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BIO-ELECTRO-FENTON: OPTIMIZATION OF ELECTROCHEMICAL ADVANCED OXIDATION PROCESS IN THE PERSPECTIVE OF ITS COMBINATION TO A BIOLOGICAL PROCESS FOR THE REMOVAL OF PHARMACEUTICALS FROM WASTEWATER

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Abstract

Water pollution is one of the biggest challenges that humanity faces as the removal of various complex pollutants is no longer effective by using conventional water treatment methods. Recently pharmaceuticals have been recognized to be contaminants of emerging environmental concern as their traces were detected in a spectrum of water bodies around the globe. The long term effects of their presence in a natural environment are not yet fully studied, but the potential outcomes can be detrimental to a sustainable future. In this regard the development of novel treatment processes is crucial for tackling this environmental problem.

Among the variety of currently rising treatment technologies, the electro-Fenton method, an electrochemical advanced oxidation process, has demonstrated an ability to eliminate pharmaceuticals as well as other types of persistent contaminants. This electrocatalytical process generates *in situ* strong oxidants species - hydroxyl radical ($^{\bullet}OH$) - which non-selectively degrade organic pollutants. As application of electrical energy is required, the operation of such process might be cost-prohibitive. A solution would be to combine electro-Fenton with a biological process which is more economically viable, but less effective in the removal of pharmaceuticals. Such a combined process is expected to have a synergetic effect and to balance cost and effectiveness. The goal of this PhD thesis is to optimize operating conditions of the electro-Fenton process for a feasible combination with a biological process as a means for eliminating pharmaceutical pollution of water.

The main objectives addressed by this work are related to the influence of operating parameters of the electro-Fenton process on (a) removal of pharmaceuticals; (b) mineralization of organic matter; (c) enhancement of biodegradability; (d) energy consumption.

The thesis has three distinct parts related to the type of treated aqueous solution. First, a mechanistic study was conducted on aqueous solutions of individual pharmaceuticals in order to understand general trends of their removal. Next, a series of experiments was carried out on a synthetic mixture of thirteen pharmaceuticals from different therapeutic classes. Lastly, two laboratory bench-scale reactors of a combined bio-electro-Fenton process were operated for the treatment of real wastewater from a pharmaceutical and cosmetic industry. The advance in the complexity of the treated solution allowed a comprehensive comparison and analysis of the influence of the operating parameters.

The main results include the optimal values of two operating parameters: the catalyst (Fe^{2+}) concentration and the applied current intensity for a given electro-Fenton setup. The effects of the operating parameters on the removal of pharmaceuticals and other organic matter were similar regardless of the treated solution. The optimal value for the Fe^{2+} concentration was concluded to be around 0.2 mM. The optimal current intensity was in the range 100-500 mA. The current efficiency in terms of the pharmaceuticals' removal was the highest with the lowest intensity (100-300 mA). At the same time the biodegradability, which was an important factor in the biological post-treatment process, improved with higher intensities of electric current (500-1000 mA). However, high current intensities resulted in an elevated energy consumption, particularly with a prolonged treatment time. Consequently a

decision should be made between improving energy saving or removal rates, and this trade-off would have to be found in any single case.

The novelty of the research presented in this PhD thesis is firstly attributed to the novelty of the combination of electro-Fenton to a biological process. A detailed study of the influence of operating parameters of the electro-Fenton process on removal rates and biodegradability enhancement contributes not only to the general knowledge on the electro-Fenton process, but also to the advancement towards its upscaling and then further towards the industrial application of this technique.

Résumé

La pollution des ressources en eau est un des défis les plus importants auquel l'Homme doit faire face. De nouvelles solutions doivent émerger car les techniques conventionnelles de traitement utilisées actuellement ne permettent pas une élimination assez efficace des divers polluants retrouvés dans les eaux. Parmi les polluants émergents, les composés pharmaceutiques ont récemment été détectés dans différentes sources d'eau à travers le monde. Leurs effets indésirables sur l'environnement et sur l'Homme ont déjà été reconnus mais doivent encore être éclaircis.

De nombreux nouveaux procédés de traitement de l'eau apparaissent. En particulier, le procédé électrochimique d'oxydation avancée appelé électro-Fenton a démontré sa capacité à pouvoir éliminer les produits pharmaceutiques et autres contaminants persistants. Ce procédé est basé sur la génération *in situ* d'une espèce oxydante très puissante, les radicaux hydroxyles ($^{\bullet}OH$), qui permettent la dégradation non-sélective des polluants. Cependant, cela nécessite l'utilisation d'une quantité d'énergie importante, relativement coûteuse. Une solution viable est de coupler le procédé électro-Fenton avec un procédé biologique. En effet, l'utilisation de ce dernier est beaucoup plus économique, mais il possède une efficacité limitée envers les polluants persistants tels que les pharmaceutiques. Ainsi, le procédé hybride bio-électro-Fenton apparaît comme un bon compromis entre le coût et l'efficacité, d'autant plus que des effets synergétiques peuvent se produire. Le but de cette thèse de doctorat a donc été d'optimiser le procédé électro-Fenton dans l'optique de le coupler avec un procédé biologique, afin d'éliminer efficacement les produits pharmaceutiques de l'eau.

Les principaux objectifs de cette étude reposent sur l'étude de l'influence des paramètres opératoires utilisés au cours du procédé électro-Fenton sur (a) la dégradation des composés pharmaceutiques ; (b) la minéralisation de la matière organique ; (c) l'évolution de la biodégradabilité de l'effluent ; (d) la consommation énergétique.

Cette thèse est composée de trois grandes parties, au cours desquelles la complexité des solutions traitées a progressivement augmentée. Premièrement, une étude a été menée sur des solutions de produits pharmaceutiques seuls afin de mieux comprendre les mécanismes impliqués au cours de leur dégradation. La seconde partie porte sur l'étude expérimentale d'une solution synthétique composée d'un mélange de 13 pharmaceutiques appartenant à différentes classes thérapeutiques. La dernière étape a consisté à mettre en place un procédé bio-électro-Fenton pour le traitement d'un effluent pharmaceutique réel. Cette démarche progressive a permis de mieux comprendre l'influence des paramètres opératoires utilisés au cours du procédé électro-Fenton.

Les principaux résultats obtenus sont notamment l'optimisation de deux paramètres opératoires importants : la concentration du catalyseur (Fe^{2+}) et l'intensité du courant. L'influence de ces paramètres s'est révélée similaire au cours du traitement de tous les types de solution testés. Il a donc été possible de conclure que les valeurs optimales sont une concentration en Fe^{2+} de 0,2 mM et une intensité entre 100 et 500 mA. L'efficacité d'élimination des composés pharmaceutiques a été plus importante en utilisant des intensités plus faibles (100-300 mA). Cependant, la biodégradabilité de l'effluent, un paramètre

important dans l'optique du post-traitement biologique, a été d'avantage augmentée en utilisant des intensités élevées (500-1000 mA). Par ailleurs, l'utilisation d'intensités élevées a aussi mené à augmenter la consommation énergétique, en particulier dans le cas de temps de traitement longs. Il apparaît donc évident qu'un compromis entre efficacité et consommation énergétique doit être trouvé pour chaque cas particulier et effluent à traiter.

Pour conclure, les avancées en termes de recherche présentées dans cette thèse de doctorat sont principalement attribuées à la nouveauté de la combinaison du procédé électro-Fenton avec un procédé biologique. L'étude détaillée de l'influence des paramètres opératoires du procédé électro-Fenton sur l'efficacité d'élimination des composés pharmaceutiques ainsi que sur l'augmentation de la biodégradabilité de l'effluent a aussi permis d'améliorer la compréhension de cette nouvelle technique de traitement de l'eau et contribue à son développement vers une application à échelle pilote ou industrielle.

Sintesi

L'inquinamento degli ecosistemi acquatici è una delle principali sfide ambientali che l'umanità deve oggigiorno affrontare per le quali bisogna sviluppare sempre nuovi processi di trattamento dato che quelli tradizionali non risultano più estremamente efficaci per la rimozione di svariati contaminanti di natura più o meno complessa. Negli ultimi anni, i composti farmaceutici sono stati riconosciuti come "contaminanti emergenti" vista la loro presenza diffusa, sebbene in tracce, in corpi idrici di tutto il pianeta. Gli effetti a lungo termine della loro presenza negli ecosistemi naturali non sono ancora ben noti ma, tuttavia, è chiaro che possono essere fortemente negativi soprattutto se si ragiona nell'ottica di un futuro sostenibile.

Tra le tecnologie che si stanno oggi sviluppando, il processo elettro-Fenton ha mostrato un'ottima capacità di rimozione di composti farmaceutici così come di altri inquinanti persistenti. Tale processo rientra nella categoria dei processi elettrochimici di ossidazione avanzata che porta alla formazione "in-situ" di specie fortemente ossidanti, i radicali ossidrili $^{\bullet}OH$, capaci di degradare contaminanti organici in maniera non selettiva. Tuttavia, a causa degli elevati costi di gestione dovuti all'applicazione prolungata di energia elettrica, l'esercizio del processo elettro-Fenton può essere fortemente limitato. Per far fronte a ciò, il processo elettrochimico può essere accoppiato ad un classico processo biologico, notoriamente più economico ma anche meno performante per la rimozione di composti farmaceutici. La combinazione dei due processi, comunque, dà luogo ad effetti sinergici positivi permettendo un ottimo equilibrio tra costi e benefici. Per queste ragioni, lo scopo principale della presente tesi di dottorato ha riguardato l'ottimizzazione delle condizioni operative per l'implementazione del solo processo elettro-Fenton e di un processo combinato elettrochimico-biologico al fine di massimizzare le rese depurative nei confronti di composti farmaceutici.

Nello specifico, il presente studio è stato indirizzato alla valutazione degli effetti dei parametri operativi del processo elettro-Fenton (a) sulla rimozione dei composti farmaceutici, (b) sulla mineralizzazione della sostanza organica, (c) sull'incremento delle caratteristiche di biodegradabilità degli effluenti e (d) sul consumo di energia.

La tesi può essere fondamentalmente suddivisa in tre parti, in base al tipo di acqua trattata nella sperimentazione. In prima istanza, sono state considerate differenti soluzioni acquose costituite ciascuna da un solo composto farmaceutico ai fini di valutare le caratteristiche individuali di rimozione di ogni composto. Successivamente, durante la seconda fase della sperimentazione, è stata condotta una serie di esperimenti su una soluzione sintetica caratterizzata da una miscela di 13 farmaci appartenenti a differenti classi terapeutiche. Infine, nella terza ed ultima fase, sono stati eserciti due reattori a scala di laboratorio per l'implementazione del processo combinato "Bio-elettro-Fenton" finalizzato al trattamento di un'acqua reflua reale proveniente da un'industria cosmetica. L'aumento graduale della complessità dello studio ha permesso un miglior confronto e un'analisi più dettagliata dell'influenza dei differenti parametri operativi sulle rese dei processi.

I valori ottimali di concentrazione del catalizzatore (Fe^{2+}) e di intensità di corrente da applicare sono stati tra i principali risultati ottenuti in questa ricerca. Gli esperimenti hanno

mostrato che questi due parametri hanno avuto sempre la stessa influenza sull'efficienza del processo elettro-Fenton, indipendentemente dall'acqua reflua di partenza utilizzata. Il valore ottimale di concentrazione di Fe^{2+} osservato è stato pari a 0,2 mM, mentre l'intensità di corrente ottimale è sempre rientrata nell'intervallo 100-500 mA. La più alta efficienza di rimozione dei composti farmaceutici è stata osservata per valori di intensità di corrente bassi (100-300 mA), mentre intensità di corrente più elevate (500-1000 mA) sono risultate più idonee per incrementare la biodegradabilità delle acque reflue utilizzate, a fronte, però, di un consumo di energia maggiore soprattutto per tempi di trattamento elevati. Un giusto compromesso tra minimizzazione dei consumi energetici e massimizzazione delle rese depurative deve essere necessariamente trovato e valutato caso per caso.

L'originalità di questa ricerca descritta nella presente tesi di dottorato è soprattutto relativa all'approccio di trattamento combinato proposto, ovvero l'accoppiamento del processo elettro-Fenton ad un processo biologico convenzionale. Lo studio dettagliato dell'influenza dei parametri operativi del processo elettro-Fenton sulle efficienze di rimozione dei composti farmaceutici e sull'aumento della biodegradabilità dei reflui contribuirà sicuramente ad incrementare la conoscenza generale su tale processo ma, inoltre, rappresenterà un importate passo verso la sua applicazione a livello industriale e su scala reale.

Samenvatting

Water vervuiling is één van de grootste bedreigingen voor de mensheid en om dit tegen te gaan moeten er zuiveringsprocessen worden ontwikkeld, omdat de conventionele methodes die tegenwoordig worden gebruikt niet meer effectief zijn voor het verwijderen van complexe verontreiniging. Recentelijk worden wereldwijd geneesmiddelen gevonden als verontreinigingen in een diverse watertypes. De lange termijneffecten van hun aanwezigheid in een natuurlijke omgeving zijn nog niet volledig bestudeerd, maar de potentiële uitkomsten kunnen bepalend zijn voor een duurzame toekomst.

Onder de momenteel opkomende zuiveringstechnologieën, is de electro-Fenton methode, een elektrochemisch geavanceerd oxidatie proces, in staat is om geneesmiddelen en andere hardnekkig verontreinigingen volledig te verwijderen. Dit electro katalytische proces genereert sterke oxidanten - hydroxyl radicaal ($^{\bullet}OH$) - welke niet-selectief organische vervuilingen degradeert. Door de hoge kosten verbonden aan het gebruik van elektrische energie zou de exploitatie de electro-Fenton methode te duur kunnen zijn. Een oplossing hiervoor is het combineren van de electro-Fenton methode met biologische processen welke economisch haalbaar zijn, maar minder effectief in het verwijderen van geneesmiddelen. De verwachting is dat het gecombineerde proces een synergetisch effect heeft op de kosten en de effectiviteit. Het doel van deze PhD thesis is om de operationele condities van het electro-Fenton proces te optimaliseren, zodat pharmaceutische polluenten in een combinatie met biologische processen volledig verwijderd worden.

De belangrijkste onderwerpen behandeld in dit werk zijn gerelateerd aan de invloed van operationele parameters van het electro-Fenton proces op (a) verwijdering van geneesmiddelen; (b) mineralisatie van organische materie; (c) verbetering van de biologische afbreekbaarheid; (d) energie verbruik.

Deze thesis heeft drie verschillende delen gerelateerd aan het type te behandelen waterige oplossing. Eerst werd er een mechanistische studie verricht aan waterige oplossingen van individuele geneesmiddelen om te algemene trend van hun verwijdering te begrijpen. Vervolgens werd een serie experimenten uitgevoerd op een synthetische mengsel van dertien geneesmiddelen uit verschillende therapeutische klassen. Ten slotte werden twee laboratorium bench-scale reactoren van het gecombineerde bio-electro-Fenton proces bedreven voor de behandeling van echt afvalwater van de pharmaceutische en cosmetische industry. De vooruitgang in de complexiteit van de behandelde oplossing laat toe om een uitgebreide vergelijking en analyse te maken van het effect van de operationele parameters.

De belangrijkste resultaten omvatten de optimale waarden van twee operationele parameters: de katalysator (Fe^{2+}) concentratie en de aangebrachte elektrische stroom voor een gegeven electro-Fenton opstelling. Het effect van de operationele parameters op de verwijdering van geneesmiddelen en andere organische materie waren vergelijkbaar, ongeacht de behandelde oplossing. De gevonden optimale waarden voor de Fe^{2+} concentratie waren rond de 0.2 mM. De optimale stroom was tussen de 100-500 mA. De efficiëntie van de stroom in termen van geneesmiddelen verwijdering was het hoogst bij een lage stroomsterkte (100-300 mA). De biologische afbreekbaarheid, welke een belangrijke factor is voor de biologisch nabehandeling, verbeterde bij hoge stroomsterkte (500-1000 mA). Echter leidden

de hoge stroomsterkten tot een verhoogd energieverbruik, vooral bij een verlengde behandelingstijd. Bijgevolg moet een afweging worden gemaakt tussen energiebesparing en de benodigde verwijderingssnelheid voor elke pharmaceutische stof afzonderlijk.

De vooruitgang van het onderzoek uit dit promotieonderzoek ligt in de eerste plaats in de combinatie van electro-Fenton met een biologisch proces. Een gedetailleerde studie van de invloed van operationele parameters van het electro-Fenton proces op de verwijderingskinetiek en de verbetering van de biologische afbreekbaarheid dragen niet alleen bij aan de algemene kennis van het electro-Fenton proces, maar ook aan de opschaling en de industriële toepassing van deze techniek.

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5-FU	5-fluorouracil
Abs(X)	Absorbance at wavelength X nm
ADMI	refers to ADMI (American Dye Manufacturers` Institute) Color Index
AO	Anodic oxidation
AOPs	Advanced oxidation processes
BDD	Boron-doped diamond
BOD	Biochemical oxygen demand
BOD5	Biochemical oxygen demand in 5 days
CAF	Caffeine
CAS	refers to CASNR (Chemical Abstracts Service Registry Number)
COD	Chemical oxygen demand
const	constant
d-COD	Dissolved chemical oxygen demand
DOC	Dissolved organic carbon
DSA	Dimentionally stable anode
EAOPs	Electrochemical advanced oxidation processes
EC	Electrocoagulation
EC_{50}	Half maximal effective concentration
ECP	Electrochemical peroxidation
EF	Electro-Fenton
GC-MS	Gas chromatography coupled to mass spectroscopy
HPLC	High-performance liquid chromatography
MCE	Mineralization-current efficiency
NSAID	Non-steroidal anti-inflammatory drug
OMW	Olive mill wastewater
OP	Orthophosphates
PPCP	Pharmaceuticals and personal care products
SAC	Spectral absorption coefficient
SBR	Sequencing batch reactor
SS	Suspended solids
TDS	Total dissolved solids
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TS	Total solids
TSS	Total suspended solids
TVS	Total volatile solids
VFA	Volatile fatty acids
VSS	Volatile suspended solids
UV	Ultraviolet radiation
WWTP	Wastewater treatment plant

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I would like to dedicate this thesis to my grandmother who was one of my greatest supporters, and who would be very proud of me, and whom I miss immensely.

INTRODUCTION

1. GENERAL PROBLEMATICS OF THE RESEARCH TOPIC

Water pollution becomes a more complex and global issue as new types of contaminants, their fate and distribution patterns are being discovered. Among the great variety of contaminants of emerging concern, pharmaceuticals take a large share of scientific and public concern. Biological methods for water treatment are the most wide-spread economic processes that employ microorganisms as removing agents. Yet their efficacy for the degradation of drugs can be erratic and inadequate. [1]. This is due to the bioactive characteristics of pharmaceuticals, which are designed to have a certain therapeutic action on target living organisms. As a result they can interfere with microbial degradation and escape treatment systems. This further leads to trace concentrations of these compounds being detected in natural water sources and sources of drinking water. As a matter of fact a search for new effective treatment technologies has been launched [2-4].

The new generation of treatment methods should be able to degrade all types of pollutant, even these that are toxic or refractory for conventional biological processes. One such method is called electro-Fenton, which stands for electrochemically assisted Fenton reaction. This process belongs to the group of advanced oxidation processes (AOPs). According to the definition these are the processes that employ strong reactive oxidant species, mainly hydroxyl radicals ($^{\bullet}OH$). Being a chemical agent, hydroxyl radical displays a non-selective degradation of organic contaminants, which is the main advantage of AOPs over other treatment techniques. Typically these processes are differentiated by the way hydroxyl radicals are produced: there can be based on ozone or UV radiation, Fenton reaction, etc. All of these processes require application of electricity, which makes AOPs quite a cost-intensive solution in comparison to conventional biological treatment. The benefits of electrochemical AOPs, specifically of electro-Fenton, is the fact that current can be used both directly and indirectly for the production of hydroxyl radicals, in case appropriate materials of electrodes are chosen. On one side the main process is indirect formation of $^{\bullet}OH$ through electrochemically assisted Fenton reaction, while on the other there is an auxiliary electrolysis of water that results in formation of additional $^{\bullet}OH$ on the surface of the anode.

A solution to the potentially elevated costs of electro-Fenton, as well as other AOPs, is a combination with conventional biological processes, which are commonly used nowadays [5, 6]. This is expected to have a synergetic effect between the non-selective and fast removal of pharmaceuticals by electro-Fenton and cost-effective degradation of residual organic pollutants by a biological method.

In this chapter all different aspects of this problematics - combined electrochemical-biological treatment of pharmaceutical pollution - are discussed in more detail.

1.1. Pharmaceutical micropollution: emerging issues

"*Emerging contaminants*", "*emerging risks*" and "*emerging concerns*" are commonly used terms in scientific literature nowadays. These terms refer normally to the newly emerging contaminants or contaminants of emerging concern. The definition of the US Environmental Protection Agency states that these are chemicals that have been recently discovered in water at unexpected concentration levels and whose risks to human health and environment are not fully known or understood. Pharmaceuticals and personal care products (PPCPs) constitute a major part of this type of pollutants. It is an extremely diverse group of chemicals, which are used by and for humans, animals and plants internally or externally and comprise "over-the-counter and prescription drugs, drug excipients (inert ingredients of pharmaceutical formulations), diagnostic agents (e.g. X-ray contrast media), food supplements, sun-screen agents, fragrances etc." [7].

One of the first reports on the presence of PPCPs in water environment was a study on clofibric acid, a metabolite of lipid regulators such as clofibrate, etofibrate, theofibrate [8]. Being published more than 30 years ago this study could had served as an indication to a world-scale problem related to the continuous release of pharmaceuticals. Nevertheless this issue started to be recognized and studied in detail only in the early 1990s [7]. A critical but behind-the-scene role in the discovery of pharmaceuticals in water was played by the analytical techniques, in particular mass spectrometry. Development and advance in analytical chemistry lowered the detection limits and enabled discovery of a myriad of xenobiotics present in water [7, 9].

1.2. Environmental and health concerns

The concentrations of pharmaceuticals present in the environment are at trace levels (ppb or ppt), which most probably cannot have any therapeutic effect on humans e.g. with consumption of contaminated water. Still in most of the cases the effect of these environmentally relevant concentrations is unknown for non-target organisms. Non-mammalian species (e.g. lower vertebrates) have different pharmacokinetics and pharmacodynamics, thus presence of micropollutants can lead to unexpected negative outcomes on their reproductive and hormone systems, immune defense, neurological behavior, etc. [10].

Apart from direct exposure, accumulation of pharmaceuticals in the environment can lead to higher concentrations inside the organisms rather than in the surface water. Therefore consumption by humans or predators of such species might cause higher levels of exposure via food chain (e.g. by eating fish) [11]. The so-called bioaccumulation factor (BAF) is defined as the ratio between the concentration of the drug in the animal tissue to its concentration in water. Bai et al. [12] have analyzed 19 commonly used antibiotics, two of which (enrofloxacin and roxithromycin) have been determined to be bioaccumulative (BAF > 5000 L/kg). Another important issue, which challenges scientific research with its high complexity, is related not only to

accumulative effect but to chronic exposure of the mixture of pharmaceuticals present in sub-therapeutics concentrations [13].

Documenting the effects of pharmaceuticals is largely based on the chemical analytical techniques. It should be stressed that the identification of an unknown compound is dependent on the extraction and purification methods, apart from the characteristics of the detection equipment. These methods are used for analysis of environmental samples and selected for compounds with certain characteristics. However, there is also a significant part of nonextractable, unresolved or overlooked compounds, which makes environmental forensics a laborious and time-consuming task [14]. This means that the research dedicated to this domain is often restricted by the employed methods.

1.3. Identification of pharmaceuticals of interest

Among all the variety of pharmaceuticals it was necessary to determine a list of interest, which could have been further used to address the objectives of this work. The composition of the effluent from hospitals, as one of the drug-containing type of wastewater, was summarized from these studies [1, 15-21].

The concentration of pharmaceuticals in water shows different patterns depending on the type of hospital, period of the day, season of the year. Therefore it was established that in order to make a representative selection of molecules, it was necessary to select the ones with highest concentrations and the ones that were most frequently detected. Table 1 represents the results of such selection: 10 pharmaceuticals with the highest concentrations were selected from each of five reference publications. This table contains 39 pharmaceuticals (instead of 50), which makes it obvious that 11 molecules were the same for some publications. In this table molecules were classified according to the class of pharmaceuticals they belong.

Table 1. Top 10 pharmaceuticals with the highest concentration from each reference (concentration in μg/L).

Pharmaceutical	[15]	[16], range	[17]		[1]	[18]		
			Ullevall hospital	Rikshospitalet		Hospital A	Hospital B	Hospital C
II-RECEPTOR ANTAGONISTS								
ranitidine							1.3	3.0
ANALGESICS								
aspirin	384.00							
paracetamol	63.10	1 - 10^2	177.67	1368.474	107.00	4.5	4.1	2.5
ANTICONVULSANT/ANTIEPILEPTICS								
carbamazepine	6.08						0.97	
ANTIBIOTICS								
ciprofloxacin	5.03	10^{-1} - 10^2	54.049	39.843	31.98	12.0	1.6	21.0
clarithromycin								11.0
erythromycin	0.86	1 - 10^2						
gabapentin					19.40			
levitiracetam					11.02			
lincomycin	4.82							
norfloxacin		10^{-2} - 10^2						
ofloxacin						19.0	3.7	31.0
oxytetracycline			3.743	2.294				
sulfamethoxazole		10^{-3} - 10^2	1.375	4.107		4.2	1.8	
tetracycline		10^{-2} - 10	1.537	4.178				
trimethoprim	7,26	10^-2 - 10	14,993	11,899				
triclosan		10-10^2						
triclosan		10-102						

Table 1. (continued)

Pharmaceutical	[15]	[16], range	[17]		[1]	[18]		
			Ullevall hospital	Rikshospitalet		Hospital A	Hospital B	Hospital C
ANTI-INFLAMMATORY								
diclofenac	6.88	10^{-1} - 10^{2}	1.63	14.934				
ibuprofen		10^{-1} - 10^{2}	0.987	8.957				2.6
indomethacin							2.2	
ketoprofen						5.0	1.1	
ANTINEOPLASTICS								
5-fluorouracil		$10\text{-}10^{2}$						
BETA-BLOCKERS								
atenolol		10^{-1} - 10^{2}				5.1	2.4	5.8
metoprolol		$1\text{-}10^{2}$	2.232	25.097				
propranolol		10^{-1} - 10						
sotalol						4.8		5.1
DIURETIC								
hydrochlorotiazide						1.8		
furosemide						14.0	7.1	5.8
OTHERS								
benzotriazole					23.57			
galaxolide		1 - 10						
tonalide		1 - 10						
RADIO CONTRAST MEDIA								
diatrizoic acid					348.70			
iomeprol					439.00			
iopamidol					2599.00			
iopromide		10^{-1} - 10^{4}			170.60			
ioxatalamic acid					342.00			
HORMONES								
estriol			0.784	0.785				
PSYCHOSTIMULANTS								
caffeine	182.00	10^{2}						

Table 2 represents the top-20 pharmaceuticals that were detected in more than one study. This list gives an idea of the most common molecules that are present in most of the effluents.

Table 2. Most detected pharmaceuticals (concentration in µg/L).

Reference / Pharmaceutical	[19]	[1]	[15] *	[17] *		[20]	[18]			[21]	
				Ullevall hospital	Riks hospital		Hospital A	Hospital B	Hospital B2	General hospital	Psychiatric hospital
II-RECEPTOR ANTAGONISTS											
Ranitidine		1.565±0.763				0.4-1.7	1.5	1.3	3		
ANALGESICS											
Paracetamol	9.3	107.0±85.7	25.5	58.372	329.852	0.5-29	4.5	4.1	2.5	64	9.61
ANTICONVULSANT/ANTIEPILEPTICS											
Carbamezapine	1.0	0.222±0.118	0.827			0.03-0.07	0.73	0.97	0.95	0.5	5
ANTIDEPRESSANTS											
Fluoxetine		<0.03					0.005	0.027	0.056		0.54
ANTI-INFLAMMATORY											
Diclofenac	2.9	0.833±0.179	1.92	0.819	2.737	0.06-1.9	0.3	0.22	0.51	2.35	73
Indometacin		0.069±0.080					2.5	2.2	0.53		
Ibuprofen	7.8			0.499	2.440	1.5-151	1.7	0.6	2.6	11.4	26.3
Mefenamic acid		6.140±1.779					0,33	0,12	0,55		5.38
Naproxen	11.6	<5.6	3.17				2.3	0.41	4.9		
ANTIMICROBIALS											
Trimethoprim		0.930±0.890	1.62	4.302	3.896	0.01-0.03					
Erythromycin		0.188±0.297	0.330			0.01-0.03	0.16	0.082	0.16	1.4	
Azithromycin		0.139±0.156					0.03	0.047	0.8	2.08	
Clarithromycin		2.555±1.558					0.06	0.058	11	5.41	
Metronidazole		3.388±1.322				1.8-9.4	0.72	0.033	0.96		
Ciprofloxacin		31.98±14.06	1.98	23.336	15.531		12	1.6	21		
Norfloxacin		5.933±3.390					0.07	0.034	0.35		
Sulfamethoxazole		3.476±4.588	25.3	0.404	1.389		4.2	1.8	2		
BETA-BLOCKERS											
Atenolol		2.315±0.632				0.1-122	5.1	2.4	5.8		
Metoprolol		1.325±0.330		1.072	5.811		0.83	0.74	1.1		
Propanolol		0.116±0.041				0.2-6.5	0.023	0.085	0.043		

Note: * - average values

A combined list of pharmaceuticals is given in Table 3. From each therapeutic class of pharmaceuticals one or two representatives were selected for this work based on the following criteria:

- availability of previous research on the molecule for proper comparison of results;
- good solubility of a chosen molecule;
- low photosensitivity and tendency to being photodecomposed.

Finally among the molecules presented in Tables 1 and 2 a selection of thirteen pharmaceuticals, which were supposed to constitute a synthetic pharmaceutical mixture, was done: 5-fluorouracil (antineoplastics), aspirin (analgesics), atenolol (beta blocker), caffeine (psychoactive), diclofenac and naproxen (non-steroidal anti-inflammatory drugs), diatrizoate meglumine (contrast agent), erythromycin (macrolide antibiotics), norfloxacin (fluoroquinolone antibiotics), paracetamol (antipyretics/analgesics), ranitidine (histamine H_2-receptor antagonist), sulfamethoxazole (sulfomanide antibiotics), tetracycline (tetracycline antibiotics). Two drugs from this list (caffeine and 5-fluorouracil) were chosen to be studied in detail for their degradation kinetics.

Table 3. Selection list of pharmaceuticals studied in the frame of this work.

Top concentrations	Top detection	Chosen for mixture	Study in detail
5-fluorouracil		■	■
aspirin		■	
atenolol	atenolol	■	
	azithromycin		
benzotriazole			
caffeine		■	■
carbamazepine	carbamezapine		
ciprofloxacin	ciprofloxacin		
clarithromycin	clarithromycin		
diclofenac	diclofenac	■	
diatrizoate meglumine		■	
erythromycin	erythromycin	■	
estriol			
	fluoxetine		
furosemide			
gabapentin			
galaxolide			
hydrochlorotiazide			
ibuprofen	ibuprofen		
indomethacin	indometacin		
iomeprol			
iopamidol			
iopromide			
ioxatalamic acid			
ketoprofen			
levitiracetam			
lincomycin			
	mefenamic acid		
metoprolol	metoprolol		
	metronidazole		

Table 3. (continued)

Top concentrations	Top detection	Chosen for mixture	Study in detail
naproxen	naproxen	■	
norfloxacin	norfloxacin	■	
ofloxacin			
oxytetracycline			
paracetamol	paracetamol	■	
propranolol	propanolol		
ranitidine	ranitidine	■	
sotalol			
sulfamethoxazole	sulfamethoxazole	■	
tetracycline		■	
tonalide			
triclosan			

2. ELECTRO-FENTON PROCESS

The electro-Fenton and other electrochemical advanced oxidation processes are discussed in detail in Chapter I. Here following matters are dealt with: advantages of electro-Fenton process over other types of treatment, as well as reactor setup and operating parameters.

2.1. Advantages and drawbacks of electro-Fenton

In contrast to conventional processes, electro-Fenton and other AOPs are able to generate hydroxyl radicals - strong oxidant species. Therefore, the advantages of the whole group of AOPs include:

- mineralization of pollutants. Likewise other AOPs, electro-Fenton process is able to break down organic pollutant molecules until carbon dioxide, water and inorganic ions. It means that these processes are destructive; consequently no other additional treatment is needed e.g. valorization and/or disposal of activated sludge as in biological processes.

- non-selective degradation. Hydroxyl radicals, being highly reactive species, are able to attack almost any type of chemical bonds, with slight preferences towards unsaturated and aromatic bonds. As it is a chemical treatment process, such terms as toxic or refractory pollutants are not applicable here.

The biggest advantage of electro-Fenton over other AOPs is related to the fact that pollutants can be destroyed by both indirect oxidation via Fenton reaction and by direct anodic oxidation (in the case a suitable anode is used).

Electro-Fenton is also a type of Fenton process that is assisted electrochemically. As a result it possesses a few important improvements of the classical Fenton oxidation.

- generation of hydrogen peroxide in the solution. Consequently, the cost related to purchase, transportation and storage of this reactant are avoided.

- re-generation of ferrous ion (catalyst) at the cathode, meaning there is no need in continuous addition of catalyst and no iron sludge generation.

The main drawbacks of this process are mainly due to the operation of electro-Fenton reactor at acidic pH and due to potentially high energy consumption when mineralization of pollutants is required.

2.2. Reactor configuration and electrodes` materials

The electro-Fenton reactor has two different cell configurations: undivided and divided. The latter consists of two compartments (anodic and cathodic) with two separate solutions (anolyte and catholyte, respectively). The separation between different compartments is ensured by cathionic/anionic membranes, grits or diaphragms. If both compartments are physically separated then the connection in-between is provided by a salt bridge. Such cells have the advantage of preventing hydrogen peroxide, which is produced on the surface of cathode, from being destroyed on the surface of anode. In an undivided cell reactor the hydrogen peroxide is oxidized to oxygen gas on the surface of anode via hydroperoxyl radical [22]:

$$H_2O_2 \rightarrow HO_2^{\bullet} + H^+ e^- \quad (1)$$

$$HO_2^{\bullet} \rightarrow O_2 + H^+ e^- \quad (2)$$

The advantage of undivided kind of cell is connected to lower cell potential and consequently lower energy consumption, as the voltage penalty of the separator is avoided. Minimization of energy consumption was one of the objectives of this work (section 1.6.), so undivided cell was preferred over a divided one. The schematic representation of the electro-Fenton reactor that was studied here is provided on Figure 3.

Materials of the electrodes have a crucial influence on the process efficiency of any electrochemical process. Cathode is the working electrode, as production of Fenton's reagents is dependent on its effectiveness. Larger cathode area favors both H_2O_2 production and Fe^{2+} regeneration leading to a higher concentration of hydroxyl radicals [22, 23]. Carbonaceous electrodes were best cathodes for electrogeneration of H_2O_2, while cathodes made from transition metals, like copper, stainless steel, lead, platinum and nickel can cause decomposition of peroxide [24]. Moreover stainless steel electrodes get gradually corroded [24]. A new area of research is elaboration of graphitic membranes, which can be used as a working electrode in electro-Fenton process [25]. Among all the variety of available materials carbon felt was preferred in this study for a number of reasons: (a) commercial availability; (b) reasonable costs and most importantly (c) large three-dimensional surface area. Regarding the anode material a boron-doped diamond (BDD) was selected due to its high O_2-overpotential, which enhances the removal rates by promoting the additional formation of hydroxyl radicals on its surface through reaction 3 [26]:

$$BDD + H_2O \rightarrow BDD(^{\bullet}OH) + H^+ + e^- \quad (3)$$

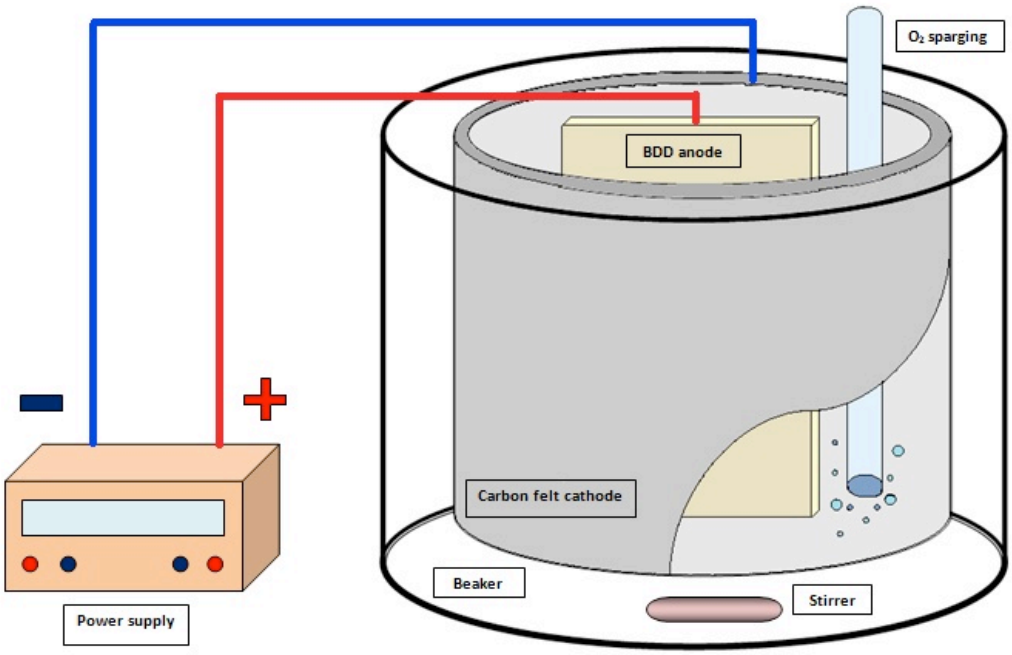

Figure 1. The schematic representation of electro-Fenton reactor used in this study.

2.3. Operating parameters of electro-Fenton

Brillas et al. [22] have defined operation parameters that influence the degradation of organics during electro-Fenton treatment: O_2 feeding, stirring rate, liquid flow rate, temperature, solution pH, electrolyte composition, applied potential or current, iron catalyst and initial pollutant concentrations. Here these parameters will be discussed shortly regarding the scale of their impact.

- Ferrous ions concentration

Concentration of hydroxyl radicals is dependent on the concentration of ferrous ions, therefore greater amount of iron increases Fenton efficiency. However excess of ferrous ions could have a scavenging effect on the radicals as showed in the reaction 4 [27, 28].

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \qquad (4)$$

Concentration of a catalyst depends on the cathode employed. At pH=3 Fe^{3+} optimal concentration is 0.1-0.2 mM for carbon felt cathode, while for carbon-PTFE GDE cathode it should be 0.5-1.0 mM Fe^{2+} [22].

- Current

Current characteristics influence directly both the cost of the treatment and its effectiveness. Higher current densities as well as oxygen sparging rate influence greatly the hydrogen peroxide production in a positive way and therefore formation of hydroxyl radicals. Higher current fosters faster regeneration of ferrous ions from ferric ions. [27]. High voltage increases the degradation at the beginning, but after a certain time (about 30 min as stated by most researchers), the rate becomes constant and much slower [29].

- pH solution

The pH of 3 is optimal according to most of the reviews on electro-Fenton [22, 27]; 2.5 - in the study of Umar et al. [30]. Variation of this parameter throughout most of the studies does not exceed the range 2-4. With pH > 5 hydrogen peroxide becomes unstable and in basic solution it decomposes [27]:

$$H_2O_2 \rightarrow H_2O + O_2 \quad (5)$$

At lower pH a termination of the Fenton reaction chain occurs due to the formation of stable Fe complexes with hydrogen peroxide, which deactivates catalyst [27].

- Temperature

Temperature effects electro-Fenton efficiency in the same way as the pH does: there is an optimal range and both higher and lower temperatures have negative impacts. Temperatures below 8 ^oC as stated by Umar et al. [30] decreases kinetics and the reaction rate. Nidheesh and Gandhimathi [27] have concluded that the optimal temperature lies in the range of 20-30^oC. At the same time high temperature favors autodecomposition of hydrogen peroxide and lower solubility of oxygen in water, therefore causing decrease in the peroxide concentration. Ultimately room temperature is a sufficient condition that allows avoiding unnecessary costs.

- Electrolyte

Electrolyte is required if the conductivity of the wastewater is not sufficient for the electrochemical reaction. Typical electrolytes are sulfate and chloride [22]. The most common one according to Jiang and Zhang [29] is sodium sulfate and its optimal concentration should not be more that 3.6 mg/L. There was no correlation found between amount of electrolyte and COD removal, therefore no excessive addition is needed as it increases the operational costs.

3. SETUP FOR COMBINED ELECTROCHEMICAL-BIOLOGICAL PROCESSES

Coupling electro-Fenton, or any other AOPs, with biological treatment can be organized in two ways: electro-Fenton as pre-treatment or as a post-treatment (Figure 4) [5]. The choice of a sequence of treatment mostly depends on the characteristics of the influent to be treated.

Generally, if the effluent has a BOD_5/COD ratio much lower than 0.33 (BOD_5: biochemical oxygen demand after 5 days, COD: chemical oxygen demand) and contains toxic substances that interfere with the microbial activity, the EAOPs as pretreatment helps to increase this ratio (i.e. biodegradability) and to decrease toxicity to the threshold level when biological methods can be applied [31, 32]. However, the toxicity of the influent should be watched carefully, as high toxic levels after electro-Fenton might slow down biological digestion [34]. On the other hand, when the wastewater contains mostly biodegradable compounds, but also some refractory organics, it makes sense to apply cost-effective biological treatment at first, and then to treat it with EAOPs to eliminate the recalcitrant compounds [35]. Hydroxyl radicals are non-selective, so in case when EAOP is done as pre-treatment, they will be used

to eliminate pollutants, which can be treated easily and at a reasonable cost using conventional methods [31, 33].

Another interesting point to be considered is the intermediate adjustment step, as operational conditions might not be the same for the processes that are coupled. For example, pH requirement for electro-Fenton is around 3 and biological treatment takes place normally at circumneutral pH [22]. In case when electro-Fenton is applied as the first step, adjustment of pH to 7 after such pre-treatment will precipitate Fe in the form of sludge. For this reason an additional intermediate step of withdrawing ferric hydroxide sludge might be necessary [36].

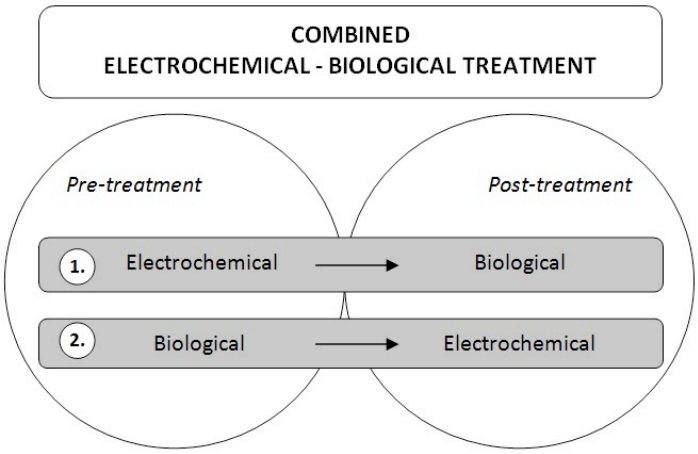

Figure 2. Ways of combining electrochemical and biological processes.

Comninellis et al. [35] gave a more specific advice on suitable treatment regarding the main wastewater characteristics: biodegradable and refractory fraction. In the case when the effluent is biodegradable, conventional biological methods are applicable. If the effluent is still biodegradable, but total organic carbon (TOC) is high (> 0.1 g/L), an advanced oxidation pre-treatment is recommended. Otherwise, if contaminants are biorecalcitrant, TOC is low (< 0.1 g/L) and high toxicity is observed, an application of AOPs is sufficient without any consequent biotreatment. Yet forwarding of the effluent to a municipal sewage treatment plant should be considered in order to eliminate the residual pollution. Providing that all the above-mentioned parameters are low (biodegradability, total organic carbon, toxicity), but other characteristics, like odor and color, are not satisfying, it is adequate to simply apply advanced oxidation.

4. RESEARCH OBJECTIVES

The two main aims of this study (Figure 5) were (1) to optimize operation of electro-Fenton and (2) evaluate a potential of EF being combined to a biological process.

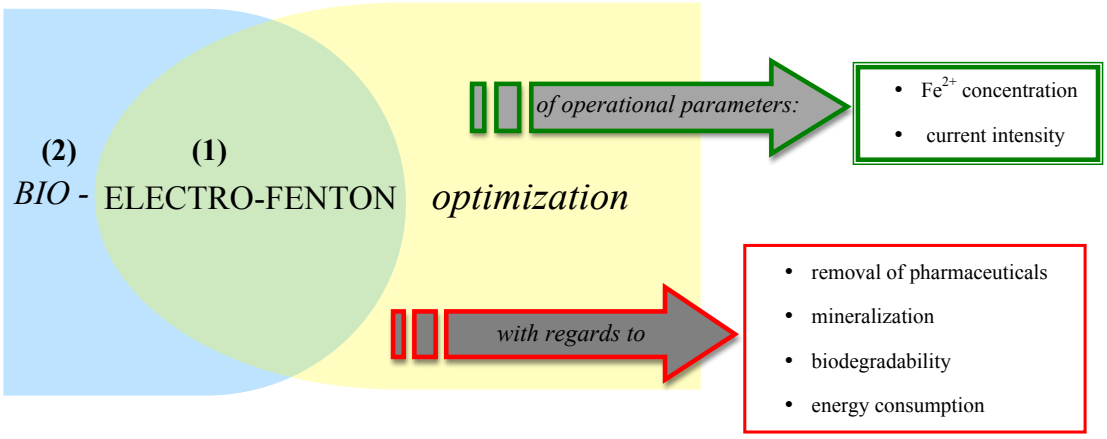

Figure 3. Research objectives of the thesis.

Process optimization was done with regards to two operating parameters: concentration of catalytical Fe^{2+} and current intensity in dependence of treatment time. The objectives of optimization were (a) maximal removal of pharmaceuticals; (b) maximal mineralization; (c) enhancement of biodegradability; (d) minimization of energy consumption. To accomplish it the thesis was divided into three distinct parts related to the type of treated aqueous solution. First, a mechanistic study was conducted on aqueous solutions of individual pharmaceuticals in order to understand general trends of their removal. Next, a series of experiments was carried out on a synthetic mixture of thirteen pharmaceuticals from different therapeutic classes (see section 1.3). Lastly, laboratory bench-scale reactors of a combined bio-electro-Fenton process were operated for the treatment of real wastewater. The advance in the complexity of the treated solution allowed a comprehensive comparison and analysis of the influence of the operating parameters.

The novelty of the research presented in this PhD thesis is firstly attributed to the combination of electro-Fenton to a biological process. A detailed study of the influence of operating parameters of the electro-Fenton process on removal rates and biodegradability enhancement will contribute not only to the general knowledge on the electro-Fenton process, but also to the advancement towards its upscaling and then further towards the industrial application of this technique.

5. STRUCTURE OF THE THESIS

The thesis consists of six chapters (Figure 6), introduction and conclusions. The Chapter 1 is dedicated to the literature review of the combined electrochemical-biological processes for treatment of different pollutants: dyes and textile wastewater; olive processing wastewater; pharmaceuticals and hospital wastewater.

The experiments are divided in three parts depending on the type of treated aqueous solution (Part II, III and IV on Figure 6).

Chapter 2 and 3 present the results from a mechanistic study conducted on aqueous solutions of individual pharmaceuticals (caffeine and 5-fluorouracil, respectively) in order to understand general trends of their removal.

Next, Chapter 4 discusses a series of experiments that was carried out on a synthetic mixture of thirteen pharmaceuticals from different therapeutic classes. The objective was to analyze the removal of pharmaceuticals as well as biodegradability enhancement and toxicity evolution after the pre-treatment by EF.

The laboratory bench-scale reactors of a combined bio-electro-Fenton process were operated for the treatment of real wastewater from pharmaceutical production. The results are given in Chapter 5. The main objective of this study was to evaluate the potential of bio-electro-Fenton for removal of pharmaceutical contamination, comparing the two configurations: EF-biological process and biological process-EF.

The last part "Conclusions" summarizes the conclusions from experimental studies, provides general discussion as well as future perspectives.

