Environmental Toxicology and Pharmacology 40 (2015) 492–497

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap

# Utilization of Fenton-like reaction for antibiotics and resistant bacteria elimination in different parts of WWTP

Tomáš Mackuľak[a,*], Kristína Nagyová[b], Milota Faberová[a], Roman Grabic[c], Olga Koba[c], Miroslav Gál[d], Lucia Birošová[b]

[a] Department of Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic
[b] Institute of Biochemistry, microbiology and health protection, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic
[c] Faculty of Fisheries and Protection of Waters, University of South Bohemia in Ceske Budejovice, Zátiší 728/II, 389 25 Vodňany, Czech Republic
[d] Department of Inorganic Technology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovak Republic

ARTICLE INFO

*Article history:*
Received 26 March 2015
Received in revised form 1 July 2015
Accepted 3 July 2015
Available online 6 July 2015

*Keywords:*
Antibiotics
Wastewater treatment plant
Antibiotic resistance
Fenton like reaction
Degradation

ABSTRACT

Utilization of relatively low-cost modification of Fenton reaction for the elimination of selected antibiotics and resistant coliforms in different part of wastewater treatment plant (WWTP) was studied. The concentration of antibiotics and occurrence of resistant gems in different stages of WWTP in the capital city of Slovakia – Bratislava was analyzed by LC–MS/MS technique. Consequently, Fenton-like reaction was applied for the elimination of chemical and biological contaminants. Comparative study with classical Fenton reaction was also done. Very high concentrations of clarithromycin, ciprofloxacin and azithromycin in influent water were found. Coliform bacteria were predominantly resistant to ampicillin, ciprofloxacin and gentamicin. After the mechanical stage, the concentration of antibiotics in water was significantly decreased because of the sorption during this step. Biological step degraded 12 types of antibiotics. Analyses of effluent water showed very bad elimination of azithromycin (919 ng/L) and clarithromycin (684 ng/L). Contrary, ciprofloxacin was removed with very high efficiency (95%). The number of resistant bacteria was also significantly decreased in effluent water. In the case of *Escherichia coli* only ampicillin and gentamicin resistance bacteria were detected. Our results show that antibiotics as well as resistant bacteria were eliminated by the modification of classical Fenton reaction with high efficiency. The modification of the Fenton reaction can decrease the process wages, environmental impact. Moreover, the degradation process was easily controlled, monitored and tuned.



## 1. Introduction

Households, hospitals, mental hospitals, retirement's homes and other health care facilities represent the main source of pharmaceuticals in wastewater. Many of these contaminants are not completely eliminated in WWTP. Degradation of hormones, pesticides, antibiotics, antihistaminic and drugs is limited and as a result they commonly turn up in the aquatic environment (Brodin et al., 2013; Kotyza et al., 2009; Thomas et al., 2012). Moreover, activated sludge is able to absorb pharmaceuticals from wastewater. After the application of the sludge into the soil these compounds can be released to the environment following rainfalls. In this way drugs that resist biodegradation, photodegradation and sorption can get into groundwater. According to the National Centers for Coastal Ocean Science (NCCOS) drugs concentration in the worldwide environment ranging from nanograms to milligrams per liter. Surface waters tend to be the most polluted in the middle and lower flows that are largely connected to major cities across the WWTP (Buser et al., 1998; Kotyza et al., 2009; Metcalfe et al., 2010; Rua-Gomez and Puettmann, 2013). The presence of broad spectrum antibiotics at concentrations below 10 $\mu$g per liter may also give rise to multi-drug resistant types of bacteria (Baquero et al., 2008). Other problem represents antibiotic resistant bacteria in influent as well as in effluent wastewater of WWTP. Several studies have pointed out that wastewater is primary contributor of bacteria to the aquatic ecosystem (Grabow et al., 1974). Many of these bacteria carrying transmissible R-factors that are responsible for dissemination of multiple antibiotic resistance (Meckes, 1982). Subinhibitory

* Corresponding author.
*E-mail address:* tomas.mackulak@stuba.sk (T. Mackuľak).

http://dx.doi.org/10.1016/j.etap.2015.07.002
1382-6689/

concentrations of antibiotics, biocides and other pharmaceuticals can also contribute to the development of antibiotic resistance in bacteria presented in WWTP (Cantas et al., 2013).

Advanced oxidation processes (AOPs) are currently extensively studied in term of an effective removing of above mentioned micro and biopollutants. Studies in this area suggest the possible applications in the treatment of wastewater containing a wide range of pollutants (Comninellis et al., 2008). AOPs are used in water pretreatment before the biological stage and/or as a tertiary treatment at the WWTP.

Ozonation, Fenton reaction, photolysis and others (Mackuľak et al., 2013) are among the frequently tested processes. AOPs are able to produce hydroxyl radicals in a large extent which may effectively remove not only the micropolutants such as drugs but also resistant bacteria.

The chlorination which is also frequently applied mainly for water disinfection, can lead to the formation of chlorinated intermediates which display potential toxicity to the environment and especially aquatic animals (Gibs et al., 2007). Several studies are interested in Fenton reaction and its modifications because of its ability of degradation and mineralization of emerging products. The combination of Fenton and photo-Fenton reaction followed by final purification of the product formed in activation is also very interesting. This method of cleaning was applied on hospital effluent in the study of Kajitvichyanukul (Kajitvichyanukul and Suntronvipart, 2006). Authors found out that applied photo-Fenton reaction was able to sufficiently pre-treat waste water in terms of toxicity studied on gram negative marine bacterium *Vibrio fischeri*. Subsequent final purification by activated sludge was able to significantly reduce the value of COD effluent from the hospital (Kajitvichyanukul and Suntronvipart, 2006). Furthermore, study by Oh et al. (2004) showed, that AOP processes are able to degrade not only *Escherichia coli* but also its endotoxin in water systems.

