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Pharmaceuticals, benzene, toluene and chlorobenzene removal from contaminated groundwater by combined UV/H_2O_2 photo-oxidation and aeration

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ABSTRACT

This study was performed to test the feasibility of several decontamination methods for remediating heavily contaminated groundwater in a real contaminated locality in the Czech Republic, where a pharmaceuticals plant has been in operation for more than 80 years. The site is polluted mainly by recalcitrant psychopharmaceuticals and monoaromatic hydrocarbons, such as benzene, toluene and chlorobenzene. For this purpose, an advanced oxidation technique employing UV radiation with hydrogen peroxide dosing was employed, in combination with simple aeration pretreatment. The results showed that UV/H_2O_2 was an efficient and necessary step for degradation of the pharmaceuticals; however, the monoaromatics were already removed during the aeration step. Characterization of the removal mechanisms participating in the aeration revealed that volatilization, co-precipitation and biodegradation contributed to the process. These findings were supported by bacterial metabolite analyses, phospholipid fatty acid analysis, qPCR of representatives of the degradative genes and detailed characterization of the formed precipitate using Mössbauer spectroscopy and scanning electron microscopy. Further tests were carried out in a continuous arrangement directly connected to the wells already present in the locality. The results documented the feasibility of combination of the photo-reactor employing UV/H_2O_2 together with aeration pretreatment for 4 months, where the overall decontamination efficiency ranged from 72% to 99% of the pharmaceuticals. We recorded even better results for the monoaromatics decontamination except for one month, when we encountered some technical problems with the aeration pump. This demonstrated the necessity of using the aeration step.

1. Introduction

Co-mingled plumes containing various organic pollutants with differing chemical nature represent a difficult task for remediation practice. Pharmaceutical companies are typical potential sources of these types of environmental pollution because of the wide range of chemical substances that they produce and precursors that they use. In this respect, pharmaceutical compounds represent typical new emerging pollutants that are not included in the lists of contaminants defined by national regulations (Verlicchi and Zambello, 2015). There are two main routes of environmental contamination by pharmaceuticals. One of these is insufficient wastewater treatment technology and the consequent diffusive contamination *via* treated water (Cajthaml et al., 2009) or application of biosolids

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(Citulski and Farahbakhsh, 2010). Another means of contamination arise from accidents and old ecological burdens where elevated concentrations are present. These localities can be treated using various remediation technologies (Alexander, 1999). The type of technology is often tailor-made and should reflect the particular properties of the site and the types of pollutants.

Advanced oxidation processes (AOPs) are widely studied methods focusing on water-soluble contaminants and their applicability has already been documented in water, groundwater and wastewater treatment (Mascolo et al., 2008; Saharan et al., 2014). Photochemical technologies can be employed *in situ* or as surface treatment for groundwater in the "pump and treat" arrangement (Cheng et al., 2016; Tuhkanen, 2004). The general principle of AOPs lies in chemical oxidation of organic contaminants to carbon dioxide, water and inorganics or their transformation into harmless products. AOP methods are usually effective, simple, clean and economically sustainable. The principle of AOPs employing UV/H_2O_2 is UV photolysis of hydrogen peroxide, generating extraordinary reactive hydroxyl radicals (OH·). These radicals subsequently attack and decompose contaminants in the water (Stefan, 2004). The UV/H_2O_2 method enables rapid degradation of various organic compounds including e.g. BTEX, chlorobenzene, nitrobenzene, cresols, phenols, chlorophenol and micropollutants (Bahmani et al., 2014; Oncescu et al., 2008; Stefan and Williamson, 2004; Tuhkanen and Marinosa, 2010). Photo-oxidation processes are generally influenced by the pH, redox potential, temperature, contact time, application rate and reactivity of the compounds (Hofman-Caris and Beerendonk, 2011).

Degradation of pharmaceuticals is a rather recent issue (Rosario-Ortiz et al., 2010). Several studies have described the efficiency of UV/H_2O_2 process towards pharmaceuticals (Benitez et al., 2009; Lester et al., 2011; Wols et al., 2013). Generally, the degradation rate depends on the water matrix, in relation to the background components that compete with the target compounds for hydroxyl radicals (Benitez et al., 2009; Wols et al., 2013).

This study concentrated primarily on testing the feasibility of several decontamination approaches for remediating heavily contaminated groundwater in a polluted locality in the Czech Republic where pharmaceutical industry has been in operation for more than 80 years. The groundwater is contaminated by recalcitrant psychopharmaceuticals and monoaromatic hydrocarbons such as benzene, toluene and chlorobenzene. The objective of the research is thus to develop and to test a tailor made effective treatment method for such the complex polluted groundwater. For this purpose one of the AOPs, namely UV radiation with hydrogen peroxide dosing was used. A secondary objective was to characterize mechanisms participating in the decontamination, including volatilization, co-precipitation and biodegradation by autochthonous microflora.

2. Materials and methods

2.1. Locality

The studied locality is situated in the town Olomouc in the area of the Farmak company, approximately 300 m from municipal drinking water reservoirs. The contamination represents a typical environmental burden in the Czech Republic, where the current owner did not cause the contamination and this is a result of the operation of the factory before 1989, when it was state-owned. The state also claimed responsibility for remediation of the site and several attempts have already been made to remediate the massively contaminated soil and groundwater in the area in the past. All the degradation experiments within this study were performed directly at the locality. First, feasibility of the used methods was tested in a discontinuous arrangement with partial optimization; subsequently, the applicability of the selected approaches was tested in a continuous arrangement. The groundwater was pumped from the SM-70 well, one of nine long-term monitored wells in the locality originating from previous remediation attempts. The treated water was returned to the groundwater system *via* the SM-72 upgradient well. The composition of the groundwater in April 2016 was as follows: pH 6.7; conductivity 3585 μS cm^{-1}; Eh −102.1 mV; T 14.4 °C; dissolved oxygen 2.2 mg L^{-1}, total organic carbon 21.2 mg L^{-1}, groundwater table depth 4.0 m. The groundwater is contaminated with pharmaceuticals that consist of three commonly used antidepressants (tricyclic antidepressants and antipsychotics), one antitussive agent and four by-products or precursors. Aromatic and chlorinated compounds consist mostly of benzene, toluene and chlorobenzene, which originated as reagents used in former production. The characteristics of the detected contaminants and their mean concentrations in the SM-70 well are listed in Table 1.

2.2. Photo-oxidation

Photo-oxidation (UV-C 254 nm) was carried out in two photo-reactors shown in Fig. 1. UV radiation was emitted by 24 UV lamps per a reactor (T8 G13 Philips, germicidal lamp length 120 cm, in total 864 W per photo-reactor). The flowrate through the reactor was set at 1.5 m^3 h^{-1} and the H_2O_2 (35 w/w %) dosing flow was 1 L h^{-1} based on our previous experience (Žebrák et al., 2014).

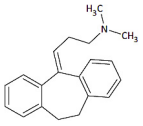
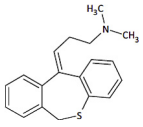
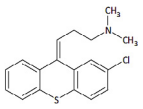
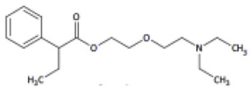
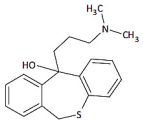
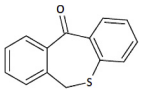
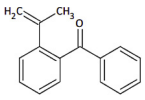
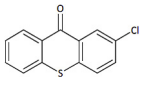
The initial testing arrangement for optimization of operation of the pilot unit was discontinuous. The duration of a photo-oxidation cycle was tested in the intervals from 30 to 150 min. Groundwater was pumped (0.3 m^3 h^{-1}; Grundfos 3″ SQ 1–80, $H_{max.}$ 27.5 m) from the borehole to the settling tank (1 m^3) and then the water flowed gravitationally into the reaction tank (1 m^3). The water was then pumped (1.5 m^3 h^{-1}) through the photo-oxidation reactors (2 × 21 L) and returned to the reaction tank. H_2O_2 (35 w/w %) was dosed in to the water prior to the entrance into the photo-reactors. The liquid samples were collected at the given times (see the results section). Physico-chemical parameters were monitored continuously using Multimeter WTW-3430. Because of residual hydrogen peroxide concentrations (ca. 250 mg L^{-1}) that could cause explosion of the sampling vessels through further reactions and O_2 and CO_2 generation, manganese dioxide (Penta, powder fraction <45 μm) was added to each collected sample to avoid any further decomposition during transport to the laboratory. At the end of the cycle, the treated water was discharged into the SM-72 borehole.