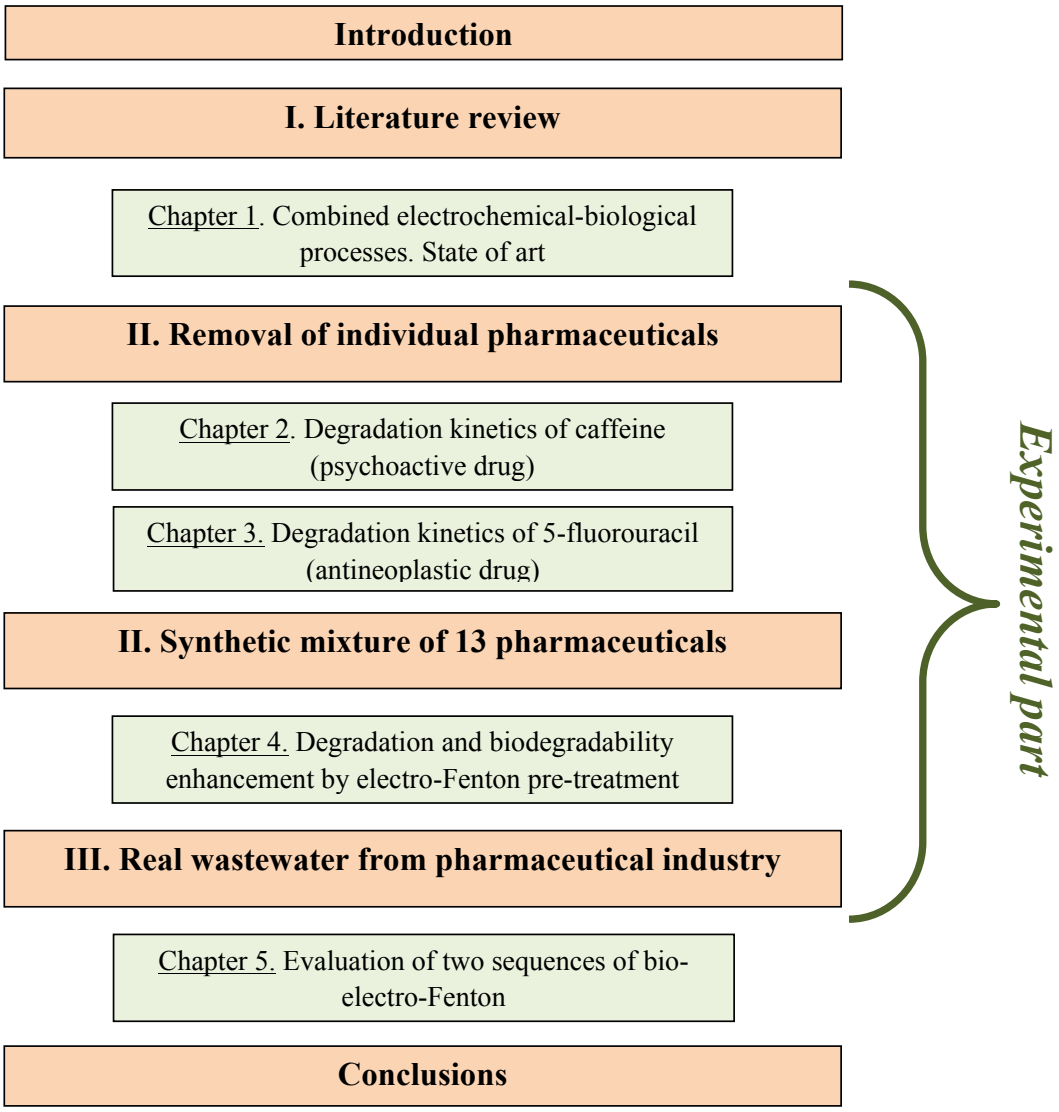

Figure 4. Structure of the thesis.

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CHAPTER I

Electrochemical advanced oxidation and biological processes for wastewater treatment: A review of the combined approaches

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ABSTRACT

As pollution becomes one of the biggest environmental challenges of the XXI century, pollution of water threatens the very existence of humanity, making immediate action a priority. The most persistent and hazardous pollutants come from industrial and agricultural activities, therefore effective treatment of this wastewater prior to discharge into the natural environment is the solution. Advanced oxidation processes (AOPs) have caused increased interest due to their ability to degrade hazardous substances in contrast to other methods, which mainly only transfer pollution from wastewater to sludge, a membrane filter or an adsorbent. Among a great variety of different AOPs, a group of electrochemical advanced oxidation processes (EAOPs), including electro-Fenton, is emerging as environmentally friendly and effective treatment processes for the destruction of persistent hazardous contaminants. Still energy consumption and related investment and operational costs slow down a large-scale implementation. This concern can be resolved by combining EAOPs with a biological treatment. In such a synergetic way, removal efficiency is maximized, while minimizing operational costs. The goal of this review is to present cutting-edge research for treatment of three common and problematic pollutants and effluents: dyes and textile wastewater; olive processing wastewater; pharmaceuticals and hospital wastewater. Each of these types is regarded in terms of recent scientific research on individual electrochemical, individual biological and a combined synergetic treatment.

Keywords: Biodegradation, Combined treatment, Dye, Electrochemical processes, Olive mill, Pharmaceutical, Wastewater

I.1. INTRODUCTION

Biological processes are by far the most widespread conventional methods for wastewater treatment. They possess important advantages that could not be overcome by any other treatment so far, as they are cost-effective, well studied and therefore easily modified according to local needs [1-3]. However, they have some serious limitations with degrading toxic and/or refractory organic pollutants, and as wastewater discharge regulations become stiffer, there is an urgent need of proper and effective treatment. For the time being the only available solution for elimination of non-biodegradable organic pollutants is the advanced oxidation processes (AOPs) [4-9]. The main agent is a hydroxyl radical, which, among its other important properties, has the second highest redox potential after fluorine, and consequently it is strongly oxidizing [10, 11]. Generally, it is non-selective in oxidation, but it reacts more easily with compounds that have non-saturated bonds like aromatic hydrocarbons [10, 12]. Hydroxyl radicals have a short life [2, 13], as a result they can be self-eliminated from the treatment system.

Among a great variety of AOPs, specifically the electrochemical advanced oxidation processes (EAOPs) have been increasingly attracting attention due to their perspective applications [14-20]. These technologies have demonstrated high-level removal of pollutants and even their complete mineralization [21-26]. EAOPs are environmentally friendly technologies that enable extensive production of hydroxyl radicals under optimal conditions of applied electric current and appropriate catalyst [27, 28]. Application of current allows for full automatization of the process on a large scale, which is one of many distinct features of EAOPs.

The main drawback, which holds back up-scaling, is relatively high costs [1], especially those related to energy consumption during extensive treatment time until the complete mineralization. There is an interesting solution to high costs i.e., a combination of EAOPs with biological treatment [29, 30]. Handling refractory organics with EAOP often leads to formation of intermediates, such as short-chain carboxylic acids, which are hardly oxidizable by $^{\bullet}OH$ radicals, but are no longer toxic to the microflora and are biodegradable [31]. Therefore their further oxidation with cost-intensive AOPs is not economically viable. Nevertheless, a consecutive biological post-treatment is a sound solution due to its low cost and well-studied technological process [32-35]. EAOPs can also be applied as a final step after biological treatment in order to remove residual refractory pollution and to make the effluent comply with discharge limits for either direct discharge into receiving waters or to a sewer system [31, 36, 37].

This review gives an overview of the cutting-edge research on individual electrochemical, individual biological processes and combined electrochemical-biological treatment, as well as basic principles behind EAOPs and their coupling with biological treatment. Such comparison between these three treatment processes for given types of effluent has not been done before.

I.2. BACKGROUND INFORMATION ON ELECTROCHEMICAL ADVANCED OXIDATION PROCESSES

Electro-Fenton is an electrochemical advanced oxidation process (EAOPs). There are different types of electrochemical treatment methods in general and EAOPs are only a part of them. There are three basic groups (see Figure I.1): electrochemically induced coagulation, electrochemical reduction and electrochemical oxidation.

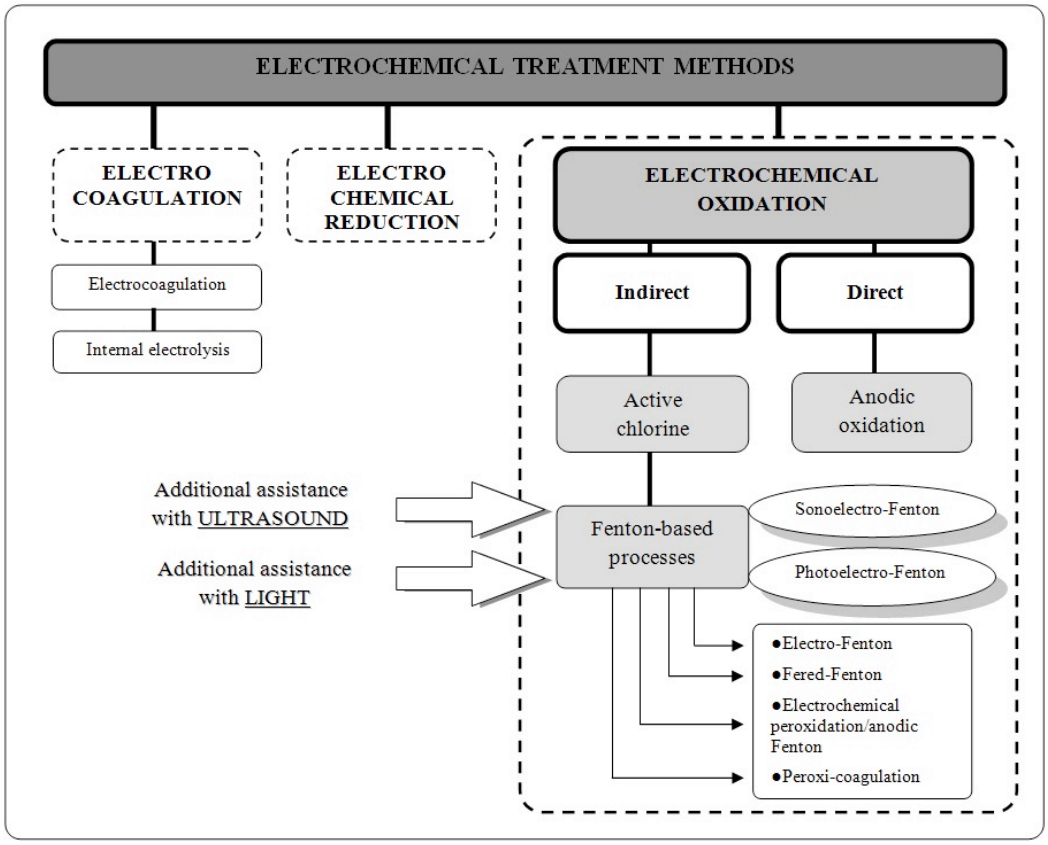

Figure I.1. General classification of electrochemical treatment processes.

Electrocoagulation (EC) is a process in which the current is applied to sacrificial Al or Fe electrodes that results in a release of metal ions which act as coagulants [22-26]. One variation of electrocoagulation is called internal micro-electrolysis. During regular EC electric power is supplied externally, whereas micro-electrolysis is run as a galvanic cell. For instance, a mixture of iron chips and granular activated carbon is added to the wastewater and their interaction with each other is regarded as microgalvanic cells providing electrons [27]. A significant number of such cells despite their small size is able to ensure considerable flow of electrons in the water matrix [28, 29].

Electrochemical reduction is a treatment process where pollutants are reduced by electron transfer at cathode. Significantly fewer papers investigating electrochemical reduction have been published, as in general it offers poor decontamination in comparison with oxidation methods [30, 31].

There are two fundamental ways in which electrooxidation process happens i.e., direct and mediated oxidation [32]. The first type is based on direct oxidation of pollutants at the

anode, so the process is limited to its surface. Mediated process grounds on the production of reactive species in the bulk solution, which consequently oxidize organic contaminants [33]. Direct oxidation essentially requires power supply and electrodes, whereas mediated oxidation implicates also addition of reagents (mostly a catalyst), depending on the type of oxidizing agent that is to be produced [34]. It is important to note that if the treatment in place is indirect oxidation, then both processes occur simultaneously in the solution [35].

2.1. Direct oxidation

Anodic, or direct, oxidation happens owing to either direct electron transfer from the electrode to the pollutant molecule or by oxidation through reactive species, which are formed on the surface of the electrode (reaction 1). The latter (oxidation of pollutant R) follows this reaction, where the electrode is denoted as M [32, 36]:

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^- \quad (1)$$

$$M(^{\bullet}OH) + R \rightarrow M + mCO_2 + nH_2O + H^+ + e^- \quad (2)$$

Hydroxyl radicals, which are known to have one of the highest redox potentials, are formed on the surface of the anode through oxidation of water. Yet, two slightly different mechanisms can be distinguished depending on the properties of the anode material, which is the key parameter for removal efficiency. If the anode is active, its interaction with hydroxyl radical produced on its surface is strong and leads to the formation of higher state oxides or superoxides (MO) [37]:

$$M(^{\bullet}OH) \rightarrow MO + H^+ + e^- \quad (3)$$

In this case reactive species are attached to the anode and defined as chemisorbed radicals. As the attraction force is strong enough, oxidation is limited to the surface of the electrode. Examples of active anode materials are Pt, oxides of Ru and Ir. Nonactive anodes such as boron-doped diamond (BDD) [38], oxides of Sn [39] and Pb [40] do not participate in the process to a great extent and their role is restricted to promotion of radical formation. Here, when the anode has a large O_2-evolution overpotential (BDD or PbO_2), the radicals formed are physisorbed, which means that the force of interaction is weak and radicals are barely attached to the electrode. Accordingly oxidation of organics occurs more effectively, since $^{\bullet}OH$ are generated in large quantities and they are available for reaction with pollutants.

2.2. Mediated oxidation by active chlorine

During the mediated oxidation process oxidizing species are generated *in situ* and are responsible for consequent degradation of organics in the bulk solution. For example, active chlorine species are produced from chloride ions, which added to the solution as a salt (e.g. sodium chloride). Chloride ions are oxidized at the anode to form molecular chlorine [32, 36]:

$$2Cl^- \rightarrow Cl_2 + 2e^- \quad (4)$$

Chlorine is further transformed into hypochlorous acid and hypochloride ions (OCl^-) (E_{redox} = 1.59 V) as a result of its dissolution in water [41, 42]:

$$Cl_2 + H_2O \rightarrow HClO + Cl^- + H^+ \quad (5)$$

$$HClO \leftrightarrows H^+ + OCl^- \quad (6)$$

2.3. Electro-Fenton and related Fenton-based processes

The Fenton chemistry is based on the Fenton's reagents, a mixture of hydrogen peroxide and Fe^{2+}, which were found to be very oxidizing by an English chemist H.J.H. Fenton [43]. Later it was discovered, that this is due to the formation of hydroxyl radicals: oxidation power of H_2O_2 is enhanced by its oxidation to $^{\bullet}OH$, following Fenton's reaction 7 [44, 45]. The chain reactions of Fenton's chemistry are presented below [46]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (7)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+ \quad (8)$$

Ferrous ions can be regenerated from Fe^{3+} through reaction 8, in order to further catalyze Fenton's reaction. However, the reaction 8 is much slower than the reaction 7 [33, 47]. As a consequence Fe^{3+} accumulates in the solution and then precipitates as $Fe(OH)_3$ sludge. The removal of Fe from solution decreases the process efficiency. In addition, it requires adding a large amount of reagents that increases operational costs. Another important drawback of classical Fenton process is consumption of formed $^{\bullet}OH$ by hydrogen peroxide and ferrous ions following wasting reactions 9 and 10 [33, 48]:

$$^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^- \quad (9)$$

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \quad (10)$$

At high reagents' concentrations, these reactions can strongly hinder the efficiency of the process since $HO_2^{\bullet}$ formed is a weaker oxidant compared to $^{\bullet}OH$.

The EAOPs, in particular electro-Fenton, have been developed to overcome the drawbacks of the classical Fenton process and to increase the efficiency of pollutant removal. One of the reagents, H_2O_2, is electrochemically generated *in situ* in a controlled way:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (11)$$

Regarding Fe^{2+}, it is initially added to the solution and then electrochemically regenerated [49]:

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (12)$$

Based on the above, the electro-Fenton process appears to be as environmentally friendly and efficient, since *in situ* generation of the Fenton's reagents allows to avoid: i) the cost of reagents and risks related to their transport and storage, ii) the formation of process sludge and iii) parasite reactions 9 and 10 due to maintenance of small reagent concentrations in the medium. As a result, the oxidation/mineralization power of the process is significantly enhanced [33].

It is really important to highlight the fact that electro-Fenton, as the most common and widely studied process, is only one type of electrochemical oxidation processes based on the Fenton's chemistry and it should not be confused with other variations. Other related processes are distinguished according to which reagents are produced in solution and/or are added externally. There can be different setups, which are graphically represented in the Figure I.2:

1) generation of iron anions using a sacrificial iron anode and external addition of hydrogen peroxide;
2) *in situ* generation of both iron anions (sacrificial cathode) and hydrogen peroxide;
3) addition of both reagents to the solution and regeneration of Fe^{2+} from reaction (12);
4) generation of hydrogen peroxide and regeneration of initially added Fe^{2+} catalyst.

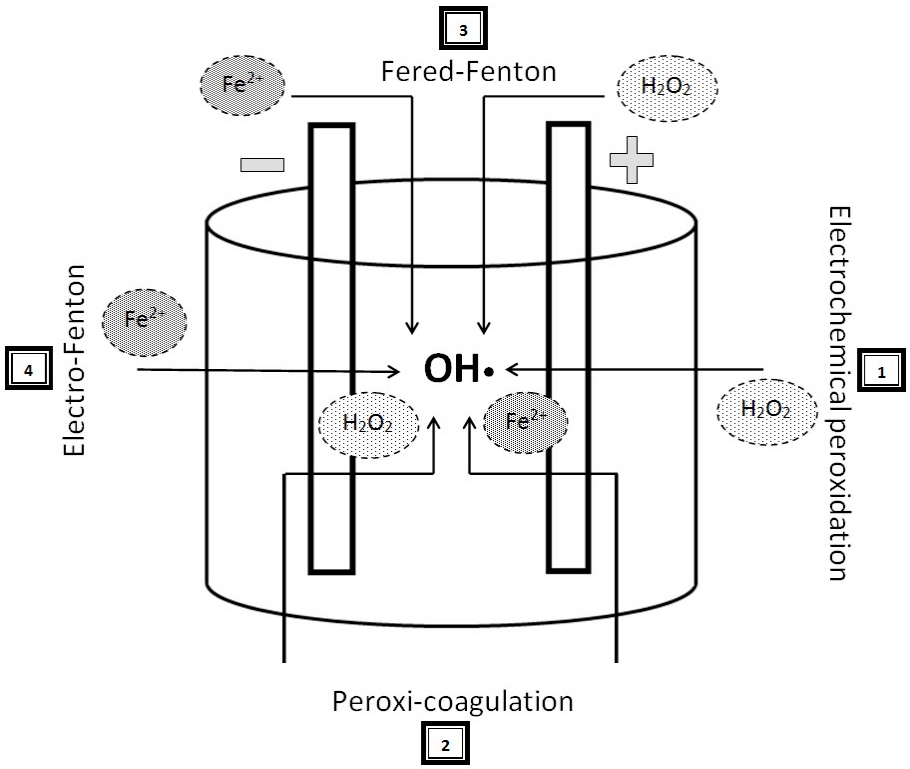

Figure I.2. Electrochemically induced Fenton process.

The first variation has two different types depending on the kind of reactor used. The undivided cell, where all reactions happen simultaneously, is used for electrochemical peroxidation (ECP) [50]. The use of a divided cell, where cathodic and anodic compartments are separated, was initially proposed as an improvement of ECP and was named anodic Fenton treatment [51].

The second variation is peroxi-coagulation. Here the sacrificial anode is the source of Fe^{2+} ions (reaction 13). H_2O_2 is produced at the carbonaceous cathode (reaction 11) [52]. During this process oxidation of pollutants by $^{\bullet}OH$ and coagulation via $Fe(OH)_3$ formation take place simultaneously: as part of organic pollutants is oxidized, while the other part is coagulated [33].

The third variation is called Fered-Fenton, which involves one-time addition of full load of Fe source (either Fe^{2+} or Fe^{3+}) and a continuous addition of hydrogen peroxide throughout the treatment progress in an undivided cell. Fe^{3+} is then reduced to Fe^{2+} at the cathode following reaction 12 [53, 54].

Finally, the last process describes classical electro-Fenton treatment. This process is by far the most popular than any other electrochemically enhanced Fenton treatment. Here H_2O_2 is electrogenerated at a carbonaceous cathode (reaction 11) from oxygen or air sparging and Fe^{2+}, which is supplied at the start in a catalytic amount, is constantly regenerated in an electrocatalytical way from reaction 12 [55].

Electrochemical Fenton processes can be enhanced by additional application of UV radiation or ultrasound. In that case they are called respectively photoelectro-Fenton and sonoelectro-Fenton [56, 57]. If the source of light is solar radiation, the process is named solar photoelectro-Fenton [58, 59]. Besides, energy consumption due to electrochemical processes,

additional energy is required for photo- or sound assistance, therefore additional installation and operational costs are involved in comparison to classical EAOPs.

I.2. COMPARISON OF TREATMENTS OF DIFFERENT TYPES OF TARGET POLLUTANTS AND WASTEWATERS

This section presents an overview on the treatment of three different effluents, which are often investigated: wastewater containing dyes, pharmaceuticals and olive oil mill wastewater.

I.2.1. Dyes and textile wastewater

Industries related to dye production or application generate a great amount of wastewater. The main consumer is textile manufacturing, which is considered to be one of the most polluting industries [38]. Moreover, a lot of dyes are used in food, pharmaceutical, cosmetic, tannery, paper and pulp, electroplating industries [39]. It is estimated that 5-10% of the dyes in total are lost in the effluent during the dying process, but when it comes to reactive dyes in particular, as much as 50% of the initial load is present in the dye bath effluent [40].

The presence of dyes in the water is visible even at low concentrations [41]. However nuisance from dyes in water is not only an aesthetic issue [42]. These compounds absorb and reflect sunlight, restricting the activity of microorganisms and plants, and as a result shifting the ecosystem's balance [39]. Regarding the influence on human health and well-being, most dyes, as well as their precursors and by-products have a toxic, mutagenic and carcinogenic nature and are able to accumulate in the food chain [40, 43].

Table I.1 highlights each of the discussed publication.

Table I.1. Examples of treatment of dyes and textile wastewater by different methods.

Reference	Method	Operating parameters	Wastewater characteristics	Removal
[44]	two stage biological treatment	anaerobic continuous flow rotating disc reactor rotation = 4 rpm temperature = *const* = 34ºC hydraulic retention time = 15 h aerobic continuous flow rotating disc reactor rotation = 4 rpm room temperature = 18-23 ºC aeration with air = 40 L/h hydraulic retention time = 7.5 h	synthetic wastewater with reactive vinyl sulfone diazo dye C. I. Reactive Black 5 [RB5] = 530 mg/L pH = 7	Color removal -65% (absorbance at 436, 525, 620 and 595 nm)
[45]	anoxic/aerobic treatment	anoxic phase volume = 15 L mixed liquor volume = 7 L dissolved oxygen = < 0.5 mg/L pH= 6.5-7.4 hydraulic retention time = 8 h aerobic phase volume = 15 L mixed liquor volume = 7 L dissolved oxygen = 8.7 mg/L pH= 6.5-7.4 hydraulic retention time = 16 h	wastewater from fiber reactive dyeing of cotton COD = 1153 mg/L Color = 2130 ADMI [Reactive Red 198] = 0.015 g/L [Reactive Yellow 86] =0.025 g/L [Reactive Black]= 0.013 g/L [Reactive Violet] = 0.029 g/L	COD – 95% Reactive Red 198 – 74% Reactive Yellow 86 – 12% Reactive Black 5 - 60% Reactive Violet -85%

Table I.1. (continued).

Reference	Method	Operating parameters	Wastewater characteristics	Removal
[46]	biological	constructed wetland vertical-horizontal-sub-surface flow 2 vertical flow beds and 1 horizontal flow area = 80 m^2 planted with common reed (*Phragmites australis*) with 5 clumps/m^2 hydraulic retention time = 24 h	real textile wastewater COD = 276-1379 mg/L BOD = 99-350 mg/L TOC = 74-530 mg/L N_{total} = 7-82 mg/L $N_{organic}$ = 6-77 mg/L NH_4-N = 0.2-4.5 mg/L SO_4 = 76-2200 mg/L Anionic surfactants = 1-10 mg/L SAC(436 nm) = 5-34 SAC(525 nm) = 9-100 SAC(620 nm) = 4-16	average values COD -84% TOC -89% Sulfate – 88% Anionic surfactants -80% N_{total} – 52% $N_{organic}$ – 87% NH_4-N +331% spectral absorption coefficient (SAC) SAC(436 nm) -88% SAC(525 nm) -93% SAC(620 nm) -89%
[47]	biological	up-flow constructed wetland planted with macrophyte *Phragmites australis* aeration temperature = 23 ± 3°C hydraulic retention time = 3 days	synthetic wastewater containing azo dyes pH = 7.7 [Acid Orange 7 (AO7)] = 100 mg/L COD = 450 ± 8 mg/L TN = 59 ± 2 mg/L TP = 6.5 ± 0.3 mg/L NH_4-N = 36 ± 4 mg/L NO_3-N = 28 ± 2 mg/L	COD – 86% AO7 – 94% TN – 54% TP – 15% NH_4-N -99% NO_3-N -22%
		same design no aeration		COD – 74% AO7 – 96% TN –49% TP – 12% NH_4-N -24% NO_3-N -99%
[48]	anaerobic	anaerobic batch reactor volume = 5 L methanogenic sludge = 0.5 L pH = 7 room temperature hydraulic retention time= 23 days biogas production = 7.2 L/day	mixture of textile dye wastewater and starch wastewater in ration 30:70 COD = 3440 mg/L	COD – 81.0% Color – 87.3%
[49]	electro-Fenton	open undivided cell Pt cylindrical mesh anode and carbon felt cathode [Fe^{3+}] = 0.2 mM current intensity = 400 mA electrolyte [Na_2SO_4] = 0.05 mM pH = 3.0 ambient temperature mixing aeration 1 L/min	synthetic solution in ultra-pure water COD = 1000 mg/L [malachite green] = 0.05 mM [crystal violet]=0.05 mM [methyl green]=0.05 mM [fast green] = 0.05 mM	COD -96%
[50]	electro-Fenton	undivided cell BDD anode and O_2-diffusion cathode pH = 3.0 [Fe^{2+}] = 1.0 mM [Na_2SO_4] = 0.05 mM current density = 33 mA/cm^2 temperature = 35.0 ºC mixing aeration with O_2 12 mL/min reaction time = 9 h	synthetic solution [Indigo carmine] = 220 mg/L TOC = 100 mg/L	decolorization -100% TOC -91%
[51]	solar photoelectro-Fenton	recirculation flow plant undivided cell BDD anode and air-diffusion cathode pH = 3.0 [Fe^{2+}] = 0.5 mM flow rate = 200 L/h current intensity = 0.5 A temperature = 35 °C aeration reaction time = 360 min	[Acid Yellow 36] = 108 mg/L TOC = 50 mg/L	decolorization -100% mineralization -95%

Table I.1. (continued).

Reference	Method	Operating parameters	Wastewater characteristics	Removal
[52]	electro-Fenton	undivided cell 2 graphite sheets electrodes electrode surface = 15 cm^2 inter-electrode gap = 6 cm pH = 2 [$FeSO_4$ * $7H_2O$] = 150 mg/L current voltage = 16V aeration with compressed air = 1L/min reaction time = 45 min	[Azure B] = 6.9 mg/L	decolorization -96.21%
[53]	electro-Fenton	undivided cell Pt anode and carbon felt cathode pH = 3.0 [Fe^{2+}] = 0.1 mM electrolyte [Na_2SO_4] = 50 mM current intensity = 250 mA ambient temperature reaction time = 6 h	[Direct Orange 61] = 0.53 mM	TOC -98%
[54]	electro-Fenton	undivided cell carbon felt cathode and BDD anode pH = 3.0 [$FeSO_4$ * $7H_2O$] = 10^{-4} M electrolyte [Na_2SO_4] = 50 mM current intensity = 250 mA stirring = 900 rpm ambient temperature reaction time = 9 h	[Acid Orange 7] = 0.53 mM	TOC – 98%
[55]	electro-Fenton	open undivided cell Pt mesh anode and carbon felt cathode [Fe^{3+}] = 0.2 mM electrolyte [Na_2SO_4] = 50 mM current density = 3 mA/cm^2 pH = 3 aeration = 1 L/min reaction time = 6 h	separate solutions of following dyes [Basic Blue 41(BB41] = [Basic Yellow 28 (BY28)] = [Basic Red 46 (BR46)] = 0.05 mM	BB41 -93% BR46 -86% BY28 -77%
			aqueous solution with all three dyes with concentration 0.05 mM	TOC -82%
[56]	electro-Fenton	undivided cell [Fe^{2+}] = 0.1 mM electrolyte [Na_2SO_4] = 0.05 M pH = 3.0 current intensity = 300 mA BDD anode and carbon felt cathode aeration stirring room temperature reaction time = 4 h	[Azure B] = 0.1 mM	complete decolorization mineralization 90%
	anodic oxidation	undivided cell current intensity = 500 mA BDD anode and carbon-felt cathode electrolyte [Na_2SO_4] = 0.05 M pH = 3.0 room temperature aeration mixing reaction time = 4 h	[Azure B] = 0.1 mM	complete decolorization
[57]	electro-Fenton	undivided cell reticulated vitreous carbon cathode and Pt gauze anode and saturated calomel reference electrode [$FeSO_4$] = 0.1 mM electrolyte [Na_2SO_4] = 0.05 M pH = 3 cathode applied potential = -1.0V vs. reference electrode temperature = *const* = 25ºC stirring and aeration reaction time = 120 min	Sunset Yellow FCF azo-dye in aqueous solution [dye] = 5 mM	complete decolorization mineralization of dye 97%

Table I.1. (continued).

Reference	Method	Operating parameters	Wastewater characteristics	Removal
[58]	electro-Fenton	undivided cell gas-diffusion cathode and a Pt grid anode $[Fe^{2+}]$ = 1.0 mM, one time addition pH = 3 current = 200 mA stirring aeration flow rate = 20 mL/s temperature = 35ºC reaction time = 4 h	Synthetic solution [Alizarin Red] = 120 mg/L	COD - 93%
[59]	electro-Fenton	undivided cell in continuous mode graphite bar electrodes $[Fe^{2+}]$ = 150 mg/L pH = 2 inter-electrode gap = 240.4 mm reaction time = 21 h	a mixture of dyes [Lissamine Green B] = 8.5 mg/L [Methyl Orange] = 1.5 mg/L [Reactive Black 5] = 70 mg/L [Fuchsin Acid] = 15 mg/L	decolorization -43% TOC -46%
[60]	electro-Fenton	divided flow-by cell in batch mode 50 graphite rings as 3D cathode and Pt/Ti plate anode $[Fe^{2+}]$ = 20 mM pH = 3 current = 68 A/m^2 aeration rate = 0.3 dm^3/min reaction time = 150 min	dyeing wastewater Color = 1094 ADMI pH = 6.2 COD = 2942 mg/L Cl^- = 238 mg/L	color -70.6%
[61]	anodic oxidation	Nb/BDD anode and stainless steel cathode inter-electrode gap = 1.55 cm mixing reaction time = 12 h	biologically pre-treated dye wastewater COD = 532 mg/L TOC = 138 mg/L BOD_5 = 80 mg/L pH = 7.76 color = 200 dilution times sulfates = 1940 mg/L chlorides = 3000 mg/L salinity = 6146 mg/L conductivity = 5.53 mS/cm	COD final = 99 mg/L toxicity negligible after 6h of treatment more than 50 % of main organic pollutants measured were not detected after treatment
[62]	active chlorine / biological	Pt anode and Zirconium spiral cathode pH = 6.3 current = 2 A density = 50 mA/cm temperature = 70 ºC reaction time = 4 h up-flow fixed bed reactor biolite as support media pH = 7.0 volume = 11 L aeration hydraulic retention time = 4.5 h	AMBI (5-amino-6-methyl-2-benzimidazolone) = 94% of TOC TOC = 5053 mg C/ L BOD_5 = 136 mg O_2/L COD = 14350 mg O_2/L conductivity = 15.8 mS/cm Cl^- =7335 mg/L NH_4^+ = 6.8 mg/L PO_4^{3-} = 0.9 mg/L	AMBI -100% DOC -100%
[63]	electro-Fenton (pre-treatment step to biological treatment)	divided cell 1st compartment contained cathode and reference electrode (saturated calomel electrode); 2nd compartment – anode in HCl solution graphite felt cathode and Pt anode $[Fe^{2+}]$ = 1 mmol/L cathode potential = -0.5 V vs. SCE aeration in 1st compartment stirring temperature = *const* = 30ºC reaction time = 4 h	[Methyl Red] = 100 mg/L	decolorization -93.8% mineralization – 8.2%
			[Orange II] = 100 mg/L	decolorization – 77.1% mineralization -7.7%
			[Biebrich Scarlett] = 100 mg/L	decolorization – 97.8% mineralization – 18.8%

Table I.1. (continued).

Reference	Method	Operating parameters	Wastewater characteristics	Removal
[64]	anodic oxidation /biological	undivided cell in batch mode anode ruthenium coated on titanium metal (RuO_X-TiO_X) and cathode – stainless steel plate current density = 7.6 A/dm^2 retention time = 3 h biological isolated bacteria from textile site soil sample (*Pseudomonas* sp. and *Micrococcus* sp.) retention time = 8 days	synthetic textile effluent containing Procion Scarlet chlorides = 6997.83 mg/L sulfates = 118.414 mg/L COD = 880 mg/L conductivity = 16.66 m mhp/cm TSS = 1100 mg/L TDS = 8980 mg/L pH = 12-13 color = purple red	Color - 98.5% COD – 90%
[65]	biological/anodic oxidation	up-flow anaerobic sludge blanket reactor thermophilic conditions = 55ºC anodic oxidation plug-flow cell in batch mode BDD anode and stainless steel foil cathode electrolyte [NaCl] = 0.1 M current density = 20 mA/cm^2 reaction time = 8 h	simulated textile effluent containing dye [Acid Orange 7] = 300 mg/L COD = 1010 ±70 mg O_2 / L TOC = 662 ± 19 mg C /L pH = 8.3±0.3 conductivity = 2.94 ±0.01	COD – 90% TOC -30% Abs (486 nm) -100% Abs (250 nm) -91%

Note. Abs(X nm) – absorbance at wavelength X nm; BOD – biochemical oxygen demand; BOD_5 - biochemical oxygen demand after 5 days; COD – chemical oxygen demand; *const* – constant; DOC – dissolved organic carbon; SAC – spectral absorption coefficient; TDS – total dissolved solids; TN – total nitrogen; TOC – total organic carbon; TP – total phosphorous; TSS – total suspended solids.

I.2.1.1. Individual biological treatment

Removal of dyes by conventional methods, such as biological processes, has certain specifications. In general aerobic treatment did not prove to be effective, as toxicity of such an effluent is too high for aerobic bacteria [41, 66, 67]. In contrast, decolorization by use of the anaerobic method is confirmed by multiple scientific publications [40]. Azo dyes as one of the most common classes (they account for around 60-70% of all dyes) are taken here as an example. A characteristic azo bond (-N=N-) responsible for color cannot be broken by aerobic treatment, but anaerobic conditions cause its cleavage [39]. The by-products are aromatic amines, which are refractory to anaerobic bacteria, but not for aerobic species. Therefore, a combined system of sequential anaerobic/aerobic treatment is suggested as a plausible option [68], where the goal of aerobic post-treatment is mineralization of dye by-products. Often an addition of co-substrate to the waste, e.g. glucose, tapioca, yeast extract or hydrolyzed starch etc., can enhance biodegradability [40, 69-71]. Such combined treatment can be implemented in two distinct manners: as a sequential application of both processes and as one integrated process, taking place in one reactor.

Such a combined anaerobic/aerobic system was evaluated in terms of decolorization, mineralization and toxicity removal of the synthetic solution containing reactive azo dye Reactive Black 5 [44]. After almost 23 h of combined treatment color removal reached 65% (measured as absorbance at four different wavelengths). A combination of sequential anoxic and aerobic treatments was also tested [45]. Solutions with four reactive dyes (Reactive Yellow 86, Reactive Red 198, Reactive Black 5 and Reactive Violet 5) were treated by a combined process and by a single aerobic process, which served as a control. High removal rates by the combined system were achieved for COD (95%) (Figure I.3.), as well as for

individual dyes: Reactive Red 198 (74%), Reactive Violet 5 (85%) and Reactive Black 5 (60%). Yet Reactive Yellow 86 was hardly degraded (12%). Authors attributed that to a different chemical structure of this specific dye. As discussed before, decolorization of azo dyes happens due to cleavage of the azo bond, which acts as an electron acceptor. In its structure Reactive Yellow 86 has, in addition to azo bond, an alternative group, -$CHONH_2$, which is more susceptible to reduction. Subsequently the azo bond, responsible for color, was left almost unchanged and low decolorization was observed. Principally, experimental data showed that the anaerobic phase of combined treatment outperformed the aerobic phase in terms of color removal.

Gnanapragasam et al. [48] analyzed a single anaerobic batch reactor for treatment of a textile effluent mixed with starch wastewater as co-substrate. An optimal ratio of two wastewaters was found to be 30:70 and with such a setup both decolorization and COD removal were significant: 87% and 81% respectively.

An alternative option for treatment of real dye-rich wastewater is a constructed wetland. A pilot plant of this technology showed good results with a retention time of 24 h [46]. Different parameters were analyzed, such as COD, TOC, sulfates, anionic surfactants and spectral absorption coefficients at different wavelengths. All in all, the removal of these parameters was in the range of 80-90%. The only relatively low criterion was removal of total nitrogen (52%), which is accredited to more than a three-fold increase in ammonia concentration after the treatment. Authors attributed that to the lack of oxygen in the constructed wetland and as a result formation of de-oxygenated nitrogen compound such as ammonia.

Another constructed wetland research work [47] also reported good removal results, but with longer hydraulic retention time (3 days). Here two different setups were compared: a constructed wetland with and without aeration for treatment of a synthetic wastewater containing azo dye Acid Orange 7 (AO7). Different dye concentrations and respective removal efficiencies were analyzed. In Table I.1 the removal results for the highest concentration of AO7 (100 mg/L) in both types of wetlands are presented. COD removal was higher for non-aerated wetland, but as the concentration of AO7 doubled, it dropped by 8%. This is probably due to an accumulation of aromatic amines, by-products of partial azo dye degradation. For aerated wetland COD removal remained constant despite a drastic increase in dye concentration and even improved with longer hydraulic retention time. This phenomenon can again be referred to the presence of aromatic amines and their enhanced degradation by aerobic microorganisms. Regarding color removal, non-aerated wetland showed constantly good (96%) results. In contrast, aerated wetland especially with higher dye concentrations showed some drastic changes in the middle of its operating period. The situation went back to normal after a few months. Generally speaking, an option of constructed wetland seems to give good results and have low expenditure, however more research especially for the continuous operating mode is required [72].

I.2.1.2. Individual electrochemical treatment

There has been growing interest in EAOPs as a pre-treatment option for degradation of dyes in wastewater. A great deal of current research investigates the application of

electrochemical processes in terms of toxicity removal and biodegradability improvement of the pre-treated effluent. Most of the studies are also following the degradation kinetics and by-products, which could lead to crucial understanding of the appropriate duration of treatment and chemical composition of the final effluent, which is important for further biological processes. EAOPs can also be a plausible standalone option. However, it is important to remark in this section that: (i) several authors have used different electrochemical methods for depollution of dyes (already reported by Martinez-Huitle and Brillas, 2009), and (ii) some papers published by other authors have proposed the electrochemical treatment as pre-treatment of real textile effluents, before biological treatment [73, 74].

Sires et al. [49] studied the degradation of four different triphenylmethane dyes (Malachite Green, Crystal Violet, Methyl Green, Fast Green) in an aqueous medium by electro-Fenton. Single-compound solutions were used to study the degradation peculiarities of each individual dye. Malachite Green was chosen as a model dye for the study of degradation kinetics and the influence of operating parameters on the COD removal and current efficiency. COD removal was found to be directly dependent on the current intensity; which is explained by generating a greater number of hydroxyl radicals. Current efficiency is a time-dependent parameter: it decreases with longer treatment time. This could be referred to the initial fast degradation of dye molecules and further formation of more hardly oxidizable compounds. This phenomenon is clearly seen from the degradation kinetics of the solution containing four dyes. The overall treatment time was 25 h and COD was finally removed by 96% (Figure I.3.). However, almost 50% of the removal was attained only after 2 h and around 70% after 8 h. During the last 10 h of treatment COD was removed further by only 13%. Current efficiency at the beginning of the treatment was almost 100% and then it gradually decreased. It is evident from such results, that after a certain reaction time, when the dyes are largely degraded, biological post-treatment is a logical solution to the efficiency and cost issue.

Basic dyes, such as Azure B, are a class of dyes used mainly to acrylic fibers and requiring a mordant for fixation. Martinez and Uribe [75], who studied its degradation, gave a brief outline of the research of other scientific groups that were studying degradation of this dye. Of the four studies, two of them were on photocatalytic methods. As this compound is recalcitrant, no studies dealing with biological degradation have been reported so far. However, there are a few publications of its removal by means of electrochemical processes. Olvera-Vargas et al. [76] studied the decolorization and mineralization of a solution of Azure B dye comparing anodic oxidation and electro-Fenton with different electrode setups. Complete decolorization of dye happened fast for both processes with the BDD anode: 10 min for electro-Fenton and 45 min for anodic oxidation. Mineralization followed a similar pattern and both processes required about 4 h to achieve 90% mineralization. After a 4 h treatment TOC removal was significantly slower and could hardly reach 100% in the next 4 h. Rosales et al. [52] experimented on electro-Fenton with graphite electrodes for degradation of Azure B solution with a concentration of more than 20 times higher than in Olvera-Vargas et al. [56]. They achieved almost complete decolorization within 45 min of treatment without monitoring TOC removal.

Sunset Yellow FCF is an example of an azo dye. Ghoneim et al. [57] treated its

synthetic aqueous solutions with electro-Fenton. After having optimized the operating parameters, they have achieved a complete decolorization and almost complete mineralization (97%) in 2 h. Another study [58] compared anodic oxidation and electro-Fenton for degradation of Alizarin Red, an anthraquinone dye, refractory for traditional treatment methods. Optimized electro-Fenton process showed total decolorization and excellent COD removal (93%).

Elimination of Acid Orange 7 from the aqueous solution by electro-Fenton was studied using Pt and BDD anode [54]. Mineralization experiments under optimal conditions showed almost total mineralization after 9 h treatment for both anodes. The main difference was a higher degradation rate during the first three hours for BDD anode. BDD was concluded to be more effective in this study, as well as in other publications dealing with treatment of different pollutants [77-81]. Hammami et al. [53] studied the degradation and mineralization of Direct Orange 61 dye. Factorial experimental design showed that the main influencing parameters were current density and initial concentration of dye. A statistical analysis was used to determine optimal operating parameters (Table I.1), which would eliminate TOC by almost 100% in 6 h of treatment. Degradation of Indigo carmine colorant was studied by Flox et al. (2006) using two types of electro-Fenton treatment: a classical and photo-enhanced. The most effective system in terms of mineralization was electro-Fenton with a BDD anode, which mineralized 91% of TOC after 9 hours and reached complete mineralization after 13 hours. However, another study that compared electro-Fenton system with solar photoelectro-Fenton, states that the latter treatment yields better mineralization results. After 6 hours of solar enhanced treatment TOC was reduced by 95%, while for classical electro-Fenton mineralization was 46%. The authors relate this to the fact that Fe^{3+} complexes are photolyzed by UV irradiation, thus producing more Fe^{2+} catalyst.

Not all the publications show such promising results, as the ones mentioned above, for dye removal with electrochemical processes, especially when switching from batch to continuous mode of operation. After optimization of some operating parameters Rosales et al. [59] tried to operate a bubble electro-Fenton reactor in a continuous mode for removal of four dyes from the synthetic solution: Lissamine Green, Methyl Orange, Reactive Black, Fuchsin Acid. After an extensive reaction time of 21 h neither mineralization nor decolorization have reached even 50% removal. It is important to note that more experimental data on continuous operation is required in order to fully understand the degradation pattern and the affecting parameters.

A comparison of different methods is an essential part of critical scientific research; it helps to place treatment processes of interest among other similar methods and to better understand their advantages and drawbacks. For the degradation of three cationic dyes, Bouafia-Chergui et al. [55] compared two technologies: electro-Fenton and photo-Fenton. Optimal operating parameters were defined for both processes. Experiments were carried out on aqueous solutions of individual dyes and a mixture of all three dyes. Individual dye solutions were quickly mineralized by more than 95% by both processes. However, the results of treatment of dye mixture (with total concentration 3 timer higher that for individual treatments) showed better TOC removal for photo-Fenton, but the reaction time was also longer: electro-Fenton removes 82% of TOC in 6 h whereas photo-Fenton required 6 h for

below 80% removal. It was concluded that electro-Fenton is a preferable treatment method due to its lower energy consumption and environmentally friendly nature. It is also worth noting that after such treatment the degradation products are biodegradable short-chain carboxylic acids, which means that there is always a possibility to apply biological post-treatment for complete removal of all organics. In fact, as all aromatics are removed from the solution after 2 h treatment, biological post-treatment can then be implemented to achieve total mineralization.

There is really little research available on electrochemical treatment of real effluents. Experiments with real dyeing wastewater from a textile plant were carried out by Wang et al. [60]. Their publication focused on the optimization of parameters for highest decolorization. After 150 min of electro-Fenton treatment in a divided cell reactor with graphite 3D cathode and Pt/Ti anode decolorization of the wastewater was reduced by 70.6%. However for a complete analysis of removal efficiency it would be necessary to follow the COD evolution.

I.2.1.3. Combined electrochemical-biological treatment

As mentioned above, hybrid processes can be combined into two basic ways depending on the sequence of each treatment: electrochemical treatment as pre- or post-treatment. Torres et al. [62] investigated degradation of 5-amino-6-methyl-2-benzimidazolone (AMBI) by electrochemically generated active chlorine/biological treatment. AMBI is an intermediate substance in dye production and is refractory to biological degradation. The samples of real wastewater from a Swiss company for socks production were diluted at a ratio of 1:5. No chlorine was added, as the concentration of chloride ions in the solution was already high (> 7 g/L). The BOD_5/COD ratio after electrolysis was extremely high (0.9) and biodegradability tests showed that there was no need for acclimation of biomass. The biological part was represented by a fixed bed reactor inoculated with activated sludge. The final removal rates for both ADMI and dissolved organic carbon were absolute.

The degradation of three different azo dyes (Methyl Red, Biebrich Scarlet and Orange II) by electro-Fenton prior to a biological step was studied by Elias et al. [63]. Among the two removal parameters that were followed for electro-Fenton − decolorization and mineralization, the first one was of greater importance, as full mineralization could be achieved during consequent biological treatment. Methyl Red and Biebrich Scarlet, were decolorized by more than 90%, whereas Orange II was hardly degraded by 80% after 4 h of electro-Fenton. Mineralization after the same reaction time was in the range of 8-18% for all three dyes. Further experiments on a model dye (Methyl Red) confirmed the trend for complete decolorization in a short time (1 h), very low mineralization, increase in biodegradability (BOD_5/COD increased to 0.24) and removal of toxicity (EC_{50} reduction by 165%). All those trends are favorable for consequent biological treatment in order to remove residual pollution.

Electrochemical pre-treatment was also studied by Senthilkumar et al. [64]. Anodic oxidation was performed prior to a biological oxidation by bacteria isolated from a textile outlet site. Four setups were compared: single biological, single electrochemical, a combined treatment and an integrated one. Among all of them, the combined treatment (electrochemical

and biological steps were operated sequentially), showed best removal results with 90% and 98.5% COD and color removal, respectively (Figure I.3.).

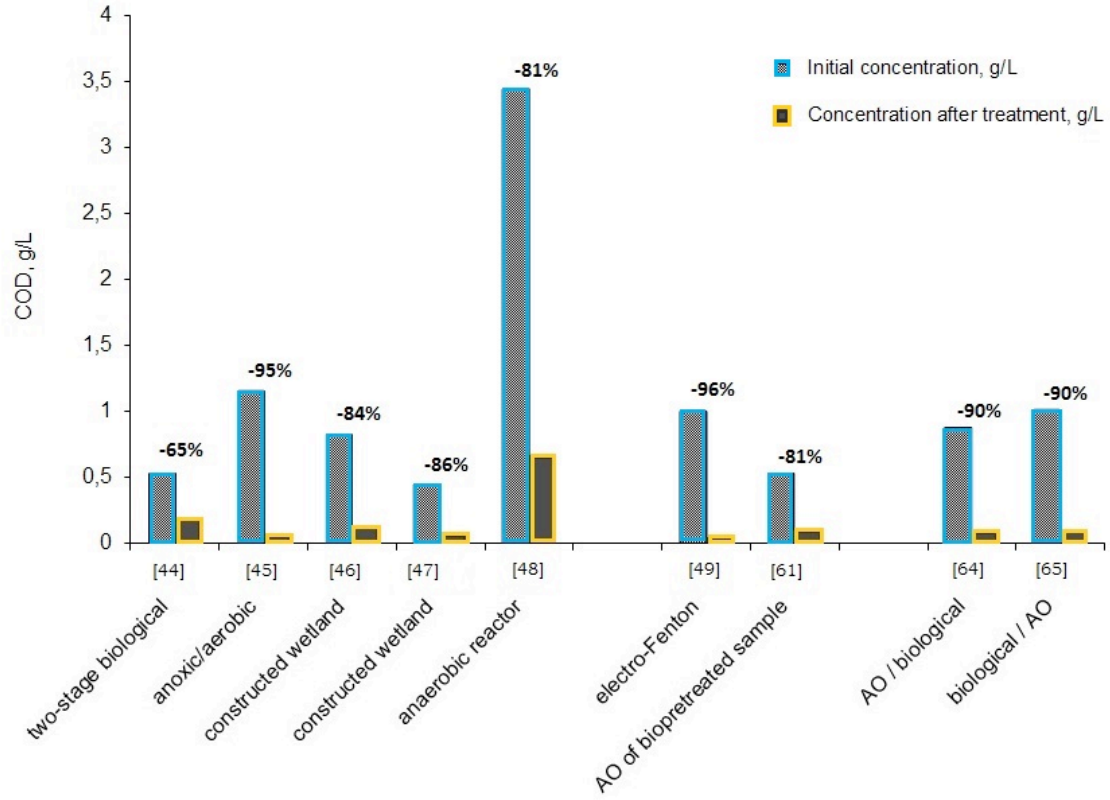

Figure I.3. Removal of COD from dye-containing effluents (COD – chemical oxygen demand, AO – anodic oxidation).

