Degradation of antibiotics by electrochemical advanced oxidation processes using an activated carbon fiber cathode

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Degradation of antibiotics by electrochemical advanced oxidation processes using an activated carbon fiber cathode

繊維状活性炭電極を用いた電気化学促進酸化法による抗生物質の分解

Presents:
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Abstract

Water pollution by pharmaceuticals is an emerging environmental issue, one of the most harmful pharmaceuticals are the antibiotics due to their high production around the world and wide use. Antibiotics are used not only to treat diseases but to enhance the growth of the animal that will be consumed as a food. The main problem of the antibiotics is that they possess low biotransformation, which means that they are excreted almost unchanged in the urine or feces. So, they keep their pharmaceutical activity that further can act over other living creatures which were not the original target organisms. The main input of antibiotics is by wastewater because they are not eliminated during its treatment in the common wastewater treatment plants. Several investigations have been reported that usually pharmaceutical substances are neither remove by the wastewater treatments nor biodegraded when they are released into the environment.

The presence of pharmaceuticals in wastewater is a concern because they alter the environment. It has been reported their presence in the sewage sludge. Also, and may be the most important effect that has been documented is that antibiotics at low concentrations can exert a mechanism in the microorganisms to develop antibiotic resistance. This resistance could be transfer among different microorganisms like pathogens. Now is already difficult to treat some infections due to the antibiotic resistance.

Some governmental instance, like the EPA, have identified and registered the most commonly found antibiotics in wastewater. And now the development of technologies to remove the antibiotics is needed because the existing methods have shown their low efficacy to remove them.

The comparative degradation behavior of cefalexin (CLX) using Advanced Oxidation Processes (AOPs) with the aim of improving CLX biodegradability was studied. Among the AOPs used, RuO$_2$/Ti anodic oxidation (AO), AO in the presence of electro generated H$_2$O$_2$ (AO-H$_2$O$_2$), and the electro-Fenton (EF) process, the EF process was the most effective. In the EF process an activated carbon fiber (ACF) was used as a cathode. Different input variables, including catalyst concentration, pH, and current density were evaluated to find the optimum conditions for the EF. The most suitable operational conditions were as follows: a current density of 6.66 mA/cm$^2$, a pH of 3, and a concentration of 1mM of Fe$^{2+}$ as the catalyst. Different CLX concentrations were analyzed as well of different reaction times to assess the degree of mineralization. The change in biodegradability was evaluated by the BOD$_5$/COD and the BOD$_{14}$/COD ratios. The EF did not effectively remove the COD, but removed enough to achieve suitable biodegradability for a further biological process.

The Activated carbon felt has been used as an electrode during different electrolysis process due to its physicochemical characteristics. It has been reported to exhibit a high yield concentration of hydrogen peroxide working under its optimal operational conditions. It has a high surface area,
Abstract
due to its high porosity of 900-2500 m²g⁻¹. In addition, it has excellent adsorption capabilities, good mechanical strength, and high conductivity, which may lead to high capacitance at a dense current and low inner resistance due to the easy ion transfer, and also it has a low cost and it is not toxic. So the production of hydrogen peroxide takes place on its surface. It was found that after several use the ACF its surface change as consequence it is necessary to be replaced after some electro-Fenton processes.

The Fenton reaction was first found to be catalyzed by iron ions, mainly ferrous ion, but further investigations found that this reaction could be also catalyzed by different metals. In this case we used transition metals which have similar chemical characteristics as the ferrous ion and that have been reported to act as good (M (n+1) / Mⁿ⁺) redox couple. Copper, Cobalt, and Manganese ions were compared with Iron ions. The maximal oxidation efficiency was for the Cobalt ion (Co²⁺), then the efficiencies were as follow: the Copper ion (Cu²⁺), the Iron ion (Fe²⁺), and the lowest was for the Manganese ion (Mn²⁺). All the experiments were carried out under the same operational conditions of a current density of 0.36 A, a pH of 3.0 and a cefalexin concentration of 200 mg/L.

Finally the degradation of the antibiotic oxidation of the sulfamathoxazole (SMX) was done by the Anodic Oxidation (AO), Anodic Oxidation with electro-generated hydrogen peroxide (AO-E-H₂O₂), (AO-E-H₂O₂-UVA) irradiated with UVA light at 365nm, electro-Fenton (EF) and photo-electro-Fenton irradiated also with UVA light at 365nm, during 6 hours.

The removal of SMX with AO less than 24% of TOC removal, with AO-E-H₂O₂ 28.4%, AO-E-H₂O₂-UVA 36.4%, with the EF 63.2% and the highest was with the PEF a 79.8% was removed.

The efficiency of the processes was enhanced with the irradiation of the UV light, due to the better decomposition of the molecule of hydrogen peroxide, also because the UV-light can break the intermediate complexes formed with the iron ions. To understand the high efficiency of the photo-electro-Fenton process, its reaction mechanism was investigated by the identification and quantification of the produced intermediates.
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Adriana Ledezma Estrada
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CHAPTER 1

General Introduction
1 General introduction

1.1 Pharmaceuticals and personal care products in the wastewater.

Wastewater treatments are facing a challenge to remove certain organic pollutants. The problem seems small because they are in small amounts but actually they represent a big threat to the environment. These substances are the Pharmaceutical and Personal Care products (PPCPs). So, besides the common organic charge, the inorganic materials like heavy metals, acids, basis and salts, the PPCPs must be removed before discharged into the environment [1].

The problem of the PPCPs has arisen from the development of the society as it is now. There is practically no human activity that does not produce waste products in addition there is a direct relationship between the life style standard with the amount of waste products produced [2]. Also the economical growth is linked to the waste production because of the industrialization. At present there are around five million of registered substances, approximately 70000 of them are world widely used. And it is estimated that every year 1000 new substances are added to the list.

The PPCPs include a diverse group of chemicals like

- Human and veterinary drugs
- Dietary supplements
- Other consumer products like: fragrances, cosmetics, sunscreens, laundry and cleaning products.
- All the inert part that functions as a vector which carry de active ingredients of the above mentioned products
- The PPCPs include the EDCs, endocrinal disrupting chemicals could be hormones or mimic molecules.

The PPCPs have been in the environment since long time ago, however the problem was minimized because they were found in low concentrations and detected only in some places. Moreover their effects in long term were unknown, but this has been changed with the advances in technology. Now is possible to detect many kinds of different structure compounds at low concentrations. As a result, now the surveys of the pollutants in the environment are more accurate, their effects on the environment are better known, and they are easily tracked. In addition, due to the findings on the environment with regard of their presence more deeply research is done about how to effectively remove them from the environment.

1.2 Pharmaceuticals

May be the most dangerous PPCPs are the pharmaceuticals due to its diversity and that they are designed to directly modify the cells behavior, in other words their targets are living creatures. It
explains their effect not only in animals but in microorganisms and plants.

Just to have an idea of the problem we can recognize that most of us take some kind of medication, whether a prescription drug or over the counter product [3]. Besides, most of the medications are not to cure disease but to give support in the disease, it means that the medication will be taken may be for the rest of life, like blood pressure medicine [4].

**1.3 Antibiotics in the environment**

The human eating habits have change. Now people consume more meat and animal products than before. Actually the traditional farming or fishing has been turned into an industry, where the animals are any longer consider as a living creatures but as products. So that to get better yields in their “production” a lot of antibiotics as well of hormones are used. The antibiotics are used to prevent diseases and sometimes to promote growth.

There are over 1800 approved animal drug formulations used in the Animal feedlot operations (AFOs). It represents about the 80 percent of the antibiotics sold in the U.S. that is 13 million kilos a year are fed to animals. Many of these drugs such as fluorquinilones and cephalosporins, are front-line treatments for humans illness such as urinary-tract infections and pneumonia. More and more of the bacteria that cause such diseases have become resistant to the drugs The metabolism of the antibiotics usually is incomplete, so they are excreted mostly unchanged in the urine or/and faces, or converted in another metabolites that could be either more toxic than the original one [5,6].

**1.3.1 Animal production as a source of antibiotics in the environment.**

Antibiotics started to be used in the U.S. in animal farming in 1950, the FDA approved it because they found that animals put on more weight in less time, what results in fatter profits.

Twenty years later, 1970, the researchers began to warn about the risk of drug-resistant organisms. Because of that some European countries banned the use of such substances for animal growth promoting [7]. On the contrary in U.S, they kept using, a further report from a research in Tufts Univ. U.S., proved in 1976 that highly resistant E. coli bacteria could pass from chickens to farm workers.

Even though the FDA continued approved the use of such substances as growth promoters, this is very important because many countries not only U.S. comply with FDA regulations. So many countries around the world indiscriminately used antibiotics to enhance their livestock [7,8].

In 1977, the FDA just made a statement saying that the use of penicillins, tetracyclines and other antibiotics was unsafe for the creation of antibiotic resistant bugs [9].

Finally, after 34 years which means in this year 2012, The FDA issued an order to prohibit certain
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ccephalosporin classes in cattle, swine, chickens and turkeys, as a growth promoter. The regulation is effective from April 5 of 2012 [8,9].

The most commonly found antibiotics are Flumequine, which has been detected in a concentration of 50 mg/L it is used in aquaculture [10]. Clarithromycin, erythromycin, roxithromycin, chloranphenicol, sulfamethoxazole, and trimethoprim have been detected in the wastewater and surface water [11] Amoxicillin, penicillin, ceftazidime, cefalexin, ciprofloxacin, ofloxacin, tetracycline, oxytetracycline, norfloxacin, and sulfadiazine [12].

1.3.2 Another sources of antibiotics

Another source of antibiotics is the wastewaters from the pharmaceutical industries. Even though the loss of product represent a loss in the gains, some factories have considerable product run offs in their effluents. It has been reported mainly in factories allocated in developing countries because there usually the laws are not so stricter or the factories are operating under any regulation. For example, in India in a cefalexin factory the wastewater effluent contains a variable amount of 185 mg/L [13] of cefalexin sometimes mixed with intermediates substances. This effluent after been treated is discharge into a river with a concentration of 30 mg/L, which is still too high to be discharge, but in that place it is allowed.

Also hospitals are an important source of pharmaceutical discharges; in addition its wastewater is commonly mixed with domestic waters. Just in a few places, hospitals have their own pretreatment process, like happens in Germany or Swiss [14]. They usually use Membrane bioreactors, but they cannot complete remove the pharmaceuticals or if so, the problem still remain there because they are not mineralized.

The last important source of antibiotics is the disposal of surplus drugs to sewage by flushing them into the toilet or throwing it by the sink. Those pharmaceuticals end up in the wastewater treatment plants.

1.4 Effect of the antibiotics in the environment.

To date, most of the research has been focused on aquatic environments. There’s a lot we don’t know yet, but what we do know is that PPCPs are blamed for various negative effects on marine life. Populations of male fish are being feminized when exposed to wastewater containing low concentrations of estrogen from oral contraceptives. They are growing ovaries, suffering from low sperm count, producing egg proteins usually found only in females, and in some cases, trying to lay eggs.

Generations of aquatic organisms have lived and continue to live in this chemical “soup”. For them there is no escape. The toxic exposure is constant in the aquatic environment. For this reason,
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even low concentrations of PPCPs can have very significant impacts on many species. Some impacts are profound and even disturbing, while others are unknown. As with humans health risks, there are many questions and few answers other than the all too familiar response—we do not know [15].

Also the PPCP side effects on humans now are very well proved. The increased use of antibiotics has created antibiotic-resistant pathogens, and trace compounds from antibiotics may exacerbate the problem. USGS found ampicillin-resistant bacteria in every U.S. river tested in 1999. Combating drug-resistant bacterial strains will require more expensive and more toxic alternatives, which, without PPCP removal, will only cause a cyclical, snowballing effect [16,17].

