during the biodegradability test of Sample 1

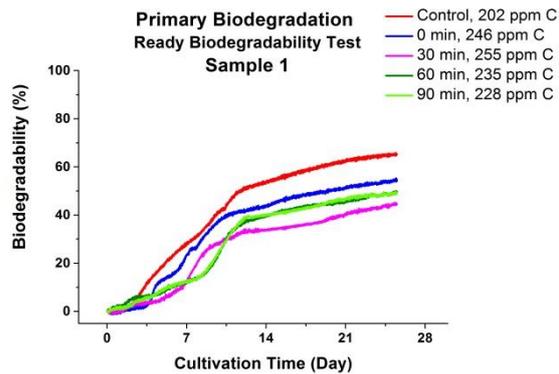


Fig. 5 Primary biodegradation of Sample 1 according to modified ready biodegradability test

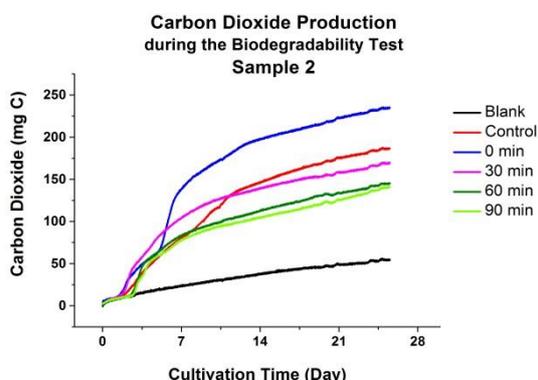


Fig. 6 Carbon dioxide production during the biodegradability test of Sample 2

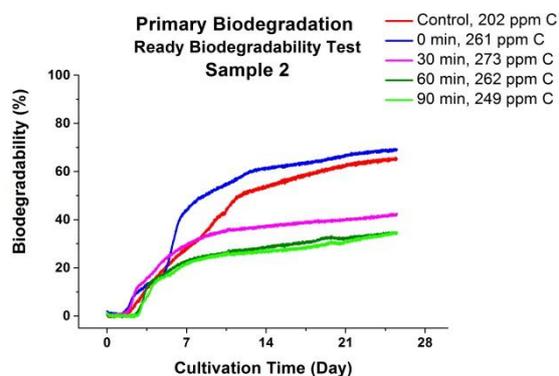


Fig.7 Primary biodegradation of Sample 2 according to modified ready biodegradability test

The degradability of both samples were harmfully affected by the O₃/UV treatment, because the achieved primary degradation level was lower when compared to the untreated sample. While in untreated sample 1 the level of the biodegradability was 55 %; the treated samples achieved only 49 % (90 min. of treatment) to 54 % (60 min.). Similar situation was observed also in sample 2. Untreated sample achieved 69 % degradation, while the sample treated for 30 min. achieved only 42 % degradation. The samples treated for 60 min. and 90 min. achieved only 34 % level of primary biodegradation.

CONCLUSIONS

The preliminary experiments with advanced oxidative processes were utilized as the pre-treatment step before biological oxidation. These experiments did not investigate the influence of different condition during the application of ozone and UV-radiation such as pH contribution, utilization of different catalyst, temperature etc. Neither any of physic-chemical pre-treatment steps were used. The preliminary findings from this study under defined conditions show that the combination of O₃/UV pre-treatment decreases the level of primary degradation in comparison to untreated sample of synthetic metalworking fluids. Then next study will focus on the investigation of the pH and catalyst addition impact on maximizing the effect of the AOPs oxidation and also its impact on final degradability of real samples.

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References:

1. Supplement to the methodology for risk evaluation of biocides Harmonisation of Environmental Emission Scenarios for biocides used as metalworking fluid preservatives (Product type 13) European Commission DG ENV / RIVM. *Eur. Comm. DG ENV / RIVM*, 2003, p. 61.
2. EI BARADIE, M., A., CUTTING FLUIDS: PART II. RECYCLING AND CLEAN MACHINING. 1996. *Mater. Process. Technol.*, vol. **0136**, pp. 798–806.
3. BENITO, M., et al. 2002. Design and construction of a modular pilot plant for the treatment of oil-containing wastewaters. *Desalination*, vol. **147**, pp. 5–10.
4. CHENG, C., et al. 2005. Treatment of spent metalworking fluids. *Water Res.*, vol. **39**, pp. 4051–4063.
5. CHANG, I., et al. 2001. Treatment of oily wastewater by ultrafiltration and ozone. *Desalination*, vol. **133**, pp. 225–232.
6. MUSZYŃSKI, A., ŁEBKOWSKA, M. 2005. Biodegradation of Used Metalworking Fluids in Wastewater Treatment. *Polish J. Environ. Stud.*, **14**(1), pp. 73–79.
7. C. J. van der GAST, et al. 2004. Temporal dynamics and degradation activity of an bacterial inoculum for treating waste metal-working fluid. *Environ. Microbiol.*, **6**(3), pp. 254–263.
8. MOSCOSO, F. et al. 2012. Assessment of a process to degrade metal working fluids using *Pseudomonas stutzeri* CECT 930 and indigenous microbial consortia. *Chemosphere*, vol. **86**, pp. 420–426.
9. Treatment and disposal of used metalworking fluids CIMCOOL® Technical Report No. J/N 00/52. *Milacron Mark. Co./ Consum. Prod. Div. / Cincinnati, Ohio 45209*, pp. 1–2.
10. HAUS, F., et al. 2001. Primary biodegradability of mineral base oils in relation to their chemical and physical characteristics. *Chemosphere*, **45**(6–7), pp. 983–990.
11. BOYDE, S. 2002. Green lubricants. Environmental benefits and impacts of lubrication. *Green Chem.*, **4**(4), pp. 293–307.
12. EKENGREN, R. B. Ö, NIEMINEN, I. Environmentally acceptable metalworking processes, No. A96291. *IVL SWEDISH Environ. VTT Res. Inst.*
13. Van Der GAST, C., J., et al. 2001. Identification and characterisation of bacterial populations of an in-use metal-working uid by phenotypic and genotypic methodology. *Int. Biodeterior. Biodegradation*, vol. **47**, pp. 113–123.
14. van der GAST, C., J., et al. 2002. Selection of microbial consortia for treating metal-working fluids. *J. Ind. Microbiol. Biotechnol.*, vol. **29**, pp. 20–27.
15. JAGADEVAN, S., et al. 2011. Harmonisation of chemical and biological process in development of a hybrid technology for treatment of recalcitrant metalworking fluid. *Bioresour. Technol.*, vol. **102**, pp. 8783–8789.
16. BIJAN, L., et al. 2005. Integrated ozone and biotreatment of pulp mill effluent and changes in biodegradability and molecular weight distribution of organic compounds. *Water Res.*, vol. **39**, pp. 3763–3772.

17. DURÁN-MORENO, et al. 2011. Assessment of Fenton's reagent and ozonation as pre-treatments for increasing the biodegradability of aqueous diethanolamine solutions from an oil refinery gas sweetening process. *J. Hazard. Mater.*, vol. **186**, pp. 1652–1659.
18. OLLER, I., et al. 2011. Combination of Advanced Oxidation Processes and biological treatments for wastewater decontamination-A review. *Sci. Total Environ.*, vol. **409**, pp. 4141–4166.
19. GOGATE, P., R. et al. 2004. A review of imperative technologies for wastewater treatment I: Oxidation technologies at ambient conditions. *Adv. Environ. Res.*, vol. **8**, pp. 501–551.
20. MUNTER, R. 2001. Advanced Oxidation Processes - Current Status and Prospect. *Proc. Est. Acad. Sci. Chem.*, **50**(2), pp. 59–80.
21. U. Von GUNTEN. 2003. Ozonation of drinking water: Part I. Oxidation kinetics and product formation. *Water Res.*, vol. **37**, pp. 1443–1467.
22. RATPUKDI, T., et al. 2010. Mineralization and biodegradability enhancement of natural organic matter by ozone-VUV in comparison with ozone, VUV, ozone-UV, and UV: Effects of pH and ozone dose. *Water Res.*, vol. **44**, pp. 3531–3543.
23. JAGADEVAN, S., et al. 2013. Treatment of waste metalworking fluid by a hybrid ozone-biological process. *J. Hazard. Mater.*, vol. **244–245**, pp. 394–402.
24. Mac ADAM, J., et al. 2012. Incorporating biodegradation and advanced oxidation processes in the treatment of spent metalworking fluids. *Environ. Technol.*, **33**(24), pp. 2741–2750.
25. NORR, C. 2001. Modification of the Zahn - Wellens test: determination of the biodegradability of poorly soluble, adsorbing and volatile substances by measurement of oxygen consumption and carbon dioxide production, vol. **44**, pp. 553–559.

Reviewers:

prof. Ing. Maroš Soldán, PhD.

doc. Ing. Jana Müllerová, PhD.