Anodic oxidation as post-treatment after anaerobic method was studied by Carvalho et al. [65]. Research material simulated textile wastewater containing the azo dye Acid Orange 7. Optimized running parameters of both steps of treatment led to almost complete removal of color and COD (Figure I.3.), but at the same time mineralization was low. In the previously discussed study [47] the synthetic effluent also contained Acid Orange 7 and was treated in the constructed wetland. It was noticed that anaerobic treatment degrades the dyes well, but the presence of oxygen enhances removal of by-products. In this study the electrochemical post-treatment aimed at removing the remaining dyes and their degradation products. Other work reported by Zhu et al. [61] focused on anodic oxidation as post-treatment option of already pre-treated dye wastewater. The effluent from a dye factory was treated using chemical (zero valent iron and coagulation) and biological (up-flow anaerobic sludge bed reactor) methods. After 12 h anodic oxidation with BDD anode, more than half of the pollutants (especially refractory humic substances) were removed, the rest degraded to carboxylic acids and short-chain alkanes. Final COD met the national discharge limits for China (< 100 mg/L) and therefore this treatment option was regarded as very promising for post-treatment of dye effluents.

As stated in a critical review of Hai et al. [41] none of the experimented methods for dye removal, neither biological nor physicochemical processes have been proven to be a sufficient and effective treatment. Biological methods are often unable to meet discharge limits and therefore require additional post-treatment [61, 82]. Even anaerobic process, especially if operated in a continuous mode can face sudden disruptions in biogas production [44], the rational explanation to the processes is yet to be found. For example, acid and reactive dyes, which follow azo dyes in their popularity, are hardly adsorbed on sludge [41], thus making microbial degradation barely achievable. On the other hand, electrochemical treatment shows better removal results with shorter reaction time. Moreover, not only is decolorization of effluent possible, but also there is a substantial COD decrease. A graphical comparison of COD removal is presented in Figure I.3. To conclude, further research should concentrate more on experimenting with real effluents and on operating a treatment system in a continuous mode and not only in batch.

I.2.2. Olive processing wastewaters

More than three quarters of the world production of olive-based products belong to the countries of the Mediterranean basin (Spain, Italy, Greece, Turkey and Tunisia are the biggest producers). The production process generates significant amounts of solid and liquid waste. Wastewater from the production of either olive oil or edible olives has a high content of polyphenols and long-chain fatty acids [83, 84]. The wastewater from the edible olive industry is similar in its constituents but less polluted in organics [85]. The solid waste is called olive pomace and consists of olive pulp, water, seeds and remaining oil, which is removed at central extraction plants and used, for instance, for soap production. Such treatment has no waste apart from the remaining pomace, but its open-air storage before disposal produces olive pomace leachate due to a high water content [86].

Despite an almost exponential increase in olive oil production, there is no appropriate regulation of its waste and there is no suitable treatment method in place. Most commonly the effluents from production are directly discharged into receiving water bodies. In the best-case scenario prior to discharge this wastewater is kept in storage ponds [87]. During the storage period of three summer months excess water evaporates and its volume reduces, although anaerobic conditions that develop in ponds cause unpleasant odors and spread of flies [88]. Such a situation of improper treatment is mainly due to lack of regulation especially in leading countries of the olive industry [88].

The summary of publications on treatment of olive wastewater is given in Table I.2.

Table I.2. Treatment of olive processing wastewater by individual electrochemical and biological methods and integrated processes.

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal
[89]	aerobic treatment	lime neutralization / aerobic digestion C/N ratio by weight = 48.08 (with NH_4NO_3) pH = 6.18 (with CaO) temperature = 25ºC hydraulic retention time = 45 days	pH = 6.18±0.01 Moisture = 80.93±0.20 TOC = 50% TKN = 1.04±0.05% COD = 104.95%	COD -4.8% Phenols -75.8% water -20.51%

Table I.2. (continued).

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal
[90]	aerobic treatment	pilot plant packed bed reactor with olive fruit bacteria (mixed culture of *Alcaligenes* and *Acitobacter)* support media – rippled plastic tubes draw-fill mode with recirculation working volume = 500 L no external oxygen supply inoculum volume = 10 L retention time = 5 days	OMW TSS = 41.01 g/L VSS = 34.03 g/L Dissolved phenols = 2.9 g syringic acid/L d-COD = 15.05 g/L BOD = 10.64 g O_2/L pH = 5.5	d-COD -70% Phenols -55%
[91]	two-phase biological treatment	aerobic pre-treatment by the yeast *Candida tropicalis* volume = 18 L temperature = 30ºC pH = 7 stirring = 400 rpm aeration = 1 L/min retention time = 12 days anaerobic phase in semi-continuous mode volume = 20 L temperature = 37ºC biogas production = 1.25 $L_{BIOGAS}/L_{REACTOR}$/day organic loading rate = 3.0 kg COD/L/day	OMW pH = 5.04 COD = 90.0 g/L TOC = 29.2 g/L TKN = 1.05 g/L Phenols = 700 mg/L moisture content = 94.8% alkalinity = 610 mg $CaCO_3$/L in mixture with cheese whey (25% v/v): COD = 58.3 g/L TOC = 4.76 g/L Phenols = 16.0 mg/L	COD -93%
[92]	biological treatment	external ceramic membrane bioreactor volume = 15 L mixed liquor = 10 L aeration = 7L/min mineral support – carbon with ZrO_2-TiO_2 Biomass TSS = 12 g/L VSS = 10.8 g/L	diluted solution of OMW COD = 1500-5300 mg/L pH = 5.12 SS = 7.65 g/L $N\text{-}NO_3^-$ = 41 mg/L $N\text{-}NH_4^+$ = 32 mg/L Total salinity = 18.46 g/L Total phenols = 6.32 g/L VFA = 9.78 g/L	Phenol ->92% COD -37-81%
[93]	aerobic treatment	trickling filter with recirculation tank mixed culture of bacteria from olive pulp porous media – high density polyethylene recirculation = 17 days	OMW pH = 5 COD = 43000 mg/L Phenol =9500 mg/L TS = 36 g/L BOD = 26 000 mg/L TKN = 909 mg/L NH_3 = 3 mg/L OP = 146 mg/L	COD -51.0% Phenols -53%
[85]	anaerobic/aerobic treatment	anaerobic treatment mesophilic conditions T = 35ºC pH = 7.15 hydraulic retention time = 50 days aerobic treatment hydraulic retention time = 5 days T = 25ºC	green olive debittering wastewater pH =12.6 TSS = 3420 mg/L VSS = 2860 mg/L d-COD = 16500 mg/L polyphenols = 1384 mg/L TKN = 500 mg/L TP = 38.8 mg/L total VFA = 294 mg/L	COD -83.5% Polyphenols -28 %
[94]	active chlorine	undivided cell 4 cathodes and 4 anode (RuO_2 coated Ti plate electrodes) inter-electrode gap = 0.8 cm electrolyte [NaCl] = 2 M current density = 135 mA/cm^2 temperature = 40º reaction time = 7 h	COD = 41 000 mg/L oil-grease 1970 mg/L phenols = 215 mg/L pH = 4.57 turbidity = 4050 NTU conductivity = 6.9mS/cm	COD – 99.6% complete conversion of phenol, oil and grease turbidity – 98.3%
[95]	anodic oxidation	undivided flow cell BDD anode and stainless steel cathode inter-electrode gap = 9 mm current density = 300 A/m^2 temperature = *const* = 25ºC reaction time = 25 min	OMW after treatment with Fenton process and sand filter COD = 700 mg/L conductivity = 2.5 mS/cm pH = 7.13	COD and TOC almost total elimination

Table I.2. (continued).

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal
[96]	active chlorine	undivided flow cell $Ti/TiRuO_2$ anode and stainless steel cathode inter-electrode gap = 0.5 cm current = 5 A electrolyte [NaCl] = 5 g/L temperature = 25ºC reaction time = 17 h	OMW pH = 5.0 conductivity = 10.2 mS/cm polyphenols = 0.96 g/L COD = 26.75 g/L BOD_5 = 10.6 g/L nitrates = 0.35 g/L sulfates = 0.1 g/L chlorides = 0.8 g/L	almost complete removal of color and aromatics and COD
[84]	electro-Fenton	undivided cell carbon felt cathode and Pt sheet anode $[Fe^{2+}] = 10^{-4}$ M pH = 3 current = 200 mA electric charge = 7000 C stirring aeration reaction time = 9 h	OMW diluted 10-fold in distilled water Initial before dilution: COD = 92000 mg/L pH = 4.85 conductivity = 12.4 mS/cm	complete mineralization complete removal of color
[97]	biological / ECP / coagulation	aerobic treatment with *Aspergillus niger* external loop air-lift bioreactor ambient temperature 3-day batch mode electrochemical peroxidation $[H_2O_2]$ (30%) = 1.6% current = 10 A 4 iron plate electrodes equal inter-electrode gap retention time = 1 h coagulant 0.4% (w/v) CaO settling time = 20 h	pH = 10-12 COD = 8-35 g/L BOD_5 = 3.5 – 20 g/L NH^{4+} = 1-2 mg/L P = 15-20 mg/L K^+ = 2000 – 3000 mg/L Cl- = 10 – 140 mg/L SO_4^{2-} = < 3 mg/L	COD -97.9% COD_{final} = 360 mg/L
[98]	electro-Fenton/anaerobic digestion/ultrafiltration	four-compartment cell in semi-continuous mode each compartment with 2 iron cathodes and 2 iron anodes inter-electrode gap = 2 cm current = 20 A reaction time = 4 h up-flow anaerobic filter hydraulic retention time = 4.5 days organic loading rate = 10 g COD/L/d	OMW pH=5.20 Abs(395 nm) = 72±5 COD = 95±5 g/L BOD_5 = 19±2 g/L TSS = 15±4 g/L lipids = 9.8 ±1.2 g/L polyphenols = 11.5 ±1.6 g/L monomers = 2740±50 mg/L	COD -94.4% color – 92.6% TSS – 100% polyphenols – 95.7% monomers -94.2%
[86]	biological/anodic oxidation	two vertical-flow pilot-scale constructed wetland retention time = 2 days organic loading = 5 g COD/m^3/d undivided cell BDD anode and cathode inter-electrode gap = 0.01 m no electrolyte addition current = 20 A ambient temperature reaction time = 360 min	olive pomace leachate from evaporation pond COD = 9740 mg/L DOC = 3535 mg/L TSS = 1900 mg/L color = 16 450 TCU conductivity = 14.3 mS/cm pH = 8.2 TN = 35.2 mg/L TP = 19 mg/L EC_{50} = 3.8%	mineralization - 95% decolorization -94% complete elimination of *V. fischeri* toxicity

Note. Abs(X nm) – absorbance at wavelength X nm; BOD – biochemical oxygen demand; BOD_5 - biochemical oxygen demand after 5 days; COD – chemical oxygen demand; *const* – constant; d-COD – dissolved chemical oxygen demand; DOC – dissolved organic carbon; EC_{50} – half maximal effective concentration; OMW – olive mill wastewater; OP – orthophosphates; SS – suspended solids; TKN – total Kjeldahl nitrogen; TN – total nitrogen; TOC – total organic carbon; TP – total phosphorous; TS – total solids; TSS – total suspended solids; TVS – total volatile solids; VFA – volatile fatty acids; VSS – volatile suspended solids.

I.2.2.1. Individual biological treatment

Biological treatment is not able to neutralize such effluents completely, mainly due to their toxicity. The main source of toxic to bacteria substances (e.g. polyphenols) comes from broken seeds [88]. The anaerobic process is normally inhibited by the presence of phenolic compounds and certain organic acids [90]. Aerobic consortia seem to be selective for certain types of phenolic species: they are able to degrade simple phenolic and monoaromatic compounds, but more complex molecules responsible for the characteristic dark color of olive wastewater are refractory for them [83]. Therefore, there is a need for a pre-treatment step in order to remove toxicity and increase biodegradability of olive mill wastewater [99].

Tziotzios et al. [90] studied treatment of olive mill wastewater (OMW) in a packed bed reactor under non-sterilized aerobic conditions on flask, bench and a pilot plant scale. Removal results after 5 days of treatment for dissolved COD (Figure I.4.) and phenols were 70% and 55% respectively. However authors noticed that the real effluent used for their experiments had a lower content of COD and phenols than typical OMW due to the larger volume of water used during the production process. Bacterial inoculum that was used for those experiments was isolated from the olive pulp, which is a certain advantage, as it ensures a more stable operation of a bioreactor. A serious limitation of such aerobic treatment of OMW is worth mentioning: an excessive sludge production connected to the presence of phenols. Batch experiments with olive pulp bacteria and phenols as a carbon source show that around 67% of phenols are transformed into the biomass. Therefore, for any aerobic treatment, an additional issue of sludge production should be considered.

El Hajjouji et al. [89] also analyzed aerobic treatment for OMW with two slight variations. The first one was aerobic digestion with addition of extra N source and natural pH, and the second one was aerobic digestion also with N addition and lime neutralization to pH 6. Experimental data showed that only the addition of lime could reduce COD by almost 25% and enhance removal of phenols (50% without adding lime against 75% with lime). Authors explained that neutralization of the solution (pH = 6) leads to a transformation of phenols into phenates, which makes them readily available for microbial degradation. Results obtained from an elemental analysis and spectroscopy show that the resulting organic matter is different for both variations. After digestion with the addition of lime, neoformation of humic substances was observed, which is a sign of polymerization of organics and consequently of waste stabilization. It implies the possibility of further use of this waste as soil amendment, which produces value-added waste [100].

An operation of full-scale trickling filter was analyzed in the work of Michailides et al. [93]. As mechanical aeration comprises a significant part of the operating cost, bioreactor had natural aeration, ensured by continuous recirculation of wastewater. After hydraulic retention time of 24 h, COD and phenol removal reached above 50%. Authors concluded that relatively short treatment time (24 h) and reduced costs due to the absence of aeration make the trickling filter a viable option for pre-treatment of OMW and that additional treatment is still required for total reduction of toxicity.

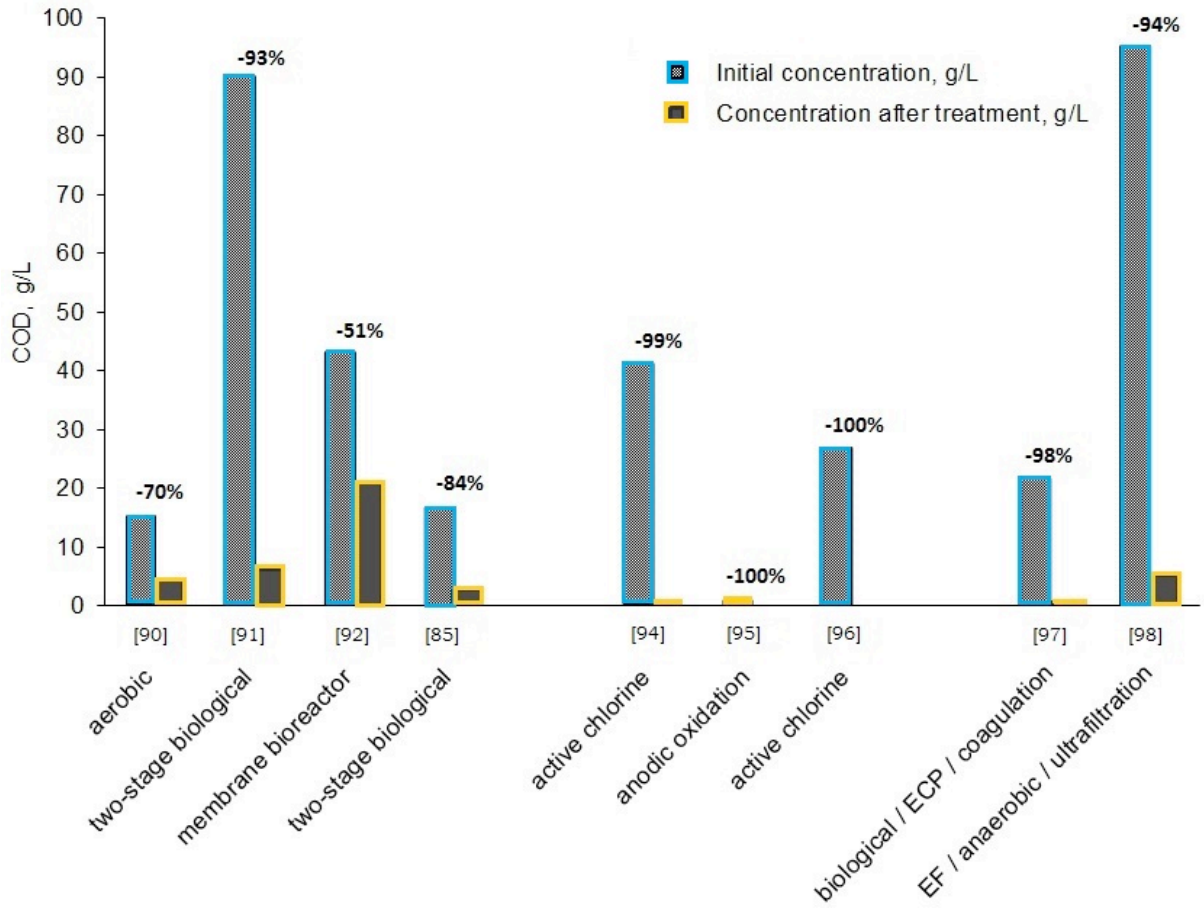

Figure I.4. Removal of COD from olive processing wastewater (COD – chemical oxygen demand, EF – electro-Fenton, ECP – electrochemical peroxidation).

As noted above, anaerobic treatment can be severely inhibited by the presence of phenolic compounds; therefore pre-treatment to remove or at least reduce this toxicity seems to be a logical solution. Martinez-Garcia et al. [91] tested an aerobic pre-treatment with yeast *Candida tropicalis* followed by anaerobic bacterial digestion. The aim was to degrade phenols and in that way to improve biomethanization. In addition, the possibility of using whey as co-substrate was considered. Whey is highly biodegradable and contains high amounts of nitrogen, which when mixed with OMW improves general COD: N: P ratio. COD removal during the anaerobic process was indeed improved in comparison to other similar studies, which were analyzed in this publication. The final COD (Figure I.4.) decreased by 93%, which is also by far the best result in this review.

Another combined biological treatment system was studied by Aggelis et al. [85]. The wastewater under investigation originated from debittering green olives. Its composition is very similar to the OMW. In this study separate aerobic and anaerobic processes were compared to the combined anaerobic-aerobic system. Anaerobic degradation was severely inhibited by toxicity of the wastewater and could hardly reach 50% COD removal and only 12% polyphenols removal. Aerobic treatment was more effective and could remove over 70% of COD, though excessive sludge production was observed. A combined system could attain almost 85% COD removal rate (Figure I.4.), and 30% of polyphenols were destroyed. Authors

highlighted that better results could be achieved by applying a pre-treatment method for removal of polyphenols, which would improve significantly the subsequent step of anaerobic digestion.

A membrane bioreactor (MBR) is a promising biological treatment, which is a combination of an activated sludge process and membrane separation. The influent primarily undergoes degradation by suspended biomass and then passes through a filtering unit (either nano- or ultrafiltration). In that way, the final effluent can be disposed of and the biomass is retained in the reactor. Dhaouadi and Marrot [92] analyzed the feasibility of such a system for diluted OMW. Their work showed that the degradation of phenols was really high (above 92% removal), but the COD removal varied greatly (37-81%) depending on the dilution factor. Authors concluded that MBR can be used as a pre-treatment option for removal of toxic phenolic compounds before conventional biological treatment.

I.2.2.2. Individual electrochemical treatment

Single electrochemical treatment can also be a sound solution to the problem of OMW as it entails no inhibiting effects and risks to operational stability as in biological methods. Active chlorine for treatment of olive waste was studied by Un et al. [94] and Panizza and Cerisola [96]. Both studies reached almost complete removal results of COD, phenols and other parameters (oil, color, grease, turbidity etc.). It is worth noticing that reaction time differed significantly: 7 h against 90 min. It could be attributed to the fact that in the first study four pairs of electrodes were used against only one pair in the second study.

Another interesting investigation was reported by Canizares et al. [95], in which anodic oxidation was used as a final step for already pre-treated OMW. Pre-treatment sequence consisted of the classical Fenton reactor, settling and a sand filter. Generated effluent still had a COD of around 700 mg/L, which could not be completely removed by use of the classical Fenton reaction. A treatment time of only 25 min was sufficient for almost complete removal of TOC and COD (Figure I.4.), therefore it was demonstrated that electrolysis could be a successful terminal treatment step. In terms of energy minimization it was established that addition of salts as electrolyte greatly influences power consumption. For example, energy consumption of electrolysis with electrolyte addition dropped from 150 kWh/kg COD to 100 kWh/kg COD, which is a substantial decrease.

Electro-Fenton was also studied for diluted olive oil wastewater [84]. The first part of this study includes oxidative degradation of vanillic acid, which was chosen as a model compound of olive waste. Its concentration in the solution decreased exponentially with treatment time, and degradation followed a pseudo-first order kinetics. The second part deals with mineralization of the real wastewater diluted with deionized water 1:50 (v/v). Complete mineralization was achieved after 9 h treatment and a current intensity of 200 mA. Degradation of pollutants was also obvious from the color change throughout the treatment process. At the end of treatment, the effluent turned from dark to transparent due to the presence of polyphenols.

I.2.2.3. Combined electrochemical-biological treatment

A combined system consisting of a biological pre-treatment with a fungal species *Aspergillus niger* and a consequent electrochemical peroxidation was studied by Kyriacou et al. [97]. Biological treatment with this particular fungus was chosen on the basis of previous research, which established that this strain can degrade phenolic compounds and decolorize different types of wastewater. Samples were real wastewater from processing of green table olives and its COD ranged from 8 to 35 g/L, which means that this effluent was extremely charged with organics. An interesting peculiarity of this investigation arises from comparing the operation of a combined system at different scales: that of a laboratory and a pilot plant. It is important to note that the laboratory results were successfully replicated on the pilot plant scale. Results from laboratory experiments showed that during initial biological treatment that had a hydraulic retention time of 3 days, COD was reduced by almost 75%. Removal of COD during electrochemical treatment displayed a dependence on pH and hydrogen peroxide concentration. Both COD and phenols removals were around 96% after 1 h of post-treatment. In addition the pilot plant was operated in semi-batch mode and consisted of an extra step of coagulation at the end. Total removal efficiency was above 98% (Figure I.4.) and final effluent complied with Greek wastewater limits for discharge into sewer system. Based on this evidence, such a combined system was found to be a good option for olive wastewater.

Another pilot scale study was carried out in Tunisia [98]. Here the pre-treatment step used electro-Fenton system with H_2O_2 addition (Fered-Fenton), which was followed by anaerobic digestion and ultrafiltration. In comparison to the previously discussed study [97], COD of these samples was significantly higher: on average 35 g/L for the first against 95 g/L maximum for the second. Electro-Fenton was chosen to be the initial treatment process in order to remove part of the refractory contaminants and to enhance anaerobic digestion afterwards. After 4 h of reaction time, it was able to remove on average around 50% of COD. It is important to mention that during a semi-continuous operation mode, the electrochemical reactor had to be stopped, as electrodes required cleaning. This was caused by an increase in monophenolic substances, which adhered to the surface of electrodes after 4 days of continuous operation. The consequent anaerobic step was used on undiluted samples from electrochemical treatment. The average COD removal was about 75% during the whole period of digester operation. An ultrafiltration step was carried out in order to remove residual pollution mainly due to polyphenolic compounds, which could not be completely removed during previous treatment steps. The final, overall removal results were very good: percentage removals for COD (Figure I.4.), polyphenols, color, monomers and total suspended solids (TSS) were all above 90%. Also toxicity (based on bioluminescence inhibition of *Vibrio fischeri* bacteria) was significantly decreased (initial 100% inhibition against final 38%). An evaluation of economic feasibility showed that production of methane gas from the anaerobic step would not only cover for all the energy needs of the whole treatment process, but would also create a surplus.

Another study investigated a pilot plant for treatment of olive pomace leachate taken from an evaporation pond [86]. Treatment methods under investigation were anodic oxidation and a constructed wetland. It is interesting that two different scenarios with different

sequences of treatment processes were compared: constructed wetland as pre- and post-treatment. In terms of COD and color removal, better results were achieved by the initial treatment in constructed wetland and a polishing step of electrochemical oxidation: 95% mineralization and 94% decolorization against 81% and 58% for electrochemical pre-treatment followed by a post-treatment in constructed wetland. Such results were likely due to the higher toxicity of electrochemical reaction products towards biological degradation. Regardless the sequence, a constructed wetland is a simple-to-operate, low-cost technology and can compensate for electrochemical oxidation, which in this case is also minimal in terms of cost, as no reagents are added to the treated solution [101].

I.2.3. Pharmaceuticals

Pharmaceuticals are one of the newly emerging pollutants that have raised a great deal of public concern. On the one hand, these chemicals constitute the basis of modern medicine, they sustain human life and health, but on the other side their uncontrolled release into the environment has numerous negative consequences [102-110]. One of the main ways of discharge is through hospital effluent [111-114]. It has a really diverse and complex composition: different classes of pharmaceuticals, which are excreted by the human body in almost unchanged form, and their metabolites, diagnostic agents, disinfectants, musks, corrosion inhibitors and other hardly degradable pollutants [114-118]. Hospital effluents are normally discharged to the common sewage system and undergo conventional treatment at municipal wastewater treatment facilities [119-121]. However, most of the pharmaceutically active compounds are not degraded at all, therefore they end up in the receiving natural waters [122-124]. Despite tiny concentrations, these pollutants affect the ecosystem's balance, as, for example, hormones from oral contraceptives shift the gender balance in fish and cease their reproduction [125]. The presence of antibiotics in small concentrations, which are not high enough to destroy bacteria, causes habituation and trigger antibiotic resistance in microorganisms [126, 127]. In the light of the above, effluents that contain pharmaceuticals require additional pre-treatment before discharge to municipal sewage or a separate treatment system in order to provide sufficient and efficient degradation of the emerging contaminants [128]. In Table I.3 the summary of discussed publications is presented.

Table I.3. Examples of treatment of pharmaceuticals and hospital wastewater by individual electrochemical and biological processes and by their combination.

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal of pollutants
[129]	biological	full-scale submerged membrane bioreactor 24 membrane modules with area 96 m^2 polyethylene membrane treatment capacity = 20 m^3/day working volume = 6 m^3 pore size = 0.4 µm hydraulic retention time = 7.2 h	hospital wastewater COD = 48 – 277.5 mg/L BOD_5 = 20-55 mg/L NH_4^+-N = 10.1 – 23.7 mg/L turbidity = 6.1 – 27.9 NTU pH = 6.2 – 7.1 bacteria 9.9 ×10^3 number/L *E.coli* > 1600 number / 100 mL	COD – 80% NH_4^+-N -93% turbidity – 83% *E.coli* – 98% average effluent quality COD < 25 mg/L NH_4^+-N < 1.5 mg/L turbidity < 3 NTU

Table I.3. (continued).

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal of pollutants
[130]	extended sludge age biological process	laboratory scale up-flow bioreactor consisting of 2 reservoirs with 20 L capacity pH = 6.4-7.6 DO = 7-8 mg/L aeration with flow rate = 10 L/min room temperature sludge age greater than 200 days hydraulic retention time = 12h	synthetic wastewater COD = 300 mg/L sulfamethoxazole = 1-5 µg/L sulfamethazine = 2-5 µg/L sulfadimethoxine = 2-5 µg/L trimethoprim = 1 µg/L acetaminophen = 1-100 µg/L ibuprofen = 5-100 µg/L naproxen = 5-15 µg/L ketoprofen = 5-15 µg/L	COD - 95±5% antibiotics -64-93% acetaminophen and ibuprofen 90-100% ketoprofen and naproxen -79-96%
[131]	electro-Fenton	undivided cell carbon felt cathode and Pt anode pH = 3.0 [Fe^{3+}] = 0.2 mM electrolyte [Na_2SO_4] = 0.05 M current intensity = 60 mA stirring aeration = 1L/min ambient temperature reaction time = 45 min	Solution of [triclosan] = 50 mg/L in a 20:80 (v/v) acetonitrile/water	triclosan – 100 %
		same reactor setup apart from BDD anode and O_2 diffusion cathode reaction time = 120 min	Solution of [triclocarban] = 5 mg/L in a 40:60 (w/w) acetonitrile/water	triclocarban -100%
[132]	electro-Fenton	open undivided cell Pt anode and carbon felt cathode pH = 3 [Fe^{2+}] = 0.2 mmol/L electrolyte [Na_2SO_4] = 0.05mol/L current intensity = 300 mA aeration = 1L/min stirring, ambient temperature reaction time = 180 min	aqueous solution [atenolol] = 0.15 mmol/L [metoprolol] = 0.15 mmol/L [propranolol] = 0.15 mmol/L TOC = 85 mg/L	TOC -95%
[133]	anodic oxidation	undivided cell 2 BDD electrodes inter-electrode gap = 0.1 cm pH = 4 electrolyte (Na_2SO_4) concentration = 0.5 mol/L current density = 235 mA/cm^2 solution flow rate = 1.42 cm^3/min temperature = *const* = 25±0.1°C	[ketoprofen] = 0.196 mmol/L	degradation = 100%
[33]	electro-Fenton	open undivided cell pH =3 current density =6.66 mA/cm2 [Fe^{2+}] = 1 mM electrolyte [Na_2SO_4] = 0.05 M both cathode and anode – activated carbon felt mixing temperature = ambient reaction time = 480 min	[cefalexin] = 200 mg/L solution BOD_5/COD = 0	cefalexin -100% COD -72% BOD_5/CODfinal = 0.26
[134]	electro-Fenton	combined BDD/ADE-Pt/CF cell pH = 3 [Fe^{2+}] = 0.5 mM electrolyte [Na_2SO_4] = 0.1 M current intensity = 3.0A (BDD/ADE) and 0.4A (Pt/CF) liquid flow rate = 250 L/h temperature = const = 35°C reaction time = 360 min	aqueous solutions of metoprolol tartate, atenolol, propranolol hydrochloride TOC = 100 mg/L of each drug	metoprolol tartate -66% atenolol – 61% propranolol hydrochloride -65%
	solar photoelectro-Fenton	electrochemical reactor (combined Pt/ADE-Pt/CF cell) coupled with a solar compound parabolic collector pH = 3 [Fe^{2+}] = 0.5 mM electrolyte [Na_2SO_4] = 0.1 M current intensity = 3.0A (Pt/ADE) and 0.4A (Pt/CF) temperature = const = 35°C reaction time = 360 min		metoprolol tartate -90% atenolol -88% propranolol hydrochloride -93%

Table I.3. (continued).

Reference	Treatment	Operating parameters	Wastewater characteristics	Removal of pollutants
[135]	anodic oxidation	undivided cell Pt mesh anode and carbon felt cathode pH = 3.0 electrolyte [Na_2SO_4] = 0.05 M current intensity = 30 mA room temperature stirring compressed air sparging at rate = 1L/min reaction time = 600 min	sulfamethoxazole solution [SMX] = 0.208 mM = 52.7 mg/L	complete degradation of SMX after 240 min TOC -7% (after 600 min)
	electro-Fenton	undivided cell BDD anode and carbon felt cathode pH = 3.0 [Fe^{2+}] = 0.2 mM electrolyte [Na_2SO_4] = 0.05 M room temperature stirring compressed air sparging at rate = 1L/min reaction time = 600 min		complete degradation of SMX after 60 min mineralization – 86% (after 600 min)
[136]	anodic oxidation	one-compartment reactor current density = 33 mA/cm^2 electrolyte [$NaClO_4$] = 0.05 M BDD anode and cathode interelectrode gap = 1 mm temperature = 20°C flow rate = 300 L/h reaction time = 210 min	[iohexol] = 3.6 mM COD = 82 mmol O_2/L	COD – about 85% complete elimination of iohexol
[137]	electro-Fenton / biological	undivided cell carbon-felt cathode and cylindrical platinum anode pH = 3 [Fe^{2+}] = 0.1 mmol/L electrolyte [Na_2CO_4] =0.05 mol/L current = 300 mA room temperature aeration reaction time = 2 h/4 h	[tetracycline] = 25 mg/L	TOC – 69% (2h electrolysis) and -86% (after 4h electrolysis)
		[activated sludge] = 0.5 g/L pH = 7 retention time = 2 days		
[138]	electro-Fenton (as pre-treatment before biological process)	carbon felt cathode and platinum anode pH = 3 [$FeSO_4*7H_2O$] = 0.1 mM electrolyte [Na_2SO_4] = 0.05 M current intensity = 300 mA temperature = 18°C aeration reaction time = 120 min	[sulfamethazine] = 0.2 mM COD = 88 mg O_2/L non-biodegradable	$BOD_{5\ FINAL}$ = 38 mg O_2/L COD_{FINAL} = 39 mg O_2/L BOD_5/COD_{FINAL} = 0.97

Notes: BOD – biochemical oxygen demand; BOD_5 - biochemical oxygen demand after 5 days; COD – chemical oxygen demand; *const* – constant; TOC – total organic carbon.

I.2.3.1. Individual biological treatment

A summary of a 6-month operation of a submerged membrane bioreactor for treatment of real hospital wastewater was reported by Wen et al. [129]. The removal efficiency was monitored for COD, ammonia, turbidity and BOD_5. Final characteristics of the effluent met Chinese standards for hospital wastewater discharge and during the whole period of performance no cleaning operation was done and no sludge was discharged. In general this technology was proved to be successful in terms of the main removal parameters; still, elimination of pharmaceutically active compounds was not followed and therefore it remains an open issue.

Removal of several antibiotics and non-steroidal anti-inflammatory drugs by means of an extended sludge age process was studied by Yu et al. [130]. They obtained quite good results, which depended greatly on the initial concentration, but were more or less steady. However, the question of the mechanisms by which those pharmaceuticals are removed is an open one: is it real biodegradation or simple sorption onto the biomass? In case of the latter molecules are removed from the liquid phase, but there is a possibility that they stay unchanged, which practically means a transfer of pollution from water onto microorganisms. A similar study by the same research group Yu et al. [139] was done to investigate biodegradation, bio-sorption, hydrolysis and volatilization during the immobilized cell biological process. Biodegradation and biosorption were found to be the main processes, whereas volatilization and hydrolysis could be ignored due to very low contributions to eliminating the selected drugs. Results show that acetaminophen was strongly biodegraded and biosorbed, while sulfamethoxazole, sulfadimethoxine, NSAIDs such as ibuprofen and naproxen exhibit strong biodegradability and weak sorption. Sulfamethazine and ketoprofen were hardly biodegraded (23% and 28% respectively) and hardly sorbed onto the biomass (20% and 18% respectively), trimethoprim also had low biodegradability (27%) but medium sorption (47%). These results show that different substances even within the same class and group of pharmaceuticals display varying behavior when treated by means of biological treatment. Due to this, treating real effluents containing a mixture of pharmaceuticals with biological methods has become a challenge. Indeed certain pharmaceuticals when being sorbed on the surface of bacteria may affect the composition of the population and may interfere with efficiency of such treatment in the long run.

I.2.3.2. Individual electrochemical treatment

EAOPs are able to neutralize the effluents containing concentrations of pharmaceutically active compounds, but they can also have an additional function. Hospital effluent, besides chemical pollutants, contains pathogenic bacteria, which need to be destroyed. As hydroxyl radicals are toxic to microorganisms by nature and cause inhibition of their activity, methods based on those chemical species can play a disinfecting role as well [1].

One of the most common classes of pharmaceuticals is antimicrobials. These chemicals are designed to kill or inhibit microorganisms. They can be specifically used for bacterial infection (antibacterials or antibiotics), for fungi (antifungal), for viruses (antivirals), etc. Sirés et al. [131] and Sirés et al. [140] investigated four different electro-Fenton systems for degradation of chlorophene, triclosan and triclocarban, common substances with antibacterial and antifungal properties. Various cell configurations of electro-Fenton were tested for removal of chlorophene with different combinations of electrode materials: Pt or BDD anode and carbon felt or O_2-diffusion cathode. Degradation of triclosan was followed in an aqueous solution and in hydro-organic medium composed of acetonitrile (20%) and water (80%) mixture. As the solubility of these compounds is very low, apart from aqueous solutions acetonitrile/water matrix was used in order to enable higher concentrations and to follow the oxidation intermediates. Under optimized conditions of the electro-Fenton total

elimination of triclosan took place after 45 min for current intensity of 60 mA and 30 min for 300 mA. A similar reactor setup but with BDD/O_2-diffusion electrodes, was used for elimination of triclocarban in 120 min. These results prove that electro-Fenton is a plausible treatment option for fast and complete degradation of both antimicrobials for saturated solutions in water and organic solvents (Figure I.5.).

The same research group [135] compared anodic oxidation and electro-Fenton treatment for degradation and mineralization of two antibiotics from the sulfonamide family: sulfamethoxazole [135] and sulfachloroprydazine [28]. Electro-Fenton was found to be more effective and faster in both degrading the pharmaceutical and mineralizing its intermediates. Glyoxylic and oxalic acids were ultimate intermediates, which have the highest accumulation and persistence. In conclusion electro-Fenton was found to be an efficient treatment method for sulfonamide antibiotics. Complete degradation and high COD removal was also showed by Estrada et al. [33] for cefalexin, a first generation cephalosporin antibiotic (Figure I.5.).
EAOPs can also degrade non-steroidal anti-inflammatory drugs (NSAID), which are used for reducing fever and pain. Ketoprofen in aqueous solution was degraded completely by anodic oxidation [133] (Figure I.5.) and electro-Fenton [141]. A review on the degradation of anti-inflammatory and analgesic drugs by EAOPs [142] states that generally anodic oxidation is a very promising technology for elimination of this class of pharmaceuticals, especially on BDD electrodes, which was the case for a study of Domínguez et al. [133]. On the other hand, the electro-Fenton process, which is widely studied for degradation of various types of pollutants, was also successfully applied for removal of NSAID such as ibuprofen [143], paracetamol [144, 145], and salicylic acid [142, 146, 147].

Another group of pharmaceuticals with a very wide medical use starting with heart problems and ending with anxiety disorders are beta-blockers. Beta-blockers target beta-receptors, which cause stress responses when stimulated. Beta-blockers bind to those receptors and in that way prevent them from further reactions. Sires et al. [132] studied the removal of three different beta-blockers. Anodic oxidation and electro-Fenton were compared in experiments on aqueous solutions. Results showed that electro-Fenton had better performance, faster removal and mineralization. After 180 min of optimized treatment the removal of total organic carbon was almost complete (95%). It is important to note that toxicity of the treated solution decreased and formed intermediates were more biodegradable, which makes biological post-treatment a plausible option for further detoxification of the effluent.

Isarain-Chavez et al. [134] compared electro-Fenton and solar photoelectro-Fenton for degradation of three beta-blockers (Figure I.5.). Comparison was also made regarding the cell configuration: different electrodes were tested as single pairs or combined pairs in one reactor. Combined cells like BDD/gas diffusion cathode and Pt/carbon felt cathode for electro-Fenton and solar photoelectro-Fenton were proven to be most effective in degradation. In general electro-Fenton enhanced by solar collector removed around 30-35% more of each beta-blocker than simple electro-Fenton. Mineralization current efficiency (MCE) values were very high (100-110%) for solar photoelectro-Fenton at the beginning, but towards the end of treatment they decreased rapidly to 50%. Authors attribute this to the formation of less degradable by-products. For electro-Fenton no drastic change in MCE was observed

throughout the whole duration of treatment, which signifies the same mineralization rate of both beta-blockers and their by-products. However, the MCE was low: less than 45% for combined cells and less than 20% for single cells.

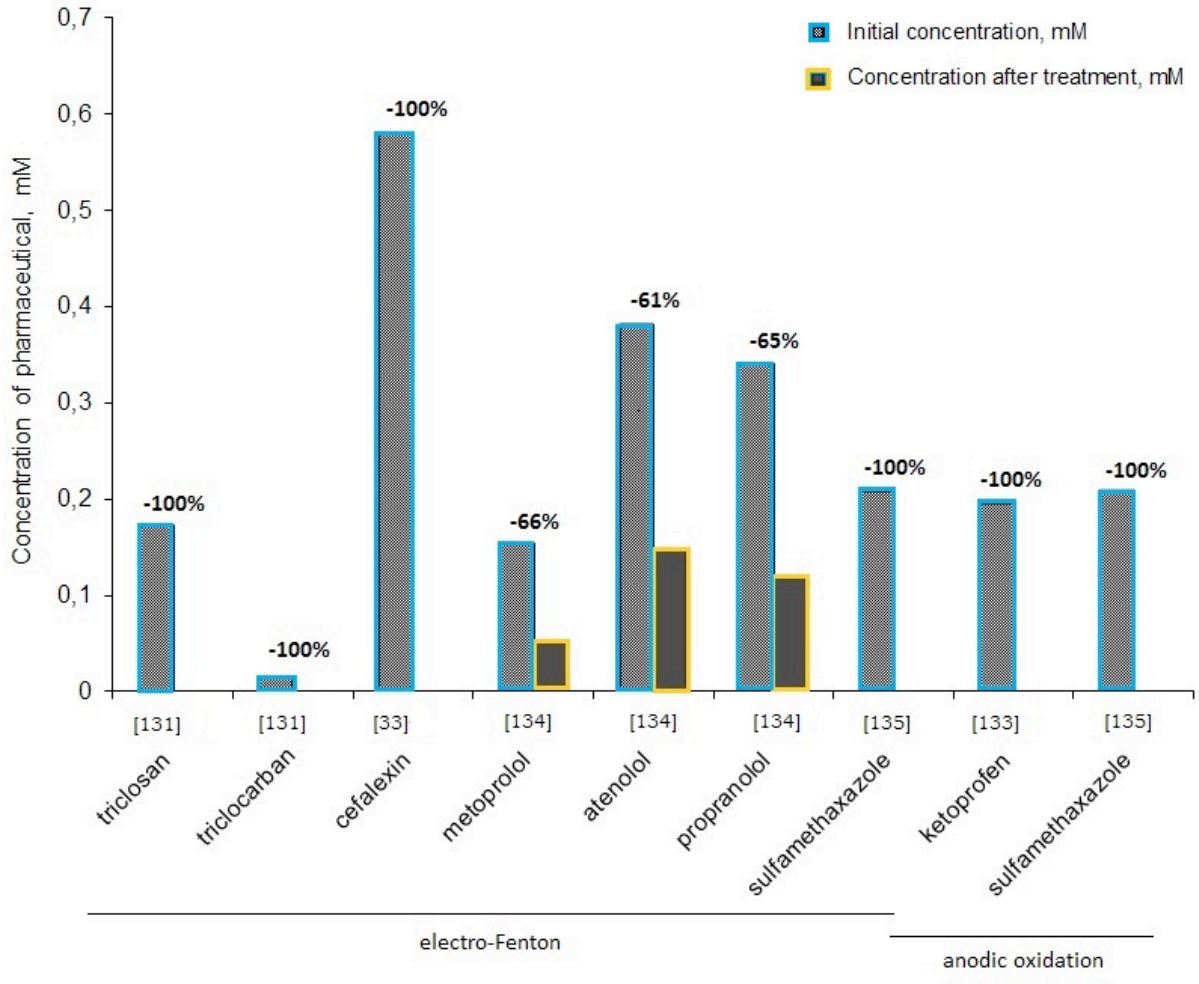

Figure I.5. Removal of pharmaceuticals by means of electrochemical processes.

Iodinated X-ray contrast media do not have any actual pharmaceutical effect, but these diagnostic agents for X-ray based imaging techniques are widely used [118]. Among all possible constituents of hospital wastewater they have the highest concentrations [115]. Inefficient removal of those compounds from water (especially by biological processes) leads to their discharge into natural waters, from where they act as absorbable organic halogens, which are notorious for their negative effects on natural ecosystems. Despite all this, there is not much research done on the removal methods of contrast media and only recently this subject has started to attract more attention. Tissot et al. [136] investigated the possibility of eliminating X-ray contrast media iohexol by means of electrolysis. The results were quite promising as degradation of iohexol after 210 min of treatment, which was complete and COD removal was around 85%. However, more research on degradation of these compounds is still needed.

I.2.3.3. Combined electrochemical-biological treatment

There are few publications dealing with a combined electrochemical-biological system for any type of pharmaceutical-containing wastewater. An article of Ferrag-Siagh et al. [137] analyzed the relevance of electro-Fenton as a pre-treatment option prior to a biological process for an antibiotic tetracycline. An optimal set of operating parameters was established, among others the treatment time, which was short enough, but still made the solution biodegradable, was found to be around 2 or 4 h. A BOD_5/COD ratio after 2 and 4 h of electro-Fenton was 0.33 and 0.44, respectively and mineralization degree 46 and 72%, respectively. Afterwards, activated sludge cultures were tried on initial tetracycline and a pre-treated solution. TOC removal of the initial solution increased from 28% to 68% and 86% for 2 or 4 h electro-Fenton treatment, respectively, which makes such pre-treatment option viable.

Another study of Mansour et al. [138] also investigated electro-Fenton as a pre-treatment before biological process for another antibiotic sulfamethazine in aqueous solution. After optimization of running parameters, the final solution of 0.2 mM drug, which was initially totally non-biodegradable, had a BOD_5/COD ratio of 0.97. This signifies that intermediates can be totally removed by a biological process and feasibility of electro-Fenton as first treatment step was proved to be successful.

It is obvious that more scientific effort is required in the area of a combined treatment system. Particular emphasis should be put on experimental data from a full cycle of such a process and not only on the pre-treatment step and verification of the biodegradability level of the effluent. Regarding the general research on pharmaceuticals and relevant wastewater, priority should be given to real effluents from hospitals or drug production, as this will ensure further up-scaling of innovative treatment technologies.

I.3. CONCLUSIONS

At the present time biological processes are dominating treatment methods for polluted water. Electrochemical processes are perspective methods for treatment of refractory, organic and hardly charged wastewater. Their coupling is a solution, which can potentially give high removal rates and have low investments.

In general, individual electrochemical treatment of dyes shows great potential as preliminary studies on aqueous solutions of dyes have good decolorization results. Different dyes show distinctive degradation patterns and require different treatment durations for both decolorization and mineralization. This could be attributed to two reasons. First of all, different classes of dyes have a distinct chemical structure, which may be susceptible to hydroxyl radicals or may complicate their degradation. Secondly, the differences in the electrochemical reactor setup and operating parameters do not allow a proper comparison between different studies. Moreover, as most of the research in this area is done for single dye solutions or, in the best case, a mixture of dyes, it gives only inkling of the treatment of real effluents. The trend in biological treatment is mostly sequential anaerobic/aerobic process, as anaerobiosis is more efficient in decolorizing dye wastewater and aerobiosis is able to better mineralize degradation products. Combined processes are studied in terms of both possible

setups: electrochemical pre- or post-treatment. In the first case the goal of advanced oxidation is to render the solution biodegradable for consequent biological treatment. In the second case, it follows anaerobic treatment with the objective of removing pollutants and their intermediates, which are refractory for anaerobic bacteria.

Research on treatment of olive processing wastewater is extensive and includes many different combinations of technologies investigated. The majority of research comes from the olive-producing countries of olives as these types of effluents cause a great deal of pollution and call for an immediate solution. Despite the fact that biological processes are not capable of neutralizing such effluents properly, scientific search in this area continues. However, even if the batch results are promising, normally such treatment in a continuous mode often faces serious disruptions. Electrochemical methods do not have limitations due to the toxicity of the effluent, but they normally have a longer reaction time, which in this case means higher operation cost. It is interesting that specifically for olive processing wastewater combined processes have been successfully studied on real effluents and this research is being upgraded to pilot plant experimenting.

Pharmaceuticals are designed to be biologically active, therefore, by definition their treatment with biological methods might not be convenient. The presence of antibiotics affects microorganisms directly and can cause their resistance. Further occasional release of these species into the environment may be a threat to human health in the long run. In contrast EAOPs studies prove that these methods are reliable in terms of efficient removal of pharmaceuticals from contaminated water. Moreover, they can play a disinfecting role, as hydroxyl radicals are toxic to bacteria. Research on the combined processes is scarce and usually based on an investigation of biodegradability improvement after electrochemical methods. It is also important to remark that normally experiments are done on significantly higher concentrations of pharmaceuticals than in real effluents. Therefore, additional research on the removal of trace amount of drugs is necessary.

Further research on these technologies should focus more on experimenting with real wastewater and operation in a continuous mode, which will facilitate gradual up-scaling. There is an urgent need for hybrid processes, where a reasonable compromise will be made between acceptable economical cost, high removal efficiency and environmental responsibility. It is also desirable to operate treatment system in continuous mode and not only batch experiments.

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CHAPTER II

Removal of psychoactive pharmaceutical caffeine from water by electro-Fenton process using BDD anode: Effects of operating parameters on removal efficiency

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O. Ganzenko, N. Oturan, D. Huguenot, E.D. van Hullebusch, G. Esposito, M.A. Oturan, Removal of psychoactive pharmaceutical caffeine from water by electro-Fenton process using BDD anode: Effects of operating parameters on removal efficiency, Sep. Purif. Technol., (2015). doi: 10.1016/j.seppur.2015.09.055.

ABSTRACT

Caffeine, as a typical representative of pharmaceutical pollution, was subjected to the removal from water by means of the electro-Fenton process. The study of a single-molecule solution was meant for better understanding of the influence of operating parameters on the removal rates and for finding their optimal values. The setup comprised a bench-scale reactor equipped with a boron-doped diamond anode and a 3D carbon felt cathode. Degradation and mineralization kinetics were monitored for different sets of two major operating parameters: current intensity (from 100 to 1500 mA) and Fe^{2+} concentration (from 0.1 to 0.5 mM). Experimental data revealed that the optimal catalyst concentration was 0.2 mM, regardless the applied current intensity. For experiments on degradation kinetics, the trend of increasing the reaction rate with an increasing current was valid up to 300 mA. In contrast, the mineralization rate increased up to 1500 mA. The absolute reaction rate constant between caffeine and hydroxyl radical was determined as (2.48 ± 0.01) × 10^9 M^{-1} s^{-1}. A follow-up of aromatic compounds, carboxylic acids and inorganic ions, enabled composition of a plausible degradation pathway for caffeine degradation by hydroxyl radicals. An analysis of the operating parameters versus evolution of degradation and mineralization showed that even small concentrations of Fe^{2+} and low current intensities led to complete degradation and almost complete mineralization.