Besides the above mentioned side effects on public health, they are harmful to the food chain because they alter the nitrification process in the aquatic environment. This effect is due to the sensitiveness of *Nitrosomonas* and *Nitrobacter* bacteria, for example to oxytetracycline [18].

It has been reported by the EPA that typical wastewater treatment facilities, secondary, and tertiary treatment plants are not designed to remove PPCPs from wastewater. Still the PPCPs disposal is not strictly regulated as a consequence they are constantly flowing into the environment with unknown cumulative and synergistic effects [17].

### 1.5 Removal alternatives for the PPCPs

Up to now, various methods have been adopted to remove PPCPs from the drinking or wastewater. Coagulation–flocculation, biodegradation, photodegradation, biofiltration, ozonation, chlorination and adsorption are some of the methods studied so far [19]. However, it is reported that conventional treatments such as coagulation, sedimentation and filtration cannot remove more than 25% of PPCPs. The removal of PPCPs by adsorption is gaining much interest as this process is simple and cost effective. So far, activated carbon is the widely used adsorbent for the removal of PPCPs. Some other adsorbents such as MCM-41, SBA-15, amberlite XAD-7 and bentonite [20] have also been investigated.

Conventional wastewater treatment processes, such as activated sludge treatment and subsequent clarification, are not effective at completely eliminating all PPCPs from wastewater [21]. Activated carbon adsorption, ozonation or advanced oxidation, and membrane separation are promising advanced treatment processes that are capable of removing many of the PPCPs commonly found in wastewater.

These advanced processes have mostly been evaluated using laboratory batch tests. Few studies have examined PPCP removal in full-scale treatment plants containing the advanced wastewater treatment processes listed above.

Endocrine disrupting chemicals (EDCs) and pharmaceuticals and personal care products (PPCPs)
have been found incompletely removed in various conventional wastewater treatment plants (WWTPs). Wide presence of EDCs and PPCPs in WWTP effluents and in receiving aquatic environments may affect water quality and pose potential risks to aquatic organisms and human health. WWTP effluents have been considered as an important source of micropollutants for aquatic environments; therefore advanced treatment technologies such as ozonation and activated carbon filtration are required to reduce the emission of micropollutants via WWTPs effluents. One alternative advanced oxidation technology is ferrate (Fe(VI)) treatment technology [22].

In view of the serious threat that the PPCPs represent to the environment different removal technologies have been tried, but the results are not satisfactory enough.

1.6 Advanced oxidation process as an alternative to remove the PPCPs

The Advanced oxidation processes (AOPs) represent a good alternative to remove the PPCPs because they can destroy the pollutant and not only remove it, like physical treatments due. Also the AOPs are better than the chemical treatments, because the AOPs generally involve the generation and use of powerful but relative non-selective transient oxygen species, primarily the hydroxyl radical. (·OH).

Also there are a wide variety of AOPs that can produce the hydroxyl radical by different ways, such as photochemical decomposition of ozone and Hydrogen peroxide, or with the Fenton reagent, beside these processes could be combined to get better hydroxyl radicals yields.

AOPs can provide effective technological solutions for water treatment. Such solutions could be combined with traditional treatments which represent a decrease in cost operation and a significant improvement of the conventional treatments [22,23].

1.7 Scope of this research study

This research work is focused in the mineralization of two antibiotics: cefalexin and sulfamethoxazole, both have been detected in many water-bodies around the world and are consider as micropollutants. The removal of Sulfamethoxazole has been widely study by different means like the AOPs, but some of the showed low removal efficiency or the cost of the electrodes are too expensive. In the case of cefalexin, even though it is of an important concern just few works has been published about its removal. The focus of this research is on the mineralization of cefalexin by different AOPs, the optimal parameters for the electro-Fenton process and the effect of different catalyst ions in the electro-Fenton process to oxidize cefalexin. Besides of the research of cefalexin, the oxidation of sulfathoxazole was analyzed by different AOPs and the degradation mechanism was investigated in terms of the formed intermediates. The specific objectives of the research were:
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1. Comparison of the oxidation of cefalexin by three different Advanced Oxidation Processes: Anodic Oxidation, Anodic Oxidation with electro-generated hydrogen peroxide, and electro-Fenton process during 8 hours.

2. The biodegradability after every treatment process was assessed by measuring the biological oxygen demand (BOD), and expressed in terms of BOD₅/COD ratio.

3. The optimal parameters for the electro-Fenton processes were investigated, such as the current density, the pH, the amount of catalyst ferrous ion concentration as well of different cefalexin concentrations.

4. The changes on the biodegradability along the electro-Fenton process at different stages during the 24 hours of oxidation were assessed.

5. Analysis of the Activated carbon felt cathode before and after the electro-Fenton process.

6. Compare and analyze the effect of different transition metal ions as catalyst, Copper, Cobalt and Manganese by different means.

7. Comparison of the oxidation of cefalexin by three different Advanced Oxidation Processes: Anodic Oxidation, Anodic Oxidation with electro-generated hydrogen peroxide, Anodic Oxidation with electro-generated hydrogen peroxide irradiated with UV light, electro-Fenton process and photo-electro-Fenton processes during 6 hours.

8. Analysis of the reaction path way for the oxidation of sulfamethoxazole by the analysis of the formed intermediates during the photo-electro-Fenton process.

1.8 Contents

This paper is composed of 6 chapters. The whole structure is illustrated in Fig. 1.1

Chapter 1 General introduction

The background of the Pharmaceuticals and personal care products, their removal options and the objective of this study will be introduced.
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Chapter 2  Literature review of the current situation of water treatments to remove the PPCPs

A general outlook of the water pollution by the PPCPs, antibiotics as pollutants, Advance oxidation process as a removal option and the explanation of important analytical procedures to assess the efficiency of the AOPs as an oxidation treatment.

Chapter 3  Biodegradability enhancement of wastewater containing cefalexin by means of the electro-Fenton oxidation process

The antibiotic cephalexin, which is considered a micropollutant by the EPA, was degraded by three different AOPs, the final biodegradability was compared. The electro-Fenton process was the most effective, thus the optimal working parameters were investigated like pH, current density, catalyst ferrous ion concentration and different cefalexin concentrations. Finally, the biodegradability at different stages of the electro-Fenton process was assessed.

Chapter 4  Variations during the Electro-Fenton process: cathode changes and different transition metals as catalyst

The efficiency of the electro-Fenton process depends on several factors not only the optimal conditions, but the electrode materials. In this case the anode, made with an activated carbon felt, was analysed before the process, after saturated with the antibiotic and after several electro-Fenton processes. The physicochemical change was assessed.

Also the effect of different catalyst was investigated; in this case the ions from transition metals with similar characteristics to the ferrous ion were used, such as Co²⁺, Cu⁺, and Mn²⁺. The efficiency of each of them were compared and analysed by different means.

Chapter 5  Mineralization of antibiotic sulfamethoxazole by photoelectro-Fenton treatment using activated carbon fiber cathode and under UVA irradiation

The mineralization of the antibiotic sulfathoxazole was analysed by different AOPs using an ACF cathode and in some of them UV-light. The tested AOPs were: RuO₂/Ti Anodic Oxidation, Anodic Oxidation with electrogenerated hydrogen peroxide (AO-E-H₂O₂), (AO-E-H₂O₂) irradiated with UVA light of 365nm, electro-Fenton process (EF) and photo-electro-Fenton process (PEF).

The mechanism pathway of the sulfamethoxazole oxidation was investigated by the identification
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the formed intermediates during the photo-electro-Fenton process (PEF).

Chapter 1 General background
The background of water contamination by Pharmaceuticals and Personal care products (PPCPs), the use of advanced oxidation processes (AOPs) to remove them and the objective of this work

Chapter 2 Literature Review
Literature review of water polluting situation, removal of PPCPs by different methods, the fundamentals for AOPs use in wastewater and important analytical procedures.

Chapter 3 Biodegradability enhancement of wastewater containing cefalexin by means of the electro-Fenton oxidation process
The antibiotic cefalexin was oxidized by the Anodic Oxidation (AO), AO-Electrogenerated H₂O₂ and electro-Fenton processes, the biodegradability after 8h of treatment was assessed. Finally, the biodegradability changes during the electro-Fenton process were analysed up to 12h of treatment.

Chapter 4 Variations during the Electro-Fenton process: cathode changes and different transition metals as catalyst
The electro-Fenton process efficiency relay on different parameters, like the electrodes material, in this case the changes on the ACF cathode were analysed. Also to improve the process the catalyst to decompose the H₂O₂ different metal catalyst were employed.

Chapter 5 Mineralization of antibiotic sulfamethoxazole by photoelectro-Fenton treatment using activated carbon fiber cathode and under UVA irradiation
The antibiotic sulfamethoxazole was oxidized by different AOPs with and without UV-light, such as AO, AO-EH₂O₂, AO-EH₂O₂-UV, EF and EF-UV. The degradation mechanism was investigated and the oxidation pathway of sulfamethoxazole under EF-UV light was elucidated.

Chapter 6 Conclusions
1. Achievements in this research: evaluation of three different AOPs to oxidize the antibiotic cefalexin. Set up the optimal conditions for the electro-Fenton process.
2. Comparison of the oxidation of two different antibiotics: cephalexin and sulfamethoxazole
3. Evaluation of five different AOPs to oxidize the antibiotic sulfamethoxazole.
4. Analysis of the ACF as a cathode
5. Comparison of different transition metals as a catalyst.
Future research: elucidation of the reaction mechanism for the oxidation of cefalexin, deeply analysis of the effect of the different transition metals employed as a catalyst or the Fenton reaction

Fig. 1.1 The whole paper structure
1.9 References


[8] FDA-2008-N-0326 at Questions and Answers on FDA’s Cephalosporin Order of Prohibition


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...Continued from the previous page...


CHAPTER

2

Literature review
2.1 Introduction

2.1.1 Water current situation

Water is an essential compound for any kind of life on the Earth. The Earth is composed by 70% of water, however only the 2.5% is fresh water. Humans, land animals and other living creatures can only use fresh water so that water is considered as a limited resource. Nowadays, we are facing the scarcity of this resource because even though it is considered as a renewal one, the pollutants that carry in make very difficult its recovery.

Water pollution is an old problem that began with the sedentarism of the human race. They demand a lot of water to survive, especially for agriculture. They started modifying the water curse and then developing substances to enhance the crops. As the time passed, the agriculture as well of the farming considerably grew and then the industry came up hand in hand with the water demand.

Currently, water is polluted every day by different substances from any kind of human activities. The problem with such substances is that usually they are anthropogenic compounds so their structures differ from the natural compounds, besides they are released in high amounts. Therefore, the natural mechanisms to remove them commonly failed or take long time if so [1].

The lack of water is a serious problem for life to thrive. Because food depends on the amount of available water, as much as the 70-90% of all the water is for agriculture.

Water has been considered as renewable source, it means that water can follow a continuous cycle and then been available at any time. This statement may be could be possible if non-recalcitrant pollutants were involved but they are. Usually, the wastewater can be clean to somehow but the quality is significantly different. As a consequence, that water cannot be used for the same purpose that was used before been polluted in most of the cases [1,2]. Fig. 2.1 shows an approximately distribution of water use.

As the civilization was developing technology new problems emerged. It was clear in Europe, London, when the industrial revolution took place in the XVIII century. The environment was seriously damage and it has a great impact on public health. A lot of diseases came up due to the dirtiness caused by the accelerated growth, which was not regulated. The objective was only to get economic growth, the same as today. However, the government in London was concerned and they established the first environmental laws. [3]

London was the first European country to design and built the first sewage channels in the city to transport the wastewater from factories to the nearest water body, like rivers. They noticed that this solution was not good enough because the polluted water in the rivers kept representing a problem. So they thought it was better to discharge in the sea. Their observations continued and
the state the first parameter for wastewater most of them are still in use with some modifications, like BOD [3,4].

During some time the wastewater problem remained static because the problems were in certain places and people there deal with it. However, in the recent times the economical growth of many countries is bringing more and worst environmental problems and now the problems are globally spread.