For practical applications, further tests were carried out in continuous arrangement. One of the reasons for this decision was also the fact that, during manipulation/pumping of the freshly pumped water from the groundwater reservoir, we observed a substantial drop in the concentrations of benzene, toluene and chlorobenzene. Therefore, a pre-treatment was incorporated into the system. The pre-treatment includes aeration in an additional aeration tank (the conditions see below). The aeration step was tested as discontinuous and subsequently incorporated into the continuous arrangement (see below).

2.3. Aeration

To determine the efficiency of the aerobic pre-treatment, the aeration test was also carried out in a discontinuous arrangement. The groundwater from the SM-70 well was pumped (0.3 m^3 h^{-1}; Grundfos 3″ SQ 1–80, H_{max} 27.5 m) to the aeration tank (1.1 m^3). The airflow to the aeration tank was 130 L min^{-1} using the Secoh JDK S 100 blower. Aeration started after the first sampling. Liquid and also solid phase samples were collected for analyses at the given times

Table 1
Characteristics of the input groundwater (well SM-70; in April 2016). The concentrations represent the means of the concentrations (n = 7; monitoring within a 3 month period before the study) and S.D. stands for the standard deviation.

Compound	Abbreviation	Conc. (S.D.) [μg L^{-1}]	CAS Number	Molecular Formula	Molecul. Weight	Log K_{ow}	Structure
Pharmaceuticals							
Antidepressants							
Amitriptyline	AMP	7.46 (6.46)	50-48-6	C20H23N	277.4	5.0	
Dosulepin	DSL	7.58 (6.72)	113-53-1	C19H21NS	295.4	4.5	
Antipsychotic							
Chloroprothixene	CPT	1.37 (1.41)	113-59-7	C18H18ClNS	315.9	5.2	
Antitussive							
Butamirate	BUT	0.92 (0.88)	18109-80-3	C18H29NO3	307.4	3.3	
By-products							
Dosulepin carbinol	DSLC	12.7 (8.8)	1531-85-7	C19H23NOS	313.5	3.4	
Synthesis precursors							
Thiepinone	THP	357 (298)	1531-77-7	C14H10OS	226.3	3.5	
2-isoprophenyl-benzophenone	IPB	35.7 (20.3)	50431-89-5	C16H14O	222.3	4.7	
2-chloroprothioxant-9-on	CPTX	20.4 (17.4)	86-39-5	C13H7ClOS	246.7	4.6	
Monoaromatics							
Benzene	B	1153 (407)	71-43-2	C6H6	78.11	2.13	
Toluene	T	3493 (1953)	108-88-3	C6H5CH3	92.14	2.69	
Chlorobenzene	CB	5561 (1401)	108-90-7	C6H5Cl	112.56	2.84	

(see the results). Pharmaceuticals, benzene, toluene, chlorobenzene, chlorophenol and catechol were determined in the liquid phase. The volatile organic compounds (VOCs) in the exhaust air during the aeration were monitored by a gas analyzer (see below).

2.4. Continuous combined long term arrangement

In the continuous tests, both the steps, i.e. the aeration and subsequent photo-oxidation treatments, were combined. The groundwater was pumped (0.3 m^3 h^{-1}) from the well to the aeration tank (1.1 m^3) and the airflow was set at 130 L min^{-1} using the blower. The pre-treated groundwater gravitationally flowed into the settling tank. Thereafter, the water flowed to the reaction tank from the upper part of the settling tank. The water then passed through the photo-oxidation reactors from the reaction tank at a flowrate of 0.3 m^3 h^{-1}. The retention time in the photo-oxidation reactors was 8.4 min and the total retention time equaled 630 min. The scheme of the combined groundwater treatment is shown in Fig. 2. The treated water was returned to the SM-72 well. Polar Organic Compound Integrative Samplers (POCIS) were installed in SM-70 and SM-72 to monitor the pharmaceuticals. A "Pharmaceutical" POCIS configuration containing a single sorbent Oasis HLB was utilized. Oasis HLB is designed for pharmaceutical sampling and it is used in most studies of drug contaminants in

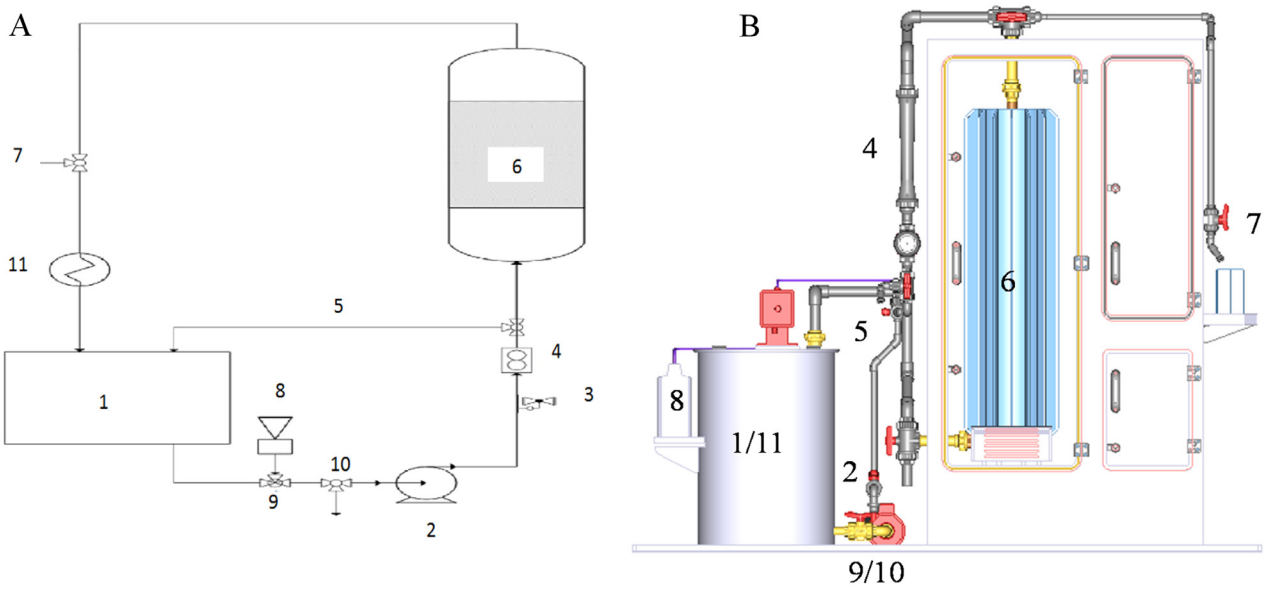


Fig. 1. Photo-oxidation reactor scheme. A – scheme of the photo-oxidation unit; B – the real appearance of the unit t: 1) storage tank, 2) centrifugal pump, 3) membrane valve, 4) flowmeter, 5) by-pass, 6) photo-reactor, 7) sampling port, 8) hydrogen peroxide dispenser, 9) mixing valve, 10) outlet valve, 11) cooler (inside the storage tank).