Keywords: Electro-Fenton, Caffeine, Pharmaceutical, Electrochemical process, Degradation

II.1. INTRODUCTION

Caffeine is the most consumed psychoactive drugs worldwide [1]. Apart from being commonly used in beverages (soft drinks, tea and coffee), it is one of the components of painkillers, medication against migraine, fatigue, drowsiness and breathing problems, etc. Unlike other psychoactive substances, utilization of caffeine is legal in most countries. It is generally recognized as safe at usual moderate doses, though it becomes toxic in excessive amounts. Several clinical studies showed a relation between caffeine and anxiogenic effect and/or panic disorders [2, 3]. Its consumption is also associated with an overall lower risk of malignant growth like hepatocellular, endometrial or colorectal cancer [4, 5]. Still the effect of caffeine and its environmental degradation products on aquatic living species is not known. Therefore, as a preventive measure a development of an effective removal method is needed.

Caffeine, like most of other pharmaceuticals, is a persistent compound and cannot be efficiently removed by municipal wastewater treatment facilities [6]. Advanced oxidation processes demonstrated an effective destruction of persistent organic pollutants through *in situ* generation of a strong oxidant such as hydroxyl radical ($^{\bullet}OH$) [7]. Among these processes, the electrochemical advanced oxidation processes (EAOPs) were recently developed and successfully applied to the removal of different refractory pollutants [7]. Electro-Fenton process is one of most popular EAOPs. It is based on the production of $^{\bullet}OH$ from the so-called Fenton′s reagent: a mixture of hydrogen peroxide and ferrous (Fe^{2+}) ion [7]. The mechanism of the reactions involved in this process and its basics are well described and analyzed in several recent review papers [8, 9]. Electro-Fenton has been rather well-researched and proved to be able to effectively remove different pollutants such as: pesticides [10], dyes [11, 12], pharmaceuticals [13, 14] and other organic pollutants [15].

Investigation of the operating parameters of electro-Fenton process and their effect on the removal rates of caffeine constitute the main goal of this study, as more knowledge is needed to determine the optimal conditions.

First and foremost, when dealing with setup optimization of the electrochemical process, the role of electrode material is of prime importance [8, 16]. Fenton's reagent is produced at the cathode: H_2O_2 from the electro-reduction of the O_2 from the compressed air, and Fe^{2+} from a reduction of Fe^{3+} (added externally as a catalyst or generated by Fenton reaction). The anode, as a place of oxidation, also plays a major role as it promotes the formation of $^{\bullet}OH$ radicals. Currently boron-doped diamond (BDD) film on a suitable support is the most satisfactory anode material [17-20]. Numerous publications claim this material provides higher efficiency due to its specific characteristics, mainly its high O_2-overpotential [21-23]. Therefore, for this study the material was preferred over others (e.g. Pt, Ti and dimensionally stable anodes (DSA), etc.).

Caffeine was chosen as a model molecule for this study based on its following characteristics: occurrence in relatively high concentration in all types of receiving waters, as well as effluents from wastewater treatment plants (Table II.1);

chemical stability due to quite high water solubility (around 20 g L^{-1} at room temperature), low volatility, as well as rather high resistivity to direct light photolysis. Moreover, its removal by conventional wastewater treatment methods is quite variable (Table II.2), meaning that efficiency of these processes is not yet satisfactory.

Table II.1. Occurrence and concentration of caffeine in different types of water environment (WWTP - wastewater treatment plant).

Concentration (ng/L)	Sample	Location	Reference
7-687	Rivers (sources of drinking water impacted by wastewater treatment plants)	USA	[24]
2.9 - 194	Surface water from three major rivers	South Korea	[25]
23 - 776	Wastewater from seven WWTPs		
7000-73000	Influent of WWTP	Wetzicon, Switzerland	[26]
28-355	Effluent of WWTP		
52-192	Sewage treatment plant	Almeria, Spain	[27]
1.4-44	Sewage treatment plant		

Table II.2. Removal efficiencies of caffeine in different wastewater treatment plants (WWTPs).

WWTP	Removal, %	References
Sewage treatment plant in Spain	85	[27]
Conventional municipal WWTP in Michigan, USA	99	[28]
Hospital WWTP (chemical flocculation/activated carbon)	69	[29]
Four conventional WWTPs in Seville, Spain	38-68	[30]

On the whole, caffeine is a well-studied molecule, especially for demonstration of the feasibility of new treatment technologies (Table II.3). Consequently present study could serve to address two issues: comparison of electro-Fenton to other treatment technologies for removal of pharmaceuticals, and process optimization of electro-Fenton technology based on a case study of caffeine, which can serve as a basis for further investigations. Besides, kinetic studies of single compounds subjected to the degradation by certain technology contribute to a better understanding of the underlying mechanisms.

Table II.3. Removal of caffeine by advanced oxidation processes.

Treatment process	Removal, %	References
Solar photo-Fenton on real effluents	36.4%	[31]
Fenton oxidation on real wastewater	80%	[32]
Heterogeneous photo-Fenton	58 - 90 %	[33]
Ozonation	100%	[34]
TiO_2 / UV system		
Fenton oxidation	> 90	[35]
UV / H_2O_2		
Photo-Fenton	100	[36]

This study is designed to clear the following questions about removal of caffeine from water using electro-Fenton technology: (i) optimization of operating parameters for degradation of caffeine; (ii) application of these optimal operating parameters for the mineralization of the target molecule; (iii) identification of the oxidation products of caffeine, such as aromatic intermediates, carboxylic acids and inorganic ions in order to propose an oxidative degradation pathway and (iv) discussion of the effects of operating parameters on degradation and mineralization in order to define general optimal values.

II.2. MATERIALS AND METHODS

II.2.1. Chemicals

Analytical grade caffeine (CAS 58-08-2) was obtained from Sigma-Aldrich. Iron (II) sulfate heptahydrate (CAS 7782-63-0) as a catalyst source, and sodium sulfate (CAS 7757-82-6) as electrolyte were obtained from Acros Organics and Sigma-Aldrich, respectively. The pH of the solutions was set using 1 M sulfuric acid. Standards for ion analysis as well as reagents for mobile phase preparation were the ACS reagents with purity over 99.5%: ammonium oxalate monohydrate (CAS 6009-70-7), sodium nitrate (CAS 7631-99-4), sodium nitrite (CAS 7632-00-0), sodium carbonate (CAS 497-19-8) and sodium bicarbonate (CAS 144-55-8). Methanol as a mobile phase for liquid chromatography was HPLC grade from Sigma-Aldrich. All the solutions were prepared with ultrapure water produced by a Millipore Milli-Q (simplicity 185) system with resistivity of >18 MΩ cm.

II.2.2. Experimental setup

All experiments were carried out in an open undivided electro-Fenton reactor in a batch mode at room temperature (T = 20±1 °C). It consisted of a glass beaker of 250 mL, which was filled with 200 mL of 0.1 mM caffeine solution containing 0.05 M sodium sulfate (supporting electrolyte) and Fe^{2+} ions (as catalyst) of a suitable concentration. The carbon felt cathode (18.5 cm × 4.5 cm physical area) was placed on the internal wall of the electrochemical reactor. The anode (6 cm × 4 cm) was a

BDD thin-film on a niobium substrate, positioned in the middle of the reactor on equal distances from the surrounding cathode. In order to avoid a short circuit between the electrodes, a teflon mesh of the same size with the carbon cathode was used around it. For the entire experiment the pH was adjusted with 1 M sulfuric acid to 2.9 ±0.1 and measured with a pH meter CyberScan pH 1500 by Eutech Instruments. The solution was stirred continuously throughout the process with a magnetic bar at a speed of 450 rpm on a magnetic stirrer CB162 from Stuart (United Kingdom). Likewise aeration with compressed air at a speed of 0.2 L/min was ensured for the whole duration of electro-Fenton and also 5 min before starting the electrolysis in order to saturate the solution with oxygen. The current intensity and voltage were monitored in real time on a power supply HM8040-3 by Hameg Instruments.

Each experiment was done in triplicate, so the data are represented by the mean value and its variation is characterized by error bars.

II.2.3. Instruments and analytical procedures

II.2.3.1. High performance liquid chromatography (HPLC)

Degradation kinetics of caffeine and kinetic experiments for determining the absolute rate constant for its oxidation by hydroxyl radicals were followed by means of reverse-phase high performance liquid chromatography (RP-HPLC). The Merck Hitachi equipment consisted of a column Purospher STAR RP-18 endcapped (5 μm), a pump (Elite LaChrome, L-2130), UV detector (Elite LaChrome, L-2400) and a thermostat (Jetstream Plus, series 140310). Analytical conditions were: T = 40°C, mobile phase 30:70 (v/v) methanol/ultrapure water, flow rate of 0.5 mL/min. Detection was performed at 275 nm wavelength. For determination of the absolute rate constant, the analytical conditions were slightly different: mobile phase 20:80 (v/v) methanol/ultrapure water, both containing 0.1% acetic acid, flow rate of 0.5 mL min^{-1}, and detection at the same wavelength. Identification and quantification of carboxylic acids as ultimate end-products before mineralization was performed by ion-expulsion chromatography using a HPLC equipped with a Supercogel H (φ = 4.6 mm × 25 mm) column at a room temperature, a LaChrom pump L-7100 and a diode array detector (LaChrom L-7455). Detection was at 210 nm wavelength, a solution of 1% sulfuric acid was as a mobile phase at a flow rate of 0.2 mL/min. The injection volume for both types of HPLC analyses was equal to 20 μL.

II.2.3.2. Ion chromatography

The analysis of inorganic ions was performed with the help of Dionex ICS-1000 ion chromatography system equipped with an ASRS-ULTRA II (for anions) or CSRS-ULTRA II (for cations) self-regenerating suppressor to improve the sensitivity of the detector. The system was equipped with a DS6 conductivity detector containing a cell heated at 35 °C. An anion-exchange column (IonPac AS4ASC, 25 cm × 4 mm) linked to an IonPac AG4A-SC, 5 cm × 4 mm column guard was used

for anions analysis. Cation (NH_4^+) analysis was performed on a cation-exchange column (IonPac CS12A, 25 cm × 4 mm) linked to an IonPac CG12A, 5 cm × 4 mm column guard. A solution of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at 2.0 mL/min, and a 9.0-mM H_2SO_4 solution at 1.0 mL/min were used as mobile phases for anion and cation analysis, respectively. Identification and quantification of ions was done by comparing the elution time and peak areas of standard solutions.

II.2.3.3. Gas chromatography coupled with mass spectrometry (GC-MS)

This specific technique was used for identification of intermediates from caffeine degradation. Identification of aromatic/cyclic intermediates of oxidative degradation of caffeine was performed by CG-MS analysis using an ISQ Single Quadrupole mass spectrometer coupled with Trace 1300 Series gas chromatograph equipped with a TraceGold TG-5MS (30 m × 0.25 mm; 0.25 μm) column. Experiments on 0.2 mM caffeine were performed with the following operating parameters: I = 50 mA, 0.2 mM $FeSO_4$, 50 mM Na_2SO_4 and electrolysis time of 2, 5, 7 and 10 min. Prior to analysis with the GC-MS system, a step of liquid-liquid extraction with dichloromethane as well as derivatization with BSTFA (N,O-Bis(trimethylsilyl) trifluoroacetamide) were performed. Analytical method for GC-MS was as follows: for the first two minutes the column was at 40°C, afterwards the temperature was increased at a rate of 10 °C/min, so at 26 min, reaching 280 °C at 26 min and then kept until the end of analysis (30 min). To compose a proposed degradation pathway of caffeine, MarkinSketch, a chemical drawing software from ChemAxon, was used.

II.2.3.4. Total organic carbon (TOC) analysis

The mineralization degree of initial and treated samples was monitored by the abatement of total organic carbon content, determined on a Shimadzu VCSH TOC analyzer. The TOC was measured by combustion with catalytic oxidation at 680 °C using high-purity oxygen gas as a carrying gas at a flow rate of 150 mL/min. A non-dispersive infrared detector was used in the system to determine TOC amount in the samples. Calibration of the analyzer was attained with standard potassium hydrogen phthalate (99.5 %) solution. The injection volumes were 50 μL.

II.3. RESULTS AND DISCUSSION

The most important operating parameters for the electro-Fenton system are certainly pH, concentration of catalyst (Fe^{2+}) and current intensity. The optimal range of pH has been studied extensively and it was proved to be definitely around the value of 3 [8, 37]. Regarding the other two parameters and their optimal values, much more discussion and research have been done here. It seems that the optimal values can fluctuate according to the setup, compound to be degraded, rate of parasitic reactions etc. [38, 39]. Therefore these two parameters were studied to

determine an optimal value for degradation of the caffeine and then for mineralization of its aqueous solution.

II.3.1. Degradation of caffeine. Determination of optimal operating parameters

II.3.1.1. Variation of Fe^{2+} concentration and current intensity

Current intensity is the main parameter in the electro-Fenton process since it governs a large number of reactions. It affects the formation rate of Fenton's reagent (through reactions 1 and 3), and consequently the rate of generation of homogeneous $^{\bullet}OH$ in the bulk solution through Fenton reaction 2. Also the formation of heterogeneous BDD($^{\bullet}OH$) on the anode surface (reaction 4) depends on the current. These homogeneous and heterogeneous hydroxyl radicals react with caffeine leading to its oxidative degradation. To clarify the effect of current intensity on oxidative degradation of 0.1 mM caffeine solution, electrolysis was carried out by applying current values from 50 to 400 mA with different catalyst concentrations (0.1 - 0.5 mM). The results were depicted in Figure II.1. It shows that the decay kinetics of caffeine is enhanced by increasing current intensity up to 300 mA, while a further increase in the current (to 400 mA) resulted in a decrease of degradation efficiency and in longer time for a complete transformation of caffeine. The same pattern is noticeable in Figures II.1, b and II.1, c for catalyst concentration of 0.2 and 0.5 mM, respectively. These results indicated that the current intensity of 300 mA constituted the optimal value under given operating conditions. On the whole, it should be noted that the degradation of caffeine was very fast for any set of current intensity/catalyst concentration, and its complete oxidation required less than 10 min.

$$O_2 + 2\,H^+ + 2\,e^- \rightarrow H_2O_2 \quad (1)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + H^+ \quad (2)$$

$$Fe^{3+} + 2\,e^- \rightarrow Fe^{2+} \quad (3)$$

$$BDD + H_2O \rightarrow BDD(^{\bullet}OH) + H^+ + e^- \quad (4)$$

The inhibition of the oxidation rate at high current values can be explained by involvement of parasitic reactions: an evolution of H_2 at the cathode inhibiting the formation H_2O_2; recombination of BDD($^{\bullet}OH$) at the anode. Regarding the catalyst concentration, the Figure II.1 shows that the degradation of caffeine was complete at about 4-6 min for all current intensities (except 50 mA) with 0.2 mM Fe^{2+}. This value agrees with previous reports [13, 40] and was taken as an optimal catalyst value for the following experiments.

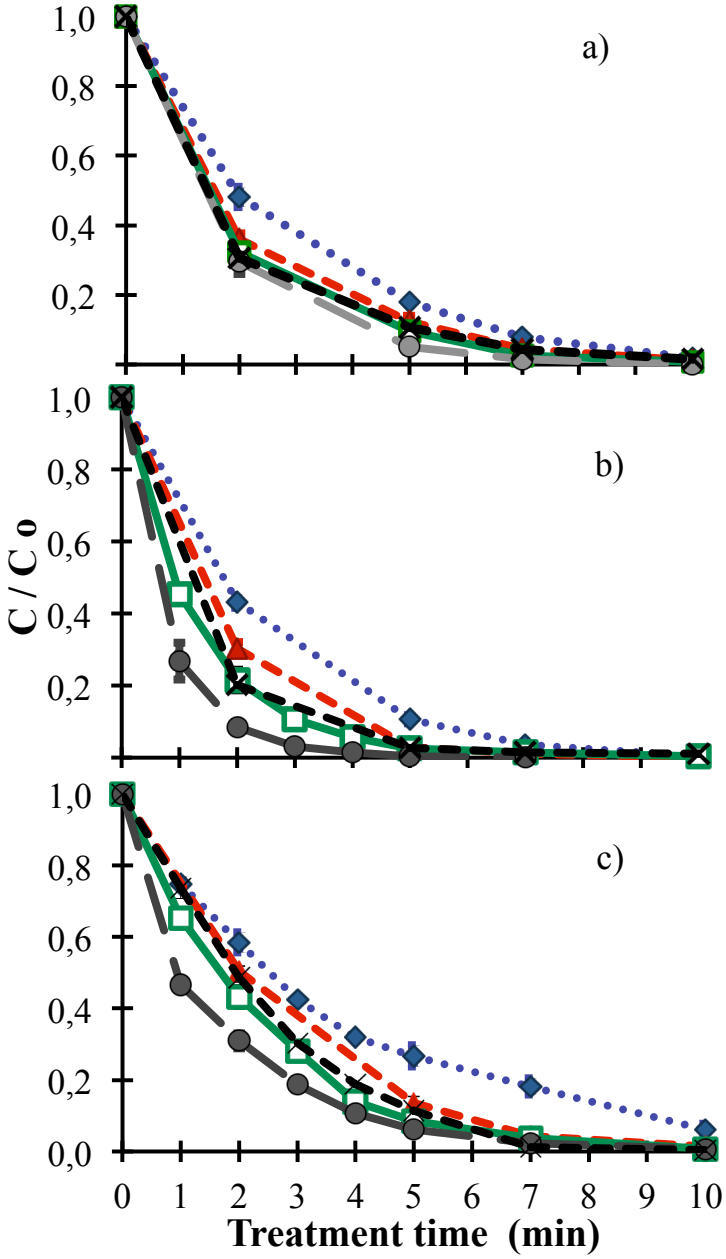

Figure II.1. The effects of applied current intensity and catalyst concentration for degradation of caffeine (CAF) during the electro-Fenton process. Catalyst [Fe^{2+}] concentration (mM): (A) 0.1; (B) 0.2; (C) 0.5. Current intensity (mA): 50 (-◆-); 100 (-▲-); 200 (-□-); 300 (-●-); 400 (-✖-). Operating conditions: $[CAF]_0$ = 0.1 mM, $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

II.3.1.2. Determination of apparent and absolute rate constants

The reaction between an organic pollutant (P) and •OH is expressed as follows:

$$P + {}^{\bullet}OH \rightarrow \text{Products} \quad (5)$$

Then the oxidation rate of P can be written as:

$$V = d[P]/dt = k\,[P]\,[{}^{\bullet}OH] \quad (6)$$

with $k = k_{abs}$ as the absolute rate constant of the second order reaction.

The second order reaction can be approximated to a pseudo-first order; because the operating parameters were constant throughout the experiment as a result the production of hydroxyl radicals was constant as well. Considering these conditions, the general rate equation can be simplified to:

$$V = d[P]/dt = k_{app}\,[P] \quad (1)$$

with $k_{app} = k_{abs}\,[^{\bullet}OH]$

Accordingly, the decay of caffeine (CAF) concentration in oxidation reaction by hydroxyl radicals generated in electro-Fenton process can be expressed as follows:

$$d[CAF]/dt = k_{abs}\,[CAF]\,[^{\bullet}OH] = k_{app}\,[CAF] \quad (2)$$

The k_{app} can then be determined from this kinetics equation. It corresponds to the slope of the line produced by plotting $\ln([CAF]_0/[CAF]_t)$ versus time. Table II.4 summarizes k_{app} values together with corresponding R^2-values for the whole set of studied parameters. From this table, it appears that the highest value of the k_{app} was obtained for 300 mA regardless the concentration of the catalyst. The summary of k_{app} confirmed the values of optimal experimental parameters determined above, i.e. I = 300 mA and $[Fe^{2+}]$ = 0.2 mM. The explanation of this phenomenon is given in the section II.3.2.

Table II.4. Reaction rate constants and their corresponding coefficients of determination (R^2 values) for the entire set of operating parameters.

I / **[Fe(II)]**	**50 mA**	**100 mA**	**200 mA**	**300 mA**	**400 mA**
0.1 mM Fe	0.380 ± 0.007 *R^2 = 0.99*	0.443 ± 0.013 *R^2 = 0.99*	0.477 ± 0.005 *R^2 = 0.99*	0.601 ± 0.024 *R^2 = 0.99*	0.460 ± 0.007 *R^2 = 0.98*
0.2 mM Fe	0.468 ± 0.017 *R^2 = 0.99*	0.651 ± 0.011 *R^2 = 0.99*	0.742 ± 0.019 *R^2 = 0.99*	1.132 ± 0.052 *R^2 = 0.98*	0.640 ± 0.012 *R^2 = 0.97*
0.5 mM Fe	0.275 ± 0.016 *R^2 = 0.99*	0.431 ± 0.007 *R^2 = 0.99*	0.460 ± 0.005 *R^2 = 0.99*	0.548 ± 0.019 *R^2 = 0.99*	0.415 ± 0.007 *R^2 = 0.99*

The absolute rate constant of caffeine degradation was then determined by the competitive kinetics method using 4-hydroxylbenzoic acid (4-HBA) as the standard competitor. The value of k for its oxidation by $^{\bullet}OH$ is well known as 2.19×10^9 M^{-1} s^{-1} [41]. Consequently the k_{abs} of caffeine was calculated from the equation:

$$k_{abs}[CAF] = k_{abs}\,[4\text{-HBA}]\,k_{app}\,[CAF] / k_{app}\,[4\text{-HBA}] \quad (3)$$

Competition experiments were carried out using equal concentrations (0.1 mM) of caffeine and 4-HBA, which competed for $^{\bullet}OH$ and BDD($^{\bullet}OH$). Evolution of their concentrations was monitored by HPLC analysis. These experiments were done at the current intensity of 50 mA, concentration of Fe^{2+} and electrolyte Na_2SO_4 equal to 0.1 mM and 50 mM respectively. As a result the k_{abs}(CAF) was found to be $(2.48 \pm 0.01) \times 10^9$ M^{-1} s^{-1}, which to our knowledge had not been reported before.

II.3.2. Mineralization of caffeine. The effect of operating parameters

In the previous section, optimal parameters for oxidative degradation of caffeine were determined as I = 300 mA and [Fe^{2+}] = 0.2 mM. Compared to other treatment technologies, mineralization of organic pollutants is one of greatest advantages of Fenton systems in general and especially of the electro-Fenton process. Thus, the effects of applied current intensity and catalyst concentration were assessed for mineralization efficiency of caffeine.

II.3.2.1. Effect of Fe^{2+} concentration

To clarify the effect of catalyst concentration, electrolysis of 0.1 mM caffeine in aqueous solutions was conducted with 0.1, 0.2 and 0.5 mM Fe^{2+} at the constant current of 300 mA. Figure II.2 shows the obtained results.

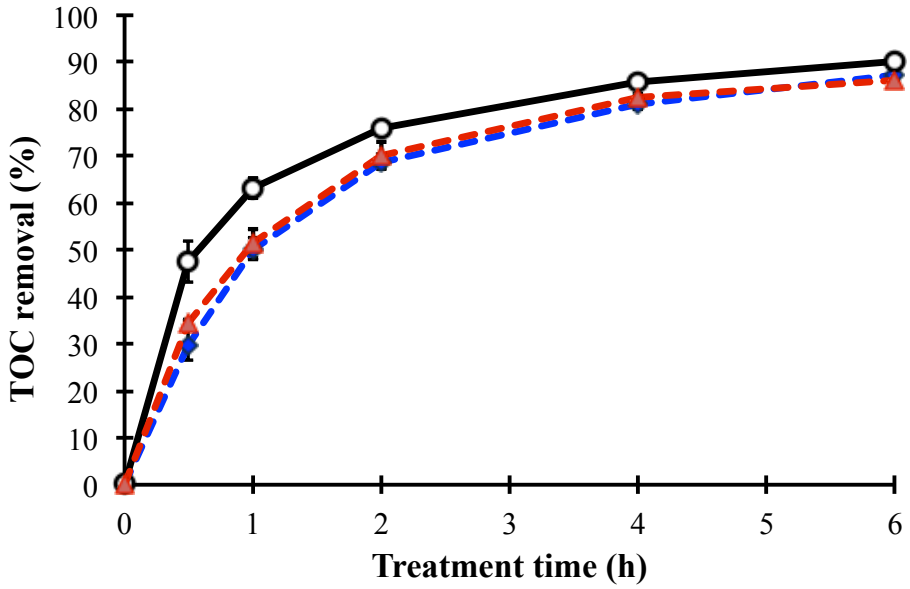

Figure II.2. Evolution of mineralization (in terms of TOC removal (%)) of the caffeine (CAF) by the electro-Fenton process in dependence on the catalyst concentration. Catalyst [Fe^{2+}] concentration (mM): 0.1 (-◆-); 0.2 (-O-); 0.5 (-▲-). Operating parameters: $[CAF]_0$ = 0.1 mM, I = 300 mA, [Na_2SO_4] = 50 mM, V = 200 mL.

It is worth noticing that here the tendency was quite similar to degradation experiments. An increase in catalyst (Fe^{2+}) concentration from 0.1 to 0.2 mM demonstrated a slightly enhanced mineralization rate. However, a further increase in concentration from 0.2 to 0.5 mM did not yield faster rates. Consequently, the value of 0.2 mM was considered as a threshold value of Fe^{2+} concentration. This phenomenon is due to enhancement of the rate of the wasting reaction (7) under excess of Fe^{2+} concentration [7, 42]. This reaction has a high rate constant (k_{abs} = 3.2 x 108 M s^{-1}) and Fe^{2+} concentration above the optimal value results in the enhancement of its reaction rate, and therefore in a competition of Fe^{2+} with caffeine for $^{\bullet}OH$.

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \qquad (7)$$

II.3.2.2. Effect of applied current intensity

The effect of current intensity on mineralization efficiency was studied using electrolysis with constant current within the range of 100-1500 mA (Figure II.3). In contrast to degradation experiments, the mineralization rate of caffeine was enhanced with an increase of current intensity of up to 1500 mA. This phenomenon can be related to the formation of reaction intermediates, which are oxidized with more difficulty and require a large amount of •OH/BDD(•OH) for mineralization purposes. High current values promote higher •OH/BDD(•OH) through reactions 1 – 4 and consequently high mineralization rates.

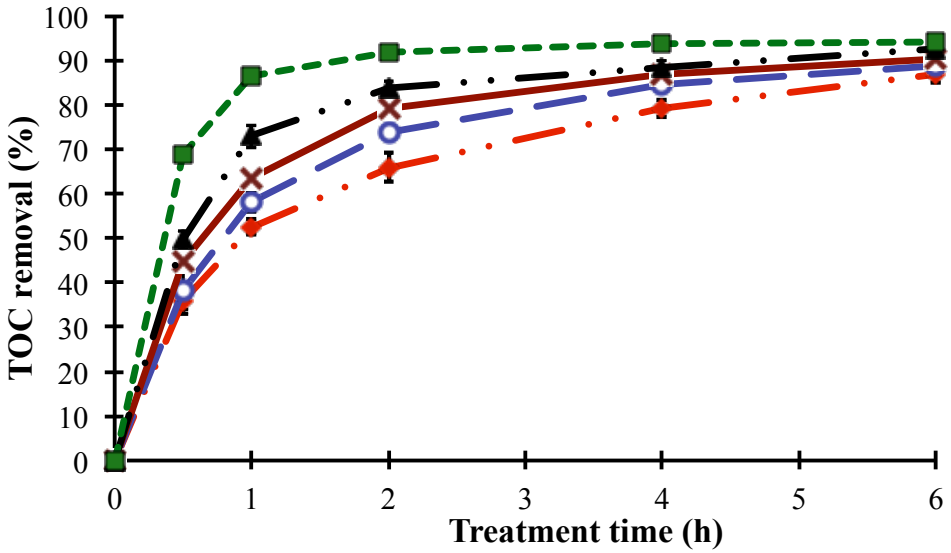

Figure II.3. Evolution of mineralization (in terms of TOC removal (%)) of the caffeine (CAF) by the electro-Fenton process in dependence on the current intensity. Current intensity (mA): 100 (-◆-); 300 (-○-); 500 (-✖-). 1000 (-▲-); 1500 (-■-). Operating parameters: $[CAF]_0$ = 0.1 mM, [Fe(II)] = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

Another observation on the overall profile of mineralization curves uncovered a change in the mineralization rate throughout the 6 h of treatment. For the intensity of 100 mA, the mineralization rates were 36%, 52% and 66% after 30 min, 1 h and 2 h, respectively. For 1500 mA the respective values were 69%, 86% and 92%. The mineralization rate was quite fast at the beginning of treatment and decelerated progressively with time. Such a decrease in the mineralization rate, especially after reaching the plateau phase, can be attributed to the following fact. After 2 h of treatment the concentration of organic matter became low, while the high rate of hydroxyl radicals production was kept. This high concentration of •OH/BDD(•OH) promoted wasting reactions 8 – 12 [15, 43]. In addition, at this stage of treatment aromatics were converted to short chain aliphatic carboxylic acids (that form the residual TOC value), which were recalcitrant to hydroxyl radicals [7, 44].

$$BDD(^{\bullet}OH) \rightarrow BDD + 1/2\ O_2 + H^+ + e^- \quad \text{(anode surface)} \qquad (8)$$

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad \text{(solution bulk)} \qquad (9)$$

$$2\ BDD(^{\bullet}OH) \rightarrow 2\ BDD + H_2O_2 \quad \text{(anode surface)} \qquad (10)$$

$$HO_2^{\bullet} + {}^{\bullet}OH \rightarrow H_2O + O_2 \quad (11)$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2^{\bullet} \quad (12)$$

II.3.3. Identification and follow up of the degradation intermediates

A follow-up of the degradation products allowed investigating a pathway of caffeine degradation by hydroxyl radicals. It enabled a better understanding of how the hydroxyl radicals and other less reactive species, which were also produced in parallel, repressed the organic molecules.

For this purpose, firstly the formation and evolution of a number of carboxylic acids were investigated using ion-exclusion HPLC (Figure II.4).

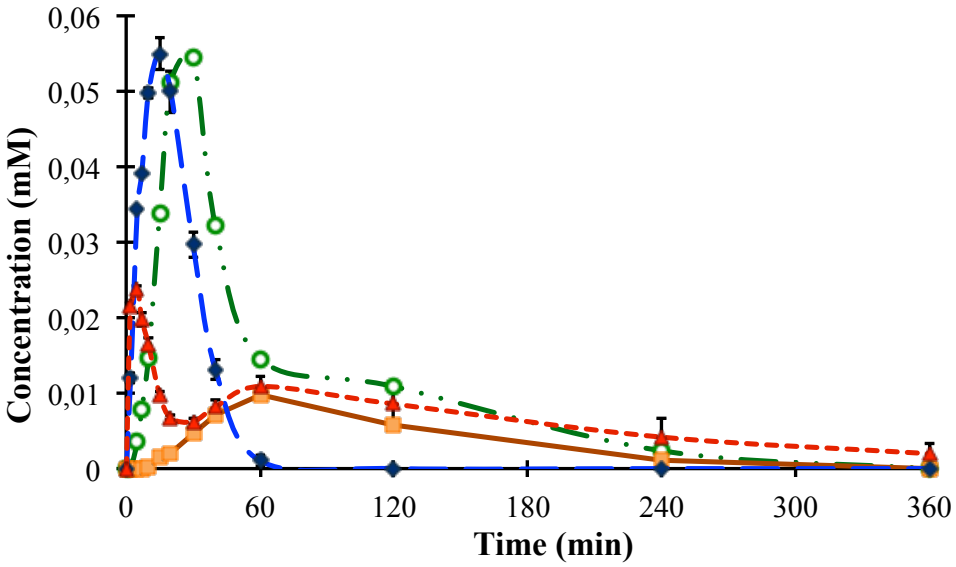

Figure II.4. Evolution of short-chain carboxylic acids during the degradation of caffeine (CAF) by electro-Fenton process: malonic (-◆-); oxalic (-O-); acetic (-▲-); oxamic (-■-). Operating parameters: $[CAF]_0$ = 0.1 mM, I = 100 mA, [Fe(II)] = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

Oxalic, malonic and acetic acids were formed from the beginning of the electrolysis and accumulated on the first 30 min, and then degraded gradually throughout the treatment. Oxamic acid, which was the only nitrogen-containing acid here, formed and accumulated much more slowly. It reached its maximum concentration at about 60 min, but persisted in the solution all along the electrolysis.

The detected acids remained even at the end of the 6-h treatment, constituting the residual TOC. For instance, after 30 min of electro-Fenton treatment the TOC of all identified acids was around 2.64 mg L^{-1}, which represented 41% of the TOC in solution (6.40 mg L^{-1}). Yet this proportion dropped to below 20% after only 1 h of treatment since these acids were formed and oxidized at the same time.

The evolution of cations (NH_4^+) and anions (NO_3^- and NO_2^-) was monitored by ionic chromatography. Having 4 atoms of nitrogen, the molecule of caffeine can yield a maximum of 0.4 mM of nitrogen ions during complete mineralization. The result of the mass balance as a percent of the total maximum concentration is depicted in Figure II.5.

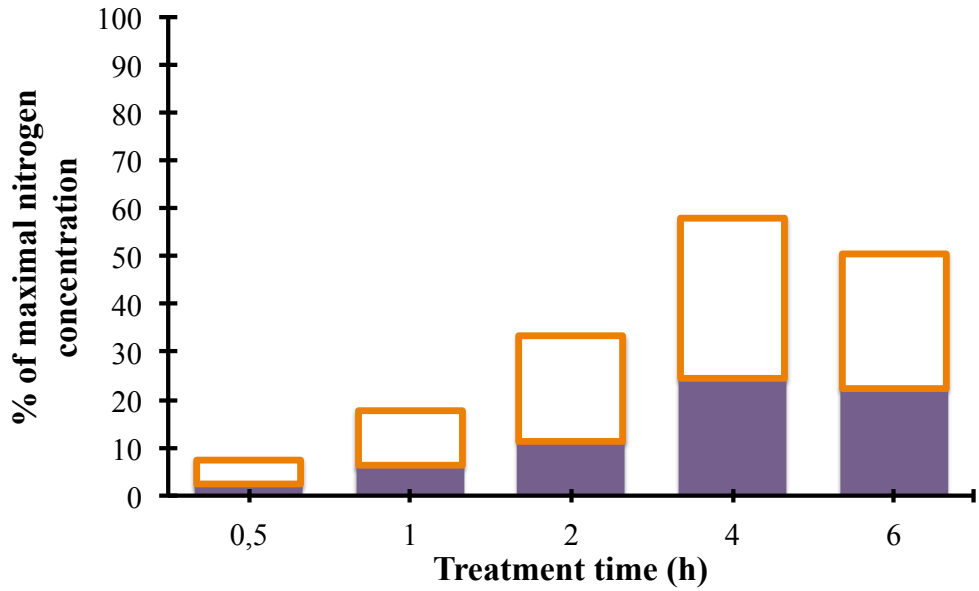

Figure II.5. Formation and evolution of NO_3^- and NH_4^+ during the treatment of 0.1 mM caffeine by the electro-Fenton process: ammonium (-■-); nitrate (-□-). Operating parameters: for nitrate/nitrite I = 100 mA, [Fe(II)] = 0.2 mM, [Na_2SO_4] = 50 mM, V = 200 mL. For ammonium same conditions apart from supporting electrolyte [$MgCl_2$] =50 mM.

The evolution of ammonium and nitrate followed the same trend: gradual increase in concentration reached the maximum value at 4 h and then slightly decreased. No nitrite ion was detected. From Figure II.5 it is seen that the mass balance was far from being complete. Such conclusions were also reported by previous studies [10, 13].

Finally, to complete the caffeine mineralization pathway, aromatic/cyclic intermediates were qualitatively identified using GC-MS analysis. Based on the identified intermediates, short-chain carboxylic acids and released inorganic ions, we suggest the degradation path of caffeine given in Figure II.6. This is a compiled pathway based on previous research and completed with our results. For example, Telo and Vieira [45] reported the formation of trimethyluric acid during degradation of caffeine by persulfate and hydroxyl radicals photocatalytically generated in a flow system. Dalmazio et al. [35] studied the advanced oxidation of caffeine by means of three different processes: UV/H_2O_2, TiO_2/UV and Fenton system. The degradation by-products were the same for all three processes: structures [1] and [2] resulting in parabanic acid and its 1,3-dimethyl derivative. Indermuhle et al. [46] monitored the degradation of caffeine by conductive diamond electrochemical oxidation and detected intermediates [4] and [5]. They suggested that these intermediates were produced from the opening of pyrimidine ring after the imidazole part had been broken. In addition, we identified by GC-MS analysis the 1,3-dimethylparabanic acid, and other aliphatic compounds such as urea, glycerol, acetamide and acetic acid. To conclude aromatic/cyclic intermediates gave small aliphatic molecules, through oxidative ring/cycle opening reactions, which are further mineralized to carbon dioxide, and inorganic ions.

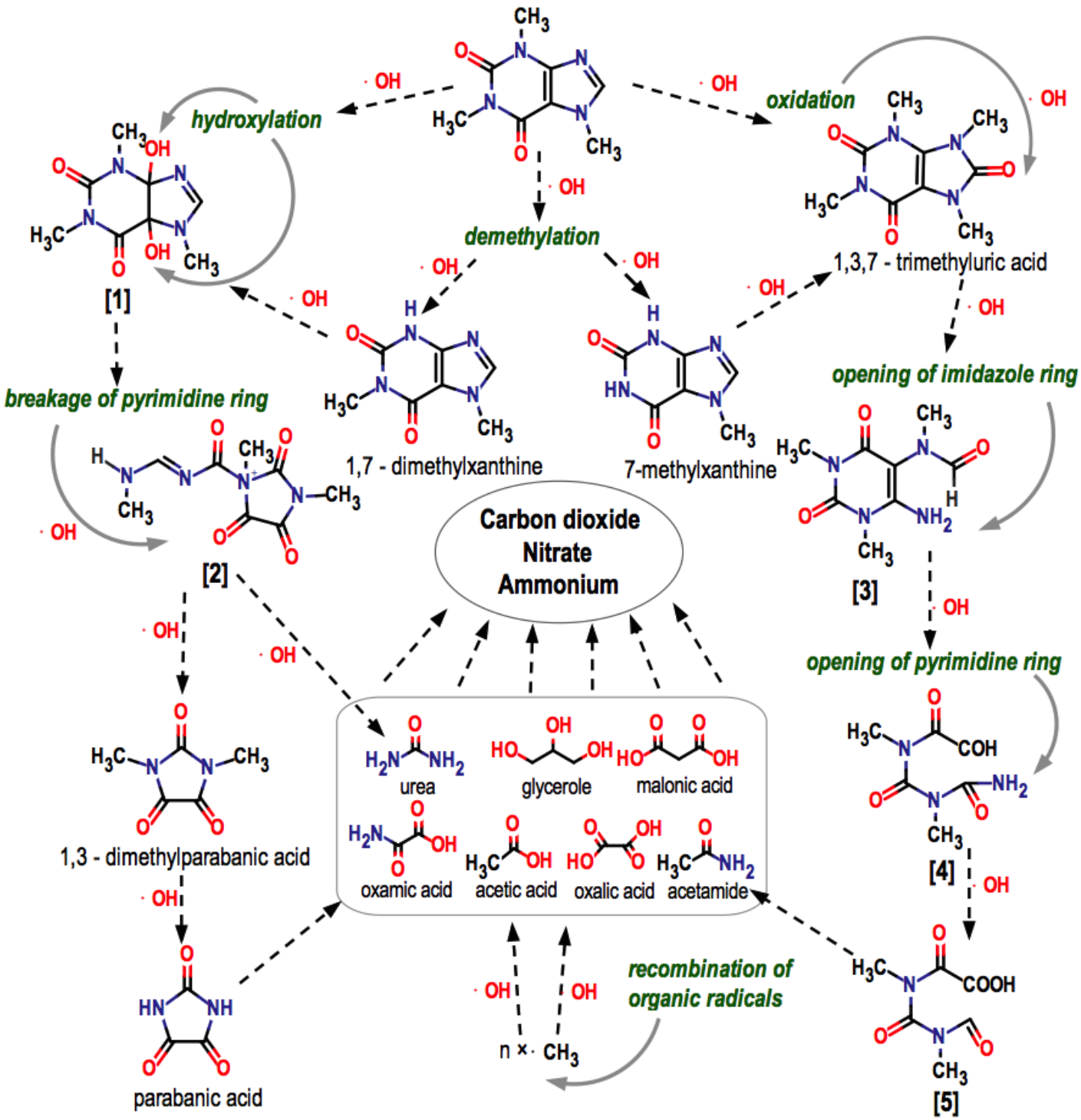

Figure II.6. Proposed pathway of oxidative degradation of caffeine by the hydroxyl radicals produced during the electro-Fenton process.

II.3.4. Optimal operating parameters: degradation vs. mineralization

Results obtained in this study revealed that the operating parameters did not influence in the same manner the degradation kinetics of caffeine and its mineralization. In terms of catalyst concentration, for both degradation and mineralization 0.2 mM Fe^{2+} led to better results. Lower Fe^{2+} concentration seemed to be insufficient, while higher concentrations promoted the wasting reaction 7. Regarding the current intensity, main difference was the existence of the limiting value for degradation kinetics. It was mentioned above that the current intensity higher than 300 mA had not led to an improvement of the degradation kinetics, while the mineralization rate increased with current intensity up to 1500 mA. This fact can be explained by the different nature of these two phenomena: degradation consists of one step (oxidation of caffeine to its primary intermediates), whereas mineralization is a significantly more complex process with numerous integral reactions. Indeed, the reactions with aliphatic carboxylic acids, which are hardly oxidizable, needed a larger amount of hydroxyl radicals [47].

A similar tendency of the limiting value for current intensity was also reported by previous studies on the electro-Fenton process with different process setups [14, 48]. It is noticeable that high current intensity may have certain adverse effects on the degradation efficiency apart from significant energy consumption, which consequently implies high operational costs. The principal parasitic reactions leading to the consumption of energy were: the evolution of H_2 (that occurred simultaneously with formation of H_2O_2 at high current values) at the cathode and evolution of O_2 at the anode that hindered BDD($^{\bullet}OH$) production.

It is worth to highlight that these trends are compound-specific and the optimal operating parameters found in this study are valid for oxidative degradation/mineralization of caffeine under given experimental conditions. The optimal operating parameters can be different for others contaminants and under different electro-Fenton setup depending on the chemical structure of the initial pollutant and that of its oxidation intermediates.

To evaluate how efficiently the applied current intensity removed organic matter, we could assess mineralization-current efficiency (MCE) from mineralization results. MCE at a given time *t* was calculated with an equation 4 [49] and presented in Figure II.7.

$$MCE\ (\%) = (n\ F\ \Delta TOC / (4.32 \times 10^7\ m\ I\ t)) \times 100 \qquad (4)$$

where F is Faraday constant (96,487 C mol^{-1}), V is the volume of the treated solution (L), Δ TOC is experimental TOC removal (mg L^{-1}), 4.32×10^7 is the conversion factor for homogenization of units (3,600 s h^{-1} × 12,000 mg mol^{-1}), m is the number of carbon atoms in the molecule (caffeine has 8 atoms), I is applied current intensity (A), t is treatment time (h), n is number of electrons consumed for mineralization of one molecule of carbon given from reaction (8).

$$C_8H_{10}N_4O_2 + 20H_2O \rightarrow 8CO_2 + 42H^+ + 2NO_3^- + 2NH_4^+ + 42e^- \qquad (13)$$

Figure II.7 shows the general MCE trends. It should be emphasized that low current intensities had high current efficiency values, which decreased throughout the treatment. Low MCE values obtained by applying a high current can be explained by the promotion of wasting/parasitic reactions, since they consume a large part of electrical energy provided in the electrochemical reactor. Therefore, when applying electrochemical advanced oxidation processes the choice is to be made between either energy saving (long treatment time) or mineralization rate (short treatment time but high energy consumption).

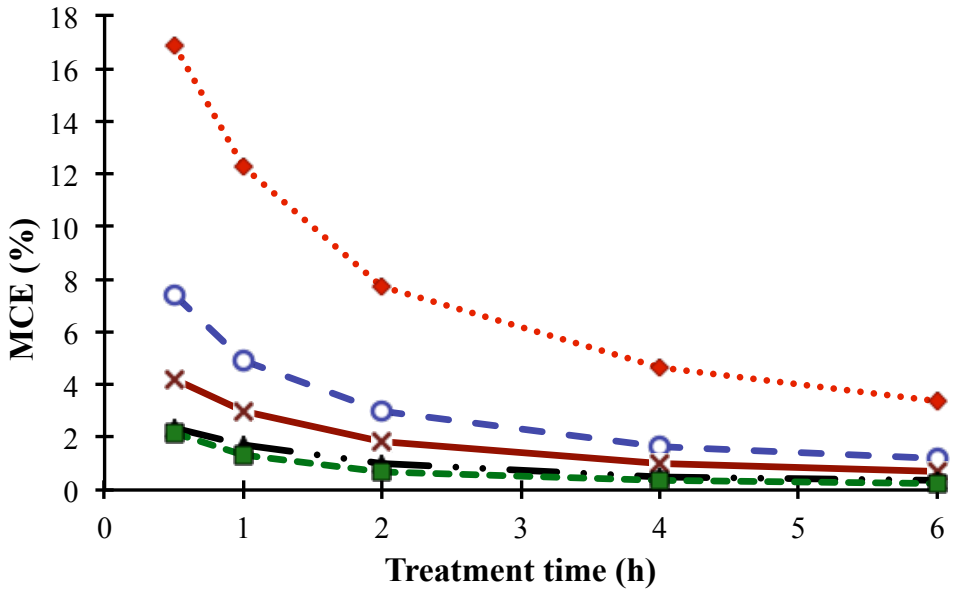

Figure II.7. Evolution of mineralization-current efficiency (MCE) during the electro-Fenton mineralization of caffeine (CAF) in dependence on the current intensity. Current intensity (mA): 100 (-◆-); 300 (-O-); 500 (-✕-). 1000 (-▲-); 1500 (-■-).Operating parameters: $[CAF]_0$ = 0.1 mM, [Fe(II)] = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

II.4. CONCLUSIONS

- Degradation of caffeine by the electro-Fenton process is very fast. Complete oxidation of this drug required 4-10 min depending on the set of operating parameters applied.
- Optimal current and catalyst concentration values for degradation of caffeine were found to be 0.2 mM and 300 mA for Fe^{2+} concentration and applied current intensity, respectively.
- Absolute rate constant of the reaction between caffeine and hydroxyl radical was determined to be $(2.48\pm0.01) \times 10^9$ M^{-1} s^{-1}.
- Almost complete mineralization of caffeine solution (>93%) was achieved after 2 h treatment with 1500 mA current intensity.

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CHAPTER III

Removal of cytostatic drug 5-fluorouracil from water by electro-Fenton process using boron-doped diamond anode

To be submitted

ABSTRACT

This study evaluates the efficiency of electro-Fenton process for the removal of cytostatic drug 5-fluorouracil (5-FU) from water. The removal efficiency was assessed with regards to kinetics of oxidative degradation and mineralization. Different ranges of operating parameters - current intensity (from 100 to 1500 mA) and Fe^{2+} concentration (from 0.1 to 0.5 mM) - were analyzed in order to determine optimal operating values with respect to maximal removal and minimal cost. The results revealed that the optimal catalyst concentration was 0.2 mM, while current intensity influenced degradation and mineralization differently. For degradation kinetics, the reaction rate was increased with an increase in applied current from 50 up to threshold intensity value of 300 mA, while the mineralization efficiency was improved up to the current value of 1500 mA. The absolute rate constant of the reaction between 5-FU and hydroxyl radical was determined by using competition kinetics method and found to be $(1.52 \pm 0.01) \times 10^9$ M^{-1} s^{-1}. The formation and evolution of mineral ions and carboxylic acids was monitored as well. The experimental data showed that even low concentrations of Fe^{2+} and low current intensities led to complete degradation of 5-FU and almost complete mineralization of its solution, showing that electro-Fenton constitutes an interesting alternative for efficient removal of pharmaceuticals from water.

Keywords: Electro-Fenton, 5-Fluorouracil, Pharmaceutical, Hydroxyl radical, Mineralization

III.1. INTRODUCTION

One of the most considerable threats for the well-being of humans and ecosystems is water pollution [1]. This issue has been steadily aggravating since its recognition and not only with regards to its increasing magnitude. Advances in analytical methods of chemistry allowed detection and identification of a previously unknown pollutants [2]. One class of those newly emerging pollutants, which are of great public and scientific concern, are pharmaceuticals and personal care products (PPCPs) [3, 4].

Conventional treatment methods are mostly based on biological processes, which employ microorganisms as removing agents. The group of PPCPs pollutants is chemically very different and possesses an important characteristic of being bioactive towards living organisms. Therefore the effectiveness of biological processes for elimination of these pollutants, which can be both recalcitrant and toxic to bacteria, becomes a serious issue. Therefore modern wastewater treatment plants are not able to properly remove such biorefractory pollutants [5-10]. As a result, the pharmaceutical residues and their metabolites can be found in surface, ground and even drinking water, but also in river sediments and activated sludge [11-15].

The most hazardous representatives of pharmaceutical pollution are those that have the most significant pharmacological action. A group of cytostatic (anti-cancer) drugs can display severe negative effects on non-target organisms, including humans [16]. Cytostatics are also called antineoplastics, i.e. preventing or inhibiting the growth of malignant cells or tumors. All these properties are a basis of a present-day cancer treatment. The drug 5-fluorouracil belongs to the sub-class of antimetabolites. These are compounds that hinder the utilization of certain metabolites, which are a part of normal metabolic pathways in the organism [17].

In order to avoid the propagation and subsequent accumulation of hazardous pollutants in the water bodies, proper preventive measures should be organized at major point sources of pollution. The sources for pharmaceutical pollution are mostly effluents from hospital and pharmaceutical production units. Therefore, new chemical methods, which can be introduced as additional effective pre- or post-treatment, are tried out and studied [18-23]. Among them, advanced oxidation processes (AOPs), which utilize strong reactive oxidant species, such as hydroxyl radicals, are taking a major part of scientific interest due to their attractive perspectives [24].