Developing countries use all their resources to get money and their water is not the exception. They use water in factories that later become dirty, yet they don’t have the technology to clean it so they polluted their water bodies. At the beginning nobody paid attention to this problem because they thought it was just a local problem, yet the local problem was spread and reached far areas.

![Fig. 2.1 Water withdrawal.](image)

### 2.2 Availability of water for humans

In 2002, the General Secretary of the UNO highlighted the importance of availability of high quality drinking water [5]. “An estimated 1.1 billion people lack access to safe drinking water, 2.5 billion people have no access to proper sanitation, and more than 5 million people die each year from water-related diseases. Even places with plentiful water supplies are increasingly at risk from pollution and rising demand. By 2025, two thirds of the world's population is likely to live in countries with moderate or severe water shortages”. Although most of the planet is covered by water, fresh water comprises just 3% of the total water on earth. Only a small percentage (0.01%) of this fresh water is available for human use. Unfortunately even this small proportion of fresh water is under immense stress due to rapid population growth, urbanization and unsustainable consumption of water in industry and agriculture. According to a UNO report, the world population is increasing exponentially while the availability of fresh water is declining. Many countries in Africa, Middle East and South Asia will have serious threats of water shortage in the next two decades. In developing countries the problem is further aggravated due to the lack of proper management, unavailability of professionals and financial constraint. [6]
2.3 Principal pollutants in wastewater.

The pollution of water has become of a great interest because it will affect its quality, and water use depends on how clean is it. Nowadays, water is considered as global concern so different instances are analyzing it in order to understand how is changing either distribution or quality. The water analysis has become more accurate for the advances in analytical chemistry, which allow to detect substances that are as low as parts per trillion. As a consequence, now the number and variety of pollutants detected in the wastewater has increased [7].

For this advances, the classification and the studies of their effects on the environment are better now. Also the regulations became stricter and punctual for every kind of pollutant. For example, the classification is based on the amount of pollutant, toxicity, use and so on.

The pollution of water is the result of the combination of a fast population growth and economic development along with wrong water management policies, spill accidents of different substances like oil, and the incorrect discharge of wastewater from agriculture, medicine or industry. The different pollution sources are shown in Fig. 2.2

In our research we are focusing on the micropollutants, those are anthropogenic substances in very low concentration, from milligrams to nanograms, disturbing the environment. They are toxic enough to produce negative adverse health effects on the organisms even their low concentration.

The most notable state-side study was conducted by the U.S. Geological Survey (USGS) in 1999-2000, where 139 rivers in 30 states were tested and found that 80 percent of streams contained 31 different drugs. After this survey, it is no longer a question of if these compounds are
in our waters. They are [8,9].

**2.4 Pharmaceutical and Personal Care Products (PPCPs)**

The PPCPs refer in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of the livestock. PPCPs comprise a diverse collection of thousands of chemical substances, including prescription and over-the-counter therapeutic drugs, veterinary drugs, fragrances, and cosmetics [8].

These PPCPs have a strong effect into the environment because they can modify the biota by different means such as intoxication, which may produce dead or at least diseases. Another side effect is to produce malformations (teratogenic), or endocrine disruptors which alter the gender of the species as well of the sex development.

![Female fish with estrogen exposure](image)

**Fig. 2.3** Fat head minnows: male exposed to estrogen in the lab loses typical male characteristics expressed by normal or control male fish.

The PPCPs are divided in other subgroups to make easier its study. The two principal groups are Pharmaceutical active compounds, such as antibiotics and the other group are the Endocrinial disruptors, such as hormones. Fig. 2.3 shows the picture of a fish that was exposed to estrogen concentration, using the same concentration reported in the sewage effluent; this experiment was done in Canada. As could be noticed the male fish became feminized, the impact of this change is that the dynamics of the population are altered. As a consequence the ecosystem is modified by changes in the biota population [9,10].

**2.5 Pharmaceutical Active Compounds (PhACs)**

Pharmaceuticals constitute a large group of human and veterinary medicinal compounds [11]. PhACs are used and produced all over the world. It is estimated that its worldwide consumption is around 100 000 tons or more per annum [12] with about 3000 different substances.

The major enter rout into the environment is from wastewater. After the intake part of them is
excreted whether unchanged or metabolized via urine or feces so they end up in the wastewater. Further this wastewater is treated by common methods, which usually failed to remove them, and the treated effluent is release in to natural water bodies like rivers. It is also true that they will be diluted and its concentration will not be high but the input is constant, which make them a potential risk for aquatic and terrestrial organism Fig. 2.4 shows the way that the PPCPs follow to enter into the environment.

They have been classified according to their therapeutic activity. For example: antibiotics, analgesic/antipyretics, CNS (central nervous system) drugs, cardiovascular drugs, Endocrinology treatments, diagnostic acid-adsorbable organic halogen compounds.

As shown in Fig. 2.4, the pharmaceuticals enter to the environment via human and animal excreta, for flushing unused medication down to the toilet, pharmaceutical production and farming practices. The 40 percent of antibiotics manufactured are fed to livestock as growth enhancers. Later on, manure which contains traces of pharmaceutical is used as fertilizer; from soil pharmaceutical can leach to local streams and rivers.

Nowadays, the threat that they represent is a serious one, these polluting problem was known since 1970 but during that time the problem was focused only into a few substances that were classified as: “persistent, bioaccumulative toxic” (PBT), “persistent organic pollutants” (POPs) and other “bioaccumulative chemicals of concern” (BCCs). However, other substances were out of the scope. The criteria has change and now all of them are consider as the PPCPs and recognize as potential threat to the environment. Both the European Union and the US Environmental Protection Agency have identified a listing of Priority Pollutants and have developed water quality criteria for them, as well of doing research in how to remove them [9].

Pharmaceuticals are not succesfully removed in common wastewater treatments a proof that
inefficacy is that they have been found in surface, underground and tap water as well as in soil. In the wastewater treatment plants two elimination processes are important: adsorption and biodegradation [13]

2.5.1 Antibiotics

Antibiotics are substances used to treat infections in humans as well as in animals, they can be used as bacteriostatics or to kill bacteria. However, in farming they are used to promote the animal growth and to prevent infections due to the bad conditions where animals are breed. [15]

Antibiotics have been detected in water bodies besides in sewage treat plants. This fact has stimulated a discussion on the origin and selection on resistant bacteria during sewage treatment.

Antibiotic resistance reduces the effect of the antibiotic treatments, which are very important because they are prescribed when the patient is more vulnerable (after surgery) so their life sometime depends on it [16].

The spread of antibiotic-resistant pathogen is a growing problem, and a lot these “super-pathogens” have been found in the environment, like water or/and soil. They have been detected mainly by molecular methods. The results showed that they have different genes, those genes are acquired because the antibiotic resistance bacteria are able to spread them into indigenous microbes, Fig. 2.5 shows the change inside the cell, how the cell acquired different genes that allow them to become resistant to some bacterial drugs.

![Fig. 2.5 Scheme of a bacteria cell attacked by antibiotic and the genetic response, which lead into a antibiotic resistant bacteria](image)

This is a big health problem because sometimes resistant strains persist in individuals so the antibiotic treatment is mostly ineffective and usually there are no other optional antibiotics to prescribe so the patient is likely to die. Actually, in the European Union antibiotic-resistant bacteria cause 25 000 death every year and contribute a yearly cost of 21-34 USD [14].

The problem of antibiotics in the environment is a big concern that is unlikely to be solved by the developing of new antibiotics in the future [17]. Thus, the problem should be solved from its origin, pollutant sources.
2.5.1.1 Cefalexin

The first known antibiotic was penicillin, which was discovered in 1928. Then 1945, the cephalosporines came up when the cephalosporin C a natural antibiotic, was isolated from Cephalosporium acremonium. Five years later, the cephalosporin family grew and cefalexin was first used. Cefalexin was introduced to the market in 1967 by Eli Lilly & Co marketed as Keflex; however, now it is produced by many other companies as a generic antibiotic and sold under several other trade names. Cefalexin (C_{16}H_{17}N_{3}O_{4}S・H_{2}O) is a common name that refers to cephadrine (C_{16}H_{17}N_{3}O_{4}S) [21]. It became popular because it started to replace penicillin since both of them are β-lactamic antibiotics because their structure contains a β-lactamic nucleus.

Most of the β-lactamic antibiotics inhibit the cell wall bio-synthesis because they inactivate the enzymes transpeptidases/transglicosylase [22]. Cefalexin can form with its β-lactamic ring, a fused structure with a dihydrothiazine system known as cephem. This structure conferred the a broad antibacterial spectrum activity mainly for Gramm negative bacteria but also for some Gram positive bacteria [23,24].

Cefalexin is an antibiotic commonly used in treating the urinary as well of respiratory tract infections (including sinusitis, otitis media, pharyngitis, tonsillitis, pneumonia and bronchitis), skin and soft tissue infections, gonorrhea, meningitis, staphylococcal and streptococcal infections [23,25,26].

Penicillins and cephalosporines constitute the largest segment of multimillion dollar antibiotic market. Cephalosporines are more available than penicillins and among antibiotic medicines cephalosporines occupy the 45 percent of the world market [27,28]. Due to its characteristics cefalexin is produced in great amounts in the world. Its world production was estimated in 1995 in US as 33 000; nevertheless due to the changes in the economy now Asia is the major producer of medicines [29] China is the largest producer of cefalexin [20], also in India [21]. Just in Vietnam their plan to develop the chemical industry, from 2005 to 2010, contemplates to produce 100 tons of cefalexin per year plus some other 100 tons different cephalosporin antibiotics [28].

In 2008, cefalexin was the most popular cephalosporin antibiotic in the United States. [29] In Europe, the second most prescribed antibiotic class is the cepahlosporines from them the most popular is the cefalexin [30]

Cefalexin has a biotransformation rate of only 10%, which means that the 90% is excreted unchanged in the urine [31-33]

According to its structure cefalexin is considered as a zwitterion, which means that has a positive and negative charges at different locations within the molecule. So it has an isoelectric point, when the molecule is neutral both poles are charged, for cefalexin in water is approximately 4.5 to
5. Its water solubility is high, cefalexin is a monohydrate compound, with white crystal morphology.

Cefalexin showed a minimal inhibitory concentration (MIC’s), this parameter provides an estimated of the susceptibility of bacteria to microbial compounds, in the range of 0.12 to 16 μg/mL. However, some microbes have already developed resistance. [34]

![Cefalexin structure](image)

**Fig. 2.6** Cefalexin structure

Cefalexin has been found ubiquitously in the aquatic environment, with one report indicating concentrations from 151 to 182ng/L in the coastal waters around Hong Kong, as well inther municipal wastewater in concentrations from 339.4 to 375 ng/L. In the sewage of Shenzhen has been also reported at low concentrations (>1μg/L). In New York City Watershed the concentrations go from 80 to 502 ng/L, and in India where the effluent concentration from a cephalosporin factory after a biological treatment is approximately 29 mg/L. These examples are evidence of one of the biggest hurdles of dealing with CLX in wastewater management: its high resistance to biodegradation.

### 2.5.1.2 Sulfamethoxazole

Sulfamethoxazole (SMX) is part of the class of synthetic antibiotics called sulfonamides or sulfa drugs. The drug is prescribed in a ratio of 5:1 with trimethoprim. The drug is used to treat different diseases in the respiratory, genitourinary and gastrointestinal tract [35]. The price of SMX is not high in comparison with other antibiotics for that reason it is highly prescribed over other antibiotics for most of the bacterial infections, from acne to toxoplasmosis. [36] (Dictionary of pharmaceutical specialties, PLM – Vandemecum. Thompson 2011. SMX is commercially known as Bactrim, Seprin or Septra. It is a very strong antibiotic used against *Streptococcus*, *Staphylococcus aureus* (including MRSA), *Escherichia coli*, *Haemophilus influenzae*, and oral anaerobes. It is commonly used to treat urinary tract infections. In addition it can be used as an alternative to amoxicillin-based antibiotics to treat sinusitis. It can also be used to treat toxoplasmosis and it is the drug of choice for *Pneumocystis pneumonia*, which affects primarily patients with HIV.