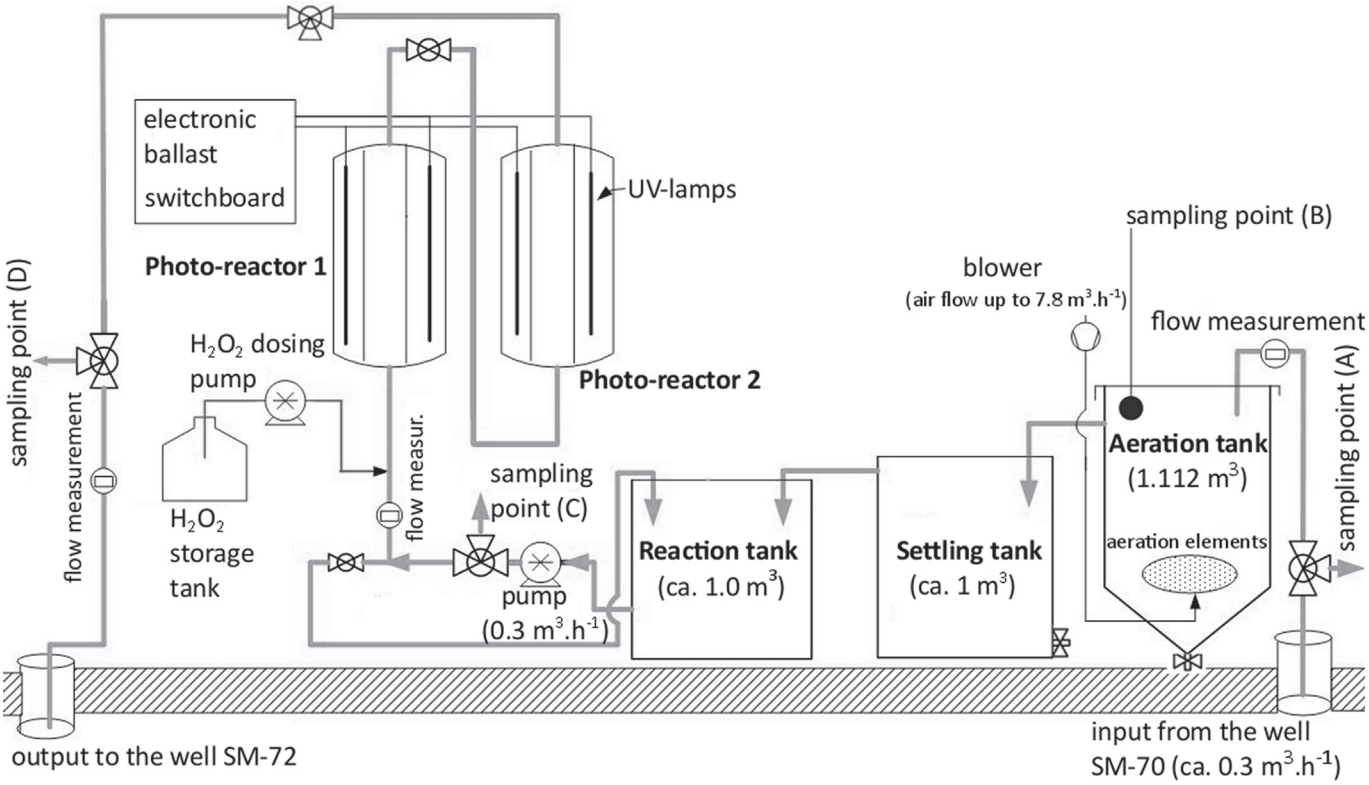


Fig. 2. Scheme of the pilot unit employed in continuous operations including aeration and UV/H_2O_2.

aqueous environments (Křesinová et al., 2016b). So called well devices (Environmental Sampling Technologies, Inc., USA) that hold six rectangular POCIS samplers were installed into the wells, where the samplers were exposed for four weeks each time. The grab sampling method was used for benzene, toluene and chlorobenzene monitoring, where the samples were collected in triplicate 4 times per a month.

2.5. Analytical methods

2.5.1. Pharmaceuticals

Determination of the pharmaceuticals, their precursors and by-products in liquid samples (1 L) after the continuous test was based on solid phase extraction (SPE) using ENVI C18-DSK SPE disks (diam. 47 mm) according to Křesinová et al. (2016a). Hydrochloric

acid (36.5–38.0%), sodium chloride, dimethyl sulfoxide ≥99.5% and ethyl acetate 99.8% were purchased from Sigma–Aldrich (Germany). Ultrapure water was prepared using a MilliQwater purification system (18.2 MΩ; Millipore; Billerica, USA).

POCIS were employed for determination of the pharmaceuticals during the continuous combined long-term arrangement. After the deployment period, the POCIS were stored at −20 °C until the analysis. Each POCIS was individually extracted as follows: 5 mL of methanol; 5 mL of methanol: dichloromethane (1:1 v/v); and 5 mL of methanol. Solvents were purchased from VWR (Czech Republic) at LCMS purity grade. Samples were concentrated using nitrogen blowdown (99.9995; Linde, Czech Republic) and resuspended in 2 mL of methanol.

The pharmaceuticals were detected using ultra-performance liquid chromatography, combined with time-of-flight mass spectrometry according to our recently developed protocol (Křesinová et al., 2016a). UHPLC-ToF analyses were performed using a Waters Acquity UPLC System (Waters; Prague, Czech Republic) consisting of Acquity UPLC Sample Manager, Acquity UPLC Solvent Manager, Acquity UPLC Column Heater and Waters LCT Premier XE orthogonal accelerated ToFMS (Water MS; Machester, UK). MassLynx V4.0 software was used for data processing. An ESI interface was employed for ionization of the analytes (operating in the positive ion mode), using the following parameters: cone voltage, 40 V; capillary voltage, +2800 V; ion source block temperature, 120 °C; nitrogen desolvation gas temperature, 350 °C; desolvation gas flow, 800 L h^{-1}; cone gas flow, 50 L h^{-1}. Fullscan spectra were acquired in the range of 100–400 *m/z* with a scan time 0.15 s and an inter-scan delay of 0.01 s. Mass accuracy was maintained by a lock spray using leucine-enkephalin (5 ng μL^{-1}; 5 μL min^{-1}). The analytes were separated on an Acquity BEH C18 column (50 mm × 2.1 mm × 1.7 μm) with a mobile phase consisting of (A) formic acid–water (0.1:99.9, v/v) and (B) formic acid–acetonitrile (0.1:99.9, v/v). A linear gradient elution program was employed as follows (min/%B): 0/5; 15/70; 18/99 followed by a 1.5 min step with 100% B and 2.0 min equilibration step. The mobile phase flow rate was 0.4 mL min^{-1}, the column temperature was 40 °C and the injection volume was 5 μL. Acetonitrile, methanol and formic acid used as the chromatographic mobile phase were of LC/MS grade and were obtained from Biosolve (The Netherlands).

2.5.2. Volatile and chlorinated organic compounds

Determination of benzene, toluene and chlorobenzene was carried out using gas chromatographic methods with FID and MS detection. Ethylbenzene and xylene concentrations were very low compared to the other pollutants; therefore only the benzene, toluene and chlorobenzene results are shown in the study. The analyses and the data processing followed the standard protocols US EPA 624, US EPA 8260, US EPA 8015, EN ISO 10301, MADEP 2004, rev. 1.1.

VOCs in the exhaust air were also monitored using the Ecoprobe 5 (RS Dynamics, Switzerland) - Infrared/PID Gas Analyzer. The photo ionization detector (PID) was calibrated using Isobutylene. The concentration range was from 0.1 ppb to 3000 ppm.

2.5.3. Chlorophenols and catechols

Chlorophenols and catechols were analyzed as typical bacterial biodegradation products of BTEX and chlorobenzene. For this purpose, the water samples were acidified using 1 M HCl to pH 4.5 and filtered on glass fiber filters (0.5 μm). The filtered water samples were extracted by SPE using HLB 6 cc columns (Waters, USA). After conditioning with 3 mL of ethyl acetate, 3 mL of methanol and 3 mL of Milli-Q water, the samples were passed through the columns. After sample loading, the column was washed with 3 mL of H_2O and dried for 30 min. The analytes were eluted with 4 mL of ethyl acetate. The extracts were dried with anhydrous sodium sulfate, the volume was reduced to 2 mL using a nitrogen stream and aliquots were derivatized for GC/MS analysis. 1 mL aliquots of the extracts were reduced to 100 μL; 50 μL of pyridine and 200 μL of derivatization agent BSTFA:TMS (99:1, v/v) were added. These reaction mixtures were incubated at 70 °C for 30 min. After the incubation, the reaction mixtures were evaporated to 100 μL and 800 μL of ethyl acetate and hexachlorobenzene (HCB) were added as the internal standard (100 μL, 10 mg L^{-1}). BSTFA:TMS was obtained from Sigma Aldrich (Germany), methanol 99.8%, hydrochloric acid 38.0%, sodium chloride and ethyl acetate 99.8% were purchased from Sigma-Aldrich (Germany).