Electro-Fenton is an electrochemically advanced oxidation process (EAOPs), which is based on the production of hydroxyl radicals from *in situ* electrogenerated Fenton's reagent: mixture of hydrogen peroxide and Fe^{2+}. The mechanism of this process and its basics are well described and analyzed in multiple fundamental reviews [25-28]. Electro-Fenton has been rather well-studied and proved to be able to remove effectively different pollutants from water: biocides [29-31], dyes [32-36] pharmaceuticals [37-39] and other organic pollutants [40-42].

There is little research achieved in the domain of electrochemical removal of antineoplastic agents. However, it is clear that the EAOPs are the ones to be applied for

an effluent containing loads of pharmaceutical originating from any possible source [43].

To sum up, this article is designed to clear following questions on electro-Fenton technology: (i) optimization of operating parameters for a degradation of 5-fluorouracil; (ii) comparison of the degradation rates of 5-fluorouracil in the individual solution to a two-compound solution with caffeine; (iii) determination of the optimal operating parameters for the 5-fluorouracil mineralization and effects of their variation on mineralization efficiency.

III.2. MATERIALS AND METHODS

III.2.1. Chemicals

Analytical grade 5-fluorouracil (CAS 51-21-8) was obtained from Sigma-Aldrich. Iron (II) sulfate heptahydrate (CAS 7782-63-0) (used as source of ferrous ions as catalyst) and sodium sulfate (CAS 7757-82-6), as supporting electrolyte, were obtained from Acros Organics and Sigma-Aldrich, respectively. The solution of 1 M sulfuric acid was used to adjust the pH. Reagents for mobile phase and salts used standards in ion chromatography were the ACS reagents with purity over 99.5%: sodium nitrate (CAS 7631-99-4), sodium carbonate (CAS 497-19-8), ammonium oxalate monohydrate (CAS 6009-70-7), sodium nitrite (CAS 7632-00-0) and sodium bicarbonate (CAS 144-55-8). Methanol, used as mobile phase for liquid chromatography, was HPLC grade from Sigma-Aldrich. All the solutions were prepared with ultrapure water produced by a Millipore Milli-Q (simplicity 185) system with resistivity of >18 MΩ cm.

III.2.2. Experimental setup

The bench-scale, open and undivided electro-Fenton reactor was used in a batch mode at room temperature (T = 20±1 °C). The electrochemical reactor of 250 mL capacity was filled with 200 mL of 0.1 mM 5-fluorouracil solution. A supporting electrolyte (0.05 M sodium sulfate) and Fe^{2+} ions (as catalyst of a suitable concentration) were added to the solution. The pH was adjusted to 2.9 ±0.1. The carbon felt cathode (18.5 cm × 4.5 cm surface area) was surrounded by the Teflon® mesh in order to avoid the short circuit and was installed around the internal wall of the electrochemical reactor. A thin film BDD on a niobium substrate was used as anode with the dimensions 6 cm × 4 cm and was placed in the center of the reactor. The solution was saturated with O_2 by bubbling of compressed air at a speed of 0.2 L/min beginning 5 min prior to the start of electrolysis to ensure oxygen saturation of the solution and likewise stirring (450 rpm) was ensured throughout the whole experiment duration. The power supply HM8040-3 by Hameg Instruments allowed real-time monitoring of current intensity and voltage. The experiments were done in triplicates. The presented data is the mean value and standard error is characterized by error bars.

III.2.3. Instruments and analytical procedures

III.2.3.1. High performance liquid chromatography (HPLC)

Degradation kinetics of 5-fluorouracil and kinetic experiments for determining the absolute rate constant for its oxidation by hydroxyl radicals were monitored by means of reverse-phase high performance liquid chromatography (RP-HPLC). The Merck Hitachi equipment consisted of a column Purospher STAR RP-18 endcapped (5 μm), a pump (Elite LaChrom, L-2130), UV detector (Elite LaChrome, L-2400) and a thermostat (Jetstream Plus, series 140310). Analytical conditions were: T = 40 °C, mobile phase 5:95 (v/v) methanol/ultrapure water both buffered with 0.1% of acetic acid, isocratic elution, flow rate of 0.4 mL/min. Detection was performed at 277 nm wavelength. For determination of the absolute rate constant, the analytical conditions were slightly different: mobile phase 30:70 (v/v) methanol/ultrapure water, both containing 0.1% acetic acid, flow rate of 0.3 mL/min, and detection at the wavelength of 270 nm. Identification and quantification of carboxylic acids as ultimate end-products before mineralization was performed by ion-expulsion chromatography using a HPLC equipped with a Supercogel H (φ = 4.6 mm × 25 mm) column at a room temperature, a LaChrom pump L-7100 and a diode array detector (LaChrom L-7455). Detection was at 210 nm wavelength, a solution of 1% sulfuric acid was as used a mobile phase at a flow rate of 0.2 mL/min.

The simultaneous quantification of 5-fluorouracil together with caffeine from their common solution was performed by the help of the gradient elution. The mobile phase was a mixture of methanol and ultrapure water both buffered with 0.1% acetic acid. Starting from t = 0 to t = 13 min the flow rate was 0.3 mL/min and the mobile phase in the proportion of 90:10 (v/v) of water to methanol respectively. Under these conditions, the 5-fluorouracil was eluted after 11 min. After 13 min the conditions changed to flow rate of 0.6 mL/min and 70:30 (v/v) water/methanol. These conditions were kept up to T=28 min, which allowed to elute caffeine after 24.5 min. After 28 min the analytical conditions reversed back to the initial 90:10 (v/v) water/methanol and flow rate of 0.3 mL/min and were kept for additional 5 min. The detection was done at the wavelength of 275 nm. The injection volume was 20 μL for both types of HPLC analyses.

III.2.3.2. Ion chromatography

The analysis of inorganic ions was performed with the help of Dionex ICS-1000 ion chromatography system equipped with an ASRS-ULTRA II (for anions) or CSRS-ULTRA II (for cations) self-regenerating suppressor to improve the sensitivity of the detector. The system was equipped with a DS6 conductivity detector containing a cell heated at 35 °C. An anion-exchange column (IonPac AS4ASC, 25 cm × 4 mm) linked to an IonPac AG4A-SC, 5 cm × 4 mm column guard was used for anions analysis. Cation (NH_4^+) analysis was performed on a cation-exchange column (IonPac CS12A, 25 cm × 4 mm) linked to an IonPac CG12A, 5 cm × 4 mm column guard. A solution of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at 2.0 mL/min, and a 9.0-mM H_2SO_4 solution at

1.0 mL/min were used as mobile phases for anion and cation analysis, respectively. Identification and quantification of ions was done by comparing the elution time and peak areas of standard solutions.

III.2.3.4. Total organic carbon (TOC) analysis

The TOC abatement was followed by a Shimadzu VCSH TOC analyzer. The analytical method was combustion with catalytic oxidation at 680 °C using high-purity oxygen as a carrying gas at a flow rate of 150 mL/min. To determine the amount of TOC, a non-dispersive infrared detector was used. Calibration of the TOC analyzer was attained with standard potassium hydrogen phthalate (99.5 %) solution. The injection volumes were 50 μL.

III.3. RESULTS AND DISCUSSION

III.3.1. Oxidative degradation of 5-fluorouracil. Effect of operating parameters

In order to evaluate the effect of operating parameters on oxidative degradation of 5-fluorouracil, the experiments were carried out by varying the current intensity in the range of 100-400 mA and catalyst (Fe^{2+}) concentration in the range of 0.1-0.5 mM. The aqueous solution of 0.1 mM 5-fluorouracil was subjected to the different set of operating parameters and the results presented on Figure III.1 and III.2. The Figure III.1 shows the evolution of 5-fluorouracil concentration as a function of treatment time for three studied catalyst concentrations. It can be seen that with an increase of Fe^{2+} concentration from 0.1 to 0.2 mM, the oxidative degradation became faster, however a further increase of Fe^{2+} to 0.5 mM demonstrated the slowest rate. This concentration of Fe^{2+} (0.2 mM) was reported as optimal by our previous study on caffeine [44].

The decay kinetics of 5-fluorouracil is faster with an increase in the current intensity, but only up to a value of 300 mA (Figure III.2). The further increase to 400 mA resulted in slower degradation, whose values are close to that of 200 mA. A threshold value of current intensity was previously remarked by other studies [45, 46] and it is mostly due to propagation of certain parasitic reactions, like an evolution of H_2 at the cathode and recombination of BDD($^{\bullet}OH$) at the anode:

$$2\ BDD(^{\bullet}OH) \rightarrow 2\ BDD + H_2O_2 \qquad \text{(anode surface)} \qquad (1)$$

$$2\ H_2O + 2\ e^- \rightarrow H_2 + 2\ OH^- \qquad (2)$$

It can be concluded that the degradation of an organic pollutant, in this case 5-fluorouracil, is dependent on both Fe^{2+} concentration and applied current. The general trend followed by both parameters is that the degradation is faster with increased Fe^{2+} concentration/current intensity only up to a certain limit value. Increased values of operating parameters are generally attributed to the higher production of hydroxyl radicals. However, it seems that the amount of hydroxyl radicals is not the only critical issue, but rather it should be viewed as a ratio between the organic matter and hydroxyl radicals. Overproduction of oxidizing species triggers a number of limiting parasitic reactions, which consume the radicals and decrease the efficiency of electro-Fenton process:

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad \text{(solution bulk)} \qquad (3)$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2^{\bullet} \qquad (4)$$

$$^{\bullet}OH + Fe^{2+} \rightarrow Fe^{3+} + OH^{-} \quad \text{(solution bulk)} \qquad (5)$$

The threshold values of 0.2 mM of Fe^{2+} concentration and 300 mA of current intensity are optimal values for oxidation degradation of 5-fluorouracil, as increasing the operating parameters up to these levels resulted in faster removal rates. In general complete oxidation of 5-fluorouracil was achieved already after 5-10 min of electro-Fenton treatment depending on the operating parameters applied.

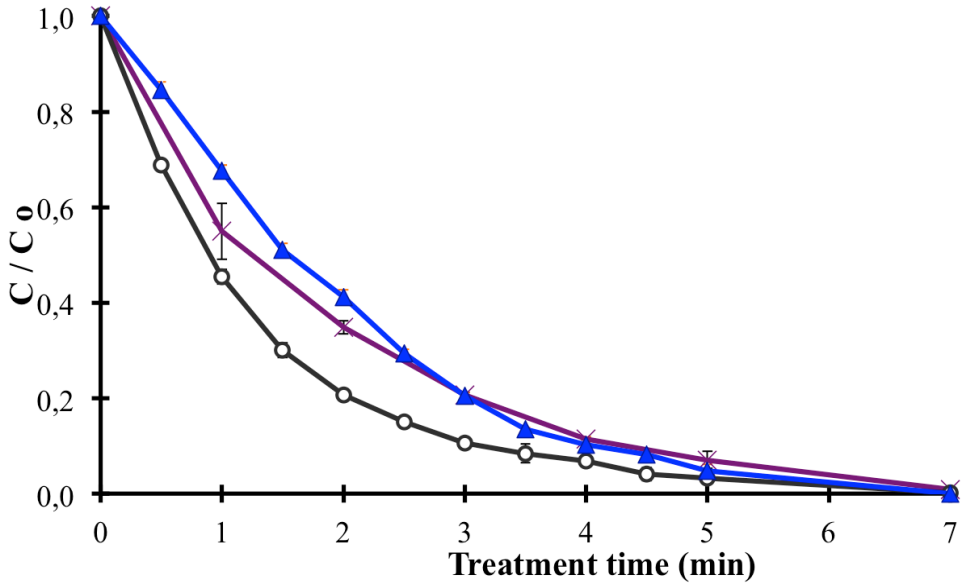

Figure III.1. The effects of the normalized Fe^{2+} concentration on oxidative degradation of 5-fluorouracil (5-FU) during the electro-Fenton process. Catalyst [Fe^{2+}] concentration (mM): 0.1 mM (-✖-); 0.2 mM (-●-); 0.5 mM (-▲-). Operating conditions: $[5\text{-FU}]_0$ = 0.1 mM, I = 300 mA; $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

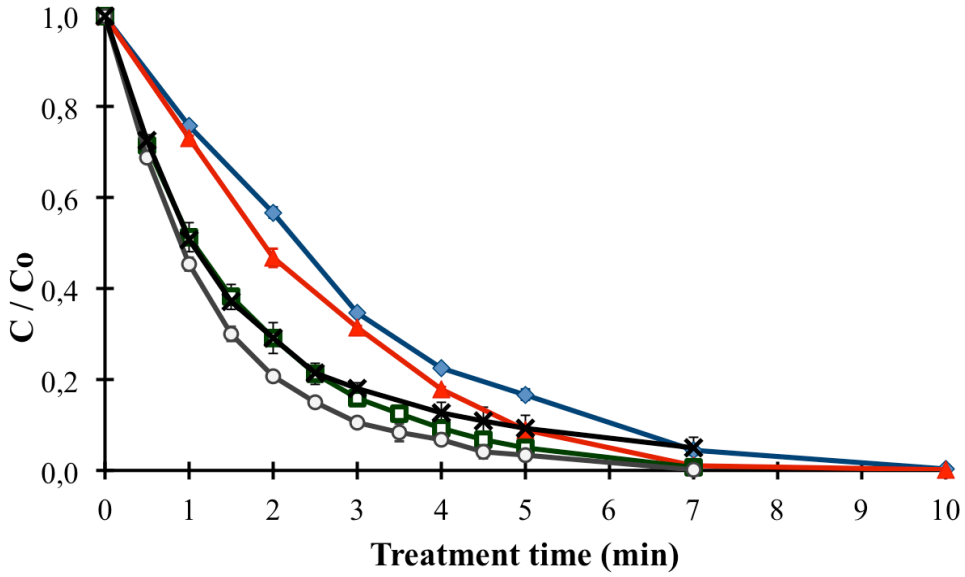

Figure III.2. The effects of applied current intensity on normalized concentration decay of 5-fluorouracil (5-FU) during the electro-Fenton process. Current intensity (mA): 50 (-◆-); 100 (-▲-); 200 (-□-); 300 (-●-); 400 (-✖-). Operating conditions: $[5\text{-FU}]_0$ = 0.1 mM, $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

III.3.2. Apparent rate constants for oxidation of 5-fluorouracil

III.3.3.1. Individual solution of 5-fluorouracil

The oxidation of 5-fluorouracil (5-FU) by •OH is a second order reaction that can be considered as a pseudo-first order reaction, since the steady state approximation can be applied to the production of •OH. Accordingly, the [•OH] in the medium remained constant due to the same operating conditions throughout the treatment process. The general rate of the reaction can be expressed as:

$$d[5\text{-FU}]/dt = k_{app}\,[5\text{-FU}] \qquad (1)$$

with $k_{app} = k_{abs}\,[^{\bullet}OH]$

The apparent constant k_{app} of a pseudo-first order reaction corresponds to the slope of the line produced by plotting $\ln([5\text{-FU}]_0/[5\text{-FU}]_t)$ versus time. The Figure III.3 demonstrates the results for the set of experiments with concentration of 0.2 mM Fe^{2+}. The summary of all k_{app} values (the slope values) for the whole set of experimental conditions is given in Table III.1. The aim of these calculations is to compare different parameters numerically. It confirms the values of optimal experimental parameters discussed previously, i.e. I = 300 mA and $[Fe^{2+}]$ = 0.2 mM.

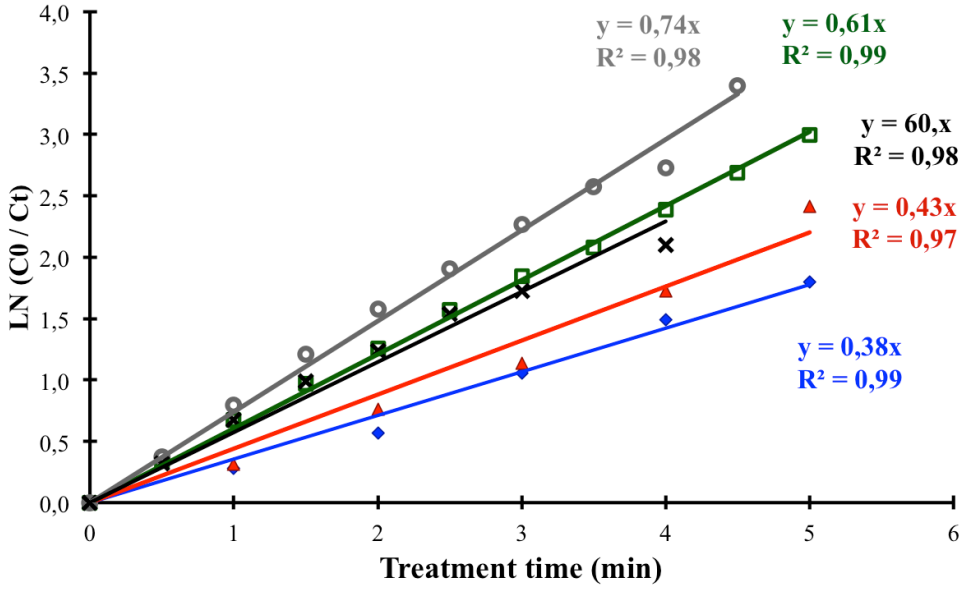

Figure III.3. The apparent rate constants for degradation of 5-fluorouracil (5-FU) during the electro-Fenton process (Ct - concentration at certain time t, C0 - initial concentration). Current intensity (mA): 50 (-◆-); 100 (-▲-); 200 (-□-); 300 (-○-); 400 (-✖-). Operating conditions: $[5\text{-FU}]_0$ = 0.1 mM, $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

Table III.1. Apparent rate constants and their corresponding correlation coefficients (R^2 values) for the entire set of operating parameters for oxidative degradation of individual 5-fluorouracil.

I [Fe(II)]	50 mA	100 mA	200 mA	300 mA	400 mA
0.1 mM	0.259 ± 0.011 $R^2 = 0.99$	0.366 ± 0.003 $R^2 = 0.99$	0.407 ± 0.012 $R^2 = 0.99$	0.567 ± 0.006 $R^2 = 0.99$	0.422 ± 0.006 $R^2 = 0.99$
0.2 mM	0.376 ± 0.002 $R^2 = 0.99$	0.429 ± 0.050 $R^2 = 0.97$	0.607 ± 0.006 $R^2 = 0.98$	0.743 ± 0.010 $R^2 = 0.99$	0.601 ± 0.004 $R^2 = 0.98$
0.5 mM	0.162 ± 0.011 $R^2 = 0.99$	0.275 ± 0.010 $R^2 = 0.99$	0.372 ± 0.004 $R^2 = 0.99$	0.542 ± 0.010 $R^2 = 0.97$	0.396 ± 0.013 $R^2 = 0.99$

III.3.3.2. Solution of 5-fluorouracil and caffeine

Further, the apparent constants were calculated for the two-component equimolar solution of 5-fluorouracil and another common pharmaceutical, i.e. caffeine, which belongs to the class of psychoactive drugs. The experiments were carried out by varying the current intensity from 200 to 400 mA. The goal was to see if the limiting threshold value of applied intensity was present in both individual solution and a two-compound mixture of pharmaceuticals. The apparent constants of the two drugs in the mixture are summarized in the Table III.2 together with the relative rates in the individual solutions. The relative rates for oxidative degradation of caffeine were calculated based on the previously reported values in Chapter III.

The results showed that the threshold value of current intensity was preserved even in the two-compound solution for both pharmaceuticals and was equal to 300 mA as reported before. The apparent degradation rate constants of 5-fluorouracil in the mixture were slightly lower than those of caffeine due to the faster reaction between caffeine and hydroxyl radicals in comparison to 5-fluorouracil. The absolute rate constant between caffeine and hydroxyl radical was reported to be 2.19×10^9 M^{-1} s^{-1} (Chapter II), while the corresponding value for 5-fluorouracil is 1.4 times lower as reported in the present study (sub-section III.3.3). In general, it can be also concluded that the individual compounds were oxidized 2.5-3 times faster than in the presence of a competitor.

Table III.2. Apparent rate constants of 5-fluorouracil and caffeine in the individual solution and in an equimolar mixture.

Current intensity	5-fluorouracil		Caffeine	
	in mixture	*alone*	*in mixture*	*alone*
200 mA	0.245 ± 0.006	faster 2.5 times	0.261 ± 0.005	faster 2.8 times
300 mA	0.325 ± 0.026	faster 2.3 times	0.364 ± 0.040	faster 3.1 times
400 mA	0.137 ± 0.007	faster 2.8 times	0.203 ± 0.007	faster 3.1 times

III.3.3. Absolute constant rate of 5-fluorouracil degradation

The method of competitive kinetics was used to determine the absolute rate constant of the reaction between 5-fluorouracil (5-FU) molecule and hydroxyl radical. A standard competitor, 4-hydroxybenzoic acid (4-HBA), was mixed together with 5-fluorouracil in equal concentrations and subjected to the electro-Fenton treatment. The absolute rate constant of 4-HBA oxidation by $^{\bullet}OH$ (k_{abs}[4-HBA]) is well known as 2.19 × 10^9 M^{-1} s^{-1} [47]. As a result the absolute rate of 5-fluorouracil (k_{abs}) was calculated from the equation [48]:

$$k_{abs}[5\text{-FU}] = k_{abs}\,[4\text{-HBA}]\; k_{app}\,[5\text{-FU}] / k_{app}\,[4\text{-HBA}] \qquad (2)$$

The experiments on competitive kinetics were performed in triplicate with following operating parameters: I = 50 mA, $[Fe^{2+}]$ = 0.1 mM, $[Na_2SO_4]$ = 50 mM. As a result the k_{abs}(5-FU) was found to be (1.52 ± 0.01) × 10^9 M^{-1} s^{-1}, which to our knowledge, has not been reported before.

III.3.4. Mineralization of 5-fluorouracil. The effect of operating parameters

III.3.4.1. Effect of Fe^{2+} concentration

In order to determine the effect of Fe^{2+} concentration on the mineralization rate, a series of experiments were conducted with a fixed current intensity of 300 mA, which was found to be an optimal threshold value for oxidative degradation of 5-fluorouracil. The results are presented on Figure III.4 and coincide with the results from degradation experiments. A two-fold increase in Fe^{2+} concentration from 0.1 mM to 0.2 mM resulted in an enhanced mineralization rate; however a further increase to 0.5 mM of Fe^{2+} showed slower rates. These results correlate with the results obtained for oxidative degradation of 5-fluorouracil.

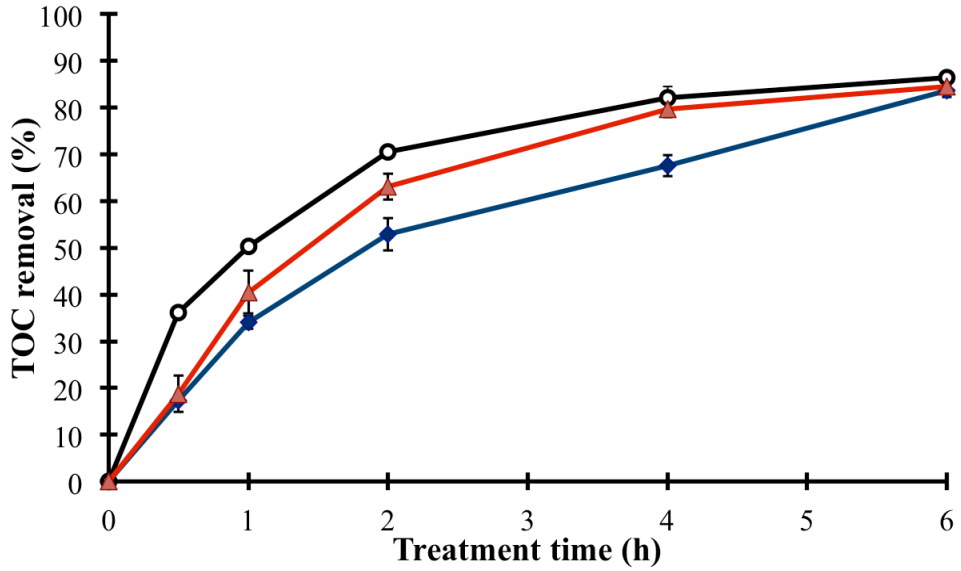

Figure III.4. Evolution of mineralization (in terms of TOC removal) of the 5-fluorouracil (5-FU) solution by the electro-Fenton process in dependence of the catalyst concentration. Catalyst $[Fe^{2+}]$ concentration (mM): 0.1 (-◆-); 0.2 (-O-); 0.5 (-▲-). Operating parameters: $[5\text{-FU}]_0$ = 0.1 mM, I = 300 mA, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

It seems that the amount of Fe^{2+} added to the solution had very similar effect on both processes: degradation and mineralization of the compound. The removal was faster with increasing the amount of Fe^{2+} up to a threshold value of 0.2 mM. The existence of such a limiting value of Fe^{2+} concentration is due to the propagation of parasitic reaction:

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \quad (6)$$

When the Fe^{2+} is supplied in excess to the solution, it starts reacting with the hydroxyl radicals, in that way reducing significantly the potential of oxidative degradation of organic matter. Therefore the amount of Fe^{2+} should be below such a threshold value, which in our experimental conditions was equal to 0.2 mM.

III.3.4.2. Effect of applied current intensity

In order to study the effect of current variation, another series of experiments was carried out with a fixed Fe^{2+} concentration (0.2 mM). The range of studied current intensities was significantly larger: from 200 to 1500 mA. The highest value was the maximal current intensity that could be applied to the given BDD anode with a surface area of 64 cm^2. The intensities above 1500 mA have caused deterioration of its surface.

The experimental results are graphically shown on Figure III.5. Unlike the oxidative degradation, the mineralization rate of 5-fluorouracil improved with an increase of current intensity. For example, the removal of TOC after only 1 h of electro-Fenton treatment was equal to 42%, 57% and 71% for the intensities of 200, 500 and 1000 mA, respectively. It can be attributed largely to the fact that the follow-up of 5-fluorouracil degradation represents only a single process among the multitude of other processes that constitute mineralization. The mother molecule of 5-fluorouracil was degraded within maximum 10 minutes, while forming a number of intermediates. As the initial compound was degraded, the number of intermediates produced became higher in that way competing with the residual 5-fluorouracil for hydroxyl radicals. Therefore for the mineralization experiments no limiting current intensity was observed, while for the oxidative degradation of one component of this complex mixture, the effect of current manifested differently.

Another point worth noticing is that the difference between the removal rates of 1000 and 1500 mA were within a range of few percentages: after 2 h the mineralization TOC removal rate was 82 and 83% for 1000 and 1500 mA respectively, and after 4 h this difference was only 4%. This can be considered as a display of a threshold value for mineralization, which is significantly higher due to the higher ratio between a number of organic molecules and hydroxyl radicals. As the organic matter got mineralized, meaning that its amount diminished, the number of molecules representing the available organics became higher due to the breakage of the molecules. It was supposed that as long as the ratio between the quantity of organic matter and radicals was adequate, the mineralization was faster with increasing the current intensity. Under conditions of an overproduction of hydroxyl radicals, consequent propagation of parasitic reactions and additional heating of the solution (for temperature evolution see Chapter IV) the

removal of TOC did not improve, as in comparison of the current intensity of 1500 mA to 1000 mA.

The Figure III.5 shows that all the trendlines of TOC removal were rather steep at the beginning of the electro-Fenton treatment, but stabilized already after 2 h and reached a plateau phase. As the operating parameters were constant throughout the whole experiment duration, the production of hydroxyl radicals was constant as well. Nevertheless, as the organic matter was being mineralized from the beginning of electro-Fenton process, the ratio between organics and radicals got lower. This means that the radicals started participating in the wasting reactions 1-6. This is a plausible explanation to the plateau phase of mineralization trend line on Figure III.5. For instance, when the intensity of 500 mA was applied, the removal of TOC in the first 1 h was equal to 57%, while after 2 h of electro-Fenton treatment it was at 74%. After another 2 h (total treatment time = 4 h), only additional 10% of TOC were mineralized.

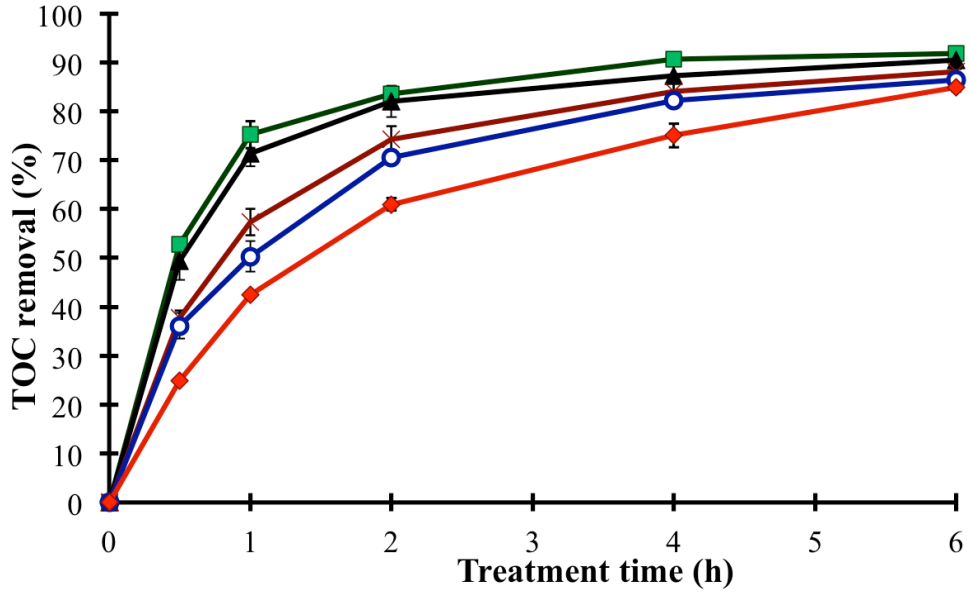

Figure III.5. Evolution of mineralization (in terms of TOC removal) rate of the 0.1 mM 5-fluorouracil solution by the electro-Fenton process in dependence on the current intensity. Current intensity (mA): 200 (-◆-); 300 (-○-); 500 (-✖-). 1000 (-▲-); 1500 (-■-). Operating parameters: $[Fe^{2+}]$ = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

III.3.4.3. Formation of carboxylic acids and inorganic ions during electro-Fenton process

The oxidation of 5-fluorouracil by •OH led to the formation of its aromatic intermediates. The oxidative reactions allowed ring opening and further formation of aliphatic molecules, including short-chain carboxylic acids. These acids are considered to be biodegradable substances. Therefore it is important to see their formation in connection with the fact that electro-Fenton can be used as pre-treatment before the conventional biological process (as a part of bio-electro-Fenton). Along the degradation of 5-fluorouracil by electro-Fenton process, two carboxylic acids were detected: acetic and oxalic. Their evolution was followed by ion-exclusion HPLC and is graphically presented in Figure III.6

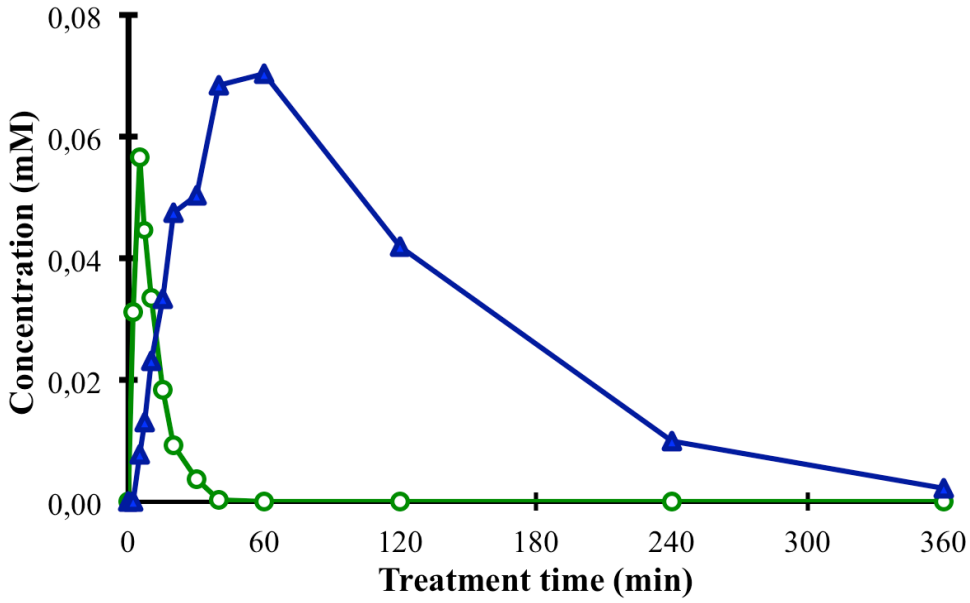

Figure III.6. Evolution of short-chain carboxylic acids during the degradation of 5-fluorouracil (5-FU) by electro-Fenton process: oxalic (-O-); acetic (-▲-). Operating parameters: $[5\text{-FU}]_0$ = 0.1 mM, I = 100 mA, [Fe(II)] = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

Both acids were formed from the beginning of the treatment process. The evolution of oxalic acid was quite rapid: it was formed and degraded within the first 30 min of electro-Fenton. Acetic acid was more persistent in the solution: it was formed more slowly and reached the peak concentration after 1.5 h. Its degradation was more gradual: it was hardly detected only after 6h of electrolysis. As 5-fluorouracil is a nitrogen-containing compounds, so it was expected to observe evolution of oxamic acid, but it could not be identified in this study. It should be therefore noted that the study of carboxylic acid formation should be confirmed by additional experiments.

The evolution of cations (NH_4^+) and anions (NO_3^-, NO_2^-, F^-) was monitored by ionic chromatography. The molecule of 5-fluorouracil has 2 atoms of nitrogen and one atom of fluorine. So the solution of 0.1 mM of fluorouracil can yield maximum of 0.2 mM of nitrogen and 0.1 mM of fluoride. The mass balance as a percent of the total maximal concentration of nitrogen is depicted in Figure III.7.

The evolution of ammonium and nitrate followed a similar trend: gradual increase with a maximum reached after 4 h and then slightly decreased. As can be seen from the Figure III.7 the mass balance of nitrogen is not complete, which can be explained by a possible formation of other nitrogen-containing organic compounds (e.g. oxamic acid). Similar results were previously reported [37, 49]. A decrease in nitrate and ammonium is most probably due to its oxidation on anode and/or reduction on cathode. In contrast, the evolution of fluoride is a gradual increase until it reaches more than 95% after 6 h of electro-Fenton treatment.

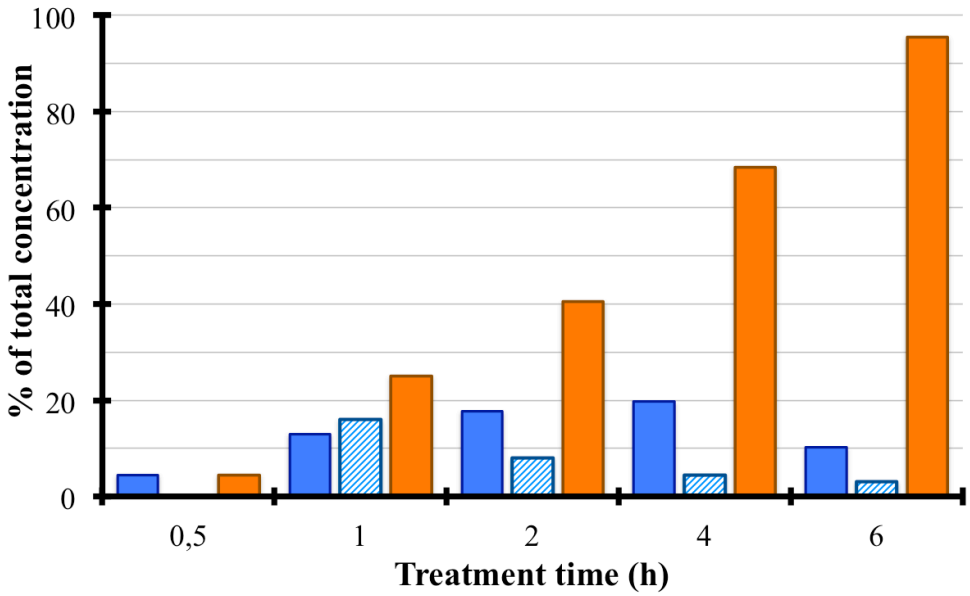

Figure III.7. Formation and evolution of NO_3^-, NH_4^+, F^- during the treatment of 0.1 mM 5-fluorouracil by the electro-Fenton process calculated as mass balance (%) of total nitrogen and fluorine: ammonium ( ); nitrate ( ); fluoride ( ). Operating parameters: for nitrate/nitrite/fluoride I = 100 mA, [Fe(II)] = 0.2 mM, [Na_2SO_4] = 50 mM, V = 200 mL. For ammonium same conditions apart from supporting electrolyte [$MgCl_2$] =50 mM.

III.4. CONCLUSIONS

The removal of 5-fluorouracil by electro-Fenton was studied in terms of oxidative degradation of the drug as well as the mineralization of its aqueous solution. The effects of operating parameters such as the Fe^{2+} (catalyst) concentration and current intensity, were analyzed. The fastest removal was achieved under the concentration of Fe^{2+} in the range 0.1- 0.2 mM for both mineralization and degradation of the drug. This result was previously reported by other studies, which allows accepting this range as optimal for electro-Fenton process with a given setup.

In the degradation experiments the threshold current intensity was 300 mA, above which no improvement of degradation rate was observed. This threshold intensity was also confirmed by the experimental data on a two-compounds mixture of electro-Fenton and caffeine. Degradation of 5-fluorouracil by means of electro-Fenton was quite fast: complete disappearance of the pollutant was achieved already after 7 min under optimal operating conditions. Absolute rate constant of the reaction between 5-fluorouracil and hydroxyl radicals was determined to be 1.52 (±0.01) × 10^9 $M^{-1}s^{-1}$.

The experimental results showed that mineralization of 5-fluorouracil solution is faster at high current intensities. However, at the end of treatment the mineralization degree was close for all studied intensities.

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CHAPTER IV

Electro-Fenton treatment of synthetic pharmaceutical mixture. Effect of operating parameters on mineralization efficiency and biodegradability enhancement

To be submitted

ABSTRACT

A combination with a biological treatment represents a solution for cost-intensive operation of electrochemical advanced oxidation processes. In the case of a combining electro-Fenton process and a biological treatment, degradation of refractory pollutants is ensured by electrochemical process in order to form biodegradable intermediates that are further removed by a conventional biological treatment. The goal of this study was to evaluate the potential of electro-Fenton process to be applied as a pre-treatment process for a synthetic pharmaceutical mixture. Mineralization experiments revealed the optimal catalyst concentration of 0.2 mM. Regarding the current intensity, a trend of increased current/increased mineralization rate was observed. For degradation kinetics of two pharmaceuticals in the synthetic mixture, the rate was increasing with an increasing current up to a threshold intensity of 400 mA, while for 700 mA the apparent rate constant was significantly lower. The formation of mineral ions and evolution of temperature during treatment were monitored. Biodegradability enhancement of the pre-treated mixture was analyzed as a ratio BOD/COD. The BOD_5/COD increased starting from 3 to 26 times in comparison to initial value after treatment by electro-Fenton process. As none of the analyzed samples reached the standard level of biodegradability BOD_5/COD = 0.33, a series of long-term assays (BOD incubated over 17 days) have been carried out. The results showed that samples after 3 h and with applied intensities of 500 and 1000 mA have reached a level of high and almost complete biodegradability. Additionally a series of toxicity analyses were performed for different current intensities to complete the evaluation of biodegradability improvement. Finally, calculations of energy consumption were done for different intensities and treatment durations.

Keywords: Electro-Fenton, Pharmaceutical, Degradation, Mineralization, Biodegradation, Toxicity

IV.1. INTRODUCTION

Electro-Fenton raises an elevated interest due to its remarkable ability to degrade hazardous substances contrarily to other physical and biological methods, which mainly only transfer pollution from wastewater to sludge, membrane filter or adsorbent [1]. This process is based on the well-known Fenton reaction (1) between hydrogen peroxide and Fe^{2+}:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^- \quad (1)$$

This reaction produces *in situ* strong oxidant species, hydroxyl radicals, which have the second largest redox potential (2.80 V at 25 °C). Such characteristics enable rapid and non-selective degradation of organic matter, which is basically a breakage of organic molecules into oxidation intermediates. If the process continues long enough, it can lead to complete transformation of organic compounds into inorganic end-products like carbon dioxide, water and other inorganic ions [2].

Classical Fenton oxidation has a number of drawbacks related to its operation: addition of significant amount of Fenton's reagent (H_2O_2 and ferrous iron), handling difficulties of hydrogen peroxide, formation of iron sludge etc. [3]. An electrochemically assisted Fenton reaction, electro-Fenton, resolves these issues. First of all, there is an electrochemical regeneration of Fe^{2+} from Fe^{3+} (which also takes place in Fenton oxidation, yet very slowly):

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad (2)$$

It means that iron catalyst is added externally only at the beginning of treatment and is steadily regenerated at the cathode throughout the treatment duration.

Secondly, aeration of the treated solution ensures continuous *in situ* production of hydrogen peroxide from the electrolysis of dissolved oxygen:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (3)$$

As a result electro-Fenton demonstrates a great potential for degradation and mineralization of different types of pollutants: dyes, pesticides, industrial contaminants, pharmaceuticals etc. [4-6]. Yet application of a treatment process on the industrial level should be preceded by a mechanism investigation and process optimization on a laboratory scale.

Most of the research on electro-Fenton is concentrated on the degradation kinetics of single compounds [7-9], which is certainly an important step to comprehend the underlying mechanisms. However, in order to upscale this electrochemical process there is a need for data on more complex solutions that should be then followed by experimentation with real effluents and engineering of pilot plants.

The current study had a goal to complement existing knowledge on electro-Fenton by optimizing this process for treatment of a synthetic pharmaceutical mixture. It comprised 13 pharmaceuticals that belong to different therapeutic and pharmacological classes (Table IV.1).

Table IV.1. Composition of a synthetic pharmaceutical mixture.

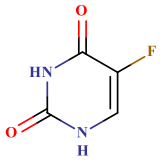
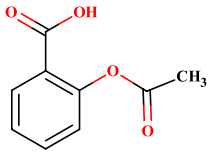
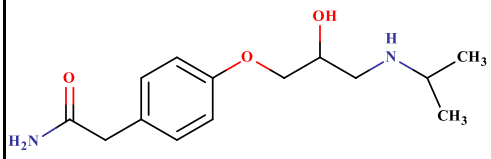
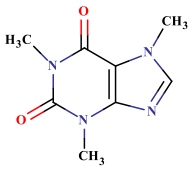
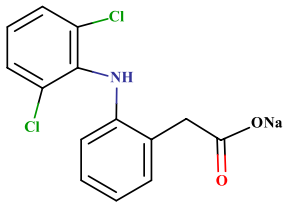
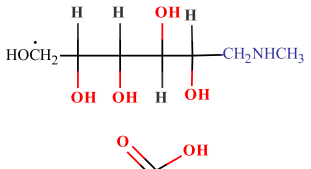
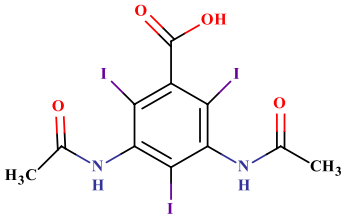
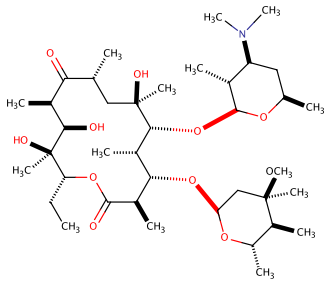
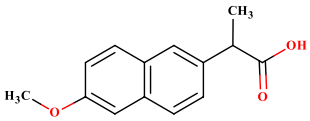
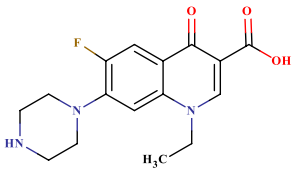
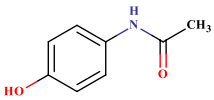
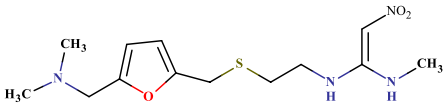
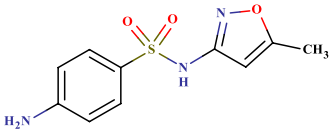
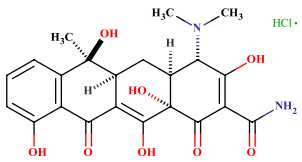
NAME	CHEMICAL STRUCTURE	PHARMACOLOGICAL CLASS
5-fluorouracil		antineoplastics
aspirin		analgesics / antipyretics
atenolol		beta blocker
caffeine		psychoactive / stimulant
diclofenac		nonsteroidal anti-inflammatory
diatrizoate meglumine	 	radio contrast media

Table IV.1. (continued)

NAME	CHEMICAL FORMULA	PHARMACOLOGICAL CLASS
erythromycin		antibiotics
naproxen		nonsteroidal anti-inflammatory
norfloxacin		antibiotics
paracetamol		analgesics / antipyretics
ranitidine		histamine H2-receptor antagonist
sulfamethoxazole		antibiotics
tetracycline		antibiotics

Though in general the concentrations of selected pharmaceuticals in different sources of water are in the range of ng/L or µg/L (Table IV.2), a combined concentration of all the representatives of a certain pharmacological class can reach significantly higher ranges. Therefore, each pharmaceutical from the list of Table IV.1 was regarded as a representative of similar drugs with same pharmaceutical action. These drugs have been selected based on several publications that investigated the composition of hospital wastewater [10-18]. Among all the multitude of identified compounds from these studies a selection was done for most frequently detected drugs with highest concentrations.

Table IV.2. Concentration of selected pharmaceuticals in the hospital effluent (μg/L).

Reference / Pharmaceutical	[14]	[15]		[13]	[17]		
		Ullevall hospital	Riks hospitalet		Hospital A	Hospital B	Hospital C
aspirin	384.00						
atenolol					5.1	2.4	5.8
caffeine	182.00						
diclofenac	6.88	1.63	14.934				
diatrizoic acid				348.70			
erythromycin	0.86						
naproxen	3.17				2.3		4.9
paracetamol	63.10	177.67	1368.474	107,00	4.5	4.1	2.5
ranitidine						1.3	3.0
sulfamethoxazole		1.375	4.107		4.2	1.8	
tetracycline		1.537	4.178				

Hospitals are one of the sources of uncontrolled release of drugs into the environment with numerous negative consequences [19]. These effluents have a really diverse and complex composition: different classes of pharmaceuticals, which are excreted by human body in almost unchanged form, and their metabolites, diagnostic agents, disinfectants, musks, corrosion inhibitors and other hardly degradable pollutants [13]. Hospital effluents are normally discharged to the common sewage system and undergo conventional treatment at municipal wastewater treatment facilities. However, most of the pharmaceutically active compounds are not degraded at all, therefore they end up in the receiving water bodies [20]. Despite their trace concentrations (Table IV.2), these pollutants affect the ecosystem balance, as, for example, hormones from oral contraceptives shift gender balance in fish and cease their reproduction [21, 22]. Presence of antibiotics in small concentrations, which are not high enough to destroy bacteria, cause habituation and trigger antibiotic resistance in microorganisms [23]. In the light of the above, hospital effluents require additional pre-treatment before discharge to municipal sewage or a separate treatment system in order to provide sufficient and efficient degradation of pharmaceuticals. In this case electro-Fenton can be applied to accomplish both objectives.

All of the selected drugs represent a certain risk or hazard for humans and ecosystems. The 5-fluorouracil was found to be genotoxic to zebrafish [24] and extremely toxic to bacterial species [25]. Environmental concentrations of acetylsalicylic acid, known by its market name aspirin, displayed significant effects on aquatic vertebrates [26]. In Table IV.1 antibiotics are represented by different families: fluoroquinolones (norfloxacin), sulfonamides (sulfamethaxozole), macrolides (erythromycin). They have been demonstrated to trigger a resistance in bacteria, which is a potential threat to propagation of resistant microbial infections and as a consequence ineffectiveness of modern medical treatment [27-29]. Diclofenac and atenolol have been demonstrated in a recent study [30] to have a negative oxidative stress response of on zebrafish, while their photolysis by-products appeared to be even more toxic. It can be interpreted as a quite alarming indication to the fact that long-term exposure of drugs to the environment might cause more severe effects due to the transformation by-products. In general terms presence of pharmaceuticals have demonstrated to have effects on: marine food webs by bioaccumulation or loss of key species due to

toxicity [31, 32]; growth and reproduction of fish [33, 34], behavioral alterations of aquatic organisms [35], etc. Despite the fact that more research is needed in this area, current state of knowledge allows getting a glimpse on a magnitude of the problem and stimulates a search for new solutions.

Considering the above-described issues, the general scope of the present study is to evaluate the potential of electro-Fenton as a pre-treatment solution for hospital wastewater. Therefore the following objectives were pursued: (i) determination of the effect of operating parameters (current intensity and catalyst concentration) on removal of organic matter; (ii) calculation of apparent rate constants of two components of the pharmaceutical mixture (caffeine and 5-fluorouracil); (iii) investigation of the evolution of inorganic ions throughout the treatment; (iv) observation of temperature increase throughout the treatment due to application of electrical energy; (v) to determine biodegradability enhancement after electro-Fenton treatment; (vi) to examine toxicity of treated solutions (vii) estimation of energy consumption in dependence of different current intensities.

Besides an apparent value of this study for environment and human health, it is worth emphasizing general novelty of studying such a treatment method in detail with a focus on the removal of pharmaceuticals.