Sulfathoxazole has been found in the surface and underground waters, as well of activated sludge in different parts of the world. It has been recognized as recalcitrant compound since it cannot be removed by biological methods also it has a low tendency to be photochemical degraded in
Literature Review

comparison with other sulfa drugs [37].

The Environmental Protect Agency of the United States (EPA) has enlisted the SMX antibiotic as a contaminant of emerging concern due to its presence and prevalence into the aquatic environment. It is a broadly studied antibiotic, for example detection methods or its effect on the ecosystem.

It has been reported that the pharmaceuticals could be degraded through direct photodegradation or sensitized photoprocess such as reactions with transient excited species such as singlet oxygen or some other formed in sunlit natural waters; however, SMX has show a great resistance to natural photodecomposition that could explain its prevalence over other sulfa drugs. Sulfonamides are structural analogs and competitive antagonists of para-aminobenzoic acid (PABA). They inhibit normal bacterial utilization of PABA for the synthesis of folic acid, an important metabolite in DNA synthesis [38].

Fig. 2.7 sulfamethoxazole structure

2.6 Wastewater treatments for “especial” pollutants.

In the wastewater treatment field, water needs to be classified according to its characteristics before been treated; it means that the treatment depends on the kind of wastewater.

Also, as explained before the regulations regarding to the water quality after been treated every day are more rigorous in order to protect the environment. Therefore this demand has boosted the development of new technologies [39].

The wastewater treatments could be classified as: Physicochemical, chemical or biological treatments. The physicochemical treatments only remove the pollutants, it means that the pollutant is still there and will need further treatment to destroy it.

2.6.1 Physicochemical treatments

- Membrane separation
  Includes filtration (micro, nano and ultra), reverse osmosis, and to some extend electrodialysis. All this methods use membranes with different pore size. The efficiency of these processes depends on the pore size and the hydrostatic pressure gradient as driving force [39]
Coagulation, flocculation and sedimentation

These processes are addressed to remove substances that are in a colloid form. These particles carry the same charges, and repulsion prevents them to combine with other particles and form large chains. However with some chemical or physical techniques they can be settled in a phenomenon known as coagulation. In this case an electrolyte is added and will interact with colloidal particles, they will be neutralized and then settled down. In flocculation a high molecular substance is added it will trap the colloidal molecules and later both will be settled down.

Sedimentation process consist in let the coagulated or flocculated particles to settle out, usually in a big tank and with enough time [40]

Adsorption by activated carbon

Treatment by activated carbon is mostly due to adsorption and absorption processes. By this method is possible to remove some particles or molecules. It is used mainly to remove colored substances like dyes, metals and odor substances. The life-span of the carbon depends on the concentration in the effluent.

Air stripping

This technique consist in a mass transference from liquid phase to gas phase by directly air input into the wastewater, the volatile compounds will be transferred. A disadvantage is that sometime toxic compounds as spill into the atmosphere and not all the toxic pollutants can be remove by this technique.

In the chemical treatments, a chemical reaction is occurred between an oxidant substance and a pollutant in order to be chemically oxidized. The oxidation standard potential of the most commonly oxidizing agents is giving in Table 1. Additionally, as these chemicals oxidize the organic material sanitized the water because they can kill pathogenic bacteria and viruses. However, sometimes the completely oxidation is not feasible and toxic compounds can be produced, like dioxins produced by chlorination which are very toxic compounds [41].

<table>
<thead>
<tr>
<th>Oxidizing agent</th>
<th>Standard potential electrode</th>
<th>Oxidizing agent</th>
<th>Standard potential electrode</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>3.03</td>
<td>HO$_2^-$</td>
<td>1.7</td>
</tr>
<tr>
<td>HO$_2^-$</td>
<td>2.8</td>
<td>MnO$_4^{2-}$</td>
<td>1.68</td>
</tr>
<tr>
<td>O$_3^-$</td>
<td>2.7</td>
<td>Cl$_2$</td>
<td>1.57</td>
</tr>
<tr>
<td>FeO$_4^{2-}$</td>
<td>2.2</td>
<td>ClO$_2^-$</td>
<td>1.49</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>1.78</td>
<td>O$_2$</td>
<td>1.2</td>
</tr>
</tbody>
</table>

Even though the fluorine ion is not used in the treatment of water it is always used as reference
Literature Review

for been the strongest oxidant.

2.6.2 Chemical oxidizing wastewater treatments

✧ Oxygen
The direct input of oxygen into the effluent, this process is barely used because the oxidation power of the oxygen by itself is too low to efficiently remove pollutants.

✧ Chlorine
It could be used as gas chlorine, which is first dissolve in water and then added to the wastewater stream. Or in form of hypochlorite, this salt is added into the water, usually the hypochlorite salts are dissolved separately. Chlorine is a very good disinfectant that can also remove odors and may be is the most used substance in wastewater treatments around the world [41].

✧ Ozone
Ozone can be used as ozonolysis or catalyst. According to the reaction it could direct oxidize the pollutant resulting from the action of an atom of oxygen. Or by ozone decomposition which produces a free radical that can oxidize any pollutant. Finally in the ozonolysis, it reacts with double bound molecules and breaks in that point that molecule.

✧ Ferrate ions
The iron (VI) and iron (V), known as ferrates are powerful oxidants they react fast and the formed by products which are not toxic. The reaction is pH dependent; it works better in acidic conditions. The iron ions have also good disinfectant properties as well of efficient coagulations properties and enhanced coagulation could also be achieved using iron (VI) as peroxidant [17].

✧ Hydrogen Peroxide
It can be used as a stand-alone treatment or as an enhancement to existing physical or biological treatment processes, depending on the situation. Hydrogen peroxide can be chemically produced from peroxigenated compounds or by the electrolysis of sulfuric acid or sulfates at the platinum anode [43]. It efficiency is not high and also it interfere with COD measurement.

Even though the variety of existing methods, the recalcitrant pollutants still represented a problem because they are difficult to oxidize. So new technologies with higher oxidation
power came up, they are the advanced oxidation process.

2.6.3 Advanced oxidation process

The advanced oxidation processes (AOPs) are based on the oxidation ability of the free radicals generate by physicochemical processes. These free radicals, usually hydroxyl radicals \( \cdot \text{OH} \), are very reactive species which can attack the pollutants and change their structures into more oxidizing substances.

The AOPs are of great interest for the diversity of technologies involved and the areas of potential application [44]. According to the generation they are divided in two: non-photo catalytic and photocatalytic process. Table 2.2, enlist some of the AOPs classified as photocatalytic or non-photocatalytic process [38]. They include heterogeneous and homogenous photocatalysis based on near ultraviolet, or solar visible radiation, electrolysis, ozonation, Fenton’s reagent, ultrasound, an wet air oxidation. Also, ionizing radiation, microwaves, pulsed plasma and ferrate reagent but these are less used [44].

<table>
<thead>
<tr>
<th>Table 2.2 Common Advanced Oxidation Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-photo catalytic processes</strong></td>
</tr>
<tr>
<td>➢ Ozonation in alkali media (( \text{O}_3/\cdot \text{OH} )) pH&gt;8.5</td>
</tr>
<tr>
<td>➢ Ozonation with hydrogen peroxide (( \text{O}_3/\text{H}_2\text{O}_2 ))</td>
</tr>
<tr>
<td>➢ Electro- Fenton (( \text{Fe}^{2+}/\text{H}_2\text{O}_2 ))</td>
</tr>
<tr>
<td>➢ Radiolysis (( \gamma ) gamma and e-beam)</td>
</tr>
<tr>
<td>➢ Non-thermal plasma</td>
</tr>
<tr>
<td>➢ Ultrasound, electrohydraulic discharge</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

The AOPs are used whether as a pretreatment in a biological process in order to enhance the biodegradability, or as a prost-treatment to polish the water after a common wastewater treatment.

The efficiency of the AOPs is due to the higher thermodynamic feasibility of the involved processes and the speed of the oxidizing reactions are higher because the \( \cdot \text{OH} \) is very reactive even higher than ozone. Table 3, shows the speed of the reaction of some organic compounds with hydroxyl radicals and ozone. It is clear the big differences between them [38].
Table 2.3 reaction rate expressed in (k L mol⁻¹ s⁻¹) for the radical hydroxyl toward some organic compounds compared with ozone reaction rates.

<table>
<thead>
<tr>
<th>Organic compound</th>
<th>'OH</th>
<th>O₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorinated alkenes</td>
<td>10⁹⁻¹⁰¹¹</td>
<td>10⁻¹⁻¹⁰³</td>
</tr>
<tr>
<td>Phenols</td>
<td>10⁹⁻¹⁰¹⁰</td>
<td>10³</td>
</tr>
<tr>
<td>Aromatics</td>
<td>10⁹⁻¹⁰¹⁰</td>
<td>2⁻¹⁻¹⁰²</td>
</tr>
<tr>
<td>Ketenes</td>
<td>10⁹⁻¹⁰¹⁰</td>
<td>1</td>
</tr>
<tr>
<td>Alcohols</td>
<td>10⁸⁻¹⁰⁹</td>
<td>10⁻²⁻¹</td>
</tr>
<tr>
<td>Alkenes</td>
<td>10⁶⁻¹⁰⁶</td>
<td>10⁻²</td>
</tr>
</tbody>
</table>

★ Ozonation at elevated pH

As the pH arises the decomposition of ozone in water increases, as showed in Eq. 2.1.

Oxidation of organic compounds may occur due to a combination of reactions with molecular ozone and the produced hydroxyl radicals at the same time hydroperoxyl radical, HO₂⁻, is formed. In addition to the reaction Eq. 2.1 hydroxide ions may react with ozone and produce the super oxide anion, O₂⁻, Eq. 2.2

2O₃ + OH⁻ + H⁺ → 2'OH + 2O₂ + HO₂⁻          (2.1)

O₃ + OH⁻ → O₂⁻ + H₂O         (2.2)

In this process the pH control is very important; otherwise the bicarbonate and carbonate ions can act as scavengers of the hydroxyl radicals that will decrease the efficiency of the process.

The process has many advantages like it doesn’t produce toxic byproducts, doesn’t leave bad or strange odors or taste and it can be electrically produced in situ. On the contrary, it needs a lot of energy not only to produce the ozone but to mix it very well, since the ozone solubility in water is low, it has temperature restrictions, and to completely mineralize the pollutants long times are needed [38,45].

★ Ozone and hydrogen peroxide

The addition of hydrogen peroxide can initiate the decomposition cycle of ozone resulting in the formation of hydroxyl radicals, which increase the availability of the 'OH radicals via Eq. 2.3-2.4. In Eq. 2.3 the hydrogen peroxide is decomposed, the produced radical further reacts with the ozone to produce the hydroxyperoxyl radical and ozonyl radical.

H₂O₂ → HO₂⁻ + H⁺                        (2.3)

O₃ + H₂O₂ → HO₂⁻ + O₃⁻                   (2.4)

The reaction continues along the indirect way described before Eq. (2.1-2.2). The combination of two oxidants increases their efficiency to oxidize the organic matter. Usually
this process is used to remove pollutants in concentrations of ppb from neutral pH to alkaline, with better results at alkaline pH.