The measurement was performed with the Scion SQ (Bruker, USA) instrument equipped with a single quadrupole MS detector. 1 μL of each the sample solution was injected into the GC/MS systems. The analyses were performed with a 30 m long × 0.25 mm I.D., 0.25 mm film thickness, Rxi-5MS column (Restek, USA). The GC oven temperature program started from 60 °C (hold 2 min), then increased to 240 °C at 15 °C min^{-1} and finally reached 280 °C at 20 °C min^{-1}, where it was held isothermally for 14 min. Helium (99.999%) was used as the carrier gas, with a constant flow rate of 1 mL min^{-1}. The injector was operated in the split/splitless mode, with a splitless time of 1 min. The injector temperature was 280 °C. The source and transfer line temperatures were 250 °C and 280 °C, respectively. The mass spectra were recorded at 3 scans s^{-1} under the electron ionization at 70 eV and the mass range 100–650 amu. Analyte quantification was carried out under the SIM mode (151, 239, 254 m/z for catechol; 253, 268 m/z for 3-methyl catechol; 185, 288, 290 m/z for 4-chlorocatechol; 185, 187, 200 m/z for 2-chlorophenol; 185, 187, 200 m/z for 3-chlorophenol; 185, 187, 200 m/z for 4-chloro phenol; and 285 m/z for the internal standard HCB). The concentrations of the analytes were calculated using external calibration in the range 0.001–1 mg L^{-1}.

2.5.4. Phospholipid-derived fatty acids analysis

Phospholipid-derived fatty acid (PLFA) analysis was used for quantification of the total biomass and to monitor broad changes in the microbial community composition in the groundwater during the aeration step. Groundwater samples for PLFA analyses were prepared using filtration (1 L) through microbial filters (0.2 μm). These filters were then extracted, fractionated and analyzed using the method described by Šnajdr et al. (2008). Briefly, phospholipids were extracted by a mixture of chloroform: methanol: phosphate buffer (1:2:0.8) and then separated by solid-phase extraction cartridges (LiChrolut Si 60, Merck). Thereafter, samples underwent mild alkaline methanolysis and the free methyl esters of phospholipid fatty acids were analyzed by gas chromatography–mass spectrometry (GC–MS; 450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA). The fungal biomass was quantified based on the 18:2ω6,9 content; the bacterial biomass was quantified as the sum of i14:0, i15:0, a15:0, 16:1ω5, 16:1ω7, 16:1ω9, 10Me-16:0, i16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 18:1ω7, 10Me-18:0, and cy19:0 (actinomycetes 10Me-16:0, 10Me17:0, 10Me-18:0; Gram + i14:0, i15:0, a15:0, i16:0, i17:0, a17:0; Gram− 16:1ω7, 16:1ω9, 18:1ω7, cy17:0, cy19:0). The sum of all the identified lipids was used to estimate the total microbial biomass (total PLFAs) (Stella et al., 2015).

2.5.5. Precipitate analysis

The precipitate created during aeration was separated using vacuum-filtration (ISOLAB, 47/50 mm diameter membranes, 1 L of a sample, filters 0.45 μm). The amount of precipitate was determined using the gravimetric method according to ISO 11465:1993.

The total iron content in the precipitate was quantified by Atomic Absorption spectroscopy (AAS) with flame ionization using

a ContrAA 300 (Analytik Jena AG, Germany) instrument equipped with a high-resolution Echelle double monochromator (spectral bandwidth of 2 p.m. at 200 nm) and with a continuous radiation source (Xe lamp). Spectrophotometry was used to determine Fe (II) according to CSN ISO 6332:1988.

^{57}Fe Mössbauer spectroscopy was carried out to analyse the valence states of iron and Fe-containing phases in the solid phase samples (precipitates) after the aeration step. Mössbauer spectrometer MS96 was employed and the analyses were performed at temperatures of 300 K and 5 K (Pechoušek et al., 2012). The spectrometer is equipped with a ^{57}Co(Rh) radiation source and a fast scintillation detector with the $YAlO_3$:Ce crystal. The spectra were captured at a constant acceleration mode in the velocity range from −10 to 10 mm s^{-1} and processed with Mosswin software. The isomer shift of the captured spectra was calibrated using α-Fe foil at room temperature.

2.5.6. Scanning electron microscopy/X-ray energy-dispersive spectroscopy

The wet precipitates were gently separated from the filtration paper and dried in vacuum desiccator for 10 h. Afterward, the dried powder samples were spread on a double sided carbon tape and measured without any additional treatment. Scanning electron microscopy (SEM) images and X-ray energy-dispersive spectroscopy (EDS) were used to analyse the qualitative composition of the precipitate. The data were recorded with a Hitachi 6600 FEG microscope. The samples were measured with accelerating voltages of 3 and 5 kV.

2.5.7. qPCR

DNA extraction and real-time polymerase chain reaction (qPCR) were carried out in order to assess changes in the abundance of specific BTEX-degrading bacteria using detection of typical representatives of their aerobic and anaerobic degradative genes.

Total environmental DNA was extracted from the aeration and settlement tank biofilm samples using the FastDNA Spin Kit for Soil (MP Biomedicals, CA, USA) following the manufacturer's protocol. Extracted DNA was quantified with a Qubit 2.0 fluorometer (Life Technologies, MA, USA). The qPCR reaction mixture contained: 5 μL LightCycler® 480 SYBR Green I Master (Roche, Switzerland), 0.4 μL of 20 mM forward and reverse primer mixture (Generi Biotech, Czech Republic) with the addition of 3.6 μL ultra-pure water (Bioline, Great Britain).

Specific primers for amplification of the functional genes encoding catechol-2,3-dioxygenase (DEG-F and DEG-R; (Mesarch et al., 2000)) and the alpha subunit of benzylsuccinate synthase (7772f and 8546r; (Winderl et al., 2007)) were used. 1 μL of DNA was added to the PCR reaction. The measurements were performed in duplicate using a LightCycler® 480 instrument (Roche, Switzerland) with reaction conditions set as follows: 5 min at 95 °C initial denaturation, followed by 45 cycles of 10 s at 95 °C, 15 s at 60 °C and 20 s at 72 °C.

3. Results and discussion

3.1. Discontinuous unit operation

We first tested the feasibility of the photo-oxidation unit for degradation of the detected pharmaceuticals and related compounds. The results are depicted in Fig. 3. More than 50% of the pharmaceutics were degraded within 60 min (ret. time in photo-oxidation reactors 2.5 min; H_2O_2 dose 1 L m^{-3}) under the discontinuous conditions of the photo-reactor. After 150 min (ret. time in photo-oxidation reactors 8.8 min; H_2O_2 dose 3.5 L m^{-3}), the observed removal efficiency was higher than 90%. The benzene, chlorobenzene and toluene removal efficiency was lower, as can be seen in Fig. 4, and the final removal reached approximately 80% after 150 min. It was documented that generally only a few minutes of retention in the photo-oxidation reactors are sufficient for oxidation of organic contaminants by OH•, which is consistent with the results of other researchers (Buxton et al., 1988; Bahmani et al., 2014).