IV.2. MATERIALS AND METHODS

IV.2.1. Chemicals

Analytical grades of 5-fluorouracil (CAS 51-21-8), aspirin or acetylsalicylic acid (CAS 50-78-2), atenolol (CAS 29122-68-7), caffeine (CAS 58-08-2), diclofenac sodium salt (CAS 15307-79-6), diatrizoate meglumine (CAS 131-49-7), erythromycin (CAS 114-07-8), naproxen (CAS 2224-53-1), norfloxacin (CAS 70458-96-7), paracetamol or acetaminophen (CAS 103-90-2), ranitidine (CAS 60357-35-5), sulfamethaxozole (CAS 723-46-6), tetracycline hydrochloride (CAS 64-75-5) were obtained from Sigma-Aldrich. Iron (II) sulfate heptahydrate (CAS 7782-63-0) as a catalyst source, and sodium sulfate (CAS 7757-82-6) as electrolyte were obtained from Acros Organics and Sigma-Aldrich, respectively. The pH of the solutions was set using 1 M sulfuric acid. The reagents used for preparation of solutions for COD analysis are listed in CEAEQ [36]. For biodegradability analysis the reagents are listed in [37]. For Microtox analysis the *Vibrio fischeri* bacteria and other reagents were purchased from Hach Lange, France. Standards for ion analysis as well as reagents for mobile phase preparation were the ACS reagents with purity over 99.5%: ammonium oxalate monohydrate (CAS 6009-70-7), sodium nitrate (CAS 7631-99-4), sodium nitrite (CAS 7632-00-0), sodium carbonate (CAS 497-19-8), sodium bicarbonate (CAS 144-55-8), sodium sulfate (CAS 7647-14-5), potassium iodide (CAS 7681-11-0), sodium fluoride (CAS 7681-49-4), sodium sulfate (CAS 7757-82-6).

IV.2.2. Preparation of pharmaceutical mixture

A synthetic pharmaceutical solution was prepared by mixing 0.1 mM of each compound listed in previous section in water. All the solutions were prepared with ultrapure water produced by a Millipore Milli-Q (simplicity 185) system with resistivity of >18 MΩ cm. Stirring of the solution was not less than for 5 hours and stored at 4℃ for maximum one week.

IV.2.3. Experimental setup

Open undivided electro-Fenton glass reactor was operated in a batch mode at ambient temperature (T = 20±1 °C) and was filled with 200 mL of the synthetic pharmaceutical solution containing 0.05 M sodium sulfate and iron sulfate of a suitable concentration. The pH of the solution was adjusted to 2.9 ±0.1 with 1 M sulfuric acid. The carbon felt cathode (18.5 cm × 4.5 cm) circled the internal wall of the reactor. Teflon mesh was used around the internal side of cathode to avoid short circuit between electrodes. The boron-doped diamond (BDD) anode (6 cm × 4 cm) was positioned in the center on equal distances from the encircling cathode. The solution was stirred at a speed of 450 rpm and aerated with compressed air at a speed of 0.2 L/min continuously throughout the experiment. Aeration started 5 min prior to electrolysis in order to saturate the solution with oxygen. The current intensity and voltage were monitored in real time on a power supply HM8040-3 by Hameg Instruments. Each experiment was done in a triplicate.

IV.2.3. Instruments and analytical procedures

IV.2.3.1. Total organic carbon (TOC) analysis

Analysis of total organic carbon was performed in order to determine the mineralization degree of initial and treated samples. It was measured on a Shimadzu VCSH TOC analyzer by combustion with catalytic oxidation at 680 °C using high-purity oxygen gas as a carrying gas at a flow rate of 150 mL/min. A non-dispersive infrared detector was used to determine the amount of organic material. Standard potassium hydrogen phthalate solution was used for calibration. The injection volume was 50 µL.

IV.2.3.2. High performance liquid chromatography (HPLC)

Experiments on degradation kinetics of caffeine and 5-fluorouracil were followed by means of reverse-phase high performance liquid chromatography (RP-HPLC). The equipment (Merck Hitachi) consisted of a column Purospher STAR RP-18 endcapped (5 µm), a pump (Elite LaChrome, L-2130), UV detector (Elite LaChrome, L-2400) and a thermostat (Jetstream Plus, series 140310). Analytical conditions were: T = 40°C, mobile phase methanol and ultrapure water both buffered with 1% acetic acid, retention time 35 min and detection was performed at 275 nm wavelength. Injection volume was equal to 20 µL. Gradient elution was used for separation of caffeine and 5-fluorouracil with following order. From the start to 11 min 90% water/10% methanol at flow rate 0.3 mL/min were applied,

then from 11 to 13 min the conditions gradually changed to 70% water/30% methanol at flow rate of 0.6 mL/min and stayed up to 28 min. From 28 to 30 min these conditions gradually changed to the initial 90% water/10% methanol at flow rate 0.3 mL/min and remained for the last 5 min. Such analytical conditions allowed eluting 5-fluorouracil at 10 min and caffeine at 25.4 min.

IV.2.3.3. Ion chromatography

The evolution of inorganic ions was followed with the help of Dionex ICS-1000 ion chromatography system equipped with an ASRS-ULTRA II (for anions) or CSRS-ULTRA II (for cations) self-regenerating suppressor to improve the sensitivity of the detector. The system was equipped with a DS6 conductivity detector containing a cell heated at 35 °C. An anion-exchange column (IonPac AS4ASC, 25 cm × 4 mm) linked to an IonPac AG4A-SC, 5 cm × 4 mm column guard was used for anions analysis. Cation (NH_4^+) analysis was performed on a cation-exchange column (IonPac CS12A, 25 cm × 4 mm) linked to an IonPac CG12A, 5 cm × 4 mm column guard. A solution of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at 2.0 mL/min, and a 9.0-mM H_2SO_4 solution at 1.0 mL/min were used as mobile phases for anion and cation analysis, respectively. Identification and quantification of ions was done by comparing the elution time and peak areas of standard solutions.

IV.2.4.4. Chemical oxygen demand (COD)

Chemical oxygen demand has been measured using a reflux method in a closed system (tubes) followed by a colorimetric dosage with potassium dichromate. The tubes with digestion and acidic solution were prepared and analyzed according to the analytical method [36]. Tubes with samples were incubated at 148 °C for 2 h. The value of COD was measured by photometric method using Spectroquant® TR 420 (Merck).

IV.2.4.5. Biodegradability tests

The tests were based on measuring biochemical oxygen demand (BOD). The analysis was performed following Rodier, Legube and Merlet [37]. All the solutions (saline, control, phosphate buffer, allylthiourea and dilution water) were prepared according to the protocol. Biomass was from activated sludge taken at a municipal wastewater treatment plant. For a set of experiments on biodegradability enhancement, the suspended solids (SS) of biomass were 1600 mg/L, whereas for a second set of experiments on long-term biodegradability it was sampled at a different location and SS was around 5000 mg/L. The SS was measured as stated by Rodier, Legube and Merlet [37].

Before the start of the experiment dilution water, sample and bacterial inoculum were aerated to reach oxygen saturation. The BOD values were measured by respirometric method using OxiTop® Control System (WTW, Germany). Each sample bottle of OxiTop® of 432 mL volume contained 100 mL of sample, 3 mL of buffer solution (amount calculated to keep the ratio COD:N not less than 100:5), 5 mL of biomass from activated sludge (regardless the set of experiments), 1 mL of allylthiourea solution (to prevent nitrification). The rest of the bottle (273 mL) was filled with dilution water. Before seeding with bacteria, the final solution was adjusted to circumneutral pH. All the bottles contained a rubber sleeve inside which

sodium hydroxide pellets were placed to absorb the carbon dioxide produced during the bacterial respiration. Continuously stirred bottles were incubated in dark place with temperature in the range 20(±1) °C. For the experiments on biodegradability enhancement the incubation lasted for 5 days and for experiments on long-term biodegradability for almost 17 days.

Each batch had a blank and a reference, or a control, which was an aqueous solution of glucose and acetamic acid [37]. A blank served to evaluate an endogenous respiration of bacteria and was ultrapure water added instead of the sample. The value of blank was subtracted from each value of sample after the end of analysis.

IV.2.4.6. Toxicity assay

Toxicity of solutions was measured by means of Microtox test on bioluminescent bacteria *Vibrio fischeri* from LUMIStock LCK-487 (Hach Lange). Microtox® Model 500 Analyzer was used for this analysis. Each sample was adjusted to the pH in the range of 6.5-7.5 and filtered with a syringe filter RC 0.2 μm in order to remove precipitated iron. A solution of sodium chloride 22% was added to the tubes with reactivated *Vibrio fischeri* for osmotic protection of bacteria. Finally, prepared samples were added to the tubes and incubated for 5 min, which gave inhibition values (%).

IV.3. RESULTS AND DISCUSSION

IV.3.1. Mineralization of the pharmaceutical mixture. The effect of operating parameters

IV.3.1.1. Concentration of Fe^{2+} catalyst

Ferrous iron as one of the two reagents of the classical Fenton reaction, acts as a catalyst, and influences greatly the formation of hydroxyl radicals. The experiments on the effects of Fe^{2+} on mineralization rates included three different concentrations: 0.1, 0.2 and 0.5 mM, and the results are presented on Figure IV.1. It can be seen that a double increase in Fe^{2+} concentration from 0.1 to 0.2 led to a faster TOC removal. However, a further increase to 0.5 mM showed that the mineralization was slower than for 0.2 mM Fe^{2+}. An existence of a threshold value of 0.2 mM has been observed previously (Chapter II and III) and is mainly attributed to the propagation of parasitic reaction 4 in the presence of excess Fe^{2+}, which scavenges hydroxyl radicals [3]:

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-} \quad (4)$$

As a result the concentration of 0.2 mM Fe^{2+} was taken as an optimal threshold value for all further experiments.

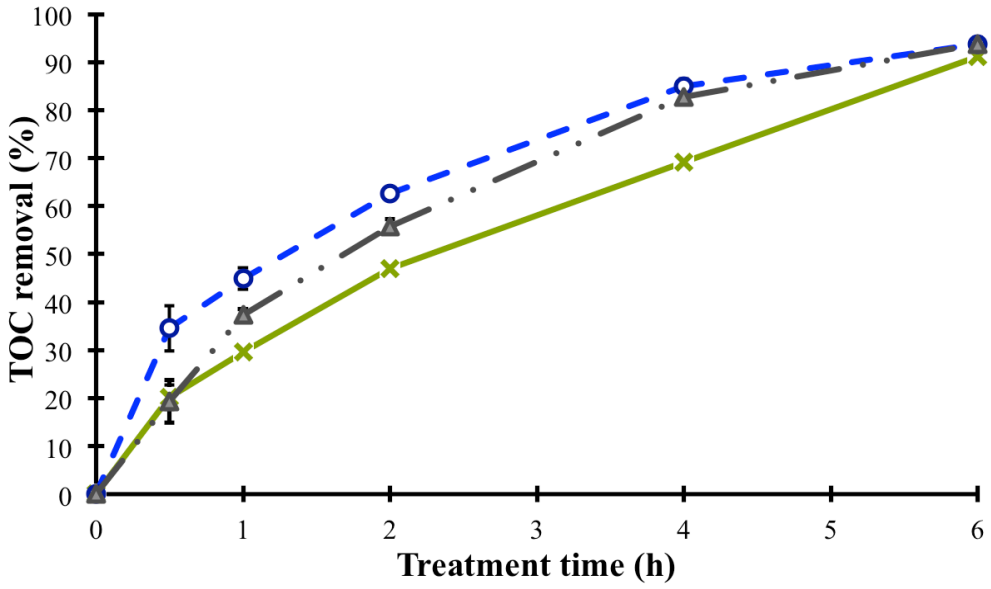


Figure IV.1. The effects of the Fe^{2+} concentration for mineralization of pharmaceutical mixture. Fe^{2+} concentration (mM): 0.1 mM (-✖-); 0.2 mM (-O-); 0.5 mM (-▲-). Operating conditions: I = 300 mA; $[Na_2SO_4]$ = 50 mM, V = 200 mL.

IV.3.1.2. Applied current intensity

The studied range was from 100 to 1500 mA and the results are graphically presented in the Figure IV.2. The general trend is that with an increase of intensity the organic matter is mineralized faster. For example, after one hour of electrolysis the mineralization rate was at 20, 33, and 50% for 100, 500 and 1500 mA, respectively. It is worth noticing, however, that the mineralization rate was faster at the beginning of electrolysis. After 1 h of electro-Fenton treatment with an intensity of 1000 mA almost 60% of organic matter was removed; however during next 1 h only additional 23% additional mineralization was reached. Such behavior can be attributed to the fact that the organic matter was being gradually mineralized, but the level of production of hydroxyl radicals was constant throughout the treatment. So the ratio between the two was becoming lower, resulting in excess of hydroxyl radicals, which started to participate in parasitic reactions 4-7 [38]:

$$^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad \text{(solution bulk)} \qquad (5)$$

$$^{\bullet}OH + HO_2^{\bullet} \rightarrow H_2O + O_2 \qquad (6)$$

$$^{\bullet}OH + H_2O_2 \rightarrow H_2O + HO_2^{\bullet} \qquad (7)$$

In addition, parasitic reactions can propagate on the surface of anode. As BDD material has a high O_2-overpotential, there is an additional formation of hydroxyl radicals physisorbed to the surface of anode owing to anodic oxidation of water. These radicals, when being in excess, can be a subject of recombination [39]:

$$2\ BDD(^{\bullet}OH) \rightarrow 2\ BDD + H_2O_2 \qquad (8)$$

$$BDD(^{\bullet}OH) \rightarrow BDD + 1/2\ O_2 + H^+ + e^- \qquad (9)$$

Consequently, proliferation of these parasitic reactions resulted in wasting of hydroxyl radicals and decreased current efficiency. Generally, in the electro-Fenton process, current plays a dual role: it assists the Fenton system and enhances it by additional formation of

hydroxyl radicals on the surface of BDD anode (as a result of anodic oxidation of water). Electrochemical assistance of Fenton reaction includes formation of hydrogen peroxide through the electrolysis of dissolved oxygen (reaction 2) and reduction of Fe^{3+} (reaction 3) into Fe^{2+} (regeneration of the catalyst). In this regard applied intensity of current is a critical operating parameter.

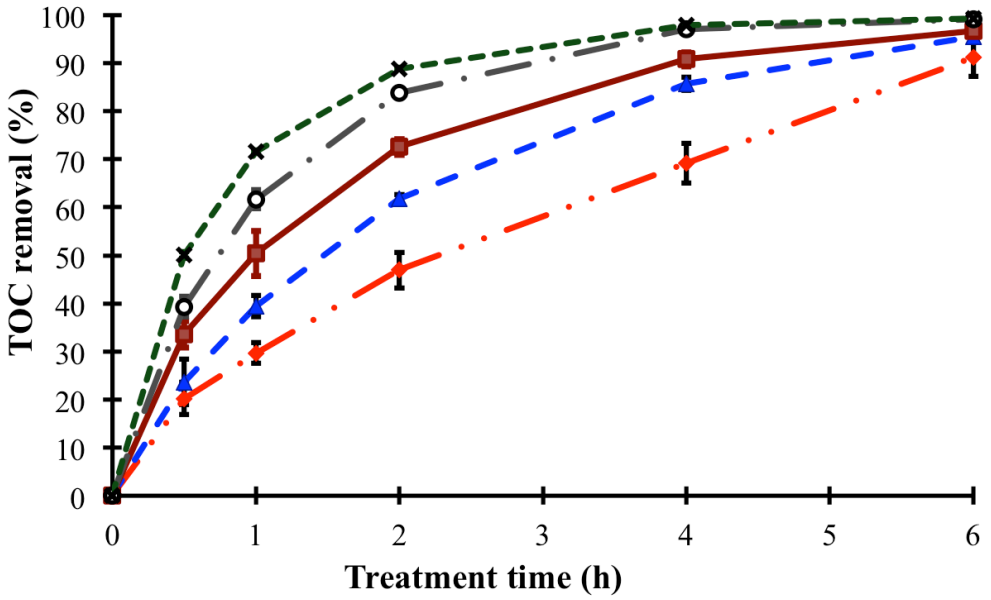


Figure IV.2. The effects of applied current intensity for mineralization of pharmaceutical mixture during the electro-Fenton process. Current intensity (mA): 100 (-◆-); 300 (-▲-); 500 (-■-); 1000 (-○-); 1500 (-✖-). Operating conditions: [Fe^{2+}] = 0.2 mM; [Na_2SO_4] = 0.05 M, V = 200 mL.

IV.3.1.3. Energy consumption of mineralization

Consumption of energy is one of the most significant criteria for process optimization and constitutes a major part of operational costs of an electrochemical treatment. It was calculated based on the following formula:

$$E = \frac{E_{cell} I t}{(\Delta TOC)_t V_s}, \quad (1)$$

where E_{cell} - average cell voltage (V), t - electrolysis time (h), I - applied current (A), $(\Delta TOC)_t$ - decay of TOC (g/L) after time t, V_s - volume of the solution (L).

The obtained values for this parameter are given in the Table IV.4 for different treatment durations and different current intensities, and represent an amount of energy consumed per kg of TOC mineralized (kWh/(kg TOC)). From these data it can be seen that energy consumption was greater with higher current intensities and this increase was roughly proportional to the increase in intensity applied. For example, for 1 h of electro-Fenton treatment, the energy consumption for 100 mA was almost 30 kWh per kg of TOC, while for a threefold increase in current intensity it was equal to 86 and for a 5-fold increase up to 500 mA the consumption of energy was 5 times more.

Table IV.3. Energy consumption E (kWh/kg TOC) for different current intensities and treatment duration.

	0.5 h	**1 h**	**2 h**	**4 h**	**6 h**
100 mA	21.2	28.8	36.5	49.5	56.3
300 mA	71.9	86.0	110.0	158.4	213.2
500 mA	97.8	130.2	180.9	289.2	407.4

The evolution of energy consumption revealed that with longer treatment time more electrical energy (in kWh) was consumed per each kg of TOC removed. After 0.5 h energy consumption for 100 mA of treatment was equal to 21 kWh/(kg TOC), while after 1 h of electro-Fenton it was 35% higher, after 2 h and 4 h it was 72% and 133% more. An explanation can be found by analyzing the mineralization trends (Figure IV.2). As was noted before, the mineralization rate dropped with longer treatment time, as the amount of organic matter available became lower and the hydroxyl radicals participated in parasitic reactions 4-7, which consequently resulted in a grow of energy consumption. Such a situation was also due to the lower reactivity of hydroxyl radicals towards formed by-products [38]. Additionally, mass control conditions played an important role for the anodic oxidation of pollutants, meaning that its reaction rate was limited by the mass transport of organic matter to the surface of anode [40].

Short treatment time can be used in case of a combined electrochemical-biological treatment (bio-electro-Fenton), where electro-Fenton is applied as a pre-treatment followed by a biological post-treatment process. In such case, during the first stage there is a degradation of refractory pharmaceuticals by means of electro-Fenton with low current intensity and short duration, while the residual contamination can be removed by application of biological post-treatment.

In general it is worth noticing that expenditure of energy for a given system was quite elevated, but so was the potential. A voltage for electro-Fenton with applied intensity of 100 mA was equivalent to 3.8 V, while for 300 mA and for 500 mA it was equal to 5 V and 5.8 V respectively. Correspondingly, energy consumption can be reduced by increasing the conductivity of the solution by adding higher concentration of electrolyte. Yet different alternatives should be compared taking into account the cost of their implementation.

IV.3.2. Degradation of caffeine and 5-fluorouracil

Degradation of two components in a synthetic mixture was followed in order to determine their apparent rate constant (k_{app}) of the reaction with hydroxyl radicals. The general rate of the reaction of the pharmaceutical compound (Pharma) can be expressed as:

$$d[\text{Pharma}]/dt = - k_{app} [\text{Pharma}], \quad (2)$$

where $k_{app} = k_{abs} [^{\bullet}OH]$

The reaction between an organic compound and a hydroxyl radical belongs to a second-order, which according to steady state approximation for OH production can be considered as the pseudo-first order. By plotting $\ln([\text{Pharma}]_0/[\text{Pharma}]_t)$ versus time it is possible to calculate k_{app} of a pseudo-first order reaction, which corresponds to the slope of the line produced.

Relevant apparent constants for caffeine and 5-fluorouracil in dependence of current intensity are given in Table IV.3. For each compound three different apparent rates were analyzed: in the pharmaceutical mixture, in a mixture of only caffeine and 5-fluorouracil and in a solution of each drug alone. The apparent rate of the pharmaceutical mixture was calculated in the frame of present studies. Two latter values were taken from the previous studies on individual molecules of caffeine and 5-fluorouracil (Chapter II and III). Based on this data it was possible to compare how much faster given pharmaceuticals were degraded in 2-component and individual solutions. Considering that the experimental setup was identical in three studies, all these data allowed visualizing a change in the degradation rate of given compounds with and without other organic competitors for hydroxyl radicals.

Table IV.4. Apparent rate constants and their corresponding correlation coefficients of determination (R^2 values) for the entire set of operating parameters for degradation of 5-flourouracil and caffeine in a synthetic pharmaceutical mixture (Pharma_mix), mixture of caffeine and 5-fluorouracil (2-component solution) and solution of individual pharmaceutical (alone).

Current intensity	**5-fluorouracil**			**Caffeine**		
	Pharma_mix	*2-component solution*	*alone*	*Pharma_mix*	*2-component solution*	*alone [41]*
200 mA	0.038 ± 0.001	faster 6.4 times	faster 16 times	0.034 ± 0.000	faster 7.6 times	faster 21.6 times
300 mA	0.040 ± 0.000	faster 8 times	faster 18.4 times	0.036 ± 0.001	faster 10 times	faster 31.6 times
400 mA	0.042 ± 0.000	faster 3.3 times	faster 14.4 times	0.038 ± 0.001	faster 5.3 times	faster 16.8 times
700 mA	0.030 ± 0.000	-	-	0.023 ± 0.000	-	-

It can be seen that the apparent constants for both molecules are close values when comparing for same current intensities. An interesting observation is linked to the fact that the degradation of both compounds in the pharmaceutical mixture became faster with increasing current intensity from 200 mA to 400 mA. However, the apparent constant for 700 mA was lower than for 200 mA for both pharmaceuticals, which means that the optimal current intensity was surpassed.

The existence of threshold current intensity, above which no acceleration of degradation was observed examined in Chapter II and III [41]. The corresponding threshold values for degradation of individual caffeine and 5-fluorouracil were 300 mA, while in the present study the threshold value was not attained even at 400 mA. Such a situation can be mainly attributed to the different ratios between the available organic matter and the production of hydroxyl radicals. Initial TOC of individual caffeine and 5-fluorouracil comprised around 10 and 5 mg/L respectively, while the TOC of the synthetic mixture was more than 220 mg/L. The threshold value of current intensity is associated with the excessive production of hydroxyl radicals, which further leads to propagation of parasitic reactions 4-7. Therefore, with elevated amount of TOC in the solution the corresponding threshold was shifted to higher current intensities, as more radicals were required for degradation of organic matter.

IV.3.3. Formation of ions during the electro-Fenton treatment

Organic matter in the synthetic solution contained heteroatoms apart from carbon, hydrogen and oxygen (Table IV.1): 13 given pharmaceutical molecules contained 5 atoms of fluorine, 2 atoms of chlorine, 3 of iodine, 2 atoms of sulfur and 28 of nitrogen. As a result the electro-Fenton mineralization of such solution produced inorganic ions, which were monitored by ionic chromatography. The evolution of nitrogen-containing ions is presented as a % mass balance of theoretical nitrogen concentration (2.8 mM) in Figure IV.3. It can be noticed that nitrogen was transformed mainly into nitrate and ammonium, while the percentage of nitrite was really low throughout the whole duration of electro-Fenton. The evolution of both NO_3^- and NH_4^+ gradually increased till the end of the treatment, when corresponding ions comprised almost 90% percent of total nitrogen.

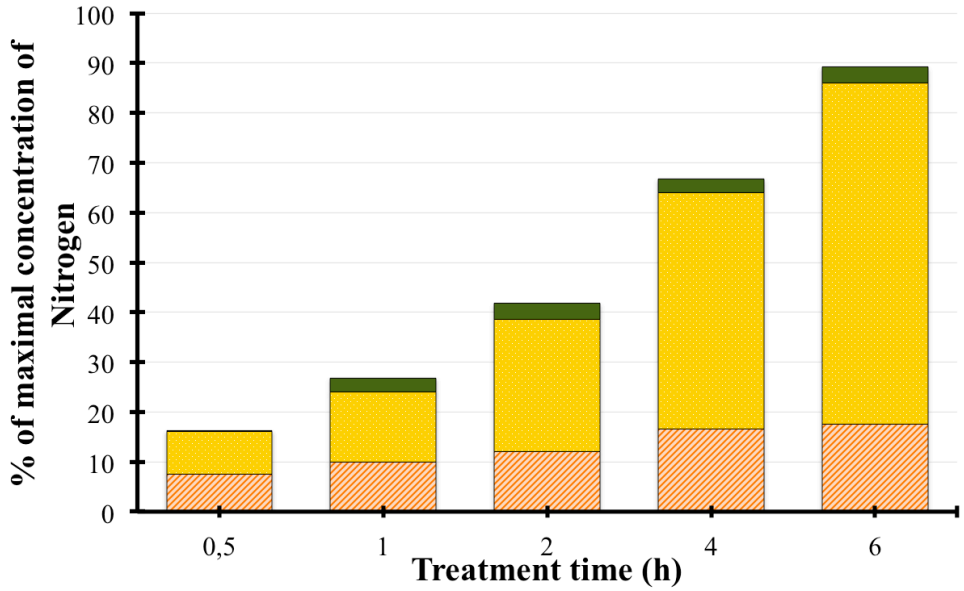


Figure IV.3. The evolution of Nitrogen containing ions during electro-Fenton treatment presented as a mass balance (%) of the initial amount of Nitrogen (2.8 mM) in the pharmaceutical mixture: nitrite ( ); nitrate ( ); ammonium ( ).
Operating conditions: I = 100 mA; $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 50 mM, V = 200 mL.

The evolution of other ions is shown on Figure IV.4 as the mass balance (%) of their respective theoretical values: 0.5 mM of F^-, 0.2 mM Cl^-, 0.3 mM I^- and 0.2 mM SO_4^{2-}. As can be seen the mass balance of chloride, fluoride and iodide reached more than 90% of the total mass balance, while sulfates hardly comprised 20% of total sulfur at the end of the electro-Fenton treatment. One of possible explanations can be formation of other sulfur-containing ions such as sulfide, thiosulfate, persulfate and even sulfate radical species ($SO_4^{-\bullet}$ and $HSO_4^{\bullet}$) [42].

The trend of gradual increase up to 6 h was observed for fluoride and sulfate. The same could be attributed to chloride and iodide but only up to 4 h afterwards their concentrations reduced, which was probably due to their oxidation on the anode to form their diatomic molecular form.

The fact that the formation of most inorganic ions almost reached the maxima of the theoretical mass balances means that the mineralization of the mixture was almost complete not only in terms of carbon transformation.

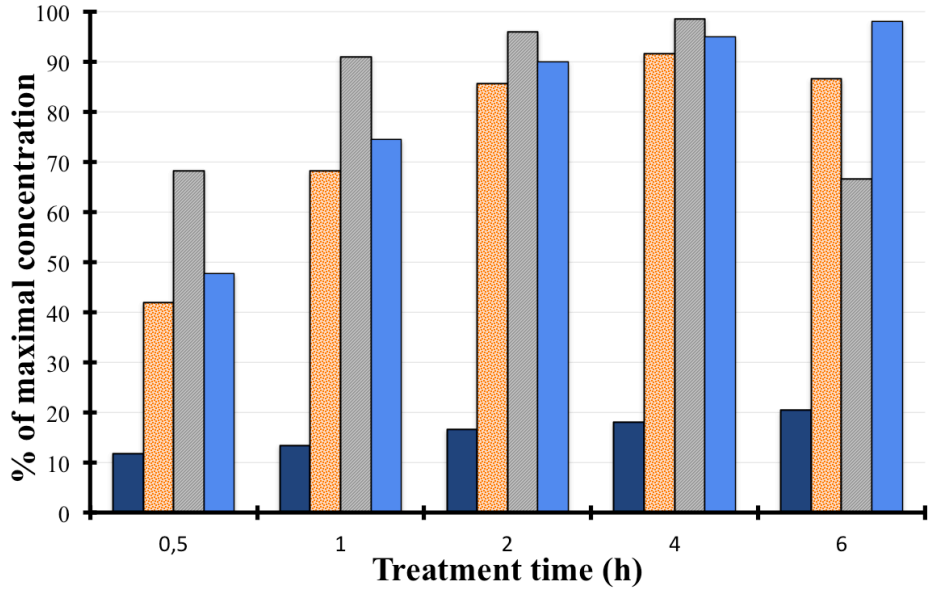


Figure IV.4. The evolution of ions during electro-Fenton treatment as a mass balance (%) of the initial concentration: sulfate ( ); iodide ( ); chloride ( ); fluoride ( ). Operating conditions: I = 100 mA; $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 50 mM, V = 200 mL.

IV.3.4. Evolution of temperature during the electro-Fenton treatment

Application of current to the electrochemical cell was transformed in additional heat in the system. As electro-Fenton reactor was not thermostated during its operation, the evolution of temperature was monitored. Results are given on Figure IV.5, where the range of ambient temperature in the reactor room is indicated for comparison. It can be observed that in general the temperature was rising during the first hour of treatment and afterwards reached the plateau phase. Even the lowest intensity applied (100 mA) demonstrated an increase of the temperature for more than 1 ^{0}C. Current intensities of up to 500 mA rose the temperature of the solution for not more than 5 °C, while the temperature at the end of the treatment under I = 1000 and 1500 mA reached 36 and 50°C respectively.

Generally an increase of the temperature favors acceleration of the chemical reactions. An increase of temperature that becomes greater with higher intensities means that part of the applied charge was used for the production of heat. Consequently it diminished the mineralization-current efficiency of the process. When considering electro-Fenton process it should be also taken into account that under elevated temperatures H_2O_2 becomes unstable and decomposes into O_2 and H_2O. Additionally oxygen becomes less soluble in water [43]. In a review paper Umar, Aziz and Yusoff [44] the range of 20-30 ^{0}C was presented as reasonable. With regards to this, it can be supposed that the removal of TOC could be theoretically higher in comparison to the one observed in Figure IV.2, if the reactor's temperature was kept in the ambient range. In order to understand the influence of temperature on the TOC removal additional series of experiments are required on the two

electro-Fenton setups with high intensities applied: a thermostated reactor and a reactor with evolution of temperature.

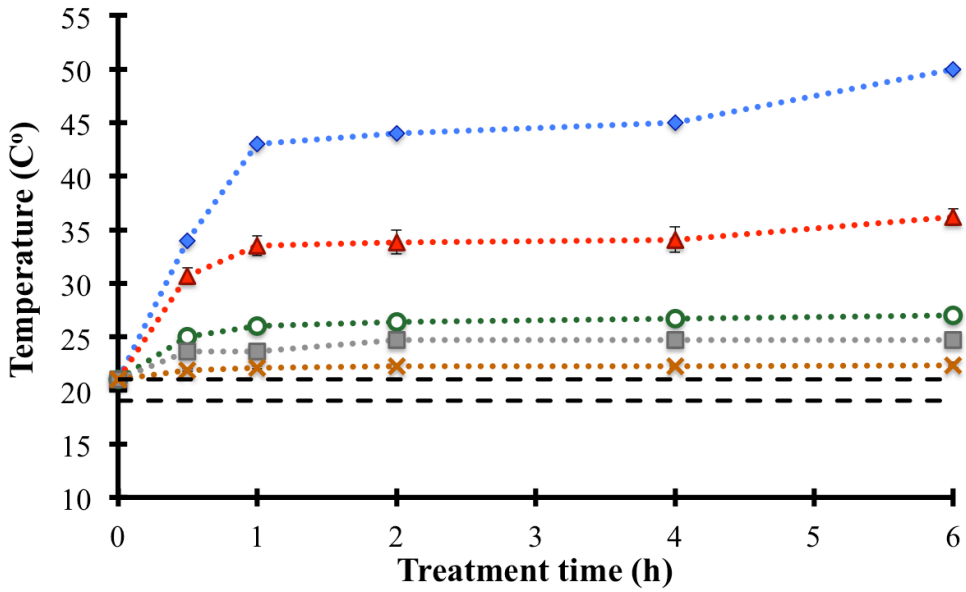


Figure IV.5. The evolution of temperature throughout the electro-Fenton treatment with different current intensities. Current intensity (mA): 1500 (-◆-); 1000 (-▲-); 500 (-O-); 300 (-■-); 100 (-✖-). Operating conditions: $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

IV.3.5. Removal of chemical oxygen demand. The effect of operating parameters

IV.3.5.1. Concentration of Iron

Analyzed concentrations of Fe^{2+} were 0.1, 0.2 and 0.5 mM and the results are presented on the Figure IV.6. Typically, by increasing amount of Fe^{2+} a production of hydroxyl radicals from Fenton reaction would also augment. It can be seen that an increase from 0.1 to 0.2 mM resulted in an enhancement of COD removal. Nonetheless a concentration of 0.5 mM Fe^{2+} did not show a positive effect on COD removal kinetics. It indicates that there is a limiting concentration of Fe^{2+} above which no improvement in removal efficiency can be attained. It confirms the previously discussed results on evolution of TOC (see section IV.3.1.).

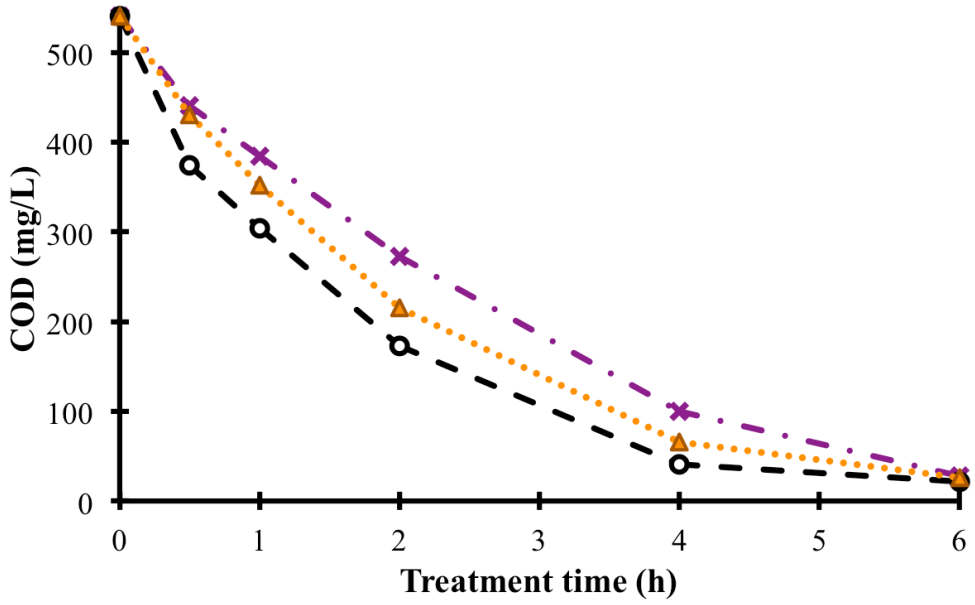


Figure IV.6. The effects of the Iron concentration for removal of chemical oxygen demand during the electro-Fenton process. Catalyst [Fe^{2+}] concentration (mM): 0.1 mM (-✖-); 0.2 mM (-O-); 0.5 mM (-▲-). Operating conditions: I = 300 mA; [Na_2SO_4] = 50 mM, V = 200 mL.

Ferrous iron in the Fenton system acts as a catalyst, meaning that only low quantity is required as it is regenerated in the process. When the optimal concentration of Fe^{2+} is surpassed, there is an excess of catalyst, which starts to inhibit the process due to the enhancement of the wasting reaction 4.

As a result Fe^{2+} does not participate entirely in the formation of hydroxyl radicals, i.e. degradation of organic matter, but is rather consumed in the parasitic reactions. On the other hand, this reaction consumes $^{\bullet}OH$ and consequently results in decrease of mineralization efficiency. Additionally the optimal amount of catalyst is a function of cathode material, more specifically of its ability to regenerate iron. The optimal concentration found in this study confirms with previously reported values for carbon felt [38].

IV.3.5.2. Applied current intensity

The effect of current intensity on COD removal was analyzed in the range of 100-1500 mA. Results are graphically presented on the Figure IV.7. As mentioned above current is responsible for the generation of hydroxyl radicals directly by the formation of physisorbed species on the surface of BDD anode and indirectly by Fenton reaction. A general trend is that elevated current intensities would produce larger amount of radicals, consequently the degradation of organic pollutants would be faster. The Figure IV.7 demonstrates a proof for such a statement. For instance, when analyzing the data after 2 h of electro-Fenton, the respective amount of COD for 100, 300, 500, 1000 and 1500 mA were 232, 173, 129, 100 and 52 mg O_2/L.

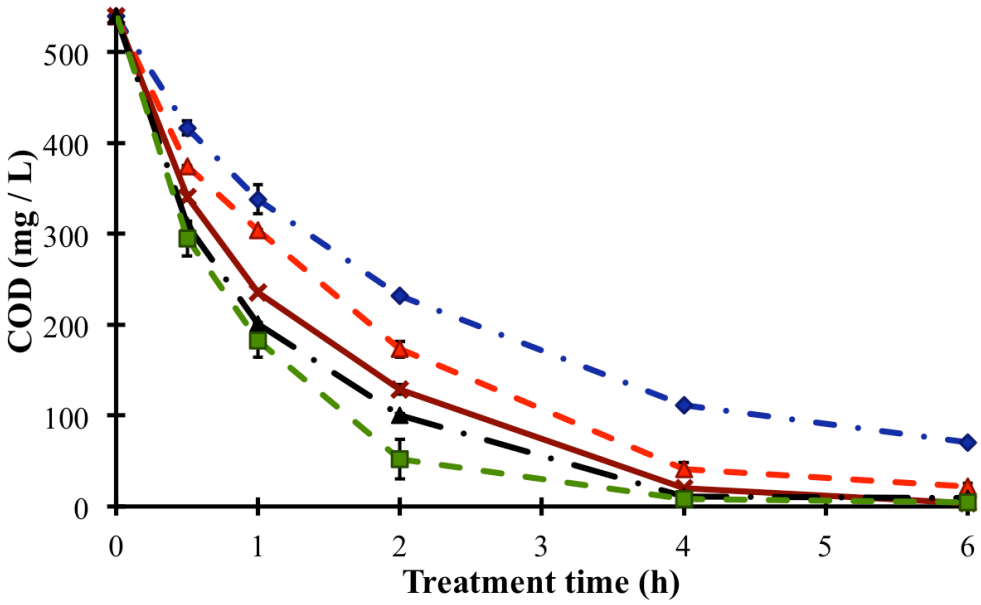


Figure IV.7. The effects of applied current intensity on removal of chemical oxygen demand during the electro-Fenton. Current intensity (mA): 100 (-◆-); 300 (-▲-); 500 (-✖-). 1000 (-▲-); 1500 (-■-). Operating conditions: $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 50 mM, V = 200 mL.

An interesting observation was that the differences between higher current intensities (500-1500 mA) were becoming insignificant towards the end of the treatment. The residual COD after 4 h was 8, 11 and 20 mg O_2/L for 1500, 1000 and 500 mA respectively. At the same time for electro-Fenton treatment with same duration but with I = 100 mA and 300 mA the corresponding values of COD were equal to 112 and 41 mg O_2/L respectively. This means that a 3-fold increase in applied current from 100 to 300 mA showed greater enhancement of removal rates than the same proportional increase of intensity from 500 to 1500 mA. It can be explained by a different ratio between amount of organic matter available and amount of hydroxyl radicals being produced. As the operating conditions of electro-Fenton were constant throughout the whole treatment duration, the production rate of hydroxyl radicals was assumed to be stable as well. Generally, higher current intensity produces greater amount of radicals, however an excess of these species in comparison to organics available will result in propagation of parasitic reactions (see section IV.3.1.2.).

IV.3.6. Biodegradability enhancement

The factor of biodegradability enhancement, E_{bio} , was calculated from the formula:

$$E_{bio} = \frac{R}{R_i} \tag{3}$$

where R is BOD_5/COD ratio at certain time and R_i is BOD_5/COD initial ratio. Calculated values are presented on the Figure IV.8.

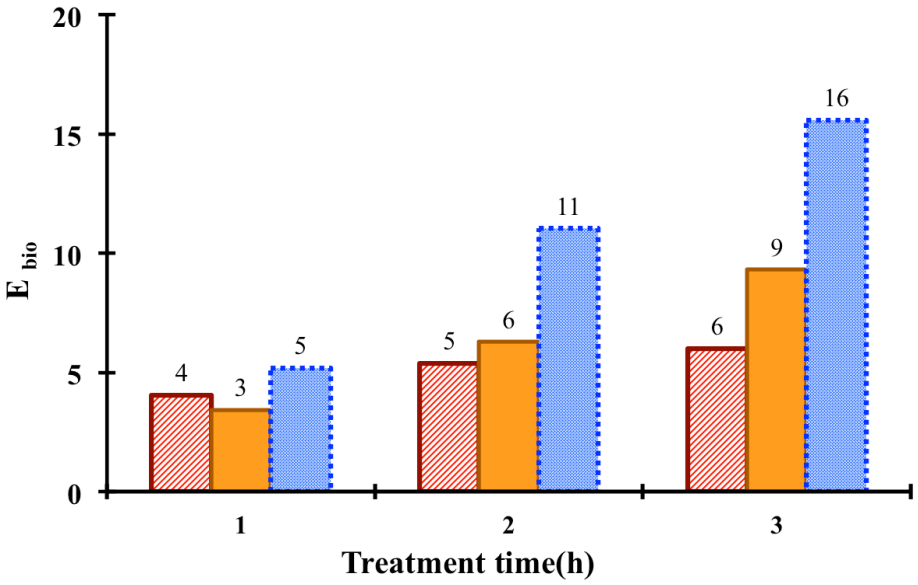


Figure IV.8. Biodegradability enhancement E_{bio} after different treatment time of electro-Fenton process in dependence on the applied current intensity (mA): 100 ( ); 500 ( ); 1000 ( ). Operating parameters: $[Fe^{2+}]$ = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

It can be seen that the biodegradability for 100 and 500 mA was enhanced in the range of 3-9 times from the initial value and tends to increase with longer treatment duration. When an intensity of 1000 mA was applied, the treated solution became 5, 11 and 16 times more biodegradable than the initial synthetic mixture after 1, 2 and 3 h treatment, respectively. To sum up, a plausible explanation for biodegradability increase is a generation of aliphatic by-products (e.g. carboxylic acids), which are easily biodegradable. Their formation required multiple steps of breaking down the cyclic pharmaceutical compounds, therefore it appears that longer treatment time and higher current intensities gave a greater enhancement of biodegradability.

IV.3.7. Evolution of BOD/COD ratio over longer time

In order to see the evolution of biodegradability over incubation time longer than conventionally accepted 5 or 7 days, a set of BOD experiments was conducted for more than two weeks and results are graphically presented on the Figure IV.9. As seen the biodegradability of initial solution of 13 pharmaceuticals did not seem to improve. The value of blank have been already subtracted from the displayed values, so the fact that they remained above zero means that these samples did not show any inhibiting effects on the microorganisms.

The dashed horizontal line represents the ratio of BOD/COD equal to 0.33, which is a standard accepted value in wastewater treatment to consider the effluent biodegradable [37]. As seen from the Figure IV.9, when we consider a standard 5-day BOD, then no sample attained this ratio. Meanwhile long-term biodegradability was gradually increasing and for two samples it overpassed the minimal requirement. After almost 17 days the ratio for 500 mA achieved a value of 0.6, which means that solution was easily biodegradable (BOD/COD >0.5). For the current intensity of 1000 mA the ratio was equal to 0.9, meaning that the

solution was completely biodegradable. By contrast the effluent after electro-Fenton with *I*=100 mA did not reach the biodegradability level even after more than two weeks.

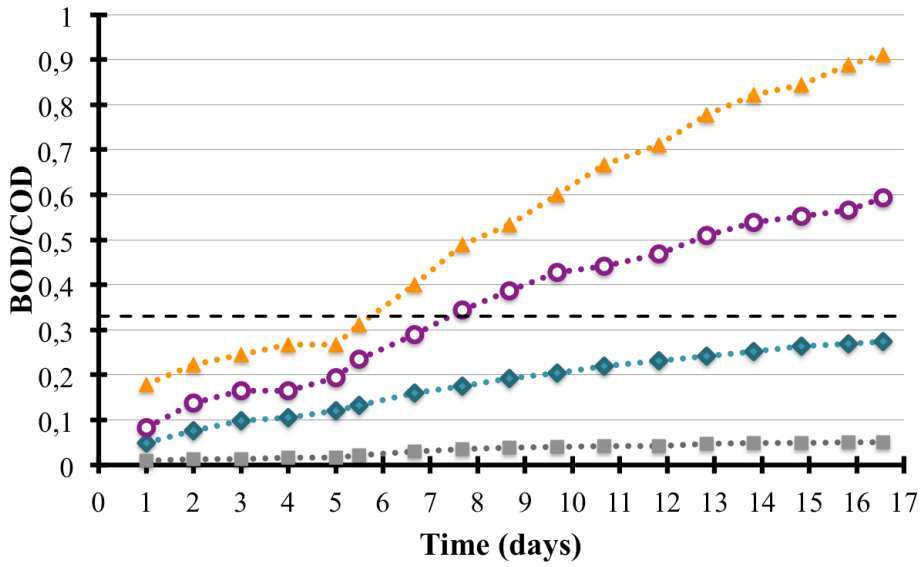


Figure IV.9. Evolution of long-term BOD/COD ratio for the electro-Fenton treated solution during 3 h in dependence on the applied intensity (mA): initial (-■-); 100 (-◆-); 500 (-○-); 1000 (-▲-). Operating parameters: $[Fe^{2+}]$ = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 200 mL.

Such results indicate that a synthetic pharmaceutical mixture after treatment with electro-Fenton under higher current intensities appeared as biodegradable with a prolonged BOD incubation. As the biomass used for this test was a typical activated sludge from a municipal wastewater treatment facility, the microorganisms required additional time for adaptation to the pharmaceutical effluent. In principle it means that when applying electro-Fenton on a larger scale as a pre-treatment before a biological process, an acclimation of biomass would be crucial. In addition, the concentration of biomass in these tests was higher in comparison to the previously discussed results on biodegradability enhancement.

IV.3.8. Evolution of toxicity

The evolution of toxicity throughout the duration of electro-Fenton was performed through a series of Microtox tests by varying the current intensity. This test is based on usage of bioluminescent bacteria *Vibrio fischeri*, which is incubated with a sample for 5 min. The results are presented on Figure IV.10. Initial solution (t = 0) had already a rather high toxicity more than 70%. As can be seen the exposition time of 5 min shows that toxicity increased after electro-Fenton treatment: samples after 0.5 and 1 h treatment inhibited almost 100% of bacteria. After 2 h and 4 h of treatment there was a slight variation in the inhibition effect in the range 86-100%, but which was still higher than the initial value. Only after 4 h a decrease of the inhibition effect comparing to the initial state was observed and solely for higher current intensities of 500 and 1000 mA.

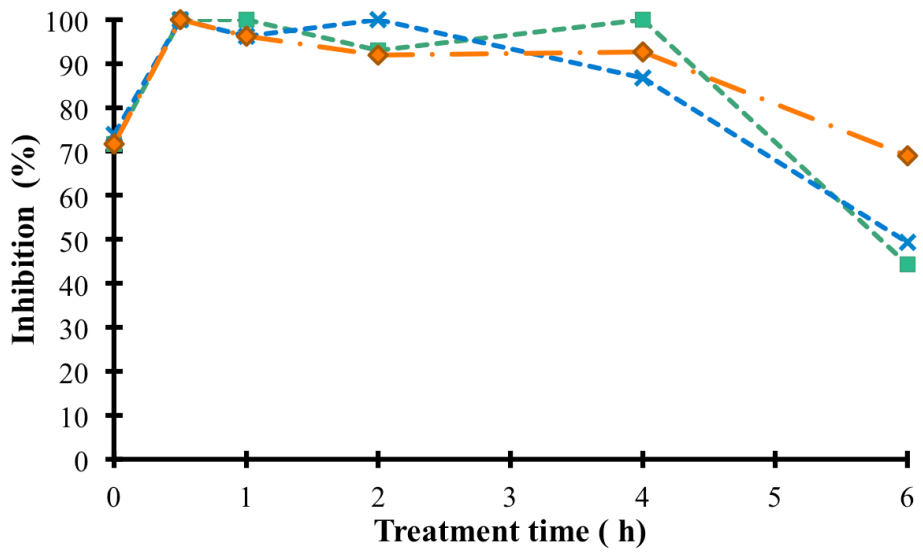


Figure IV.10. Evolution of toxicity as a function of luminescence inhibition of *Vibrio fischeri* during electro-Fenton process in dependence of current intensity (mA): 100 (-◆-); 500 (-✖-); 1000 (-■-). Operating conditions: $[Fe^{2+}]$ = 0.2 mM; $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

Conclusively, the Microtox assay with *Vibrio fischeri* demonstrated that the toxicity of the treated samples were mostly higher than of the initial solution. The only samples that displayed lower toxicity, but still higher than 50%, were samples of longest treatment times and highest current intensities. However, the previous results on biodegradability should be considered here as well. A factor of E_{bio} showed that there was an enhancement of biodegradability for all the analyzed current intensities and treatment duration. Moreover, a long-term biodegradability results showed that effluent after electro-Fenton could reach almost complete biodegradability for the stated above operating conditions and after a lag-phase of microbial adaptation. Therefore it is important to remember that bacterial species used in both Microtox and biodegradability tests are different. Activated sludge is a mixture of microorganisms used for degradation of organic material in the wastewater treatment, whereas *Vibrio fischeri* is a marine bacteria, which is sensitive for different types of toxic substances, including inorganic ions. In section IV.3.3 a constant and gradual release of inorganic ions, including chloride, fluoride and iodide, was discussed. On top of that observed toxicity could be a result of residual hydrogen peroxide, which was one of the Fenton`s reagent produced *in situ* [45]. Consequently, it is necessary to further confirm and complement presented results by a Microtox test with improved protocol for removal of residual H_2O_2 as well as by other relevant toxicity assays.