**Radiolysis (γ gamma and e-beam)**

These processes are based on the generation of highly reactive electrons, radical ions, and neutral radicals by the exposition of water to mass particles or electromagnetic waves of high energy, like gamma rays (Gammacell, $^{60}$Co), X rays or electron accelerator like Van-de-Graaf. When the electron beam pass through water, the electrons lose energy by non-elastic collisions with water molecules, as a result reactive species are generated as shown in Eq. 2.5:

$$\text{H}_2\text{O} \rightarrow e^{-}_{\text{aq}} + \text{H}^{+} + \cdot\text{OH} + \text{H}_2 + \text{H}_2\text{O}_2 + \text{H}^{+}$$ (2.5)

The three first generated species are the main products of the water radiolysis. The $e^{-}_{\text{aq}}$ and the $\text{H}^{+}$ are powerful redactor agents, that attack the organic matter by different mechanisms. $e^{-}_{\text{aq}}$ produce hydrogen abstraction, while $\text{H}^{+}$ could either abstract hydrogen or add to the molecule. On the other hand $\cdot\text{OH}$ act as strong oxidant.

This process is very expensive and only has been used as pilot project is still under development.

**Non-thermal plasma**

Plasma is considered the fourth state of the matter; it is called the electric gas because it contains ions and electrons. In this case the plasma is generating by a non-thermal way, it is by the application of strong electric fields (electrohydraulic discharges)\[46\]. The main formed reactive species are $\text{O}^{(3P)}$, $\cdot\text{OH}$, $\text{N}$, $\text{H}$, $\text{NH}$, $\text{CH}$, $\text{O}_2$, $\text{O}_2^{(1\Delta)}$

This process has many advantages like the generated by products are no toxic, they are good to remove VOCs, $\text{SO}_x$, $\text{NO}_x$, halogenated compounds. They could also be used in underground water as well in solids.

In Europe this technique is at great scale. They use the pulsed corona (at $10^2$-$10^3$ Hz) or pulsed arch systems ($10^2$-$10^3$Hz) to generate the plasma state.

**Electrohydraulic discharge (Ultrasound)**

This technology also known as sonolysis use the ultrasound of high potency (from 15 kHz to 1 MHz) utilized the acustic cavitation, which means the cyclical growth and collapse of gas bubbles. As a result of cavitation bubble implosion, extreme temperatures and pressure are generated at the center of the collapsed bubble [45]. The organic degradation occurs through three processes: reactions of supercritic water, direct pirolysis and reactions with the generated free radicals. This process usually needs short times (minutes or hours) \[38\]. The process includes the generation of hydroxyl radicals and hydrogen peroxide. The first by the water thermolysis, the $\cdot\text{OH}$ reacts with another molecule to produce the $\text{H}_2\text{O}_2$. Furthermore, other free radicals are formed, it also depends on which gas is contained in the atmosphere
that the reaction takes places like oxygen, argon, etc. [47].

\[
\begin{align*}
H_2O & \rightarrow \cdot OH + \cdot H \quad (2.6) \\
2 \cdot OH & \rightarrow H_2O_2 \quad (2.7)
\end{align*}
\]

★ Sub and super critic water oxidation process
This process is also known as Wet Air Oxidation. In this technique water is mixed with oxygen or air at high pressure and temperature. The temperature rank is about 150-370 °C, and pressure between 10 -220 bar. Under these conditions water behaves very different from that of normal liquid or vapor. In this condition the organic compound and the oxygen are all present in a single, dense fluid phase, thus the reaction occurs very fast [48].
The mechanism include the carbonization of the substrate that later reacts with the \( \cdot OH \) radical. Nitrogen as well of sulfur and halogens are mineralized [38].
This process is already in use because it can treat any kind of pollutant, in Japan it has been used in distillery industry [49].
The main disadvantage of this process is the cost of the construction materials. In super critic conditions catalyst are necessary, like heavy metals. Those catalysts become a problem at the end of the process because they are not easily recovered.

★ Photochemical process
Those processes combine the conventional ozone or hydrogen peroxide to produce hydroxyl radicals with higher yields.
They use ultraviolet light, thus UV lamps must have a maximum radiation output at 254nm for an efficient ozone photolysis. Many organic compounds absorb UV in the range of 200-300nm and may decompose due to the direct photolysis, which improves the oxidation process [45].

★ Ozone-UV
Ozone absorbs UV radiation at 254nm wavelength (the extinction coefficient \( \varepsilon_{254nm} = 3300 \text{ M}^{-1} \text{ cm}^{-1} \)) producing \( H_2O_2 \) as intermediate, which then decompose in \( \cdot OH \). Reaction Eq. 2.8-2.9

\[
\begin{align*}
O_3 + h\nu & \rightarrow O_2 + O(^{1}D) \quad (2.8) \\
O(^{1}D) + H_2O & \rightarrow H_2O_2 \rightarrow 2\cdot OH \quad (2.9)
\end{align*}
\]
However, this method is considered as an expensive technique to produce \( H_2O_2 \) that subsequently would be photolyzed into \( \cdot OH \)

★ Hydrogen peroxide-UV
An alternative is to irradiate a solution bulk which already contains hydrogen peroxide to produce \( \cdot OH \). The process occur via reaction Eq. (2.10-2.12)

\[
H_2O_2 + h\nu \rightarrow 2\cdot OH \quad (2.10)
\]
Also \( \text{HO}_2^- \) is produced, which is an acid-base equilibrium with \( \text{H}_2\text{O}_2 \), absorbs the UV radiation of the wavelength 254nm [45].

\[
\begin{align*}
\text{H}_2\text{O}_2 & \rightarrow \text{HO}_2^- + \text{H}^+ \quad (2.11) \\
\text{HO}_2^- + \text{h}_\nu & \rightarrow ^*\text{OH} + \text{O}^- \quad (2.12)
\end{align*}
\]

\* Ozone-Hydrogen peroxide- UV radiation
This is also called heterogeneous system that combines the two above explained methods, its oxidizing efficiency is high but the cost is also high.

\* Electrochemical oxidation
This process refers to the direct application of a current density of 2-20 Amperes between the correct electrodes to produce \(^*\text{OH} \) radicals, that further will oxidize the organic matter. The reaction of water could be anodic oxidation Eq. 2.13 or cathodic reduction Eq. 2.14.

\[
\begin{align*}
\text{H}_2\text{O} & \rightarrow ^*\text{OH} + \text{H}^+ + \text{e}^- \quad \text{anodic oxidation} \quad (2.13) \\
\text{O}_2 + 2 \text{H}^+ + \text{e}^- & \rightarrow \text{H}_2\text{O}_2 \quad \text{cathodic reduction} \quad (2.14)
\end{align*}
\]

In this research we used electrochemical oxidation processes to oxidize antibiotics. They were: Anodic Oxidation. Electrogenerated Hydrogen peroxide, Electro-Fenton and Photo-electro-Fenton processes.

The oxidation of organic compounds by \( \text{O}_2 \) into \( \text{CO}_2 \) and \( \text{H}^+ \) (or \( \text{H}_2\text{O} \)) is a spontaneous transformation Eq. 2.15; however, the rate of this reaction is only appreciable at high temperatures or in the presence of a catalyst. [50]

\[
\text{C}_{n}\text{H}_{2n+1} + \text{O}_2 \begin{array}{c}\Delta\end{array} \rightarrow \text{CO}_2 + \text{H}_2\text{O} \quad (2.15)
\]

Thus, the electrochemical techniques represent an option to make faster this oxidation process, such processes are carried out in aqueous phase using different electrodes, in these systems the oxidation of the organic compounds is faster than the spontaneous oxidation in the nature.

**2.6.3.1 Anodic Oxidation:**
This is the simplest process to oxidize the organic pollutants. Also it is known as electrochemical oxidation or direct oxidation and it was the most common Electrochemical Advanced Oxidation Process (EAOP) for the treatment of wastewaters with low organic contents (COD < 5 g / L) for
been easy to use [51].

In the AO process the oxidation of the pollutants occur directly at the anode. The efficiency of the oxidation process depends on the electrode material.

This method consists in the use of a high $O_2$ overvoltage anode because the oxygen transfer reaction must be encouraged while minimizing the unwanted co-discharge of oxygen. The oxidation of the pollutants occur directly at the anode by the application of an electric current between two properly electrodes through the generation of physically absorbed “active oxygen” (adsorbed hydroxyl radicals, $^{•}OH$) or chemisorbed “active oxygen” (oxygen in the oxide lattice, $MO_{x+1}$). The complete destruction of the organic substrate or its selective conversion into oxidation products is schematically represented in Fig 2.8

![Mechanistic scheme of anodic oxidation of organic compounds](image)

**Fig. 2.8** Mechanistic scheme of anodic oxidation of organic compounds with simultaneous oxygen evolution on non-active anodes (reactions 4,5,10) and on active anodes (reactions 4,6,7,11). (eq. 4) Formation of hydroxyl radicals, $^{•}OH$; (Eq. 5) oxygen evolution by electrochemical oxidation of hydroxyl radicals; (Eq. 6) formation of the higher metal oxide, MO; (Eq. 7) oxygen evolution by chemical decomposition of the higher metal oxide; (Eq. 10) electrochemical combustion of the organic compound via hydroxyl radicals; (11) electrochemical conversion of the organic compound, R, via the higher metal oxide.

According to the nature of the anode, there are two main approaches for pollution abatement in wastewater by the Anodic Oxidation process.

- The electrochemical conversion method, in which the organic pollutants are not completely oxidized. Usually this method is used to enhance the biodegradability of the pollutant since the oxidation products are mostly organic acids.
- The electrochemical combustion (or electrochemical incineration) method. In this case the organic pollutants are completely mineralized into $CO_2$ and inorganic ions.

Nevertheless the feasibility of the AO process depends on three parameters.

A) The generation of chemically or physically absorbed hydroxyl radicals.
B) The nature of the anionic material.
C) The process competition with oxygen evolution reactions.

This process can be carried out whether in a divide or undivided cell. The commonly used electrocatalytic anodes are Pt, IrO$_2$, RuO$_2$, undoped and doped PbO$_2$, doped SnO$_2$, etc. Another common kind of anode consists in a Ti substrate coated; all of them are considered high O$_2$-overvoltage anodes. Thermodynamically, the electro chemical mineralization (EM) of any soluble organic compound in water should be achieved at low potentials, widely before the thermodynamic potential of water oxidation to molecular oxygen ($E^\circ = 1.23$ V/SHE under standard conditions) as it given in Eq. 2.16. [52,53]

$$2 \text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4e^- \quad (2.16)$$

These process generates $^\cdot$OH radicals at the anode from water oxidation Eq. 2.17

$$\text{H}_2\text{O} \rightarrow ^\cdot\text{OH}_{\text{ads}} + \text{H}^+ + e^- \quad (2.17)$$

Fundamentals

In general, water can be activated by mainly two possibilities strongly affected by electrode material. The electrode could be considered as “active” or “non-active” anode.

In both cases, the first reaction is the oxidation of water molecules, leading the formation of adsorbed hydroxyl radicals Eq 2.18.

It is important to understand that the oxygen evolution refers to a process of generating oxygen through chemical reaction. In the case of water electrolysis, the mechanism evolution include the oxidation of water into oxygen and hydrogen [52]

Comninellis has proposed a mechanism which considers that the anode surface is formed by a metallic oxide MOx and assume the existence of two kinds of anodes, “active” and “non-active”. So the activation of water in acidic media can be done by a) dissociative adsorption of water in the potential region of thermodynamic stability of water (fuel cell regimen); and b) electrolytic discharge of water at potentials above its thermodynamic stability (electrolysis regimen). In both cases, the first reaction that takes place is the oxidation of water molecules leading the formation of adsorbed hydroxyl radicals [53]:

$$M + \text{H}_2\text{O} \rightarrow M(^\cdot\text{OH}) + \text{H}^+ + e^- \quad (2.18)$$
2.6.3.1.1 Activation of water by electrolytic discharge

With active electrodes there is a strong reaction between the electrode (M) and the hydroxyl radical (\(^{•}\)OH). According to this mechanism, in acidic media, water is discharged above the thermodynamic potential for the oxygen evolution (1.23 V/SHE under standard conditions) on the electrode producing adsorbed hydroxyl radicals, alternatively known as chemisorbed “active oxygen”. They may interact with the anode, and form a higher oxide Eq.2.19. However, in order to achieve the dissociative activation of water electrocatalytic electrodes (M) are needed. In fact, the dissociative adsorption of water can be achieved only at (M) on which the binding energy of the M-OH and M-H exceeds the dissociation energy of water to H\(^{+}\) + \(^{•}\)OH. This is the case of Pt-Ru based electrodes on which water activation can be achieved at 0.2 -0.3 V/SHE.

\[
M(\cdot{\text{OH}}) \rightarrow MO + H^{+} + e^{-} \tag{2.19}
\]

With active electrodes, when an oxidable organic specie R is present in the solution the redox couple MO/M acts as a mediator in the oxidation of organics Eq.2.20. This reaction is in competition with the side reaction of oxygen evolution, which is due to the chemical decomposition of the higher oxide.

\[
MO + R \rightarrow M + RO \tag{2.20}
\]

The oxidative reaction via the surface redox couple MO/M Eq. 2.20 is may be much more selective than the reaction involving hydroxyl radicals. Also the “active oxygen” participates in the formation of partially oxidized products RO.