As stated above, during manipulation with the groundwater (pumping from the well into the settling tank), we observed a substantial effect on the concentrations of monoaromatics, probably due to their partial volatilization. Therefore, we also tested a simple aeration pretreatment step. The results showed that 90% of the VOCs (benzene, toluene and chlorobenzene) were removed within 180 min (Fig. 5), while aeration had no significant effect (ANOVA, $P > 0.05$) on the concentrations of the pharmaceuticals (Fig. 6). The advantages of UV/H_2O_2 in combination with stripping have already been reported in the literature, Tuhkanen (2004). However, in our case, the removal was rather rapid and we observed the formation of a precipitate. Therefore, we employed several other techniques to characterize the mechanisms involved in the removal, focusing on possible adsorption (co-precipitation) of the compounds onto the precipitate as well as the participation of biodegradation.

3.2. Other removal mechanisms and by-products

One way to confirm a biodegradation process is the detection of typical microbial pollutant metabolites. Therefore, we focused on detection of oxidative transformation products, such as hydroxyl derivatives of the monoaromatics. The course of the concentrations of the detected intermediates during the aeration test is depicted in Fig. 7A–C. Surprisingly, we did not record only catechols as typical bacterial dioxygenases products but also mono hydroxy-derivatives, suggesting the possible involvement of mono-oxygenases (El-Naas et al., 2014) and chlorophenols, resulting from chlorobenzene degradation under insufficient oxygen conditions (Cooper et al., 2004). It is worth noting that the mono hydroxy-derivatives are not very specific oxidative biodegradation products. On the other hand, the results of PLFA (Fig. 7B) revealed that the microbial biomass in the treated groundwater increased 30 times within the first 20 min of aeration, documenting substantial microbial growth. The course of the gram-negative bacterial biomass corresponds with elevated concentrations of the transformation products. On the other hand the gram-positive bacteria, as well as actinobacteria, were close to the detection limit of the method during the course of the experiment. Surprisingly, after the maximum around 20 min, the biomass dropped down again and recovered after 120 min. A possible explanation is a change in the microbial community due to the significant decrease in concentrations of the present monoaromatics (Fig. 5). Noteworthy, common physico-chemical parameters did not changed substantially within this period. In general, the data support our hypothesis about the involvement of bacteria in the degradation of the monoaromatics (van Agteren et al., 1998). Microbial biodegradation activity was also confirmed using qPCR analysis of genes encoding catechol 2,3-dioxygenase and benzyl succinate synthase, two genes involved in bacterial aerobic and anaerobic benzene transformation, respectively.

Catechol 2,3-dioxygenase and benzyl succinate synthase genes were detected by qPCR, proving presence of microorganisms with ability to degrade monoaromatic hydrocarbons. The abundance of these genes is approximately 85–125 times higher compared to the number of the same genes in the soil samples taken from the

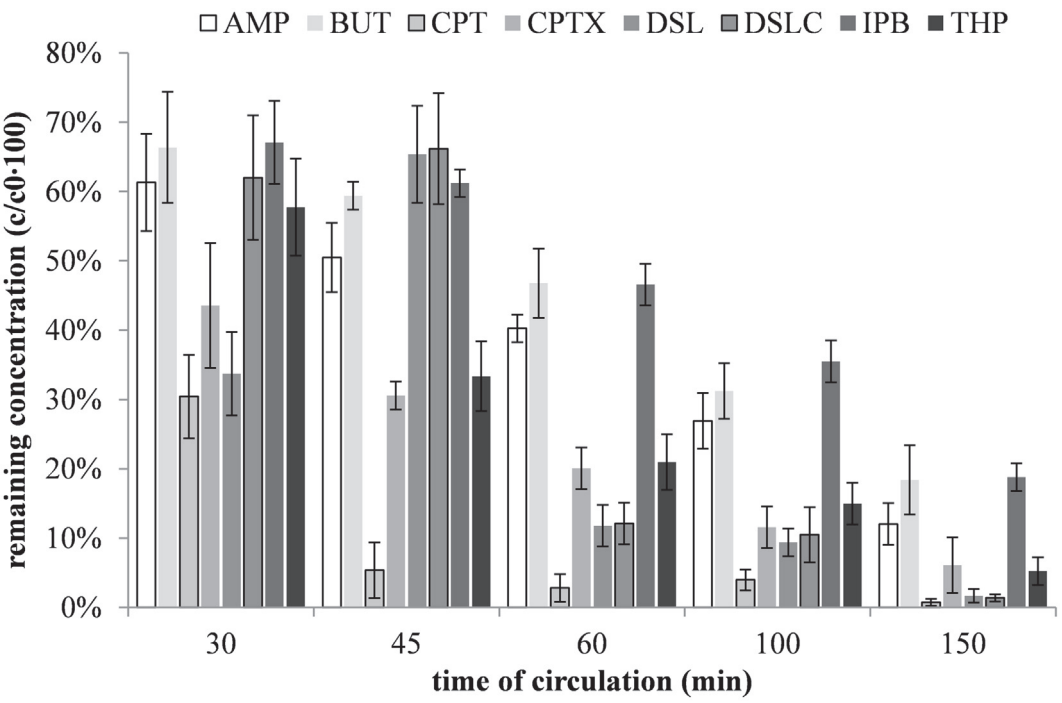


Fig. 3. Residual concentrations (c/c0) of pharmaceuticals during circulation of water from reaction tank into the photo-oxidation unit.

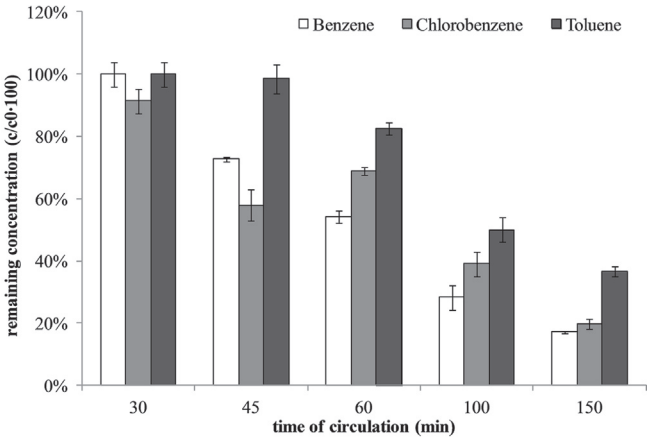


Fig. 4. Residual concentrations (c/c0) of monoaromatics during circulation of water from the reaction tank into the photo-oxidation unit.

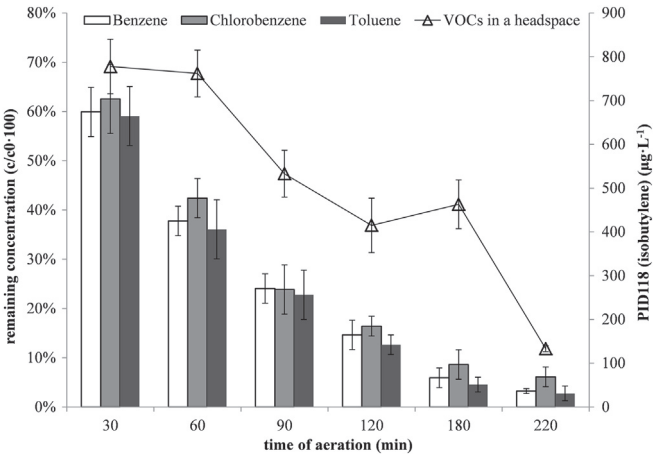


Fig. 5. Residual concentrations (c/c0) of benzene, toluene, chlorobenzene and concentration of VOCs in a headspace during the aeration pre-treatment.

underground aquifer (data not shown). The detection of both the aerobic and anaerobic degradation pathways could be explained by internal stratification of the biofilm, where the anaerobic bacteria can be present in the internal part of the biofilm layer (Li et al., 2008). The aerobic pathway markers represented by catechol 2,3-dioxygenase were also detected in water samples from both the tanks (data not shown).

It is clear that biodegradation need not be the only removal mechanism and the aeration could cause stripping of the volatile pollutants. Therefore, we monitored VOCs in the exhaust air of the aeration tank. These results are also incorporated into Fig. 5. The VOC concentrations in the exhaust air (130 L min^{-1}) were rather high and provided strong evidence for volatilization of the present contaminants.