IV.3.9. Energy consumption

Consumption of energy is calculated here in terms of kWh consumed in the process per kg COD removed [6]:

$$E = \frac{E_{cell} I t}{(\Delta COD)_t V_s} \qquad (4)$$

where E_{cell} - average cell voltage (V), t - treatment time (h), I - applied current (A), $(\Delta COD)_t$ - decay of COD (g/L) at time t, V_s - volume of the solution (L).
The results are presented in the Table IV.5 for different current intensities and treatment time used in this study for evaluation of biodegradability enhancement.

Table IV.5. Energy consumption E (kWh/(kg COD)) for different current intensities and treatment duration.

Duration / Intensity	**1 h**	**2 h**	**3 h**
100 mA	9.3	12.2	15.8
500 mA	47.5	70.2	92.6
1000 mA	113.1	176.7	262.0

From the results presented above, it could be concluded that the biodegradability enhancement was in the range of 3-16 times. The results from evolution of long-term biodegradability showed that the ratio BOD/COD > 0.33 was achieved with intensities of 500 and 1000 mA and 3 h treatment time. Despite that the energy consumption for higher current intensities and especially longer treatment time (3 h) was significantly higher in comparison to other values. Electro-Fenton treatment with 100 mA at 3 h consumed almost 16 kWh/(kg COD), while a five time increase in current (500 mA) resulted in a greater increase of the energy consumption - almost 100 kWh/(kg COD). When applied 1000 mA for 3 h, the consumption was 262 kWh/(kg COD), which is 2.5 times more energy-intensive that for 500 mA. In order to make a trade-off between maximal biodegradability and minimal energy consumption a current intensity of 500 mA should be considered as a working value under the operating conditions applied in this study.

IV.4. CONCLUSIONS

This study investigated the potential of electro-Fenton process for the removal of pharmaceuticals from a synthetic mixture and the effect of operating parameters (current intensity and Fe^{2+} concentration) on removal rates. The optimal concentration of Fe^{2+} was found to be 0.2 mM, which was also determined to be a threshold value, above which no enhancement of mineralization rate was observed.

The analysis of current intensity revealed a trend of increasing current/increasing mineralization rate. Apart from it, the degradation kinetics of two components of the mixture (caffeine and 5-fluorouracil) was analyzed in terms of apparent rate constant k_{app}. This data uncovered the threshold value of current intensity below 700 mA.

Formation of inorganic ions (sulfate, chloride, fluoride, iodide, nitrate, nitrite and ammonium) was close to total mass balance (almost 90% of initial concentration), except that of sulfate ion. This confirmed the fact that complete mineralization could be achieved with electro-Fenton process.

The experiments on temperature evolution showed that even lowest current intensities heated up the solution (extra 1 °C to the ambient level), while higher intensities (1000 and1500 mA) showed a significant temperature rise: 36 °C and 50 °C respectively.

The experimental data on pharmaceutical mixture presented here led to important conclusions on the biodegradability of effluents after electro-Fenton treatment. The assays on biodegradability gave evidence to the fact that electro-Fenton treatment can be effectively applied to enhance the biodegradability in 3-16 times from the initial value depending on the applied current intensity and treatment time. By increasing the incubation time of samples and increasing the biomass concentration almost complete biodegradability could be attained.

For toxicity analysis of samples after electro-Fenton treatment, a marine bioluminescent bacteria *Vibrio fischeri* was used and the showed an elevated toxicity due to the probable formation of more toxic oxidation intermediates. Another reason could be residual hydrogen peroxide remained at the solution after treatment. With regards to presented results, a current intensity of 500 mA should be considered as a convenient value under experimental conditions used in this study.

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CHAPTER V

Bio-electro-Fenton:
evaluation of a combined biological - advanced oxidation treatment for pharmaceutical wastewater

To be submitted

ABSTRACT

Electro-Fenton (EF), an advanced oxidation process, can be combined with a biological process for efficient and economical treatment of wastewater containing refractory and persistent pollutants such as pharmaceuticals. In this study, a biological process was performed in a Sequencing Batch Reactor (SBR), which was either preceded or followed by EF. The main goal was to evaluate the potential of two possible sequences of a combined electrochemical-biological process: EF/SBR and SBR/EF for the treatment of a real pharmaceutical wastewater spiked with 0.1 mM of caffeine and 5-fluorouracil. The biological removal of COD and pharmaceuticals could be improved by extending the acclimation time of bacteria and increasing biomass concentration in the SBR. Hardly biodegradable caffeine and COD were completely removed during the EF post-treatment (SBR/EF). During the EF/SBR sequence, complete removal of pharmaceuticals was achieved by EF within a 30 min-2 h treatment time depending on the applied current intensity (200-800 mA). With a current intensity of 500 and 800 mA, the BOD_5/COD ratio increased up to 0.38 and 0.58, respectively, after already 30 min of EF. The biological post-treatment was influenced by the level of biodegradability enhancement after EF pre-treatment. The choice of an adequate sequence of such combined process is significantly related to the wastewater characteristics as well as the treatment objectives.

Keywords: Electro-Fenton, Combined process, Pharmaceuticals, Caffeine, 5-Fluorouracil, Biological treatment

V.1. INTRODUCTION

A modern traditional health care system is founded on the usage of pharmaceuticals. The circulation of medicinal products is not simply restricted to producers, distributors and users, but has been observed to extend to the total of the environment as a global environmental concern [1]. Pharmaceuticals consumed by humans or animals find their ways into all types of surface, ground and drinking water and this disturbs not only the natural balance of ecosystems, but has also multiple devastating effects on its inhabitants [2-4].

There is a multitude of sources by which pharmaceuticals enter the water environment, mainly from human excretion and discharge of drug-containing effluents. However, the main cause of pollution remains the ineffective treatment of wastewaters [5, 6]. An apparent solution to the problem is improving the existing treatment technologies, especially for the wastewater from pharmaceutical companies and hospitals.

A typical wastewater plant includes a sequence of different stages, where water is gradually decontaminated through physicochemical and biological processes. The core of conventional treatment is typically a biological process, which employs microorganisms for degradation of pollutants. Biological processes have several advantages, especially the favorable relation between cost and efficiency. However, as some pharmaceuticals are generally refractory, non-biodegradable or toxic to bacteria, their biological removal is erratic and inadequate. In order to improve the modern treatment methods, new advanced technologies are needed.

Electro-Fenton (EF) is an advanced oxidation process based on the *in situ* production of hydroxyl radicals (•OH) through electrochemically assisted Fenton reaction [7, 8]. It is a destructive process, where the strong oxidants •OH are able to degrade non-selectively persistent/toxic organic pollutants, contrarily to the biological processes [9]. EF has been demonstrated to be effective in degradation of different pharmaceuticals [10-12], as well as other types of pollutants [13-15]. The main drawback that holds back its industrial application is the cost-intensive operation due to a relatively high energy consumption. Therefore, a combination of electrochemical and biological processes – called "bio-electro-Fenton" - is supposed to create a synergetic effect by coupling the advantages of both processes. With regard to the order of processes, bio-electro-Fenton can be performed in two different modes: EF as either pre- or post-treatment. In the first case, the removal of refractory and/or toxic pharmaceutical contaminants can be achieved by means of the electrochemical process, which breaks down mother molecules of pollutants to more biodegradable intermediates facilitating the subsequent biological process. On the contrary, if the influent wastewater is biodegradable, the application of a biological pre-treatment represents an appropriate alternative followed by EF process as a polishing step for the removal of residual refractory pollutants.

The main objective of this study was to evaluate the potential of bio-electro-Fenton for removal of pharmaceutical contamination, comparing the two above-mentioned configurations, i.e. EF-biological process and biological process-EF.

Biological process was performed in a conventional Sequencing Batch Reactor (SBR), which is simple in operation and requires only one reactor, where all the stages of biological treatment take place: i) wastewater feeding, ii) biological reaction with microorganisms, iii) biomass settlement and iv) discharge of the treated solution. When the four stages are completed, a new treatment cycle starts in the same reactor.

The research questions that were addressed in this study are: (i) to analyze the removal of chemical oxygen demand (COD) as well as pharmaceuticals during a SBR biological process alone and combined to EF post-treatment; (ii) to examine the removal of COD and pharmaceuticals during EF pre-treatment followed by a SBR biological process; (iii) to determine the optimal operating parameters of EF pre-treatment based on biodegradability enhancement and energy consumption; (iv) to compare the two different treatment strategies (SBR/EF and EF/SBR).

V.2. MATERIALS AND METHODS

V.2.1. Chemicals

Analytical grades of 5-fluorouracil (CAS 51-21-8) and caffeine (CAS 58-08-2) were purchased from Sigma-Aldrich. Iron (II) sulfate heptahydrate (CAS 7782-63-0) as a catalyst source, and sodium sulfate (CAS 7757-82-6) as supporting electrolyte were obtained from Acros Organics and Sigma-Aldrich, respectively. The pH was adjusted using 1 M and 5 M sulfuric acid as well as 5 M and 10 M sodium hydroxide. The reagents used for preparation of solutions for analysis of COD and for biodegradability tests as well as for bioreactors are as reported in [16] and [17], respectively.

V.2.2. Wastewater characteristics

A real effluent was taken from a pharmaceutical factory in the Campania region, Italy. Its composition is presented in Table V.1. The effluent was spiked with 0.1 mM of psychoactive drug caffeine (CAF) and 0.1 mM of antineoplastic drug 5-fluorouracil (5-FU), two common pharmaceuticals which are widely used worldwide and were previously studied by in detail [18].

Table V.1. Characteristics of the real wastewater from pharmaceutical production.

PARAMETER	VALUE	UNITS
pH	7.10 ± 0.05	
Total suspended solids	19 ± 1	mg/L
BOD_5	4	mg/L
PARAMETER	VALUE	UNITS
COD	120 ± 10	mg/L
Aluminum	0.071 ± 0.007	mg/L
Free active chlorine	< 0.05	mg/L

Table V.1. (continued).

PARAMETER	VALUE	UNITS
Chloride	235 ± 9	mg/L
Total phosphorous	0.40 ± 0.02	mg/L
Ammonium	8.2 ± 0.3	mg/L
Nitrous N	< 0.01	mg/L
Nitric N	0.13 ± 0,01	mg/L

V.2.3. Sequencing batch reactor (SBR)

Two separate bioreactors were maintained for a 3-month period in order to acclimate the microbial cultures to the real wastewater and subsequently investigate the potential of the SBR/EF and EF/SBR sequences. The biomass was an activated sludge taken from the municipal wastewater treatment plant in Cassino, Italy. Acclimation of biomass proceeded in two stages. Initially, the microbial activity was stimulated by adding nutrients and sucrose (1 g/L) to the wastewater diluted in 1:1 ratio with tap water in order to minimize the initial shock to the microorganisms. The solution was replaced with a fresh medium twice per week and the dilution with tap water was gradually decreased. Subsequently, caffeine and 5-fluorouracil were added to the microbial culture solution in order to acclimate the biomass to the presence of these pharmaceuticals. In this case, the replacement with a fresh solution was performed every 5 d.

The operational conditions of both reactors were similar: continuous aeration with air ensured constant oxygen saturation of 8.7 ± 0.2 mg O_2/L; pH and temperature were in the range of 7.8 ± 0.2 and 22 ± 2^{o}C, respectively. The amount of nutrients was kept within the ratio COD:N:P = 100:5:1. In addition, nutrients (buffer and saline solutions) were prepared and added as reported elsewhere [17]. The operation followed the standard steps of a SBR. After pH adjustment, the reactor was fed with the influent and all the nutrients were added. Then, the treatment was performed with continuous mixing and aeration with biological reaction time ranging between 1 and 3 d. Finally, after biomass settlement for 30 min the treated solution was withdrawn from the SBR. This SBR operational design was applied for both SBR/EF and EF/SBR sequences.

In order to investigate the effect of biomass concentration and microbial acclimation time on the biological process, the SBR used in the SBR/EF sequence was maintained under two different operating conditions. Initially, SBR-I was operated with a volatile suspended solids (VSS) concentration of 850 mg/L using a biomass adapted for 2 weeks with the wastewater spiked with pharmaceuticals. A higher VSS concentration (1310 mg/L) and a longer acclimation time (1 month) were used during SBR-II operation.

The working volume of the SBRs used for the SBR/EF and the EF/SBR sequences were 1.5 and 1 L, respectively.

V.2.4. Electro-Fenton (EF) setup

An undivided EF 1.4 L reactor was operated at ambient temperature (T = 22 ± 1 °C) in batch mode. The reactor was fed with the initial pharmaceutical wastewater spiked with caffeine and 5-fluorouracil during the EF/SBR sequence. During the SBR/EF sequence, the reactor was fed with the biologically pre-treated solution. In both cases, the EF influent contained 0.05 M sodium sulfate (electrolyte) and 0.2 mM iron sulfate (source of Fe^{2+} catalyst), according to the optimal operating conditions previously determined [18]. The pH was adjusted to 2.9 ± 0.1 with 1 M sulfuric acid. A carbon felt cathode with dimensions of 12.5 cm × 38 cm (corresponding immersed area of 475 cm^2) was used and placed in order to cover the inner area of the reactor. The boron-doped diamond (BDD) anode with coating on both sides had dimensions of 15 cm× 7.5 cm and immersed area in the solution of 193 cm^2. BDD was placed in the center of the reactor on equal distances from the cathode. Continuous stirring at 300 rpm was ensured throughout the process and aeration started 5 min prior to electrolysis in order to saturate the solution with oxygen. The current intensity was applied and monitored in real time with a power supply DM30005E III (MKC, Italy).

V.2.5. Bio-electro-Fenton setup

To ensure proper functioning of both processes as a part of one combined treatment, pH adjustment was needed between the two steps. In the sequence EF/SBR, pH was adjusted from 2.8 to 7.8 ± 0.2 with 5 M NaOH, while for implementing SBR/EF it was necessary to decrease pH to 2.9 ± 0.1 with 1 M H_2SO_4. No other transitional actions were taken in order to approximate the operating conditions to future large-scale setup.

The samples taken during SBR operation were left for 30 min in order to settle the biomass. Afterwards, the supernatant was withdrawn and filtered with 0.45 μm Millex cellulose membranes (Merck Millipore, USA) for analysis.

V.2.6. Instruments and analytical procedures

V.2.6.1. Chemical oxygen demand (COD)

A reflux method in a closed system (tubes) and a colorimetric dosage with potassium dichromate was used to determine COD. Digestion and acidic solution were prepared according to the analytical method [16] and filled in the tubes. Samples were added to the pre-made tubes and incubated at 148°C for 2 h. The value of COD was measured at 445 nm by photometric method using a WTW PhotoLab (WTW, Germany) using a COD range between 0 and 100 mg/L.

V.2.6.2. Biodegradability tests

The tests were based on measuring biochemical oxygen demand over 5 d (BOD_5). The dilution method from [17] was performed with slight modifications. All the solutions (saline, control, phosphate buffer, allylthiourea and dilution water) were prepared according to the protocol. Biomass was the same activated sludge used to seed the SBR with a VSS concentration of 4.2 g/L.

Prior to analysis, dilution water, samples and bacterial inoculum were aerated to ensure saturation of oxygen. An OxiTop® control system (WTW, Germany) was used as automated system for BOD_5 measurement by respirometric method. Each OxiTop® bottle had a working volume of 432 mL and contained 100 mL of sample, 3 mL of buffer solution (necessary amount to keep the COD:N ratio around 100:5), 5 mL of activated sludge and 1 mL of allylthiourea solution (to prevent nitrification). The remaining volume (273 mL) was filled with dilution water, prepared as in [17]. Before seeding with bacteria, the final pH was ensured to be circumneutral. A rubber sleeve with sodium hydroxide pellets was placed inside each bottle to absorb the carbon dioxide produced during the bacterial respiration. The continuously stirred bottles were incubated in a dark place at 20 ± 1°C. Each batch of analysis also included one blank and one reference control. The blank control bottle was filled with ultrapure water instead of the sample, in order to evaluate the microbial endogenous respiration to be subtracted from the final BOD_5 obtained in the biodegradability tests. The control bottle contained glucose and acetamic acid to keep the reference of microbial activity.

V.2.6.3. High performance liquid chromatography

Concentration of caffeine and 5-fluorouracil were determined by means of a reverse-phase high performance liquid chromatography (RP-HPLC) system. The equipment (Merck Hitachi) consisted of a column Purospher STAR RP-18 endcapped (5 μm), a pump (Elite LaChrome, L-2130), an UV detector (Elite LaChrome, L-2400) and a thermostatic oven (Jetstream Plus, series 140310). Detection limit for both pharmaceuticals was 10^{-6} M. Temperature was kept at 40°C and the mobile phase consisted of a mixture of methanol and ultrapure water both buffered with 1% acetic acid. Detection was performed at 275 nm with an injection volume of 20 μL. Gradient elution was used for the separation of the mixture components with the following order: from the start to 11 min, a 90% water and 10% methanol eluent was used at flow rate of 0.3 mL/min ; then, from 11 to 13 min the conditions were gradually changed to 70% water and 30% methanol at a flow rate of 0.6 mL/min and maintained till 28 min. From 28 to 30 min the eluent composition was gradually changed to the initial 90% water and 10% methanol at a flow rate of 0.3 mL/min and remained for the last 5 min. Such analytical conditions allowed eluting 5-fluorouracil at 10 min and caffeine at 25.4 min.

V.2.6.4. Volatile suspended solids (VSS)

VSS concentration was measured during the SBR operation by using the standard protocol as reported in [17]. Filtration of samples was performed with 47 mm glass microfiber filters (Whatman, UK).

V.3. RESULTS AND DISCUSSION

V.3.1. SBR as pre-treatment

As specified in 'materials and methods' section the two bioreactors operated for the SBR/EF sequence differed by the VSS concentration and microbial acclimation time. The experiments performed with SBR-I showed that after a biological reaction time of 24 h only 25% of COD and 40% of 5-fluorouracil (5-FU) were removed (Figure V.1, part I). The concentration of caffeine decreased only by 3%, indicating its lower biodegradability compared to 5-fluorouracil. The biologically pre-treated solution was then treated by EF with a current intensity of 500 mA and the results are presented in Figure V.1, part II. The residual concentrations of pharmaceuticals were below the detection limit after already 1 h of electrocatalysis. The COD removal was 99% after 4 h of EF treatment.

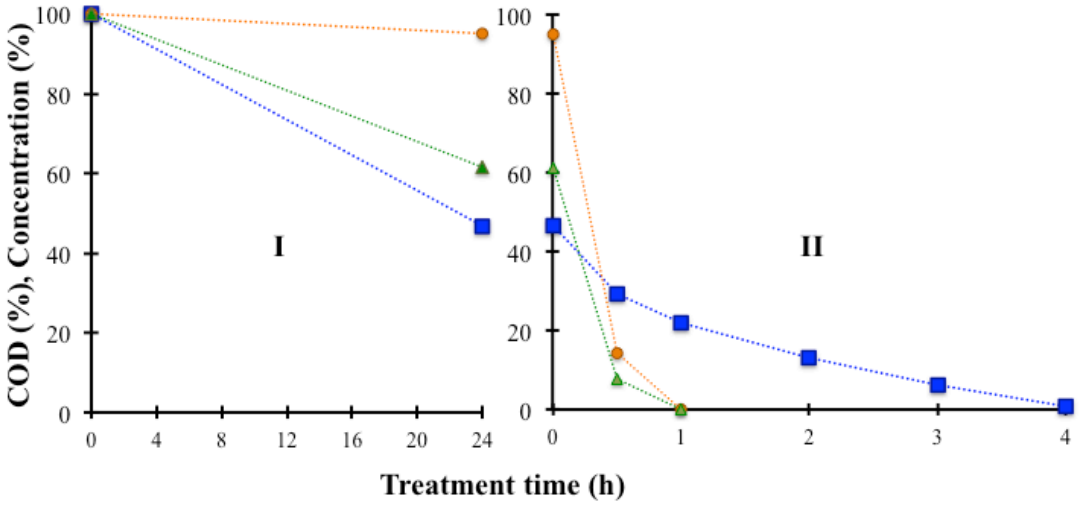

Figure V.1. Evolution of COD and pharmaceuticals` concentration during a batch experiment of combined treatment: the SBR (I) followed by EF (II). COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-). Operating conditions: (I) microbial acclimation 2 weeks; VSS = 850 mg/L; (II) I = 500 mA.

In order to increase efficiency of pharmaceutical removal, SBR-II was used with a longer microbial acclimation time and a higher biomass concentration. Experimental results are presented in Figure V.2. The evolution of COD and concentration of caffeine and 5-fluorouracil were monitored for a longer biological reaction time up to 3 d. Removal of both pharmaceuticals improved: after 24 h, 5-fluorouracil was only at 12% of the initial concentration and was removed by 43%, showing a significantly higher degradation efficiency than during SBR-I operation.

Both drugs were not detected after 3 d, which is a rather long and cost-intensive reaction time, if compared to the operation of large-scale bioreactors. A slightly enhanced COD removal was observed in SBR-II in comparison with SBR-I. After 24 h, 39% of initial COD remained in SBR-II against 47% in SBR-I. However, a longer retention time of 3 d only resulted in additional 10% of COD removed. This was most likely due to the recalcitrance of some organics to be biologically degraded even after longer microbial acclimation and reaction time.

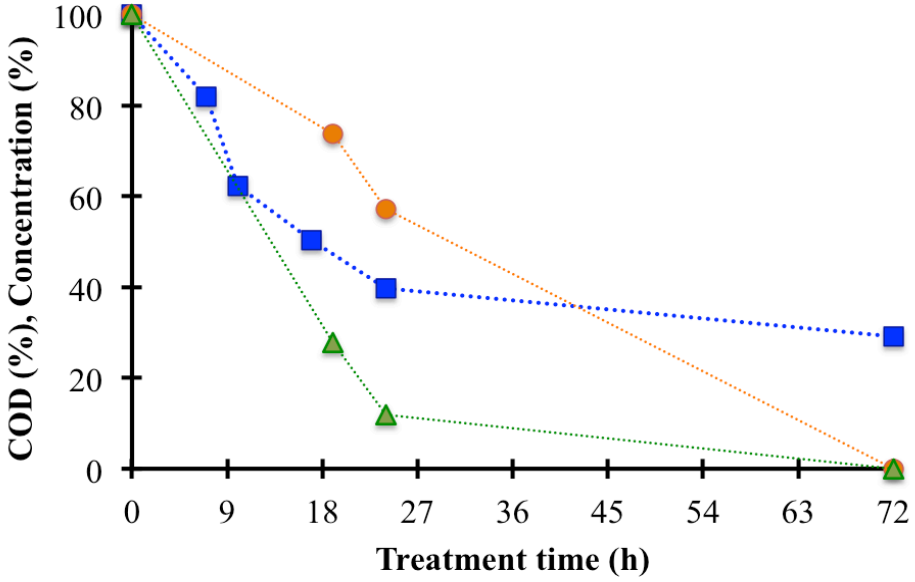

Figure V.2. Evolution of COD and pharmaceuticals` concentration in a SBR operated with acclimation time of 1 month and VSS = 1310 mg/L: COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-).

Figure V.3 shows the COD and pharmaceuticals profiles obtained when SBR-II was coupled to EF post-treatment.

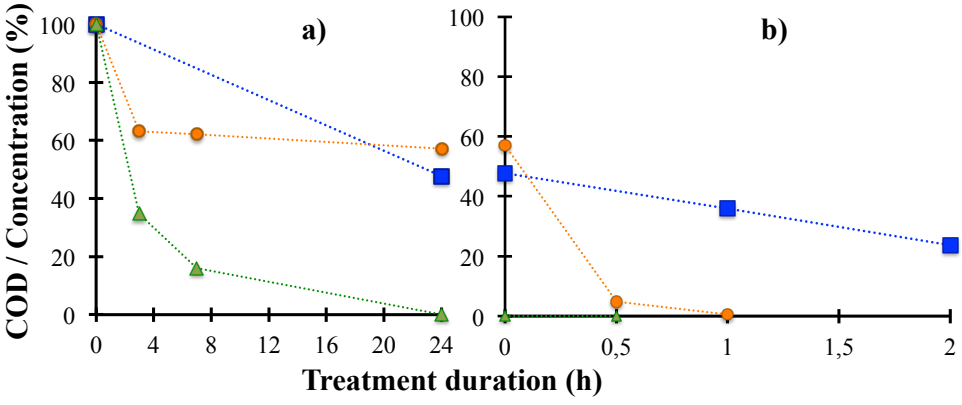

Figure V.3. Evolution of COD and pharmaceuticals` concentration during a batch experiment of a combined treatment: the SBR (I) followed by EF (II): COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-). Operating conditions: [CAF] = 0.1 mM, [5-FU] =0.1 mM; COD_0 = 120 mg/L, (I) $[O_2]$ = 8.7 mg/L, pH = 7.8, V = 1.5 L, SS = 1000 mg/L; VSS/SS = 0.85; (II) I = 500 mA, $[Fe^{2+}]$ = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 1.4 L.

With this configuration, a biological reaction time of 24 h was used which is more similar to a typical reaction time of a large-scale bioreactor. The removal during biological pre-treatment was similar to the one reported in Figure V.2. COD and were removed by 52% and 43%, respectively, whereas 5-fluorouracil was below the detection limit (Figure V.3, part I). The residual amount of caffeine was almost completely degraded by the EF post-treatment after 30 min with a current intensity of 500 mA (Figure V.3, part II).

V.3.2. EF as pre-treatment

V.3.2.1. Removal of COD and pharmaceuticals

The BOD_5/COD ratio of the feed wastewater was < 0.05, indicating a very low biodegradability. However, 57% and 82% of spiked caffeine and 5-fluorouracil, respectively, were removed when the biological process was used as pre-treatment with a longer microbial acclimation and a higher biomass concentration. This section presents the results of EF as a pre-treatment, which is considered as a more suitable treatment strategy for non-biodegradable effluents [19]. Initially, the influence of the current intensity on the evolution of COD, caffeine and 5-fluorouracil was investigated. This operating parameter is extremely important in terms of energy consumption and, consequently, treatment costs [20]. The range of the studied intensities was 200-800 mA and the results are given in Figure V.4.

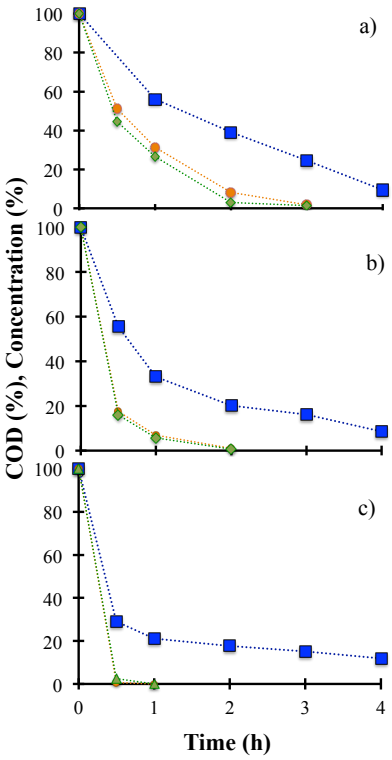

Figure V.4. Evolution of COD and pharmaceuticals` concentration by EF process in dependence of applied current intensity (mA): (a) 200; (b) 500; (c) 800; COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-).

The removal of both COD and the two pharmaceuticals was strongly influenced by the applied current intensity. After 1 h of EF, COD removal was 44%, 68% and 79% at 200, 500 and 800 mA, respectively. Caffeine and 5-fluorouracil removal rates were very similar. At 200 mA, both pharmaceuticals were below the detection limit after 3 h, while at 500 mA and 800 mA their degradation was faster resulting in complete removal after 2 h and 1 h, respectively.

V.3.2.2. Biodegradability of the EF-treated effluent

Biodegradability enhancement is an important condition for proper functioning of a biological post-treatment. In order to optimize EF as economically viable pre-treatment, it is important to evaluate the effects of current intensity and retention time on biodegradability. The results of this analysis are illustrated in Figure V.5. The biodegradability enhancement was higher with longer treatment time and higher current intensities. Nevertheless, the BOD_5/COD ratio reached 0.33, a standard level of biodegradability [17], even after only 30 min of EF. Such results can be explained by the degradation of the initially refractory compounds by hydroxyl radicals to more biodegradable intermediates, which are more easily degradable by microorganisms.

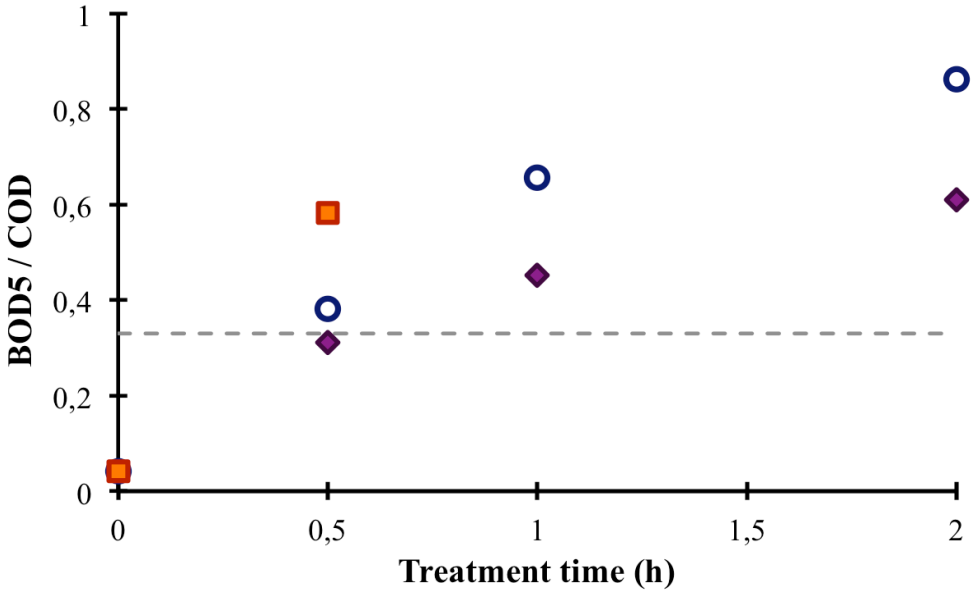

Figure V.5. Biodegradability of effluent after EF treatment in dependence on treatment duration and applied current intensity (mA): 100 (-◆-); 500 (-O-); 800 mA (-■-). Dashed line - BOD_5/COD = 0.33. Operating conditions: $[Fe^{2+}]$ = 0.2 mM, $[Na_2SO_4]$ = 50 mM, V = 1.4 L.

V.3.2.3. Energy consumption

An important parameter for the evaluation of an electrochemical process is energy consumption, which also constitutes a major part of operational costs. It is normally calculated in terms of kWh consumed per removal of kg of COD:

$$E = \frac{E_{cell} I t}{(\Delta COD)_t V_s} \quad (1),$$

where E_{cell} - average cell voltage (V), t - electrolysis time (h), I - applied current (A), $(\Delta COD)_t$ - decay of COD (g/L) after time t, V_s - volume of the solution (L).

Table V.2 shows the energy consumption for different treatment durations and current intensities. The lowest current intensity of 200 mA displayed the smallest cell potential, which is directly proportional to the energy consumption. At the same time the lowest energy consumption was observed for the shortest treatment time (0.5 h), which can be explained by a changing rate of COD removal during the treatment. Figures V.2 and V.4 show that COD removal rate was the highest at the beginning of the EF treatment prior to gradually decreasing during the experiments. This was mainly attributed to the decreasing amount of organic matter and the propagation of parasitic reactions between •OH and scavengers. Consequently, the efficiency of the process was reduced and the removal of COD slowed down. This made the ratio of energy consumed per unit of COD higher towards the end of treatment for all the intensities.

Table V.2. Energy consumption (kWh used per removal of kg of COD) of electro-Fenton process in dependence on applied current intensity and treatment duration.

Duration / Intensity	**0.5 h**	**1 h**	**2 h**
200 mA	5	6	10
500 mA	25	31	55
800 mA	37	48	94

V.3.2.4. EF/SBR combination

As shown in the section V.3.2.1, a complete degradation of pharmaceuticals required different treatment duration depending on the applied current intensity. The effect of two different current intensities (200 and 500 mA) on EF used as pretreatment stage is illustrated in Figures V.6 and V.7. At 200 mA, a removal of both pharmaceuticals higher than 90% was observed after 2 h. Caffeine and 5-fluorouracil degradation was accompanied by 60% removal of COD (Figure V.6, part I). Additional 30% of COD was removed during the SBR biological post-treatment with a biological reaction time of 3 d (Figure V.6, part II).

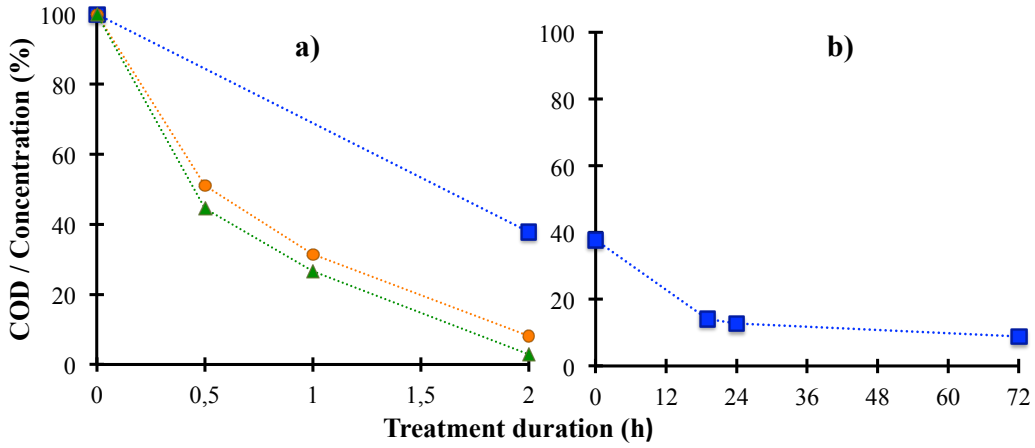

Figure V.6. Evolution of COD and pharmaceuticals` concentration during a batch experiment of combined treatment: EF (I) followed by a SBR (II): COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-). Operating conditions: (I) I = 200 mA, t = 2h.

When 500 mA was used as current intensity, both pharmaceuticals were completely degraded after 30 min (Figure V.7, part I).

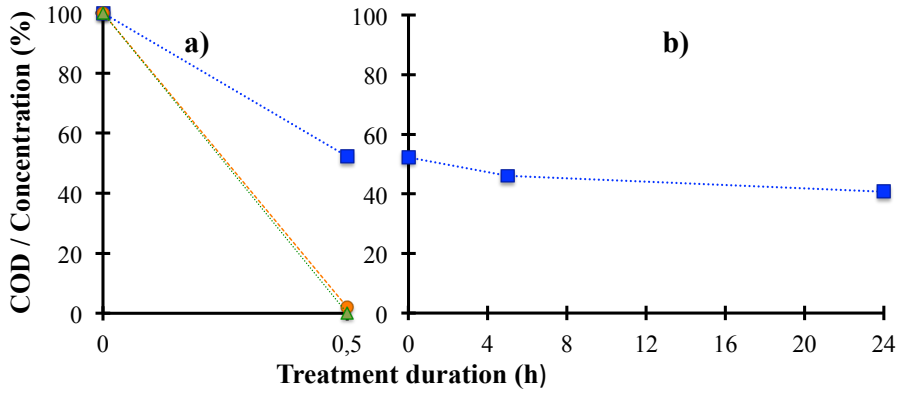

Figure V.7. Evolution of COD and pharmaceuticals` concentration during a batch experiment of combined treatment: EF (I) followed by a SBR (II): COD (-■-); 5-fluorouracil (-▲-); caffeine (-●-). Operating conditions: [CAF] = 0.1 mM, [5-FU] = 0.1 mM; COD = 120 mg/L, (I) I = 500 mA, [Fe^{2+}] = 0.2 mM, [Na_2SO_4] = 50 mM, V = 1.4 L; (II) SS = 2330 mg/L, VSS/SS = 0.62, V = 1 L.

During the 24 h biological post-treatment, only 12% of COD was removed. In comparison, 25% of COD was degraded in the first 24 h of the biological post-treatment of the previous EF/SBR combination (Figure V.6, part II). Such a difference was due to a different biodegradability level reached during the EF pre-treatment step. Figure V.5 shows that the BOD_5/COD ratio increased up to 0.61 after 2 h of EF at 200 mA, which was higher than 0.38 obtained at 500 mA after 0.5 h. This indicates that, although the pharmaceuticals could not be detected in the solution after both EF pre-treatments, the biodegradability of the solution significantly differed and influenced the effectiveness of the biological post-treatment. In the second case, the biological treatment was less efficient because of the lower biodegradability of the pre-treated solution.

V.3.3. Comparison between SBR/EF and EF/SBR sequences

Bio-electro-Fenton as combined electrochemical-biological treatment can be performed in two modes depending on which process is applied as pre-treatment. The choice of the treatment sequence significantly depends on the type and characteristics of the wastewater to be treated. In this study, the wastewater under investigation was initially non-biodegradable ($BOD_5/COD < 0.05$), with a low initial COD (120 mg/L).

Regarding the SBR/EF sequence microbial acclimation time and biomass concentration significantly affected COD removal efficiency during the biological treatment. Almost complete degradation of pharmaceuticals and more than 60% of COD removal was achieved with a biological reaction time of 3 d. When a lower biological reaction time of 24 h was used, COD was removed by 50%, 5-fluorouracil was not detected, while caffeine persisted. EF post-treatment was demonstrated to remove the residual amount of caffeine. At the same time, 2 h of EF post-treatment removed 34% (Figure V.1, part II) and 23% (Figure V.2, part II) of COD after pre-treatment in SBR-I and SBR-II, respectively. This discrepancy in terms of COD removal after EF was most likely attributed to the presence of suspended microbial cultures in the EF reactor after SBR-II operation. Part of the hydroxyl radicals produced during EF acted as neutralizers for bacteria by attacking their outer membrane and resulting in a lower COD removal [21, 22]. A possible solution is represented by the use of "immobilized-cell bioreactors" such as a membrane bioreactor, where the biomass is retained on the membrane surface and is not washed out to the electrochemical post-treatment at significant concentrations.

Further discussion is also needed regarding the way the pharmaceuticals can be biologically removed in a SBR. Here, three mechanisms are possible: biotransformation (degradation of pollutant molecule), volatilization and biosorption [23, 24]. The latter process can play an important role in the removal of a pollutant from an aqueous phase, but it is not a desirable outcome. Pharmaceuticals sorbed onto the surface of bacterial cells will be disposed with the sludge and, depending on the further sludge treatment [24, 25], their contamination will not only affect the hydrosphere but also soils [26]. Biosorption of pollutants necessarily requires further attention within future studies on a combined process of bio-electro-Fenton.

The application of EF as pre-treatment is particularly suitable when the initial wastewater is non-biodegradable. The key concerns here are efficient degradation of pharmaceuticals, biodegradability enhancement and minimal energy consumption. In the present work, both the pharmaceuticals studied were removed with similar rates depending on the current intensity. Lower intensities required logically longer treatment times for complete removal of caffeine and 5-fluorouracil, as the production of hydroxyl radicals is strongly dependent on the current intensity. At the same time longer treatment times and higher current intensities resulted in an enhanced biodegradability of the effluent, in spite of a higher energy consumption. The key choice was to be made between a higher current intensity/shorter treatment time or a lower intensity/longer treatment time. In this study, both variants were

implemented experimentally (500 mA/30 min and 200 mA/2 h). The results revealed that the biological process was more efficient after EF at 200 mA and 2 h treatment, because of the higher biodegradability enhancement. Therefore, the economic feasibility of EF as pre-treatment can be achieved by applying low current intensities and longer treatment times, making the process more energy saving and ensuring a proper biodegradability level in order to increase the efficiency of a biological post-treatment.

V.4. CONCLUSIONS

The main findings of this study are related to the combination of EF and a biological process as an example of bio-electro-Fenton. Both sequences (EF/SBR and SBR/EF) were analyzed in terms of COD and pharmaceutical removal. Experiments were carried out with a real effluent from pharmaceutical production spiked with 0.1 mM of caffeine and 5-fluorouracil.

The SBR demonstrated that an enhanced removal of organic matter and pharmaceuticals was achieved during the biological pre-treatment in case of higher biomass concentration, prolonged acclimation and longer reaction time. Complete removal of both pharmaceuticals was observed after 3 d of biological reaction time, whereas COD decreased by 70%. Caffeine was demonstrated to be more recalcitrant than 5-fluorouracil to the microbial degradation. When EF was applied as post-treatment, a lower biological reaction time (1 d) was used. EF removed residual COD and caffeine that remained in solution after SBR operation.

Regarding the EF/SBR sequence, EF was able to completely degrade pharmaceuticals after 0.5 - 2 h depending on the applied intensity. The enhancement of biodegradability to a standard level of $BOD_5/COD = 0.33$ was achieved even after 30 min of treatment at all the current intensities. Calculation of energy consumption revealed that the process was more energy demanding with prolonged treatment time and higher current intensities. The combination of EF/SBR with different operating conditions of EF (500 mA/30 min and 200 mA/2 h) demonstrated that in both cases pharmaceuticals were completely degraded. However, the subsequent biological process resulted in higher removal efficiencies when EF pre-treatment was performed with a lower intensity (200 mA) and a longer treatment time (2 h) because of the higher biodegradability enhancement. Moreover, these operating conditions are less energy-intensive and consequently more economical.

Finally, the novelty of this research addresses future studies on different aspects such as: a) a combined process with the biological step maintained in an immobilized-cell bioreactor; b) the role of biosorption in pharmaceuticals removal; c) a continuous-flow combined treatment with possible recirculation of the effluent between EF and biological process.

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GENERAL DISCUSSION AND PERSPECTIVES

1. GENERAL DISCUSSION AND CONCLUSIONS

The research hypothesis of this thesis was based on the assumption that electro-Fenton process could be a potentially attractive treatment solution to handle the spreading pharmaceutical pollution of water. The idea was to integrate the electro-Fenton into the existing conventional treatment systems, which are commonly based on biological processes. Bio-electro-Fenton is a combined electrochemical-biological process, which has a perspective to balance the efficient removal of pharmaceuticals (as well as other pollutants) and the reasonable operational cost. The goal of this PhD work was to evaluate the electro-Fenton as a part of bio-electro-Fenton and to optimize its operational parameters with regards to such combined treatment.

In combined electrochemical-biological treatment electro-Fenton can be applied in two different modes: as pre- or post-treatment. Electro-Fenton pre-treatment of wastewater originating from hospitals, pharmaceutical or cosmetic industries could be carried out in order to destroy the refractory and persistent pollutants such as pharmaceuticals and personal care products and to increase the biodegradability of the effluent. This effluent could be either further discharged into the municipal sewage system and afterwards undergo typical treatment steps at a municipal wastewater treatment plants (WWTPs); or else it could be directly treated by a biological process on site as a part of a combined treatment and further discharged into the receiving water environment. If the industrial or hospital wastewater is originally biodegradable with a certain concentration of refractory or persistent compounds, then applying electro-Fenton after a biological process is a sound solution. The goal of electro-Fenton post-treatment would be to eliminate remaining persistent pollutants. Apart from being a part of a separate on site handling of wastewater, it could be also applied at WWTPs as a tertiary treatment, which is generally addressing the issue of disinfection. Chlorination, ozonation and UV radiation are typical processes used for this purpose, however, none of them helps to combat the pharmaceutical micropollution effectively. Hydroxyl radicals - the chemical agents produced during electro-Fenton process - apart from being able to degrade organic contaminants, react with the outer shell of the bacteria in that way ceasing the bacterial growth and activity. Consequently, electro-Fenton could serve as both a destructive process for micropollutants and a disinfection method.

1.1. Optimization of operating parameters

Optimization of operating conditions of electro-Fenton was done regarding two most influential parameters in dependence of treatment time: concentration of Fe^{2+} (catalyst) and current intensity. The criteria of optimization corresponded to the previously identified objectives: 1) removal of pharmaceuticals; 2) mineralization (transformation of organics to CO_2); 3) biodegradability enhancement and 4) energy consumption.

1.1.1. Removal of pharmaceuticals

The oxidative degradation of two pharmaceuticals have been followed throughout all the studies of the thesis: caffeine (psychoactive) and 5-fluorouracil (antineoplastic). Their absolute rate constant of the reaction with hydroxyl radicals have been determined to be $2.48 \pm 0.01 \times 10^9$ M^{-1} s^{-1} for caffeine and $1.52 \pm 0.01 \times 10^9$ $M^{-1}s^{-1}$ for 5-fluorouracil. As can be seen the radicals have a closer affinity to the molecule of caffeine, therefore reacting with it faster than with 5-fluorouracil. However, the molecular geometry of caffeine is more complex, as two cyclic structures, instead of one as in 5-fluorouracil, are present. Yet its three methyl groups provide more reaction pathways for degradation of caffeine by demethylation, which can explain the difference in the absolute rate constant.

The apparent rate constant of both compounds has been followed for a set of varying operating parameters: current intensity (100-700 mA) and Fe^{2+} concentration (0.1-0.5 mM). The influence of the latter followed a similar trend in all the studies. There was an increase in degradation rate with an increase of iron concentration from 0.1 to 0.2 mM, however a further increase did not results in any improvement (Chapter II, Table II.4 and Chapter III, Table III.1). It was consequently concluded that the value of 0.2 mM is both a threshold value and an optimal concentration, which was used in experiments on the effects of current intensity.

The current intensity plays a key role in the generation of hydroxyl radicals via Fenton reaction in the bulk solution and in formation of physisorbed $^{\bullet}OH$ on the surface of BDD anode. The general trend observed during experiments on degradation kinetics was an increasing rate of degradation of pharmaceuticals with an increasing applied intensity until a threshold value. It can be explained by intensified production of hydroxyl radicals. Yet, when in excessive amounts in relation to the amount of organic matter, the radicals participate in a number of parasitic reactions. In addition, under higher current intensities evolution of H_2 at the cathode and generation of O_2 at the anode cause the drop of the process efficiency. This explains the threshold value, which was observed for a number of experiments on degradation kinetics. The Table 1 summarizes the apparent rate constants of caffeine and 5-fluorouracil in dependence of applied current and relatively to the analyzed solution of: individual pharmaceutical (Chapter II and III), a mixture of two pharmaceuticals (Chapter III) and a mixture of 13 pharmaceuticals in total (Chapter IV). By analyzing the apparent rates for these different solutions, it can be seen that the degradation rate slowed down with an increased number of other organic pollutants, which is explained by the greater competition for radicals. When comparing the individual and two-component solution, the limiting current intensity was reached at 300 mA. An intensity of 400 mA showed lower apparent degradation rates. An interesting point is related to the fact that the limiting intensity for the pharmaceutical mixture was higher (around 400 mA): application of 700 mA resulted in slower rate than 200 mA. It can be explained by the two factors: mass transfer control (anodic generation of $^{\bullet}OH$) and amount of organic matter. Bigger amount of organics (in comparison to individual

and two-component solution) requires a larger quantity of radicals, which means that the limiting intensity is reached at higher values. Moreover, under such conditions the mass transfer of molecules to the surface of anode for their oxidation by physisorbed radicals is comparatively better.

Table D.1. Apparent constant rates of caffeine and 5-fluorouracil in individual, two-compound solutions and in pharmaceutical mixture.

Current intensity	**5-fluorouracil**			**Caffeine**		
	Pharma_mix	*2-component solution*	*alone*	*Pharma_mix*	*2-component solution*	*alone*
200 mA	0.038 ± 0.001	0.245 ± 0.006	0.607 ± 0.006	0.034 ± 0.000	0.261 ± 0.005	0.74 ± 0.019
300 mA	0.040 ± 0.000	0.325 ± 0.026	0.743 ± 0.010	0.036 ± 0.001	0.364 ± 0.040	1.13 ± 0.052
400 mA	0.042 ± 0.000	0.137 ± 0.007	0.601 ± 0.004	0.038 ± 0.001	0.203 ± 0.007	0.64 ± 0.012
700 mA	0.030 ± 0.000	-	-	0.023 ± 0.000	-	-

1.1.2. Mineralization

The mineralization of organics is one of the biggest advantages of advanced oxidation processes including electro-Fenton. The transformation of organic pollutants into carbon dioxide, water and other inorganic ions would mean the elimination of toxicity. This process (expressed as total organic carbon (TOC) removal) takes place from the very beginning of the treatment. In general mineralization trends observed in this work had two main characteristics. First of all, TOC during electro-Fenton treatment was displayed by exponential decrease during short treatment duration. Its removal was fast at the beginning of treatment and slowed down towards the end. It is attributed to the fact that the organic matter was gradually mineralized, while the production of hydroxyl radicals stayed constant throughout the process. As a consequence the excess of radicals, which became more pronounced till the end of treatment, participated in parasitic reactions, which limited the removal efficiency. Secondly, the trend of mineralization depended on the current intensity applied as well as concentration of Fe^{2+}. The catalyst concentration of 0.2 mM was found to be optimal in the experiments on degradation kinetics of caffeine and 5-fluouracil and pharmaceutical mixture. The mineralization rate of the treated solutions improved by increasing the applied current intensity. On the contrary to the degradation of pharmaceuticals no obvious limiting intensity was observed until 1500 mA (7.9 mA/cm^2), however the difference between higher intensities applied was minimal. The mineralization-current efficiency, calculated for the individual solution of caffeine, was higher for lower current intensities, which means that in order to have

a more beneficial utilization of generated radicals it is preferable to use lower current intensities (100-300 mA) with prolonged treatment time.

A comparison of mineralization trends of individual caffeine, individual 5-fluorouracil and pharmaceutical mixture revealed their similarity (Figure 1). The greatest amount of TOC was in the synthetic pharmaceutical mixture (220 mg/L) followed by the 0.1 mM solution of 5-flourouracil (around 10 mg/L) and 0.1 mM solution of caffeine (around 5 mg/L). A large difference in TOC values resulted only in a small difference in the mineralization trend. It could be explained by the extent to which parasitic reactions propagated in the solution. When treating a solution highly charged with organic matter the hydroxyl radicals are reacting more with the pollutant molecules rather than with the scavengers: hydrogen peroxide, ferrous iron, other radical species, etc.