2.6.3.1.2 Activation of water by dissociative adsorption

According to this mechanism, in acid media, water is discharged (1.23 V/SHE under standard conditions) on the electrode producing physisorbed hydroxyl radicals Eq. 2.21. which are the main intermediates for the oxygen evolution Eq. 2.22. This reaction takes place with a non-active electrode due to weak interactions exist between the hydroxyl radicals and the electrode surface.

\[
H_{2}O + M \rightarrow M(\cdot{\text{OH}}) + H^{+} + e^{-} \tag{2.21}
\]

\[
M(\cdot{\text{OH}}) \rightarrow M + \frac{1}{2} O_{2} + H^{+} + e^{-} \tag{2.22}
\]

In this case, the oxidation of the organics is mediated by hydroxyl radicals. It may results in a fully oxidized reaction products such as CO\(_{2}\) Eq. 2.23

\[
M(\cdot{\text{OH}}) + R \rightarrow M + mCO_{2} + H_{2}O + H^{+} + e^{-} \tag{2.23}
\]

In the above schematic reaction equation Eq. 2.23, R is a fraction of an organic compound containing no heteroatoms, which needs one oxygen to be fully transformed into CO\(_{2}\). This reaction competes with the side reaction of hydroxyl radicals to oxygen Eq.2.24.
A non-active electrode does not participate in the anodic reaction and does not provide any catalytic active site for the adsorption of the reactants and/or the products formed from the aqueous medium. In this case the anode serves as an inert substrate, which can act as sink for the removal electrons.

Thus, electrochemical conversion is favored by anodes having a concentration of $\ce{M(\cdot OH)}$ near to zero. This condition is possible if the rate of transition of oxygen into metallic oxide lattice by reaction Eq. 2.23. is much faster than that of hydroxyl radical formation by reaction Eq. 2.18.

As a general rule, the weaker the interaction, the higher the anode reactivity for organics oxidation (fast chemical reaction). The electrochemical oxidation is mediated by hydroxyl radicals, either adsorbed at the surface, in the case of active electrodes or free, in the case of non-active electrodes.

The feasibility of this process depends on three parameters [52]:

- The generation of chemically or physically adsorbed hydroxyl radicals
- The nature of the anodic material
- The process competition with the oxygen evolution reaction.

### 2.6.3.1.3 Electrogenerated Hydrogen Peroxide

It is known since 1882 that hydrogen peroxide can be formed in aqueous NaOH by the cathodic reduction of dissolved oxygen at high surface area carbon electrodes [54]. More recently, the electrogeneration of $\ce{H2O2}$ in acid medium has been applied to wastewater remediation. In this scenario the reduction of dissolved $\ce{O2}$ on reticulated vitreous carbon (RVC), graphite, and carbon felt cathodes and directly injected $\ce{O2}$ or air to gas diffusion have been mainly reported. Carbon-based cathodes are commonly used because other materials in which $\ce{H2O2}$ can be generated with high yield are either toxic (Hg) or expensive (Au).

Indirect electro-oxidation methods with $\ce{H2O2}$ electrogeneration are performed in an electrolytic cell where this product is continuously supplied to the contaminant solution from the two-electron reduction of injected $\ce{O2}$ mainly using a carbon felt. Eq. 2.25. [55]

$$\ce{O2(g) + 2H^+ + 2e^- \rightarrow H2O2} \quad (2.25)$$

Hydrogen peroxide production and stability depend on several factors like the cell configuration, the cathode used and the experimental conditions applied. Electrochemical reduction on the cathode surface by reaction Eq. 2.26 and in much lesser extent disproportion in the bulk solution by reaction Eq. 2.27 are general parasite reactions which results in the loss of product or a
lowering of observed current efficiency.

\[ \text{H}_2\text{O}_2 + 2 \text{e}^- \rightarrow 2 \text{OH}^- \]  \hspace{1cm} (2.26)

\[ 2 \text{H}_2\text{O}_2 \rightarrow \text{O}_2\text{(g)} + 2 \text{H}_2\text{O} \]  \hspace{1cm} (2.27)

In an open and undivided cells, when \( \text{H}_2\text{O}_2 \) is produced, it is gradually accumulated during the initial 3 h of electrolysis up to reach a steady value. The steady \( \text{H}_2\text{O}_2 \) content increases with increasing the current intensity and it is attained just when its electrogeneration rate becomes equal to its oxidation rate to \( \text{O}_2 \) via hydroperoxyl radical (\( \text{HO}_2^- \)) at the anode surface by the following reactions Eq. 2.28-2.29):

\[ \text{H}_2\text{O}_2 \rightarrow \text{HO}_2^- + \text{H}^+ + \text{e}^- \]  \hspace{1cm} (2.28)

\[ \text{HO}_2^- \rightarrow \text{O}_2\text{(g)} + \text{H}^+ + \text{e}^- \]  \hspace{1cm} (2.29)

So, the anodic decomposition of \( \text{H}_2\text{O}_2 \) with a formation of \( \text{HO}_2^- \) from reactions Eq.15 and 16. a much weaker oxidant than \( \text{HO}_2^- \) [55,56].

### 2.6.3.2 Anodic Oxidation with Electrogenerated \( \text{H}_2\text{O}_2 \)

This method combines the two processes, the Anodic Oxidation and the electrogenerated hydrogen peroxide, what makes it more efficient or powerful for water remediation [40]. This method abbreviated as AO-\( \text{H}_2\text{O}_2 \) involves the destruction of contaminants with adsorbed \( \text{HO}_2^- \) generated at the surface of a high \( \text{O}_2 \)-overpotential anode as intermediate during water oxidation to \( \text{O}_2 \). Eq. 2.18

The advantage of this method is that the \( \text{H}_2\text{O}_2 \) can be electrogenerated in the cell where the oxidation occurs with the added advantage of easy control of dosage by simple means, such as current or voltage presetting [50]. Another advantage is that since \( \text{H}_2\text{O}_2 \) is produced in situ, the problem of been decompose before its addition to the experiment due to its high reactivity can be avoided.

In this process the degradation of the organics is done by different species such as M(\( \text{HO}_2^- \)) and other weak oxidizing agents like \( \text{H}_2\text{O}_2 \) and a hydroperoxyl radical \( \text{HO}_2^- \) produced from \( \text{H}_2\text{O}_2 \) oxidation at the anode. However, this method is not more useful in practice than the direct anodic oxidation (without \( \text{H}_2\text{O}_2 \)) for wastewater treatment, since pollutants undergo similar destruction rate in both cases because their main oxidant is the \( \text{HO}_2^- \) formed at the anode surface from reaction Eq. b. and the weaker oxidants above mentioned which are produced in a low concentration in both cases.

So, the disadvantage of this method is the difficulty of achieving the total mineralization of intermediates. [51]
2.6.3.3 Electro-Fenton Process

The anodic oxidation process with electrogenerated H$_2$O$_2$ could be improved with the addition of a catalyst like ferrous ion, this is the electro-Fenton method.

The experiments of H. J. H. Fenton during in the late XIX century showed that the mixture of H$_2$O$_2$ and ferrous salts was able to oxidize the tartaric and malic acid among other organic substances. [44] The mixture of H$_2$O$_2$ and Fe$^{2+}$ is known as the Fenton’s Reagent (EFR). [57]. Then Manchot studied the stoichiometric between these two reactants. Later on, Haber and Weiss suggested a radical mechanism for the decomposition of the H$_2$O$_2$ to produce hydroxyl radicals [51,58] via reaction Eq. 2.30. However, the research on the mechanism reaction is still ongoing with special attention to the oxidizing process of toxic organic compounds.

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{HO} + \cdot\text{OH} \quad ; \quad k = 76 \text{L mol}^{-1} \text{s}^{-1} \quad (2.30)
\]

So, the anodic oxidation with electrogenerated hydrogen peroxide combined with the EFR is now known as the electro-Fenton process. Since the reaction of the electro-Fenton process takes place in acidic medium it can be expressed as shown in Eq. 2.31:

\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^- \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \cdot\text{OH} \quad (2.31)
\]

The efficiency of the process relays on different operational parameters, the pH is important because in acidic conditions, at pH < 3, the reaction is considered as autocatalytic. In this pH the ferrous ion can be regenerated from the so-called Fenton-like reaction [38,51]. In this reaction the ferric ion reacts with the H$_2$O$_2$ to decompose it into the hydroperoxyl radical and the ferrous ion can be regenerated [51] Eq. 2.32.

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO}_2^- + \text{H}^+ \quad (2.32)
\]

This process is potentially useful to destroy the pollutants in the wastewater due to the hydroxyl radicals (·OH), which have the second higher oxidation power after flourine, among the commonly used substances to treat water (ozone and chlorine) as shown in Table 1. Besides the hydroxyl radicals another free radical is formed, the hydroperoxyl radical (HO$_2^-$) yet it has a low oxidation power that is quiet unreactive toward organic matter.

The electro-Fenton process is already in use to clean water, for example to treat the wastewater from the textile industry, the olive oil production, and etcetera. Even though the advantages of the electro-Fenton process, it has just an inconvenient point for the pollutants which are difficult to oxidize: the process will need longer time to completely oxidize the pollutant. As a consequence, the process will be very expensive to complete mineralize the pollutants. So, in order to make it economically operative usually it is combined with another treatment process such as a biological
Fig. 2.9 Diagram of the chemical and biological oxidation evolution of the pollutants into the treated influent as a function of molecular size.

Fig. 2 shows a diagram of how the AOPs can be combined with a biological treatment. Generally, the AOPs are used as a pre-treatment to enhance the biodegradability of the wastewater to a point where the biological treatment successfully used. As could be seen, as the molecule is oxidizing the molecule is breaking down into smaller molecules that usually are more easy to be biodegraded by microorganisms in the wastewater treatments.

### 2.6.3.4 Photo-Electro-Fenton Process

This process is similar to the above explained hydrogen peroxide-UV. When Fe$^{2+}$ are added, the Fenton reaction also takes place. It considerably enhances the efficiency. At pH 3.0, the Fe(OH)$^{3+}$ is formed because of the acidic environment:

\[
\text{Fe}^{3+} + \text{H}_2\text{O} \rightarrow \text{Fe(OH)}^{2+} + \text{H}^+ \quad (2.33)
\]

\[
\text{Fe(OH)}^{2+} \rightarrow \text{Fe}^{3+} + \cdot\text{OH}^- \quad (2.34)
\]

When exposed to UV radiation, the complex is further subjected to decomposition and will produce $\cdot$OH and Fe$^{2+}$ ions.

\[
\text{Fe(OH)}^{2+} + \text{h}^0 \rightarrow \text{Fe}^{2+} + \cdot\text{OH} \quad (2.35)
\]

The application of UV light considerably increases the hydroxyl radicals production, by this method the completely mineralization of some substances has been reported [38,45].