As mentioned above, we observed the formation of a precipitate during the aeration process; therefore another possible removal mechanism could be adsorption of the contaminants. We quantified the precipitate using gravimetry and the results are depicted in Fig. 7D together with other parameters during the course of the aeration. Detailed analysis of the precipitate using SEM/EDS revealed that the material consisted of iron, calcium, silicon oxide and chlorine (Fig. 8). Moreover, characterization of the valence states by Mössbauer spectroscopy showed that the precipitate is formed of the mineral akaganeite. Targeted analysis of the pollutants of concern was performed in the long-term accumulated precipitate from the settling tank, with detection of 4325 µg g^{-1} of chlorobenzene, 556 µg g^{-1} of benzene, 493 µg g^{-1} of toluene and 63 µg g^{-1} of the pharmaceuticals (sum of concentrations). The adsorption of these contaminants has been previously documented by a number of researchers (Hackbarth et al., 2014; Uchrin et al., 2012). In fact, the ratio between the concentrations of the pharmaceuticals and the monoaromatics corresponds to their input

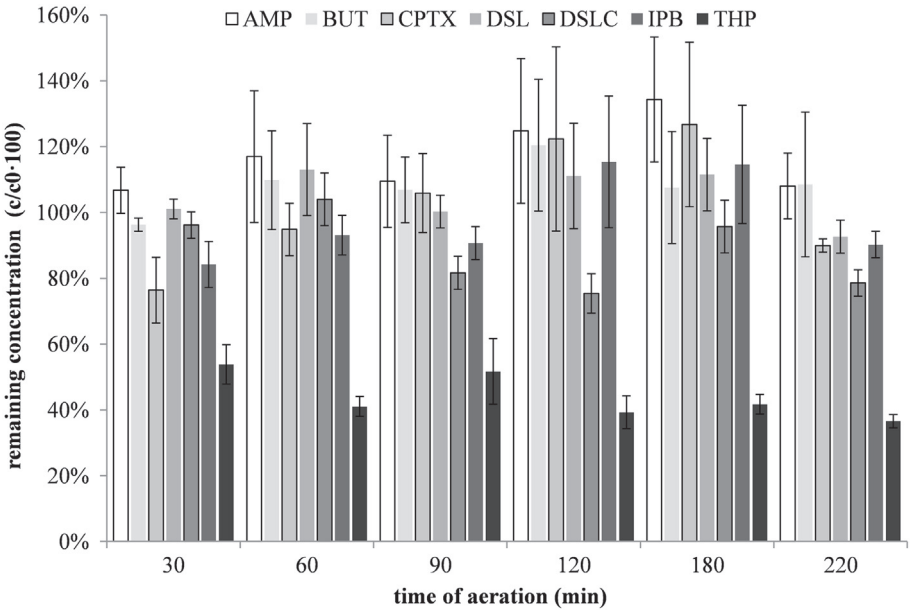


Fig. 6. Residual concentrations (c/c0) of pharmaceuticals during the aeration pre-treatment.

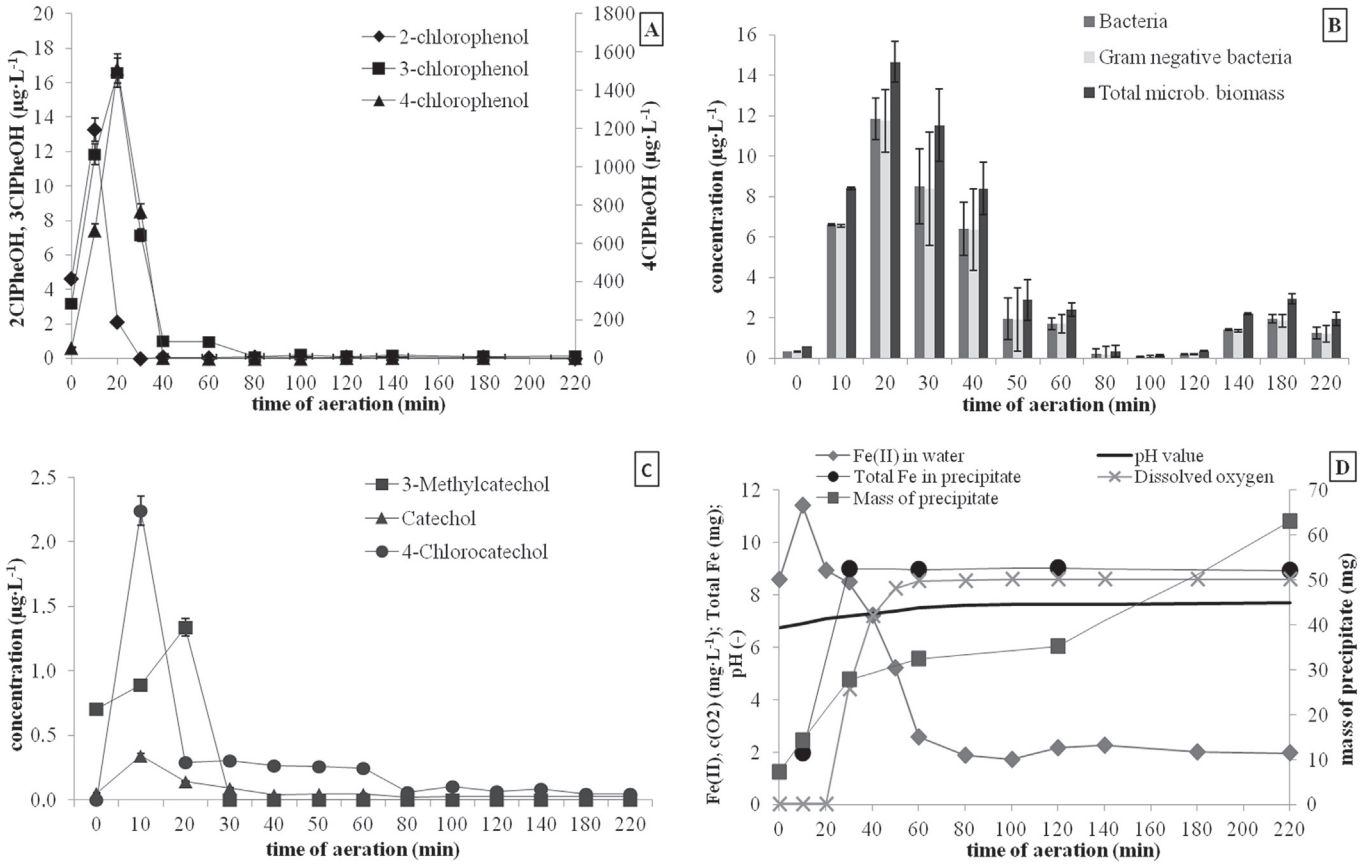


Fig. 7. A – the formation of chlorophenols during the aeration; B – the results of PLFA analysis during the aeration, species such as Fungi, Actinobacteria, Gram positive bacteria and Anaerobic bacteria were below the respective limits of quantification (0.5 µg L^{-1}); C – Catechol, the BTEX and monochlorobenzene degradation product, during the aeration process; D – precipitate characterization during the aeration process.

amounts in water except for chlorobenzene. It is impossible to quantify the extent to which the individual mechanisms contribute to removal of the pollutants. However, the discrepancy between the amounts of adsorbed chlorobenzene and toluene are consistent with the higher recalcitrance and lower volatility of chlorobenzene compared to toluene (Henry's Law constant 0.3 and