There is a lack of information about the fate of pharmaceuticals in the individual steps of WWTP cleansing. Recent studies show that bioaerosols produced during biological processes could contribute to the air contamination. Bioaerosols usually contain micropollutants such as antibiotics and antibiotic resistant bacteria (Korzeniewska et al., 2013). Therefore, it is necessary to control not only the effluent but every step of WWTP.

In this study, we have focused on both monitoring of antibiotics and resistant coliform bacteria at the particular stages of WWTP and their subsequent degradation by Fenton-like reaction. Thirty three types of antibiotics were analyzed using the LC–MS/MS technique. Consequently, Fenton-like reaction and classical Fenton reaction were applied as the degradation method of identified drugs and presented antibiotic resistant coliforms in the effluent water of WWTP. In our approach waste iron shavings from machining processes were used as an iron source instead of traditional $FeSO_4{\cdot}7H_2O$ reagent. This modification significantly decreases the cost of the overall degradation process by ca 60%. The process can be easily and precisely controlled by removing or addition of iron material into the system. Moreover, our approach enables utilization of waste and/recycled iron in the sewage water post-purification processes.

## 2. Materials and methods

### 2.1. Wastewater treatment plants

WWTP is dimensioned for 490,000 p.e., currently loaded to a level of 125,000 p.e. WWTP consists of a mechanical (contains coarse and fine rakes, grit chamber and grease catcher) and a biological (nitrification) degree, the sludge is anaerobically stabilized, the generated biogas is energetically recovered. Water flow during our analyses ranged from 29,500 to 33,000 $m^3$/day. Wastewater pH was 7.2–7.4. The process scheme includes primary settling (1600 $m^3 \times 4$); oxidation–nitrification (5500 $m^3 \times 3$); and secondary settling (3600 $m^3 \times 4$). Additional characteristics were: dissolved oxygen concentration in aerated tanks 2.8–6 mg $L^{-1}$; total suspended solid concentration in biological reactors 4.0–4.6 g/L. The effluent characteristics were: 20 mg COD/L, 3 mg $BOD_5$/L, 11.3 mg TSS/L, 10 mg $N_{TOT}$/L; 2 mg $NH_4^+$-N/L, and 0.6 mg $P_{TOT}$/L. The rest of samples were collected in plastic bottles and during 2 h after sampling were frozen (−20 °C). Sampling was performed via grab sampling at 10 a.m., October 23, 2013. Samples were collected at the inlet point of WWTP, after the mechanical treatment, after the biological treatment, in the sludge water returning to the biological stage from anaerobic processes and at the effluent point of WWTP.

### 2.2. Antibiotic resistance detection

Bacteria were detected in wastewater samples collected in sterile tubes and transferred to laboratory within 1 h. 0.1 mL aliquots of samples from each stage were spread on selective diagnostic agar plates. 10 mL of effluent water sample was filtrated through GH polypro membrane (0.2 μm, Pall Corporation, USA). Filter membrane was placed on antibiotic and antibiotic free selective diagnostic media (Chromocult Coliform Agar). Antibiotic resistant strains were detected after cultivation on the plates containing diagnostic media with different antibiotics. Before bacteriological counting, plates were incubated for 24 h at 37 °C. Each experiment ran in triplicate and was repeated 6 times. Applied antibiotics (ampicillin, ciprofloxacin, gentamicin, tetracycline and chloramphenicol) were purchased from Sigma–Aldrich (Germany).

### 2.3. Solvents, chemicals

LC–MS grade methanol and acetonitrile (LiChrosolv, Hypergrade) were purchased from Merck (Darmstadt, Germany). Formic acid, used to acidify mobile phases, was purchased from Labicom (Olomouc, Czech Republic). Ultrapure water for HPLC analysis was obtained from an Aqua-MAX-Ultra system (Younglin, Kyounggi-do, Korea). Standards origin and preparation were described in details in article of Fedorova et al. (2013). All chemicals used for degradation experiments (iron sulphate ($FeSO_4{\cdot}7H_2O$, 98%), hydrogen peroxide 30% solution, sulphuric acid and sodium hydroxide) were obtained from the Lachema a.s. (Brno, Czech Republic) and were of p.a. quality. pH was measured with pH probe (Sentek, United Kingdom). Stirring was carried out by an electromagnetic stirrer MM2A (Czech Republic).

### 2.4. In line SPE/LC – MS/MS analysis

Frozen sewage samples were let thaw at room temperature, homogenized and split to aliquots for analysis and degradation experiments. The mixture of isotope labeled internal standards was added to 10 mL of homogenized and filtered (regenerated cellulose syringe filter 0.45 μm pore size) water prior to analysis. The extraction and analysis was performed in one step using in line SPE liquid chromatography coupled with hybrid mass analyzer Q-Exactive (quadrupole coupled to Orbitrap high resolution MS, Thermo Scientific). Isotope dilution and internal standard methods together with matrix matched standards were used to eliminate matrix effects. Detail description of the method and its performance is given in paper of Fedorova (Fedorova et al., 2013). Wastewater samples were analyzed in triplicate, whole degradation experiments were performed in pentaplicates. The reported value for each experiment was obtained as an average of corresponding results.