### 2.6.4 Optimization of the experimental conditions for the EF process

To achieve higher oxidation efficiencies is important to find the optimal conditions to carry out the process.
Fenton and related reactions encompass reactions of peroxides (usually $\text{H}_2\text{O}_2$) with iron ions to form active oxygen species that oxidize organic or inorganic compounds when they are present. Fenton’s reaction involves the $\cdot\text{OH}$ as the main as the key step, it mechanism is a chain reaction known as the classical or free radical Fenton chain reaction. The Fenton and related reactions are viewed as a potentially convenient and economical ways to generate oxidizing species for treating chemical wastes [60].

From a conceptual stand point, the electrochemical alternatives seem more appealing since it allows the continuous electrogeneration and/or regeneration of the reactants ($\text{H}_2\text{O}_2$ and $\text{Fe}^{2+}$), thus increasing the efficacy of the process and enhancing their environmental compatibility. The EF was the first proposed EAOP based on the continuous generation of $\text{H}_2\text{O}_2$ at suitable cathode fed with pure $\text{O}_2$ or air by reaction Eq. 2.36

$$\text{O}_2 + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O}_2 \quad (2.36)$$

Along with the addition of an iron catalyst to the threaded solution to form $\cdot\text{OH}$ via Fenton’s reaction Eq. 2.37 [1]

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \cdot\text{OH} \quad (2.37)$$

The parameters that considerably affect the electro-Fenton process are the pH, because it will affect the catalyst behavior thus the amount of the catalyst as well of its effect in the reaction will depend on it. Then the catalyst amount is also important, since it is necessary to determine the relationship between the pH/Fe$^{3+}$. Another important parameter is the current density, due to the directly dependence of the hydroxyl radicals production, also it is the factor that gives the cost to the process. Finally, the amount of the pollutant is important in order to get better removal yields with input of energy and do not waste the energy on parasitic reactions [38,51]. Fig 1. Shows the schematic of the electro-Fenton process.
Mechanism:

Electro Fenton

\[
O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad E^0 = 0.69V/NHE
\]

\[
Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + \cdot OH
\]

\[
Fe^{3+} + e^- \leftrightarrow Fe^{2+} \quad E^0 = 0.77V/NHE
\]

Reacting with organic compound

\[
Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH
\]

\[
RH + \cdot OH \rightarrow R^- + H_2O
\]

\[
R^- + H_2O_2 \rightarrow ROH + \cdot OH
\]

2.6.4.1 Effect of Fe^{2+} concentration.

The ferrous ion plays the role of catalyst in the electro-Fenton reaction. It is necessary to understand that catalyst term referrers to a substance that mainly speeds up a chemical reaction, but is not consumed by the reaction. The catalyst is used in low concentrations and can be recovered at the end of the reaction. A catalyst works by providing a different route, with lower \(Ea1\) for the reaction in a given time interval. The presence of a catalyst allows a greater proportion of the reactants species to acquire sufficient energy to pass through the transition state and become products.

The amount of the ferrous ion depends on the \([Fe^{2+}/H_2O_2]\) relationship.

Henry J. H. Fenton described the oxidation of small chain organic acids, in 1896, and he suggested the importance of the relationship between the \(Fe^{3+}\) ion and the \(H_2O_2\); however, he was not concerned about the mechanism. Later, in 1901, Manchot state the stoichiometric of the reaction between these two chemical species (\(Fe^{2+}/H_2O_2\)). He found that an excess of Fe(II), two \(Fe^{2+}\) are oxidized per hydrogen peroxide and when the hydrogen peroxide is in excess more than two \(Fe^{2+}\) are oxidized. Haber, Wilstatter and Weiss described the mechanism of the Fenton’s reaction. They discovered that the reaction between \(H_2O_2\) and \(Fe^{3+}\) is a chain reaction. The general state of the mechanism is named as the cycle of Haber-Weiss, as follows [58]:
The Fenton reaction initiates the chain Eq. 2.38

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH} \] (2.38)

Which is followed by the chain reactions 2.39 and 2.40,

\[ \cdot\text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2 \cdot + \text{H}^+ \] (2.39)

\[ \text{O}_2 \cdot + \text{H}^+ + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{H}_2\text{O} \] (2.40)

While chain termination is caused by reaction 2.41

\[ \text{Fe}^{3+} + \cdot\text{OH} + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} \] (2.41)

The Fenton reaction is based on the transfer of electrons between the hydrogen peroxide and the ferrous catalyst. The mechanisms has been described as an inner sphere electron transfer or bonded electron-transfer, it proceeds via a covalent linkage between the two redox partners. In a transitional complex referred as [Fe(II)-H$_2$O$_2$]. In this mechanism the coordinated ferrous ion (LFe$^{2+}$) is oxidized by H$_2$O$_2$ to form LFe$^{3+}$, *OH radical, and HO$^-$ ion Eq. 2.42a (Fenton reaction) or an oxoiron (2+) compound (reaction 2.42b). Either reactive species is a powerful oxidant.

\[
\begin{align*}
\text{LFe} + \text{H}_2\text{O}_2 &\rightarrow \text{LFe}^{3+} + \text{HO}^- + \text{HO}^- \quad (2.42a) \\
&\rightarrow \text{LFe}=\text{O}^{2+} + \text{H}_2\text{O} \quad (2.42b)
\end{align*}
\]

For Fenton reaction Eq.2.43 only small amount of Fe$^{2+}$ is required because this ion is regenerated form the Fenton-like reaction between Fe$^{3+}$ and H$_2$O$_2$. This reaction is associated with a two-step transformation in which the adduct [Fe$^{3+}$(HO$_2$)]$^{2+}$ formed in the equilibrium reaction Eq. 2.44 is subsequently converted into Fe$^{2+}$ and hydroperoxyl radical (HO$_2^-$) following the first order reaction Eq.2.46.

\[
\begin{align*}
\text{Fe}^{2+} + \text{H}_2\text{O}_2 &\rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^- \quad k_2 = 63 \text{ M}^{-1} \text{s}^{-1} \quad (2.43) \\
\text{Fe}^{3+} + \text{H}_2\text{O}_2 &\rightarrow \text{Fe}^{2+} + \text{HO}_2^- + \text{H}^+ \quad (2.44) \\
\text{Fe}^{2+} + \text{H}_2\text{O}_2 &\rightarrow \text{[Fe}^{3+} (\text{HO}_2)]^{2+} + \text{H}^+ \quad k = 3.1 \times 10^{-3} \quad (2.45) \\
\text{[Fe}^{3+} (\text{HO}_2)]^{2+} &\rightarrow \text{Fe}^{2+} + \text{HO}_2^- \quad k = 2.7 \times 10^{-3} \text{s}^{-1} \quad (2.46)
\end{align*}
\]

The Fenton-like reaction Eq. 2.44 is much slower than Fenton`s reaction. Fortunately, Fe$^{2+}$ can be regenerated more rapidly by the reduction of Fe$^{3+}$ with Ho$_2^-$ from reaction Eq. 2.47, with an organic radical R$^*$ from reaction Eq. 2.48 and/or superoxide ion (O$_2^-$) from reaction Eq. 2.49

\[
\begin{align*}
\text{Fe}^{3+} + \text{Ho}_2^- &\rightarrow \text{Fe}^{2+} + \text{O}_2 + \text{H}^- \quad k_2 = 2 \times 10^{3} \text{ M}^{-1} \text{s}^{-1} \quad (2.47) \\
\text{Fe}^{3+} + \text{R}^* &\rightarrow \text{Fe}^{2+} + \text{R}^- \quad (2.48) \\
\text{Fe}^{3+} + \text{O}_2^- &\rightarrow \text{Fe}^{2+} + \text{O}_2 \quad k_2 = 5 \times 10^{7} \text{ M}^{-1} \text{s}^{-1} \quad (2.49)
\end{align*}
\]

Some authors suggested that the rate of the Fenton`s reaction Eq. 2.38 is strongly affected by the
presence of radical scavengers. However, the most important is the relationship between the Fenton’s reagents: \([\text{Fe}^{2+}] / [\text{H}_2\text{O}_2]\) [51]. As can be notice from Fig. 2, the interaction between the hydrogen peroxide and the ferrous ion will lead in the formation of the hydroxyl radicals and the conversion of ferrous ion into ferric ion.

![Schematic of the reaction between the ferrous ion and the hydrogen peroxide.](image)

**Fig. 2.11** Schematic of the reaction between the ferrous ion and the hydrogen peroxide.

In the EF and most related technologies, soluble \(\text{Fe}^{3+}\) can be cathodically reduced to \(\text{Fe}^{2+}\) by reaction Eq. 2.50, which is known as electrochemical catalyst, with \(E^* = 0.77\) V/SHE

\[
\text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+} \quad (2.50)
\]

The fast regeneration of \(\text{Fe}^{2+}\) by reaction Eq.2.50 accelerates the production of \(\cdot\text{OH}\) from Fenton’s reaction Eq. 2.38.

To ascertain the catalytic behavior of the \(\text{Fe}^{3+}/\text{Fe}^{2+}\) couple considering the cathodic regeneration of \(\text{Fe}^{2+}\) which depends on factors such as the electrode potential and area, pH, temperature, and catalyst content [61].

On the other hand, the selection of the iron source strongly relies upon the nature of the cathode. The findings proved that \(\text{Fe}^{2+}\) is preferred catalyst to accelerate the production of \(\cdot\text{OH}\) at the early stages of the process, because this ion is progressively removed from the solution. Although, the \(\text{Fe}^{2+}\) and the \(\text{Fe}^{3+}\) ions can be used as iron sources depending on the cathode material which will regenerate the ion during the process.

The availability of the ferrous ion depends on the pH, because it can modify its oxidation state and thus precipitate or support its dissolution in the media bulk. Fig. 12 shows the oxidation state of the ferrous species according to the pH [51].
Fig. 2.12 Speciation of soluble Fe(III) species in acidic aqueous medium with 0.1 M NaClO₄ at 25 °C: (a) in the absence of H₂O₂ and presence of 1 mM Fe³⁺ and (b) in the presence of 0.5 M H₂O₂ and 0.5 mM Fe³⁺.

2.6.4.2 Effect of pH

The pH of the reaction medium played an important role in the oxidation process via Fenton’s reaction because it directly affects the amount of the hydroxyl radical, since it influence the oxidation state of the ferrous ion which is the catalyst as shown in Fig. 2.12 [51].

The strict control in pH is also needed because in the pH is lower than 2.5 the protons in the media bulk will surround the hydroxyl radicals, thus they will not be able to react with the pollutant [62].

For the Fenton’s reaction Eq. 2.38 the acidic condition is favorable because the hydroxyl radicals can be efficiently formed. The value of pH of the system has been observed to significantly affect the degradation of pollutants [51, 63]. At pH lower than 3, the formation of [(Fe)²⁺(H₂O)]²⁺ occurs, which are considerably less Fenton-reactive, as a consequence they react more slowly with H₂O₂ [63, 64]. Hence less amount of reactive hydroxyl radicals are produced and the degradation efficiency is reduced [51, 65]. In addition, at very low pH the scavenging effects of hydroxyl radicals by hydrogen ions become important. (Reaction (23)) [51]

\[ \cdot \text{OH} + \text{H}^+ + e^- \rightarrow \text{H}_2\text{O} \quad (23) \]

Even though, the ferrous salts are quite soluble in water even at neutral pH. Ferrous ion will tend to coprecipitate with Fe(III) oxyhydroxides if the two ions are present and the pH is brought up above 3 [63]. So, at pH above 3 the oxidation rate markedly decreases due to the formation of Fe(OH)₃ precipitate that restricts the course of the Fenton’s reaction. [66]. It is known also that the oxidation potential of \( \cdot \text{OH} \) radicals decreases with an increase in pH and at alkaline pH the H₂O₂ is unstable and loose its oxidizing potential [38].