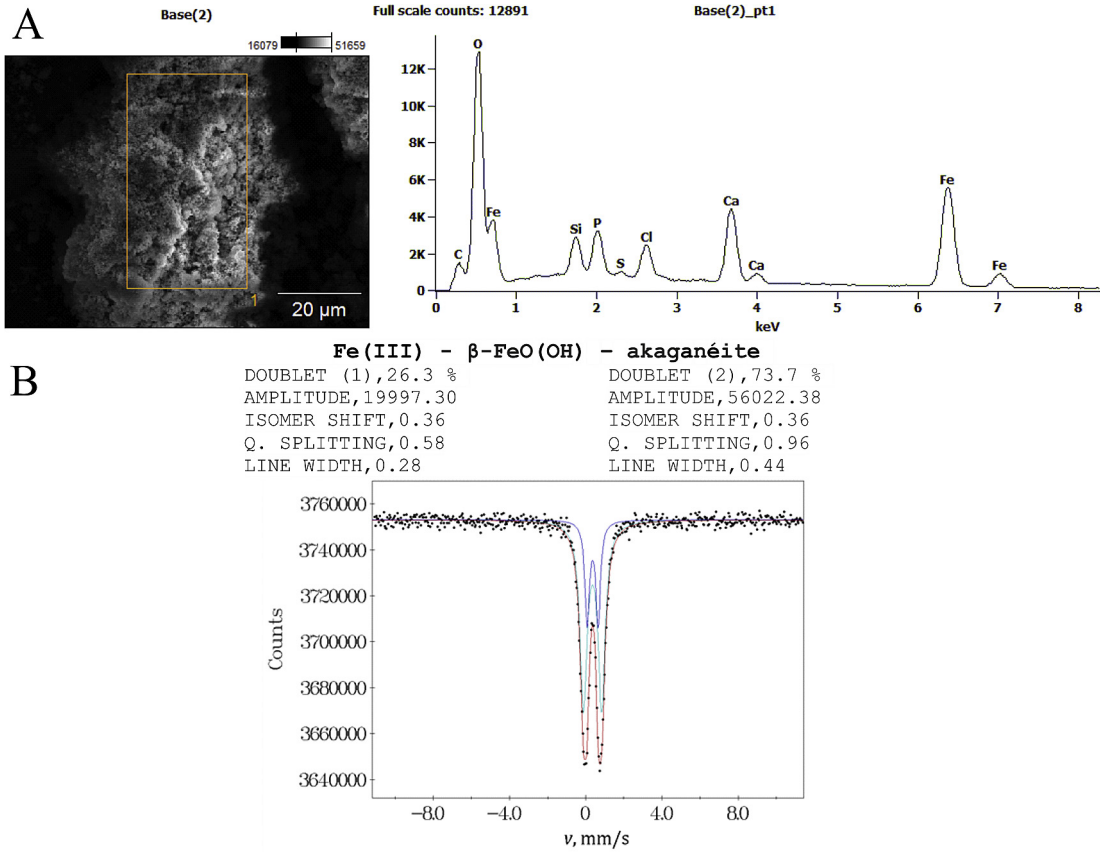


Fig. 8. A – Elemental analysis of the precipitate by EDS; B – Mössbauer spectroscopy.

0.15 mol bar kg^{-1}, respectively). In other words, co-precipitation/adsorption onto the precipitate was probably a more relevant chlorobenzene removal mechanism than for the other monoaromatics.

3.3. Continuous unit operation

A combined reactor according to the scheme depicted in Fig. 2 was installed in the locality and operated from May to September 2016. The results are shown in Figs. 9 and 10. The results clearly document that the combined reactor unit was very effective in the removal of both the types of pollutants, where the removal efficiency ranged from 72 to 99% of the pharmaceuticals and the related compounds. The results of the monoaromatics decontamination were even better except for June 2016, when we encountered some technical problems with the aeration pump that negatively affected the results of chlorobenzene removal and the efficiency dropped to 66%. In fact, these results confirmed the importance of the aeration step. It is noteworthy that the overall efficiency also depends on the input concentrations, which varied during the long term test, as well as the chemical composition of the groundwater, which can influence the removal mechanisms. The results also proved that passive sampling could be a very useful tool for monitoring groundwater pollution, including applications for site remediation.

4. Conclusion

The results of this study document the feasibility and high efficiency of both the techniques, i.e. UV/H_2O_2 treatment and simple aeration for removal of the organic contaminants from the groundwater. UV/H_2O_2 as a very efficient decomposition method was proven to decompose both the types of pollutants; however, the results of this study showed that aeration can strongly promote the removal yield of monoaromatic compounds including chlorobenzene. The extensive set of various analyses revealed that several types of mechanisms contributed to the removal, including biodegradation and co-precipitation, and the results clarified their

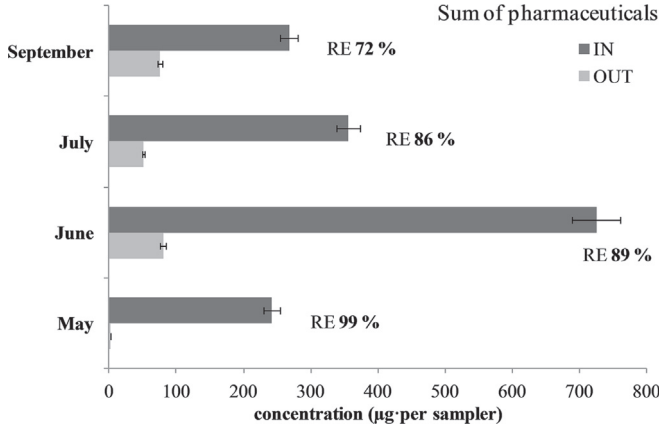


Fig. 9. Long-term monitoring of the treatment pilot unit, sum of initial (IN) and final concentrations (OUT) of the pharmaceuticals and the removal efficiency (RE). The data are the means of analyses using three POCIS samplers and the error bars represent the standard deviations.

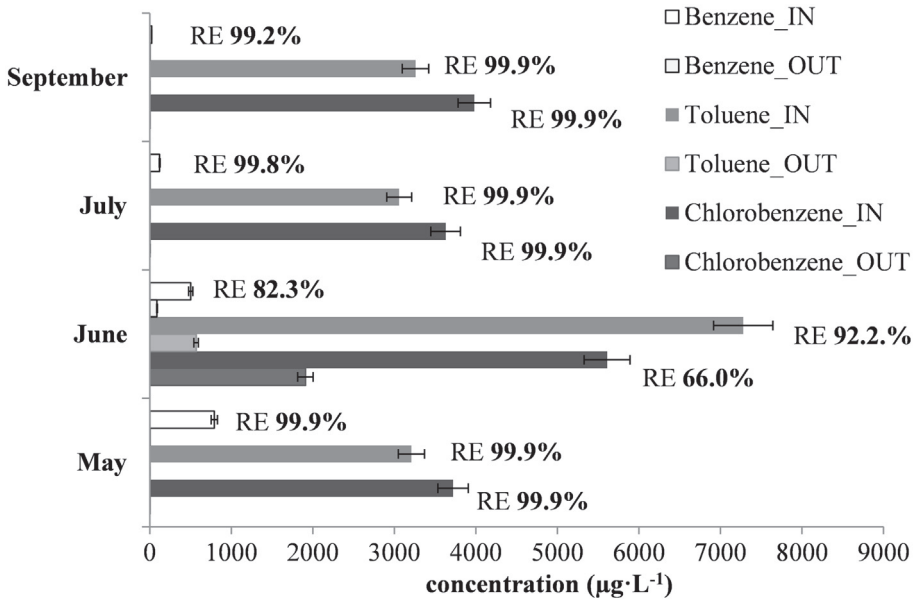


Fig. 10. Long-term monitoring of the treatment pilot unit, the initial (IN) and final (OUT) concentrations of benzene, toluene and chlorobenzene with the removal efficiencies (RE). The data are the means of four independent samplings and the error bars represent the standard deviations.

involvement in the process. Combination of the approaches was also demonstrated to be very effective and offers an alternative for application in water treatment practice. The employed technology is described as a moderate-scale pilot unit for limited water volumes; however, its configuration can also be employed for a small transportable cell unit.