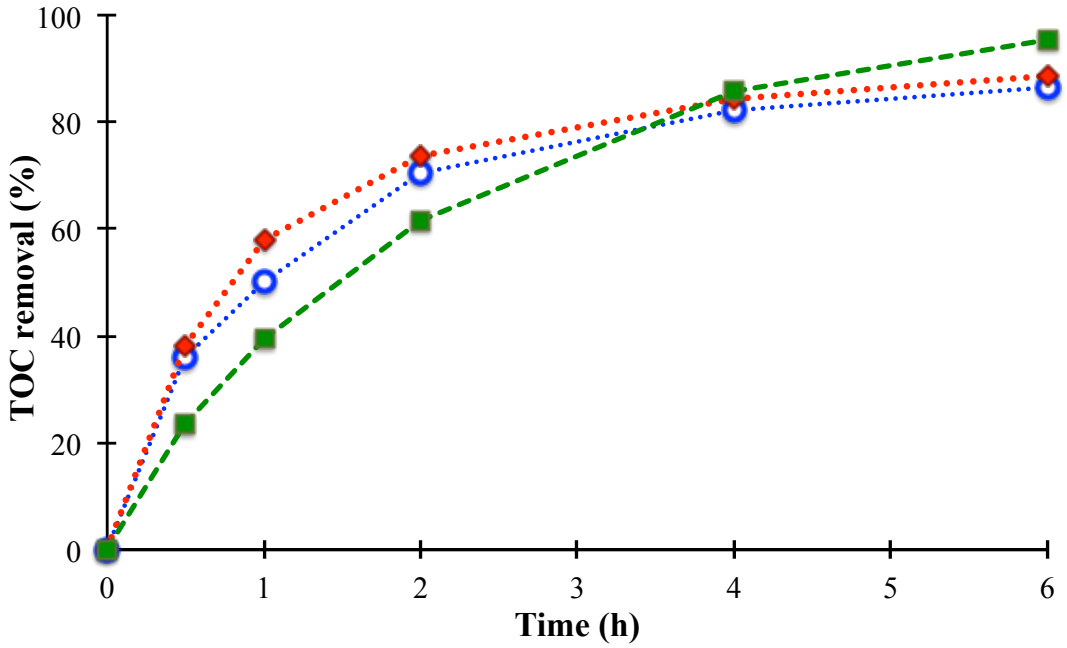


Figure D.1. Comparison of TOC removal for the same setup of electro-Fenton reactor with applied current intensity of 300 mA for solution of: individual caffeine (TOC_0 = 5 mg/L) (-◆-); individual 5-fluorouracil (TOC_0 = 10 mg/L) (-O-); pharmaceutical mixture (TOC_0 = 220 mg/L) (-■-). Operating conditions: $[Fe^{2+}]_0$ = 0.2 mM, $[Na_2SO_4]$ = 0.05 M, V = 200 mL.

1.1.3. Biodegradability enhancement

Biodegradability was studied for a synthetic pharmaceutical mixture (Chapter IV) as well as real wastewater (Chapter V) as a ratio of biochemical oxygen demand over chemical oxygen demand (BOD/COD). The results on the synthetic mixture correlated with the conclusions on real effluent. The increase of BOD/COD ratio was positively dependent on the applied current intensity, as well as the treatment time. The results on synthetic mixture revealed that the biodegradability enhancement after electro-Fenton pre-treatment was in the range of 3-16 times. The BOD values over 5 days gave rather a prompt value of biodegradable part in the effluent without

accounting for the biomass acclimation. Therefore, the BOD of the effluent after electro-Fenton with varying current intensity (100, 500 and 1000 mA) was followed over the period of 17 days. The standard ratio BOD/COD = 0.33 was accepted as a lowest limit for biodegradable effluent. This ratio was reached after 7 days by the sample treated by EF-1000 mA and 8 days by the sample EF-500 mA. By continuing the incubation up to 17 days, the ratio BOD/COD for these samples increased up to almost 0.9 and 0.6, respectively.

Experiments on the real effluent from pharmaceutical production revealed a similar trend. Biodegradability was enhanced by longer treatment duration and higher current intensity. At the same time the ratio of BOD_5/COD equal to 0.33 was reached already after 30 min of electro-Fenton pre-treatment with an applied intensity of 500 mA and 800 mA. An interesting observation was related to the ratio BOD_5/COD equal to around 0.6 which could be reached by following operating conditions: 800 mA / 0.5 h; 500 mA /1 h and 200 mA / 2h. As can be seen the choice can be done between two options: (a) higher intensity / shorter treatment time or lower intensity / longer treatment time.

1.1.4. Energy consumption

Consumption of electrical energy consumed was directly dependent on the applied current intensity and treatment time. With the higher current intensity and longer treatment time, high quantity of energy was consumed per g of TOC or COD. When analyzing the results obtained from the synthetic pharmaceutical mixture, two operational conditions resulted in enhanced biodegradability (BOD/COD > 0.33) after long-term incubation of 17 days: 500 mA / 3 h and 1000 mA/ 3 h. The energy consumption of these operating conditions were 92.6 and 262.0 kWh/ kg COD, respectively. Applying intensity of 1000 mA displayed significantly higher biodegradability enhancement BOD_{17}/COD = 0.9 against 0.6 for intensity of 500 mA, but it also consumed 2.8 times more energy.

Experiments on the real effluent in electro-Fenton reactor of a bigger volume showed that biodegradability level of BOD_5/COD = 0.33 could be reached already after 0.5 h of treatment time with intensities of 500 and 800 mA (not to be compared with intensities applied to synthetic mixture as the volume of reactors and electrode sizes were different). As discussed in the previous section on biodegradability, it seems that the choice in terms of operating parameters should be done with regards to two options: lower intensity applied for longer time (e.g. 200 mA / 2 h) or higher current intensity during shorter duration (800 mA / 0.5 h and 500 mA / 1 h). The preliminary decision can be made based on the energy consumption. The first option consumed 10 kWh per kg COD, while applying the current intensity of 500 mA for 1 h and 800 mA for 0.5 h had energy consumption of 31 and 37 kWh / kg COD, respectively. Such comparison reveals that the electro-Fenton process would be a more cost-effective treatment when used with lower current intensities and prolonged treatment duration.

1.2. Combined electrochemical-biological process: choice of the sequence

As stated previously two sequences of the combined bio-electro-Fenton process exist: electro-Fenton as pre- or post-treatment. The choice of the sequence is mainly dependent on the type of effluent to be treated. In this PhD work both sequences were analyzed for the same effluent with a purpose of experimental demonstration of both possibilities.

In the case when the effluent is toxic and/or contains high concentrations of refractory pollutants, then the application of biological treatment will have an uncertain and erratic efficiency. Electro-Fenton process, during which non-selective hydroxyl radicals are produced, represent a sound solution. As complete mineralization of organic pollutants have been shown to be energy- and time-consuming process, than the idea of removing the toxicity and enhancing the biodegradability is the key point in applying electro-Fenton as pre-treatment. The experimental results of present work have shown that the electro-Fenton pre-treatment significantly enhances the biodegradability, but can also enhance the toxicity of solution (with short treatment time) according to Microtox analysis. Increase in biodegradability proves the formation of more biodegradable intermediates that could be further degraded by a biological process. Results on degradation kinetics of individual pharmaceuticals demonstrated that in average a treatment time of 1-2 hours is sufficient for removal of parent molecules from the solution. This treatment time has also the least energy consumption and is enough for biodegradability enhancement.

When the biological process is to be applied as a first step then the effluent should necessarily be biodegradable and non-toxic for the microorganisms. Moreover, even if toxic or refractory compounds are present then their concentration should be below the level that could cause inhibitory action on the microbial degradation. For example, the microconcentrations of pharmaceuticals present in real municipal effluent would most probably not display any significant pharmacological action on the bacteria of activated sludge. As most of these micropollutants have been demonstrated to be able to escape the conventional biological treatment, then their removal could be ensured by an electrochemical post-treatment.

In general, biological process as pre-treatment when applied to biodegradable effluent with pharmaceutical micropollutants seems like a reasonable treatment option at first sight. However, it should be born in mind that the microbial removal is not always equal to degradation, but rather its combination together with other processes such as volatilization or sorption to biomass. The latter poses certain risks to the re-use of activated sludge as fertilizers due to possible contamination of soil and groundwater by the release pharmaceuticals from the microorganisms. Therefore, from an environmental point of view (in terms of removal of pharmaceutical micropollution) application of electro-Fenton pre-treatment is regarded as preferred option. Still a compromise is to be found in each separate case based on the balance of environmental and economical benefits.

2. PERSPECTIVES

In order to further advance the research on electro-Fenton process, following issues should get their attention:

- *influence of other operating parameters and reactor setups.*

Among all the variety of different factors that have effect on the electro-Fenton process only two were studied in details (catalyst concentration and current intensity) and one partially (temperature). In order to optimize the process for industrial applications, effects of other operating parameters should be studied especially in terms of the related investment. One such parameter, which would take a relatively large share of the operating costs, is aeration. Dissolved oxygen is the source of hydrogen peroxide, which is produced by oxidation at the surface of cathode. The solubility of oxygen in water is therefore a limiting factor for the generation of H_2O_2. With regards to this, different rates of aeration can be analyzed and compared by respective enhancement in the degradation rate and involved costs. Such comparison concerns also other operating parameters, e.g. stirring rate, feeding rate, temperature, etc.

Apart from operating conditions, the reactor setup is an important issue including: configuration of the reactor vessel; positioning, number and size of electrodes; electrode gap; type of aeration and stirring systems; temperature and pH control, etc. All these parameters are directly related to the type of the operational mode that is discussed below.

- *operation in continuous or batch mode.*

In this work, only batch electro-Fenton reactor was investigated as it gives an opportunity to monitor closely degradation and mineralization kinetics. At the same time continuous operation of electro-Fenton reactor will provide an interesting solution that can be easier integrated to a typical wastewater treatment plant at an industrial scale. An engineering challenge will be connected to the pH adjustment in the continuous reactor, as most of the different types of wastewater are at the circumneutral pH. In such case, pH adjustment should take place at the beginning of electrolysis as well as at the end, because the discharge or biological post-treatment of acidic water cannot be regarded as possible. In that case two additional units of pH adjustment systems are required. The same requirement is applied to the batch electro-Fenton reactor.

It should be noted that the choice of the operational mode should be primarily based on the flow and the chemistry of the influent. If the flow of the wastewater is relatively constant and there are no large changes in the composition and pH of the influent, continuous mode of both electro-Fenton reactor and two pH adjustment systems can be considered as more optimal. Continuous treatment systems are generally characterized by higher productivity (unit of wastewater treated per unit of time) and also smaller size, as there is no need to store big volumes of influent to be treated. This can also potentially reduce the costs of a continuous system. Nonetheless continuous operation is typically less capable and more sensitive to fluctuations. The

operation of a batch system can be relatively more easily adapted to more demanding applications with fluctuating flows or chemical composition. At the same time, it should be taken into consideration that a batch electro-Fenton reactor and batch pH adjustment system could have a more significant land footprint.

- bio-electro-Fenton: full application

This study had a goal to make a preliminary assessment of electro-Fenton as a part of a combined electrochemical-biological process. However, a full application of combined process, including a biological reactor and pH adjustment equipment, should be operated for considerable time period in order to observe the stability and long-term effectiveness of such system. As discussed in Chapter VI, if electro-Fenton is studied as post-treatment then the choice of the bioreactor type should fall within the attached-cell reactors and in best-case scenario membrane bioreactors. This will allow minimizing the quantity of biomass transferred to the electro-Fenton reactor and as a result, the fluctuation of removal rates due to the additional degradation of microorganisms by hydroxyl radicals.

-environmental and economical analysis

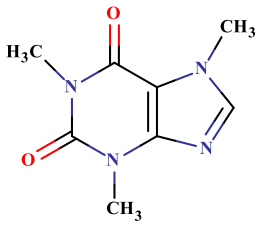
An ultimate step to the large-scale application of electro-Fenton should be environmental (e.g. life-cycle assessment) and economical analyses (e.g. cost evaluation). Life cycle analysis (LCA) is not simply a technique for assessment of the environmental impacts, but can also serve for decision-makers and stakeholders to make a comprehensive inventory analysis, comparison between alternatives, to detect weakness and drawbacks. In terms of economical aspects there is a multitude of different cost analyses, such as a simple concept of cost allocation, which allows to determine the cost per unit of service (in our case it can be a cost per unit of volume of water treated or per mass unit of pollutants removed). More sophisticated evaluations of costs are cost-effectiveness and benefit-cost analyses. These studies consider different aspects. The first is a rather comparative evaluation, which helps to decide which of the options/alternatives is more efficient in order to get a certain benefit. For example, such comparison can be carried out for two possible sequences of bio-electro-Fenton: is electro-Fenton more efficient for removal of pharmaceutical micropollutants as a pre- or post-treatment? Or else it would be interesting to compare electro-Fenton to other advanced oxidation process, e.g. O_3/UV or H_2O_2/UV, which are already applied on the industrial level. Benefit-cost analysis is used to evaluate one technology/service and to compare the total economic benefits to the total economic costs related to its application. By combining both aspects - environmental and economical analyses - it would be possible to evaluate fully the electro-Fenton technology and in case of promising outcomes to accelerate its industrial application.

APPENDIX A. Physico-chemical characteristics of pharmaceuticals.
Sources: chemical databases ChemSpider, PubChem and Drug Bank.

Pharmaceutical	Therapeutic class	Chemical formula	Molecular weight (g/mol)	pK_a	log K_{ow}	Light sensitivity
Acetaminophen	analgesic/antipyretic	$C_8H_9NO_2$	151.17	9.38	0.46	slightly
Acetylsalicylic acid	analgesic/NSAID	$C_9H_8O_4$	180.157	3.49	1.19	N/A
Atenolol	beta blocker/a selective β1 receptor antagonist	$C_{14}H_{22}N_2O_3$	266.336	9.6	0.16	N/A
Caffeine	psychoactive/stimulant	$C_8H_{10}N_4O_2$	194.19	14.0	-0.07	N/A
5-flourouracil	antineoplastics/antimetabolites	$C_4H_3FN_2O$	130.077	8.02	-0.89	slightly
Diatrizoate meglumine	radio contrast media/ ionic monomer	$C_7H_{17}NO_5 \times C_{11}H_9I_3N_2O_4$	809.127	9.52	N/A	N/A
Diclofenac	NSAID	$C_{14}H_{11}Cl_2NO_2$	269.148	4.15	4.5	sensitive
Erythromycin	antibiotics/macrolide	$C_{37}H_{67}NO_{13}$	733.93	8.88	3.06	
Naproxen	NSAID	$C_{14}H_{14}O_3$	230.259	4.15	3.18	susceptible
Norfloxacin	antibiotics/flouroquinolones	$C_{16}H_{18}FN_3O_3$	319.331	6.34	0.46	sensitive
Ranitidine	histamine H2-receptor antagonist	$C_{13}H_{23}ClN_4O_3S$	314.4	8.08	0.27	N/A
Sulfamethoxazole	antibiotic/sulfonamide	$C_{10}H_{11}N_3O_3S$	253.279	5.64	0.89	N/A
Tetracycline	antibiotic/tetracyclines	$C_{22}H_{24}N_2O_8$	444.435	3.3	-1.37	slightly

APPENDIX B. Additional information on mechanism of action, metabolism and effects of the pharmaceuticals studied in present work

CAFFEINE



Systematic IUPAC name	1,3,7-Trimethylpurine-2,6-dione
CAS Number	58-08-2
Chemical formula	$C_8H_{10}N_4O_2$
Molecular mass	194.19 g/mol
Density	1.23 g/cm^3
Boiling point	178 °C
Melting point	235 to 238 °C
Stability	Stable. Incompatible with strong acids, strong bases, strong oxidizing agents, iodine, silver salts, tannins. Weakly light sensitive in solution.
Solubility in water	Slightly soluble
Solubility	Soluble in ethyl acetate, chloroform, pyrimidine, pyrrole, tetrahydrofuran solution; moderately soluble in alcohol, acetone; slightly soluble in petroleum ether, ether, benzene.

Caffeine is a xanthine alkaloid compound that acts as a stimulant in humans. It is sometimes called guaranine, mateine and theine, and can be found in the beans, leaves, and fruits of over 60 plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding upon them.

Effects

Caffeine is a central nervous system and metabolic stimulant, and is used both recreationally and medically to reduce physical fatigue and restore mental alertness when unusual weakness or drowsiness occurs. Caffeine stimulates the central nervous system first at the higher levels, resulting in increased alertness and wakefulness, faster and clearer flow of thought, increased focus, and better general body coordination, and later at the spinal cord level at higher doses.

Metabolism

Caffeine is metabolized in the liver into three primary metabolites: paraxanthine (84%), theobromine (12%), and theophylline (4%). It is completely absorbed by the stomach and small intestine within 45 minutes of ingestion. After ingestion it is distributed throughout all tissues of the body and is eliminated by first-order kinetics.

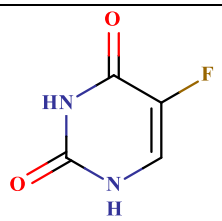
The half-life of caffeine varies widely among individuals according to such factors as age, liver function, pregnancy, some concurrent medications, etc. In healthy adults, caffeine's half-life is approximately 3-4 hours. This drug is metabolized in the liver by the cytochrome P450 oxidase enzyme system (specifically, the 1A2 isozyme) into three metabolic dimethylxanthines presented above. Each of these metabolites is further metabolized and then excreted in the urine.

Mechanism of action

The principal mode of action of caffeine is as an antagonist of adenosine receptors in the brain. Its molecule is structurally similar to adenosine, and binds to adenosine receptors on the surface of cells without activating them (so-called "antagonist" mechanism of action). Therefore, caffeine acts as a competitive inhibitor. The reduction in adenosine activity results in increased activity of the neurotransmitter dopamine, largely accounting for the stimulatory effects of caffeine. Caffeine can also increase levels of epinephrine/adrenaline, possibly via a different mechanism. Acute usage of caffeine also increases levels of serotonin, causing positive changes in mood.

Sources: About the Caffeine Molecule. Science of Cooking. Available: http://www.scienceofcooking.com/caffeine.htm
Chemspider. Database of chemicals of Royal Society of Chemicals. Available: chemspider.com

5-FLUOROURACIL



Systematic IUPAC name	5-Fluoro-1*H*,3*H*-pyrimidine-2,4-dione
CAS Number	51-21-8
Chemical formula	$C_4H_3FN_2O_2$
Molecular mass	130.077 g/mol
Density	1.23 g/cm^3
Melting point	230 to 238 °C
Stability	Solutions of 5-fluorouracil are expected to be stable in solution 7 days at 37 °C, several weeks at 25°C, and at least 4 months at 0-4°C. It is recommended to store 5-fluorouracil in airtight containers protected from light.
Solubility in water	Slightly soluble
Solubility	Soluble in dimethyl sulfoxide (5 mg/ml), dimethyl formamide (5 mg/ml), methanol (1 mg/ml) or hot water (1 mg/ml).

The 5-fluorouracil is a potent agent against solid tumors that was introduced in 1957 for clinical use. It remains one of the most effective chemotherapeutic agents in such conditions as colorectal cancer, even at its limited response rates (10 - 30%)

Pharmacodynamics

Fluorouracil is an antineoplastic anti-metabolite. Anti-metabolites masquerade as purine or pyrimidine - which are the building blocks of DNA. They prevent these substances from becoming incorporated into DNA during the "S" phase (of the cell cycle), stopping normal development and division. Fluorouracil blocks an enzyme that converts the cytosine nucleotide into the deoxy-derivative. In addition, DNA synthesis is further inhibited because fluorouracil blocks the incorporation of the thymidine nucleotide into the DNA strand.

Metabolism

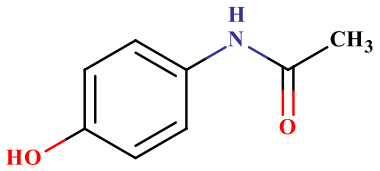
Its metabolism takes place in liver (hepatic). The catabolic metabolism of fluorouracil results in degradation products (e.g., CO_2, urea and α-fluoro-ß-alanine) that are inactive.
Around 7 to 20% of the parent drug is excreted unchanged in the urine in 6 hours; over 90% of it is excreted in the first hour. The remaining percentage of the administered dose is metabolized, primarily in the liver. Half-life is about 10-20 min.

Mechanism of action

The 5-fluorouracil acts in several ways, but principally as a thymidylate synthase (TS) inhibitor. Interrupting the action of this enzyme blocks synthesis of the pyrimidine/thymidine, which is a nucleoside required for the DNA replication. Thymidylate synthase methylates deoxyuridine monophosphate (dUMP) to form thymidine monophosphate (dTMP). Administration of 5-fluorouracil causes a scarcity in dTMP, so that rapidly dividing cancerous cells undergo cell death via thymineless death.

Sources: Drug Bank. Available: http://www.drugbank.ca
Chemspider. Database of chemicals of Royal Society of Chemicals. Available: chemspider.com
SIGMA Product Information on 5-flourouracil. Available: https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma/Product_Information_Sheet/f6627pis.pdf
*Longley D. B., Harkin D. P., Johnston P. G. (May 2003). "5-fluorouracil: mechanisms of action and clinical strategies". Nat. Rev. Cancer **3** (5): 330–8. doi:10.1038/nrc1074*

ACETAMINOPHEN (PARACETAMOL)



Systematic IUPAC name	1*N*-(4-hydroxyphenyl)ethanamide *N*-(4-hydroxyphenyl)acetamide
CAS Number	103-90-2
Chemical formula	$C_8H_9NO_2$
Molecular mass	151.163 g/mol
Density	1.263 g/cm^3
Boiling point	169 °C
Melting point	420 °C
Stability	Stable up to 45 °C
Solubility in water	12.78 mg/mL (20 °C)
Solubility	Soluble to 100 mM in ethanol and to 100 mM in dimethyl sulfoxide, also soluble in alcohols, N,N-dimethylformamide, dimethyl sulfoxide, and diethylamin

Acetaminophen is an active ingredient in hundreds of over-the-counter and prescription medicines. It relieves pain and fever. And, it is also combined with other active ingredients in medicines that treat allergy, cough, colds, flu, and sleeplessness. In prescription medicines, acetaminophen is found with other active ingredients to treat moderate to severe pain. Acetaminophen can cause serious liver damage if more than directed is used.

Effects

Acetaminophen is a p-aminophenol derivative with analgesic and antipyretic activities. Although the exact mechanism through which acetaminophen exert its effects has yet to be fully determined, acetaminophen may inhibit the nitric oxide pathway mediated by a variety of neurotransmitter receptors that results in elevation of the pain threshold. The antipyretic activity may result from inhibition of prostaglandin synthesis and its release in the central nervous system.

Usage

The paracetamol's place on the analgesic ladder of World Health Organization (WHO), which precisely defines the rules for application of analgesic drugs, is impressive. This drug has been placed on all three steps of pain treatment intensity (weak, moderate and severe). In different pains of moderate intensity, paracetamol, as a weak analgesic together with nonsteroidal analgesic drugs or coanalgesics (e.g., caffeine), is a basic non-opioid analgesic (the first step of the analgesic ladder). When pain maintains or increases, paracetamol is used as an additional analgesic with weak (e.g., caffeine, tramadol) or strong (e.g., morphine, phentanyl) opioids from the second and third step of the analgesic ladder, respectively. It is a drug of choice in patients in whom application of nonsteroidal anti-inflammatory drugs (NSAIDs) are contraindicated, e.g., in the case of gastric ulcers, hypersensitivity to aspirin, impairments in blood coagulation, in pregnant women, nursing mothers and children with fever accompanying a disease.

Metabolism

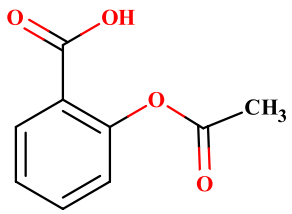
The liver is the primary site in the body where acetaminophen is metabolized. It first undergoes sulphation (binding to a sulphate molecule) and glucuronidation (binding to a glucuronide molecule) before being eliminated from the body by the liver. The parent compound, acetaminophen, and its sulphate and glucuronide compounds (metabolites) are themselves actually not harmful. An excessive amount of acetaminophen in the liver, however, can saturate the sulphation and glucuronidation pathways. When this happens, the acetaminophen is processed through another pathway, the cytochrome P-450 system. From acetaminophen, the P-450 system forms an intermediate metabolite referred to as NAPQI, which turns out to be a toxic compound. Ordinarily, however, this toxic metabolite is detoxified by another pathway, the glutathione system.

Mechanism of action

Although paracetamol was discovered over 100 years ago and has been widely used in medical practice for more than half the century, its mechanism of action has not been elucidated until now. It has analgesic and antipyretic properties similarly to NSAIDs, but contrary to them, it does not possess any anti-inflammatory activity. When applied in recommended doses, it does not induce typical for NSAIDs gastrointestinal side effects. However, it suppresses prostaglandin production likewise NSAIDs.

Chemspider. Database of chemicals of Royal Society of Chemistry. Available: chemspider.com
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
Marta Jewiak-B Benista, Jerzy Z. Nowak. Acetaminophen: mechanism of action, applications and safety concerns. Acta Poloniae Pharmaceutica ñ Drug Research, Vol. 71 No. 1 pp. 11-23, 2014
Dennis Lee. How is acetaminophen processed (metabolized) in the body? Available: http://www.medicinenet.com/script/main/art.asp?articlekey=17062

ACETYLSALICYLIC ACID (ASPIRIN)



Systematic IUPAC name	2-(acetoxy)benzoic acid
CAS Number	50-78-2
Chemical formula	$C_9H_8O_4$
Molecular mass	180.157 g/mol
Density	1.40 g/cm^3
Boiling point	135 °C
Melting point	140°C
Solubility in water	3 mg/mL (20 °C)
Solubility	Soluble in methanol (50 mg/ml), DMSO, alcohol, chloroform, ether, ethanol and dimethyl formamide

Aspirin is used in the treatment of mild to moderate pain, inflammation, and fever. It is also used as an antiplatelet agent to prevent myocardial infarction, stroke and transient ischemic episodes.

Mechanism of action

Aspirin is absorbed rapidly from the stomach and intestine by passive diffusion. Aspirin is a prodrug, which is transformed into salicylate in the stomach, in the intestinal mucosa, in the blood and mainly in the liver. Salicylate is the active metabolite responsible for most anti-inflammatory and analgesic effects (but acetylsalicylate is the active moiety for the antiplatelet-aggregating effect). Gastrointestinal intolerance to salicylate observed in some patients has prompted the development of formulations with enteric coating.
Salicylate distributes rapidly into the body fluid compartments. It binds to albumin in the plasma. With increasing total plasma salicylate concentrations, the unbound fraction increases. Salicylate may cross the placental barrier and distributes into breast milk.

Metabolism

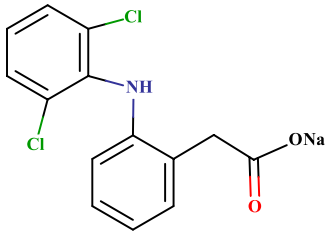
As mentioned above, aspirin is rapidly biotransformed into the active metabolite, salicylate. Therefore, aspirin has a very short half-life. Salicylate, in turn, is mainly metabolized in the liver. This metabolism occurs primarily by hepatic conjugation with glycin or glucuronic acid, each involving different metabolic pathways. The predominant pathway is the conjugation with glycin, which is saturable. With low doses of aspirin approximately 90% of salicylate is metabolized through this pathway. As the maximum capacity of this major pathway is reached, the other pathways with a lower clearance become more important. Therefore, the half-life of salicylate depends on the major metabolic pathway used at a given concentration and becomes longer with increasing dosage. Salicylate is said to follow nonlinear kinetics at the upper limit of the dosing range. Studies have shown that there is much inter-subject variation with respect to the relative contribution of the different salicylate metabolic pathways.

Excretion

Urinary excretion of unchanged salicylate accounts for 10% of the total elimination of salicylate. Excretion of salicylate results of glomerular filtration, active proximal tubular secretion through the organic acid transporters and passive tubular reabsorption. Urinary excretion is markedly pH dependent and as the urinary pH rises from 5 to 8, the amount of free ionized salicylate excreted increases from 3% of the total salicylate dose to more than 80% (by ion trapping in the urine). Salicylate metabolites are also excreted in the urine.

Chemspider. Database of chemicals of Royal Society of Chemistry. Available: chemspider.com
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
The DrugBank Database. Available: www.drugbank.ca

DICLOFENAC SODIUM



Systematic IUPAC name	2-(2,6-dichloranilino) phenylacetic acid sodium salt
CAS Number	15307-86-5
Chemical formula	$C_{14}H_{11}Cl_2NO_2 \bullet Na$
Molecular mass	318.14 g/mol
Density	0.63 g/cm^3
Boiling point	178 °C
Melting point	275 °C
Stability	Stable
Solubility in water	Slightly soluble
Solubility	Soluble phosphate-buffered saline pH 7.2, ethanol, dimethylformamide, dimethyl sulfoxide, methanol and acetone.

A non-steroidal anti-inflammatory agent (NSAID) with antipyretic and analgesic actions. It is primarily available as the sodium salt.

Effects

Diclofenac works by blocking the effect of chemicals in the body, called cyclo-oxygenase (COX) enzymes. These enzymes help to make other chemicals in the body, called prostaglandins. Prostaglandins are produced at sites of injury or damage, and cause pain and inflammation. By blocking the effect of COX enzymes, fewer prostaglandins are produced, which means pain and inflammation are eased.

There are two forms of diclofenac - diclofenac sodium and diclofenac potassium. The main difference between the two is that diclofenac potassium is absorbed into the body more quickly than diclofenac sodium. A quick action is useful where immediate pain relief is required, and a prolonged action is more useful in reducing inflammation.

Metabolism

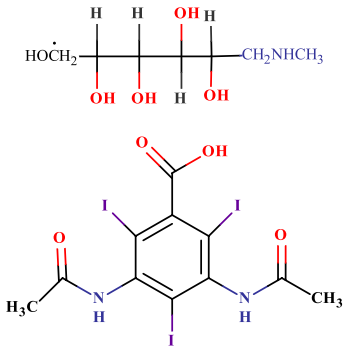
Biotransformation of diclofenac following oral administration involves conjugation at the carboxyl group of the side chain and single or multiple hydroxylations resulting in several phenolic metabolites, most of which are converted to glucuronide conjugates. Two of these phenolic metabolites are biologically active, however to a much smaller extent than diclofenac. Metabolism of diclofenac following topical administration is thought to be similar to that after oral administration. The small amounts of diclofenac and its metabolites appearing in the plasma following topical administration makes the quantification of specific metabolites imprecise.

Excretion

Diclofenac is eliminated through metabolism and subsequent urinary and biliary excretion of the glucuronide and the sulfate conjugates of the metabolites. Little or no free unchanged diclofenac is excreted in the urine. Approximately 65% of the dose is excreted in the urine and approximately 35% in the bile as conjugates of unchanged diclofenac plus metabolites.

Chemspider. Database of chemicals. Available: chemspider.com
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
The DrugBank Database. Available: www.drugbank.ca

DIATRIZOATE MEGLUMINE



Systematic IUPAC name	3,5-bis(acetylamino)-2,4,6-triiodobenzoic acid meglumine salt
CAS Number	131-49-7
Chemical formula	$C_{11}H_9I_3N_2O_4 \bullet C_7H_{17}NO_5$
Molecular mass	809.13 g/mol
Density	1.23 g/cm^3
Melting point	250 °C
Stability	Protect from sunlight
Solubility in water	Freely soluble

Diatrizoate meglumine is the meglumine salt form of diatrizoate, an organic, iodinated, radiopaque X-ray contrast medium used in diagnostic radiography. The iodine moiety of diatrizoate meglumine is not penetrable by X-rays, therefore blocks the X-ray film exposure by radiation. This makes it possible to distinguish on X-ray film, body parts that contain diatrizoate meglumine from body parts that do not contain this agent and allows for visualization of different body structures. Both diatrizoate meglumine and diatrizoate sodium are used for gastrointestinal studies, angiography, and urography.

Mechanism of action

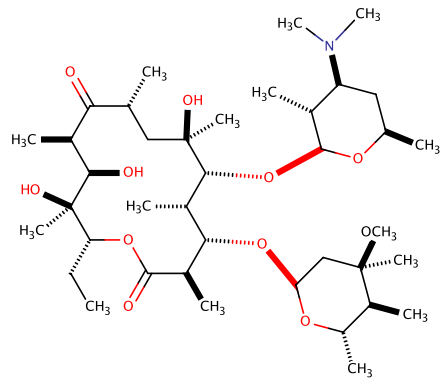
Diatrizoate is an iodine-containing X-ray contrast agent. Iodated contrast agents were among the first contrast agents developed. Iodine is known to be particular electron-dense and to effectively scatter or stop X-rays. A good contrast agent requires a high density of electron-dense atoms. Therefore, the more iodine, the more "dense" the X-ray effect. Iodine based contrast media are water soluble and harmless to the body. These contrast agents are sold as clear colorless water solutions, the concentration is usually expressed as mg I/ml. Modern iodinated contrast agents can be used almost anywhere in the body. Most often they are used intravenously, but for various purposes they can also be used intraarterially, intrathecally (the spine) and intraabdominally - just about any body cavity or potential space.

Excretion

Diatrizoate meglumine is not metabolized but excreted unchanged in the urine, each diatrizoate molecule remaining bonded to its sodium moiety. The liver and small intestine provide the major alternate route of excretion for diatrizoate. Injectable radiopaque diagnostic agents are excreted unchanged in human milk. Saliva is a minor secretory pathway for injectable radiopaque diagnostic agents.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
The DrugBank Database. Available: www.drugbank.ca

ERYTHROMYCIN



Systematic IUPAC name	(3*R*,4*S*,5*S*,6*R*,7*R*,9*R*,11*R*,12*R*,13*S*,14*R*)-6-{[(2*S*,3*R*,4*S*,6*R*)-4-(dimethylamino)-3-hydroxy-6-methyloxan-2-yl]oxy}-14-ethyl-7,12,13-trihydroxy-4-{[(2*R*,4*R*,5*S*,6*S*)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy}-3,5,7,9,11,13-hexamethyl-1-oxacyclotetradecane-2,10-dione
CAS Number	114-07-8
Chemical formula	$C_{37}H_{67}NO_{13}$
Molecular mass	733.94 g/mol
Melting point	191 °C
Stability	Stable in solution at 37 °C for 3 days
Solubility in water	4.2 mg/L at 25°C
Solubility	Freely soluble in alcohols, acetone, chloroform, acetonitrile, ethyl acetate; moderately soluble in ether, ethylene dichloride, amyl acetate

Erythromycin is a macrolide antibiotic. Macrolides are products of actinomycetes (soil bacteria) or semi-synthetic derivatives of them. Erythromycin is an orally effective antibiotic discovered in 1952 in the metabolic products of a strain of *Streptocyces erythreus*, originally obtained from a soil sample.

It inhibits bacterial protein synthesis by binding to bacterial 50S ribosomal subunits; binding inhibits peptidyl transferase activity and interferes with translocation of amino acids during translation and assembly of proteins. Erythromycin may be bacteriostatic or bactericidal depending on the organism and drug concentration.

Antimicrobial activity

Against Gram-positive bacteria, *Mycoplasma pneumoniae, Chlamydia trachomatis, Chlamydia pneumoniae, Clamydia psittaci, Ureaplasma urealyticum, Legionella pneumophila, Campylobacter jejuni, Bordatella pertussis.*

Mechanism of action

Erythromycin acts by penetrating the bacterial cell membrane and reversibly binding to the 50 S subunit of bacterial ribosomes or near the donor site, so that binding of tRNA (transfer RNA) to the donor site is blocked. Translocation of peptides is prevented, and subsequent protein synthesis is inhibited. Erythromycin is effective only against actively dividing organisms.

Metabolism

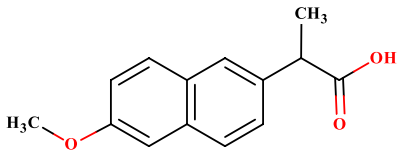
Most of erythromycin is metabolized by demethylation in the liver by the hepatic enzyme CYP3A4. Its main elimination route is in the bile with little renal excretion, 2%-15% of unchanged drug. Erythromycin's elimination half-life ranges between 1.5 and 2.0 hours. Erythromycin levels peak in the serum 4 hours after dosing. It crosses the placenta and enters breast milk. The American Association of Pediatrics determined erythromycin is safe to take while breastfeeding. Absorption in pregnant patients has been shown to be variable, frequently resulting in levels lower than in non-pregnant patients.

Excretion

Hepatic. Extensively metabolized - after oral administration, less than 5% of the administered dose can be recovered in the active form in the urine..

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
*"Transfer of drugs and other chemicals into human milk". Pediatrics **108** (3): 776–89. 2001.*

NAPROXEN SODIUM



Systematic IUPAC name	(+)-(*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid
CAS Number	22204-53-1
Chemical formula	$C_{14}H_{14}O_3$
Molecular mass	230.259 g/mol
Boiling point	402 °C
Melting point	154 °C
Solubility in water	>3 mg/ml at 25 °C
Solubility	Soluble in methanol, dimethyl sulfoxide, dimethylformamide (100 mg/ml), chloroform, dichloromethane, ether, ethylacetate or tetrahydrofuran

An anti-inflammatory agent with analgesic and antipyretic properties. Both the acid and its sodium salt are used in the treatment of rheumatoid arthritis and other rheumatic or musculoskeletal disorders, dysmenorrhea, and acute gout. Naproxen is a member of the arylacetic acid group of nonsteroidal anti-inflammatory drugs (NSAIDs).

Metabolism

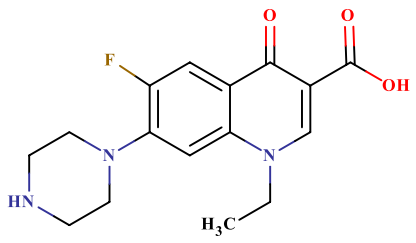
Naproxen is extensively metabolized to 6-0-desmethyl naproxen and both parent and metabolites do not induce metabolizing enzymes.
Once dissolved in biologic fluids, naproxen and naproxen sodium are chemically identical species and have the same biologic properties. Administration of naproxen as the sodium salt (Anaprox), however, permits more rapid absorption from the gastrointestinal tract. In either form, the drug is essentially completely absorbed. Its metabolic half-life averages 13 hours. The metabolism of naproxen is quite simple: it is excreted almost entirely in the urine as the native molecule, its oxidative 6-desmethyl metabolite and their respective conjugates.

Mechanism of action

The mechanism of action of naproxen, like that of other NSAIDs, is believed to be associated with the inhibition of cyclooxygenase activity. Two unique cyclooxygenases have been described in mammals: (1) the constitutive cyclooxygenase, COX-1, synthesizes prostaglandins necessary for normal gastrointestinal and renal function; (2) the inducible cyclooxygenase, COX-2, generates prostaglandins involved in inflammation. Inhibition of COX-1 is thought to be associated with gastrointestinal and renal toxicity, while inhibition of COX-2 provides anti-inflammatory activity. Naproxen works by reversibly inhibiting both the COX-1 and COX-2 enzymes.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
Segre EJ, Naproxen sodium (Anaprox): pharmacology, pharmacokinetics and drug interactions. J Reprod Med. 1980 Oct;25(4 Suppl):222-5.

NORFLOXACIN



Systematic IUPAC name	1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid
CAS Number	70458-96-7
Chemical formula	$C_{16}H_{18}FN_3O_3$
Molecular mass	316.33 g/mol
Melting point	221 °C
Stability	Stable. Light sensitive.
Solubility in water	0.28 mg/mL at 25 °C. Solubility in water is pH dependent.
Solubility	Slightly soluble in alcohols.

Norfloxacin is a broad-spectrum quinolone/fluoroquinolone antibiotic that is active against both gram-positive and gram-negative bacteria. It is bactericidal used for the treatment of urinary tract infection.

Mechanism of action

Norfloxacin has a bactericidal action and it causes inhibition of bacterial DNA synthesis. It inhibits the DNA gyrase enzyme present in the bacteria which leads to hindrance in the normal supercoil formation of the DNA. It also inhibits the process of relaxation of the DNA, which has undergone supercoiling and leads to increased damage to the DNA.

The cells of mammals have the enzyme topoisomerase II instead of DNA gyrase or topoisomerase IV, which possesses very little affinity for norfloxacin resulting in minimal damage to the host tissue.

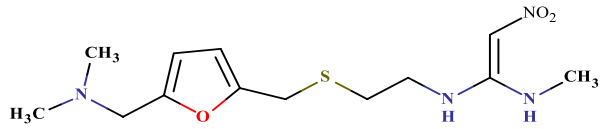
The fluorine atom at the 6th position increases potency against gram-negative organisms, and the piperazine moiety at the 7th position is responsible for anti-pseudomonal activity.

Metabolism and excretion

The half-life of norfloxacin is three to four hours. Approximately 30% of a dose is eliminated unchanged in the urine within 24 hours, thus producing high urinary concentrations. Urinary excretion is mainly by tubular secretion and glomerular filtration. Norfloxacin undergoes little metabolism, probably in the liver, and a number of metabolites have been reported in urine, some showing antibacterial activity. Approximately 30% of an oral dose is excreted in the feces.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/

RANITIDINE HYDROCHLORIDE



Systematic IUPAC name	*N*-(2-[(5-[(dimethylamino)methyl]furan-2-yl)methylthio]ethyl)-*N'*-methyl-2-nitroethene-1,1-diamine
CAS Number	6657-59-3
Chemical formula	$C_{13}H_{23}ClN_4O_3S \bullet HCl$
Molecular mass	350.86 g/mol
Melting point	134 °C
Solubility in water	24.7 mg/mL
Solubility	Soluble in acetic acid, methanol, ethanol and dimethyl sulfoxide.

Used in the treatment of peptic ulcer disease, dyspepsia, stress ulcer prophylaxis, and gastroesophageal reflux disease.

Effects

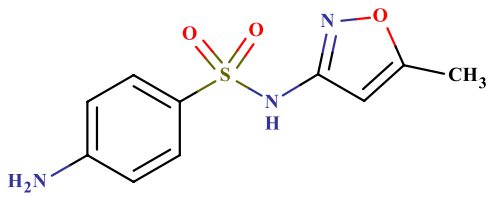
Ranitidine is a histamine H2-receptor antagonist similar to cimetidine and famotidine. An H2-receptor antagonist, often shortened to H2-antagonist, is a drug used to block the action of histamine on parietal cells in the stomach, decreasing acid production by these cells. These drugs are used in the treatment of dyspepsia. Like the H1-antihistamines, the H2-antagonists are inverse agonists rather than true receptor antagonists.

Mechanism of action

Ranitidine is an oral drug that blocks the production of acid by acid-producing cells in the stomach. H2-blockers inhibit the action of histamine on the cells, thus reducing the production of acid by the stomach. Histamine is a naturally-occurring chemical that stimulates cells in the stomach (parietal cells) to produce acid. Since excessive stomach acid can damage the esophagus, stomach and lead to inflammation and ulceration, reducing stomach acid prevents and heals acid-induced inflammation and ulcers. These type of drugs accomplish this by two mechanisms: histamine released by ECL cells in the stomach is blocked from binding on parietal cell H2 receptors which stimulate acid secretion, and other substances that promote acid secretion (such as gastrin and acetylcholine) have a reduced effect on parietal cells when the H2 receptors are blocked.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/

SULFAMETHOXAZOLE



Systematic IUPAC name	4-Amino-*N*-(5-methylisoxazol-3-yl)-benzenesulfonamide
CAS Number	723-46-6
Chemical formula	$C_{10}H_{11}N_3O_3S$
Molecular mass	253.279 g/mol
Melting point	170 °C
Stability	Stable in air
Solubility in water	<1 mg/mL
Solubility	Soluble in dimethyl sulfoxide and ethanol.

Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic that is most commonly used in combination with trimethoprim. Studies have shown that bacterial resistance develops more slowly with the combination of the two drugs than with either Trimethoprim or Sulfamethoxazole alone. Sulfamethoxazole competitively inhibits dihydropteroate synthase preventing the formation of dihydropteroic acid, a precursor of folic acid, which is required for bacterial growth.

Sulfamethoxazole has been used since the 1960s in the treatment of various systemic infections in humans and other species. The main use has been in the treatment of acute urinary tract infections. It has also been used against gonorrhoea, meningitis and serious respiratory tract infections etc. Despite its relatively unfavorable pattern of tissue distribution, it is the sulfonamide antibiotic most commonly used around the world in combination with trimethoprim or pyrimethamine for the treatment of various systemic infections.

Effects

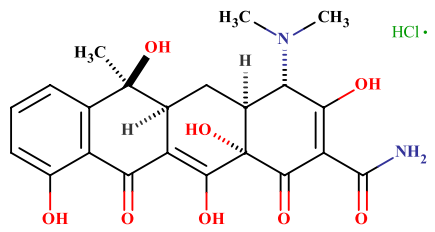
Sulfamethoxazole inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid (PABA) for binding to dihydropteroate synthetase (dihydrofolate synthetase). Inhibition of dihydrofolic acid synthesis decreases the synthesis of bacterial nucleotides and DNA.

Metabolism

Hepatic. The metabolism of sulfamethoxazole occurs predominately by N4-acetylation, although the glucuronide conjugate has been identified.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/
Monograph of International Agency for Research on Cancer "Evaluation of carcirogenic risks to humans". Volume 79 (2001)

TETRACYCLINE HYDROCHLORIDE



Systematic IUPAC name	(4S,4aS,5aS,6S,12aR)-4-(dimethylamino)-1,6,10,11,12a-pentahydroxy-6-methyl-3,12-dioxo-4,4a,5,5a-tetrahydrotetracene-2-carboxamide;hydrochloride
CAS Number	64-75-5
Chemical formula	$C_{22}H_{24}N_2O_8$ • HCl
Molecular mass	480.90 g/mol
Melting point	172 °C
Stability	This product should be frozen below 0°C and protected from light and moisture. In these conditions, the product has been shown to retain activity for 4 years. Stock solutions should be stored at -20°C and are stable at 37°C for 4 days.
Solubility in water	231 mg/L (at 25 °C)
Solubility	Soluble in methanol and ethanol, but is insoluble in ether and hydrocarbons.

Tetracycline is a broad spectrum polyketide antibiotic produced by the Streptomyces genus of Actinobacteria.

Metabolism

Not metabolized. It is concentrated by the liver in the bile and excreted in the urine and feces at high concentrations in a biologically active form.

Mechanism of action

Tetracycline exerts a bacteriostatic effect on bacteria by reversible binding to the bacterial 30S ribosomal subunit and blocking incoming aminoacyl tRNA from binding to the ribosome acceptor site. It also binds to some extent to the bacterial 50S ribosomal subunit and may alter the cytoplasmic membrane causing intracellular components to leak from bacterial cells. Additionally tetracycline may alter the cytoplasmic membrane of bacteria causing leakage of intracellular contents, such as nucleotides, from the cell.
Tetracyclines can also inhibit protein synthesis in the host, but are less likely to reach the concentration required because eukaryotic cells do not have a tetracycline uptake mechanism.

Chemspider. Database of chemicals. Available: chemspider.com
The DrugBank Database. Available: www.drugbank.ca
PubChem. Chemical database of National Center for Biotechnology Information. Available: https://pubchem.ncbi.nlm.nih.gov/

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E-mail: ganzenko.oleksandra@gmail.com

Linkedin: Oleksandra Ganzenko

PROFESSIONAL EXPERIENCE

October 2012 - December 2015

PhD researcher

Laboratory of Geomaterials and Environment, Université Paris-Est Marne-la-Vallée; Paris, France

Project: «Combination of electrochemical advanced oxidation to a biological process for removal of pharmaceuticals from wastewater»

Responsibilities: literature review, planning of research steps and experiments, operation of electro-Fenton reactor, analysis of samples by different techniques of analytical chemistry, preparation of publications, presentation of results during conferences and meetings, realization and operation of bench scale electrochemical and biological reactors; supervision of the Master student

November 2011- June 2012

Intern research assistant

Laboratory of Microbial Systems Ecology, Technische Universität München; Munich, Germany

Project: «Identification of metal-reducing lichens and microorganisms in the meteorites of Oman»

Responsibilities: literature review, analysis of meteorite samples with molecular biology techniques

September - October 2010

Intern research assistant

Department of System Ecotoxicology, Helmholtz Zentrum für Umweltforschung; Leipzig, Germany

Project: «Assessment of pesticide effects on fresh water communities and efficiency evaluation of buffer strips and forested areas for mitigation»

Responsibilities: application of Geographical Information Systems, statistical data analysis

Other jobs: interpreter (Russian-English), event manager, member of presidential election commission

EDUCATION

2012 - 2015

Joint doctoral degree in Environmental Technologies

Université Paris-Est Marne-la-Vallée, Università degli Studi di Cassino e del Lazio Meridionale and UNESCO-IHE Institute of Water Education; France, Italy, Netherlands

Thesis: «Bio-electro-Fenton: optimization of electrochemical advanced oxidation process in the perspective of its combination to a biological process for the removal of pharmaceuticals from wastewater»

2009 - 2011

Master degree in «Sustainable Resource Management»

Technische Universität München; Munich, Germany

Concentration: waste management, management of landscape and protected areas

Thesis: «Usage of hog fuel for temporary road construction. Investigation of recycling possibilities»

2005 - 2009

Bachelor degree in Environmental Sciences

National University “Kyiv-Mohyla academy”; Kyiv, Ukraine

Concentration: ecology, waste management, environmental law and policy

Thesis: «Disposal of nuclear waste in the deep geological structures»

SPECIFIC INFORMATION

Languages Ukrainian (mother tongue), French (proficient), English (proficient), Russian (proficient), German (intermediate), Italian (intermediate)

Computer skills Microsoft Office, Prezi, ArcGIS, R, Endnote, MarvinBeans

Hobbies yoga, parkour, traveling, literature