Acknowledgements

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References

Alexander, M., 1999. Biodegradation and Bioremediation. Academic Press, San Diego, California.
Bahmani, M., Bitarafhaghighi, V., Badr, K., Keshavarz, P., Mowla, D., 2014. The photocatalytic degradation and kinetic analysis of BTEX components in polluted wastewater by UV/H_2O_2-based advanced oxidation. Desalination Water Treat. 52 (16–18), 3054–3062.
Benitez, F., Real, F., Acero, J., Roldan, G., 2009. Removal of selected pharmaceuticals in waters by photochemical processes. J. Chem. Technol. Biotechnol. 84, 1186–1195.
Buxton, G., Greenstock, C., Helman, W., Ross, A., 1988. Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals (·OH/O·-) in aqueous solution. J. Phys. Chem. Ref. Data 17 (2), 513–586.
Cajthaml, T., Křesinová, Z., Svobodová, K., Sigler, K., Řezanka, T., 2009. Microbial transformation of synthetic estrogen 17 alpha-ethinylestradiol. Environ. Pollut. 157 (12), 3325–3335.
Cheng, M., Zeng, G., Huang, D., Lai, C., Xu, P., Zhang, C., Liu, Y., 2016. Hydroxyl radicals based advanced oxidation processes (AOPs) for remediation of soils contaminated with organic compounds: a review. Chem. Eng. J. 284, 582–598.
Citulski, J.A., Farahbakhsh, K., 2010. Fate of endocrine-active compounds during municipal biosolids treatment: a review. Environ. Sci. Technol. 44 (22), 8367–8376.
Cooper, W., Gehringer, P., Pikaev, A., Kurucz, C., Mincher, B., 2004. In: Radiation processes, Parsons, S. (Eds.), Advanced Oxidation Processes for Water and Wastewater Treatment. IWA Publishing, London, UK, pp. 209–246.
El-Naas, M.H., Acio, J.A., El Telib, A.E., 2014. Aerobic biodegradation of BTEX: progresses and prospects. J. Environ. Chem. Eng. 2, 1104–1122.
Hackbarth, F.V., Vilar, V.J.P., De Souza, G.B., de Souza, S.M.A.G.U., de Souza, A.A.U., 2014. Benzene, toluene and o-xylene (BTX) removal from aqueous solutions through adsorptive processes. Adsorption 20, 577–590.
Hofman-Caris, C., Beerendonk, E., 2011. General overview and conclusions. In: Hofman-Caris, C., Beerendonk, E. (Eds.), New Concepts of UV/H2O2 Oxidation. KWR Water research institute, Nieuwegein, Netherlands, pp. 187–194.
Křesinová, Z., Linhartová, L., Petrů, K., Krejčová, L., Šrédlová, K., Lhotský, O., Kameník, Z., Cajthaml, T., 2016a. Method for analysis of psychopharmaceuticals in real industrial wastewater and groundwater with suspended organic particulate matter using solid phase extraction disks extraction and ultra-high performance liquid chromatography/time-of-flight mass spectrometry. J. Chromatogr. A 1440, 15–22.
Křesinová, Z., Petrů, K., Lhotský, O., Rodsand, T., Cajthaml, T., 2016b. Passive sampling of pharmaceuticals and personal care products in aquatic environments. Eur. J. Environ. Sci. 6, 43–56.
Lester, Y., Avisar, D., Gozlan, I., Mamane, H., 2011. Removal of pharmaceuticals using combination of UV/H_2O_2/O_3 advanced oxidation process. Water Sci. Technol. 64 (11), 2230–2238.
Li, T., Bai, R., Liu, J., 2008. Distribution and composition of extracellular polymeric substances in membrane-aerated biofilm. J. Biotechnol. 135, 52–57.
Mascolo, G., Ciannarella, R., Balest, L., Lopez, A., 2008. Effectiveness of UV-based advanced oxidation processes for the remediation of hydrocarbon pollution in the groundwater: a laboratory investigation. J. Hazard. Mater. 152 (3), 1138–1145.
Mesarch, M.B., Nakatsu, C.H., Nies, L., 2000. Development of catechol 2,3-dioxygenase-specific primers for monitoring bioremediation by competitive quantitative PCR. Appl. Environ. Microbiol. 66 (2), 678–683.
Oncescu, T., Nitoi, I., Oancea, P., Mihai, S., Lucian, C., Laurentiu, D., 2008. Photosensitized chlorobenzene degradation by UV/H_2O_2 oxidation. J. Adv. Oxid. Technol. 11 (1), 149–154.
Pechoušek, J., Jančík, D., Frydrych, J., Navařík, J., Novák, P., 2012. Setup of Mössbauer spectrometers at RCPTM. In: Tuček, J., Machala, L. (Eds.), Proceedings of the International Conference Mössbauer Spectroscopy in Materials Science, Olomouc, CZ, 2012 Jun 11–15. American Institute of Physics, New York, USA, pp. 186–193.
Rosario-Ortiz, F.L., Wert, E.C., Snyder, S.A., 2010. Evaluation of UV/H_2O_2 treatment for the oxidation of pharmaceuticals in wastewater. Water Res. 44, 1440–1448.
Saharan, V.K., Pinjari, D.V., Gogate, P.R., Pandit, A.B., 2014. Advanced oxidation technologies for wastewater treatment: an overview. In: Ranade, V.V., Bhandari, V.M. (Eds.), Industrial Wastewater Treatment, Recycling and Reuse. Butterworth-Heinemann, Oxford: Elsevier Ltd, pp. 141–191.
Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T., Baldrian, P., 2008. Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. Soil Biol. Biochem. 40, 2068–2075.
Stefan, M.I., 2004. UV photolysis: background. In: Parsons, S. (Ed.), Advanced Oxidation Processes for Water and Wastewater Treatment. IWA Publishing, London, UK, pp. 7–48.
Stefan, M.I., Williamson, C.T., 2004. UV light-based applications. In: Parsons, S. (Ed.), Advanced Oxidation Processes for Water and Wastewater Treatment. IWA Publishing, London, UK, pp. 49–80.
Stella, T., Covino, S., Burianová, E., Filipová, A., Křesinová, Z., Voříšková, J., Větrovský, T., Baldrian, P., Cajthaml, T., 2015. Chemical and microbiological characterization of an aged PCB-contaminated soil. Sci. Total Environ. 533, 177–186.
Tuhkanen, T.A., 2004. UV/H_2O_2 processes. In: Parsons, S. (Ed.), Advanced Oxidation

Processes for Water and Wastewater Treatment. IWA Publishing, London, UK, pp. 86–110.
Tuhkanen, T.A., Marinosa, P.C., 2010. UV irradiation for Micropollutant removal from aqueous solutions in the presence of H_2O_2. In: Virkutyte, J., Varma, R.S., Jegatheesan, V. (Eds.), Treatment of Micropollutants in Water and Wastewater. IWA Publishing, London, UK, pp. 295–320.
Uchrin, C.G., Koshy, K., Wojtenko, I., 2012. Sorption of benzene, toluene, and meta-xylene to contaminated site soil. In: Pawłowski, L., Lacy, W.J., Uchrin, C.G., Dudzinska, M.R. (Eds.), Chemistry for the Protection of the Environment, vol. 2. Springer Science & Business Media, Berlin, Germany, pp. 303–310.
van Agteren, M.H., Keuning, S., Janssen, D.B., 1998. Handbook on Biodegradation and Biological Treatment of Hazardous Organic Compounds. Springer Science+Business Media B.V., Dordrecht, Netherlands.
Verlicchi, P., Zambello, E., 2015. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – a critical review. Sci. Total Environ. 538, 750–767.
Winderl, C., Schaefer, S., Lueders, T., 2007. Detection of anaerobic toluene and hydrocarbon degraders in contaminated aquifers using benzylsuccinate synthase (bssA) genes as a functional marker. Environ. Microbiol. 9 (4), 1035–1046.
Wols, B., Hofman-Caris, C., Harmsen, D., Beerendonk, E., 2013. Degradation of 40 selected pharmaceuticals by UV/H_2O_2. Water Res. 47 (15), 5876–5888.
Žebrák, R., Mašín, P., Klusoň, P., Krystník, P., 2014. Using of photochemical H_2O_2/UVC decontamination cell for heavily polluted waters. Waste Forum 2, 55–61.