It is often stated, incorrectly, the reaction Eq. 7 is optimum at pH 3. On the contrary, reaction Eq. 7 for ferrous ion Fe²⁺ is pH independent below pH ~ 3 and increases in rate with higher pH, at pH 4 it becomes 7 times greater than a pH 3 due to the formation of Fe(OH)₂ which is about 10 times
more reactive than Fe$^{2+}$. However, the general observed pH optimum around 3 for the Fenton reaction is due to the effects on Fe$^{3+}$ speciation. At pH 2.54, Fe$^{3+}$ and Fe(OH)$^{2+}$ exist in equal concentrations of Fe(OH)$_2^{+}$ and Fe$_2$(OH)$_4^{4+}$. [51] So the ferrous ion is stable and will not be precipitated as a ferric ion. According to the results obtained by [63], the optimal pH value was 2.8 where approximately half of the Fe(III) exists as Fe$^{3+}$ ion and half as Fe(OH)$^{2+}$.

In addition, it has been reported in several cases that the pH of the solution decreased rapidly in the initial stages of the experiment, this phenomena is owed to two main reasons: the formation of acidic intermediates like carboxylic acids [67]. And the reaction at the anode, where at least three simultaneous reactions take place: water electrolysis, oxygen evolution, and the organic compounds oxidation. There is the formation of H$^+$ species therefore the pH decreases [63] Along with the two mentioned factors, when the catalyst is FeSO$_4$ which contains residual H$_2$SO$_4$, it shows a synergy interaction with the produced H$_2$O$_2$ and it fragments the organic material into organic acids. [51,63]

Although the optimum pH must be determined in each experiment in order to have the higher removal efficiency: moreover, if the pH value is not the suitable the efficiency can be strongly negative affected.

Finally, the pH can influence on the catalyst amount.

### 2.6.4.3 Effect of current density

Another important parameter for the electro-Fenton process is the amount of DC current applied to the electrodes. The DC current refers to the unidirectional flow and the electric charge is constant it is also known as galvanic current. The galvanic term involves that the electrochemical energy was produced by an oxidation-reduction or redox reaction within a cell and the reaction occurs spontaneously. The oxidation occurs at the anode and the reduction occurs at the cathode. The anode of a galvanic cell is negative, since the spontaneous oxidation at the anode is the source of the electrons in the cell, so the cathode is positive.

The process also could be carried out under potenciostatically conditions, (a constant applied potential) either it has been reported that the reduction of oxygen into hydrogen peroxide needs a potential of $E = -0.3$ V vs. SCE ($E = 0.24$ V vs. SHE) in acidic media saturated with oxygen.

Commonly the electro-Fenton process is performed under galvanostatic conditions, the current can be expressed in the term of intensity as Ampere (A) or density involving the electrode area ($A/m^2$) [68].

These two operation parameters, current and voltage, are related directly to the cost of electro-Fenton process. With the increase of the voltage or current applied, the degradation of substrate increases at the beginning, yet after been stable it turn to worse because of the
appearance of side reactions. This side reactions tend to polarize the reaction, which means change the equilibrium potential of the electrochemical reaction. In the electro-Fenton process, the current density is always low. Some researchers suggested that the current density in the electro-Fenton process should be no larger than 10 A/m², while others thought that the upper limit value should be 6.4 A/m²[68].

The current density affects two processes, the ferrous ion regeneration and the H₂O₂ generation. As the current applied to the electrodes increases, the regeneration of the ferrous ion from the ferric ion becomes higher. [69] also the Fenton chain reactions are increased, it occurs via reaction

2.6.4.4 Effect of pollutant concentration

The oxidation ability of the electro-Fenton process depends on some reaction factors, like any other chemical reaction, such as: temperature, the concentration of the reactants, and the solubility of the reactants in the reaction media bulk.

For a reaction to happen the reactant particles need to collide, usually the rate of collisions is about 10⁹ per second, yet the collisions are not successful because just small fractions of the particle have enough kinetic energy to break the chemical bonds. The minimum kinetic energy for the reaction is the activation energy. The collision possibilities are directly related with the reactant concentration. If the reactant concentration is increased usually the reaction is also increased. The explanation for this phenomenon is that with higher concentration the collision probabilities are increased because there are more particles in the volume reaction. To stir the reaction will also affect the reaction rate, thus it is necessary to keep the same stirred conditions in all the experiments that further will be compared.

It has been reported that decomposition rates are faster at lower concentrations because the reactive radicals have a great opportunity to react with the pollutant’s molecule.

On the other hand, it has been reported that the removal efficiency increased with higher concentration of the pollutant because only a smaller reaction of •OH is wasted in parasitic reactions thus the oxidizing species interact with larger number of organic molecules that are available. [70]

2.6.4.5 Effect of different catalyst ions

The Fenton reaction has been studied not only to remove pollutants from water as well of soil, but
in the biological reactions. Fenton reaction plays an important role in the cell oxidizing reactions. It was discovered that such reaction can be occurred with different catalyst than ferrous ion, since in the biological systems other ions are available like Fe, Mn, Co, Ni, or Cu ions. In theory, the Fenton reaction can be catalyzed by any transition metal. So the classical electro-Fenton process can be carried out with any appropriate homogenous redox couple \( M^{(n+1)} / M^n \) according to the general reaction [71]:

\[
H_2O_2 + M^{n+} \rightarrow M^{(n+1)} + OH^- + \cdot OH
\]  

(2.51)

The transition metals refer to the d-block in the periodic table. They are denominated as “d-block” because they are filled in the d-orbital subshell of the second outmost shell. According to the IUPAC they are elements with partly filled d subshells either in the neutral atom or in ions in common oxidation states. The properties of the elements in the “d-block” vary linearly more than vertically as the s and p-blocks. Actually the d-block is subdivided in rows designated as first, second and third row respectively, in each row the elements posses similar features. The exception is the Group XII, because its d-subshell is full so their characteristics are different [72]. The first row of the transition metals is the most widely study, this row includes: Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu and Zn. Their properties could be explained by their electronic configuration which describes the way the electrons are distributed in the atom. See Table 2.4. It is based on the Bohr model of the atom, which assumes that electrons can be located at different shells or subshells. In the case of the “d” subshell 5 pair of electrons can be placed, but when this orbital is incomplete the element may have different oxidation states. The unpaired electrons make them more reactive, thus they can easily form bonds with other elements or molecules. Also the before mentioned characteristic of possessing unpaired electrons makes them a good catalyst, either as the metal itself or as some of their compounds [73]. The electronic properties of the elements used in this experiment are described in Tab. 2.4. According to their electronic configuration they can have many oxidation states, however some states will be more stable than others. In Tab. 4, the most common oxidation states are remarked in red color. The oxidation state will confer different properties to the metal, such as color or solubility, for example it has been reported that generally at oxidation states higher than +3 the metals do not exist in aqueous solution. In this experiment all the used metals were at +2 oxidation state [74].
Table 2.4 Properties of the transition metals

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Name</th>
<th>Atomic number</th>
<th>Electronic Configuration</th>
<th>Oxidation states</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>Iron</td>
<td>26</td>
<td>1s(^2), 2s(^2), 2p(^6),3s(^2),3p(^6),4s(^2),3d(^6)</td>
<td>+1 +2 +3 +4 +5 +6</td>
</tr>
<tr>
<td>Mn</td>
<td>Manganese</td>
<td>25</td>
<td>1s(^2), 2s(^2), 2p(^6),3s(^2),3p(^6),4s(^2),3d(^5)</td>
<td>+1 +2 +3 +4 +5 +6 +7</td>
</tr>
<tr>
<td>Co</td>
<td>Cobalt</td>
<td>27</td>
<td>1s(^2), 2s(^2), 2p(^6),3s(^2),3p(^6),4s(^2),3d(^7)</td>
<td>+1 +2 +3 +4 +5 +6</td>
</tr>
<tr>
<td>Cu</td>
<td>Copper</td>
<td>29</td>
<td>1s(^2), 2s(^2), 2p(^6),3s(^2),3p(^6),4s(^1),3d(^{10})</td>
<td>+1 +2 +3 +4 +5 +6</td>
</tr>
</tbody>
</table>

Finally, the researches in the use of different catalyst ions in the electro-Fenton process are few, most of the experiments that use a different catalyst use copper.
2.7 Summary

The development of the society came up with a high cost to the environment.

Water has been affected since the beginning of the human civilization. However, nowadays the problem turned for the worst due to the development of new substances used in our daily life.

Those substances are referred as Pharmaceuticals and Personal Care Products (PPCPs). The PPCPs include any chemical used for agriculture, farming, cosmetic and pharmaceutical purposes.

The problem with those substances is that they are not easily destroyed by natural means, so that they remain for long periods in the environment. Moreover, some of them have biological activity, like the Pharmaceutical Active compounds (PhACs). Within this group the most important are the antibiotics because they are widely used in humans and animal, in the last ones not only for health reasons but for growth promoting during farming.

It has been reported that the biotransformation of the antibiotics is usually very low, so they are excreted unchanged or broken into still active metabolites in the urine or the feces. Therefore, they end up in the wastewater.

The consequences of the antibiotics in the environment are the promotion of antibiotic resistant microbes, besides of bioaccumulation and may be allergy promoting.

For example, cefalexin has been detected in natural water bodies, like a watershed in New York, or in costal of Hong Kong as well in other places. It has been tried to remove by Membrane Bioreactor or sonochemical process, but the efficiency was low. Sulfamethoxazole also has been detected in many natural water bodies as well of sewage sludge. Their presence is an indicative that wastewater treatment plants are ineffective.

An alternative to solve this problem are the advanced oxidation processes (AOPs). Cefalexin has been tried to oxidize by one AOP, sonochemical process but it could only remove a low concentration (ng). In the case of sulfamethoxazole, it has been studied by different AOPs, but still they need to be improved.

This research is focused on the removal of antibiotics by AOPs, we analyzed which are the must effectives and then we tried to improved. We found that the most effective is the electro-Fenton process and that it could be improved by changing some operational parameters, applying UV-light, or using different catalyst metals.

Water problem is today’s problem that cannot wait for the future to be solve and the AOPs are good alternative to recover its quality after been polluted.
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Literature Review


CHAPTER 3

Biodegradability enhancement of wastewater containing cefalexin by means of the electro-Fenton oxidation process
3.1 Introduction

Nowadays, it has become more difficult to treat infections because of the increase in antibiotic resistant bacterial strains [1]. It has been reported that at subinhibitory antibiotic concentrations, the transfer of genes for resistance is promoted [2]. The major source of antibiotics into the environment is the wastewater from households, hospitals and pharmaceutical factories. Even treated effluent poses a serious problem since common wastewater treatments are unable to efficiently remove the Pharmaceutical and Personal Care Products (PPCPs) such as antibiotics. Indeed, antibiotics have been detected at low concentrations, up to the μg/L-level, in municipal sewage and surface waters located downstream from municipal sewage treatment plants (STPs) [3, 4].

The presence of PPCPs in STPs effluents is a clear indication that common treatment systems need to be improved. A great deal of research has been done over the past 20 years, to develop more comprehensive treatment systems capable of removing these potentially harmful substances from the environment. Among the most promising techniques being developed are the advanced oxidation processes (AOPs) [5-7].

AOPs are based on the chemistry of hydroxyl radicals (·OH, $E^\prime = 2.8V$ versus SHE). They are powerful technologies to treat wastewater containing recalcitrant organic compounds [8-11]. Since hydroxyl radicals react in a non-selective way with the pollutant, the pollutant is oxidized into mineral end-products, yielding CO$_2$ and inorganic ions [9, 12, 13]. While the studies into AOPs have been encouraging, their efficiency has been shown to decrease considerably when treating high concentrations of organic matter. In addition to this significant drawback, their operation cost is quiet high compared with biological treatments [9].

To overcome these problems, a tempting alternative is to combine AOPs with a biological process, with the AOPs as either a pretreatment or post treatment step. As a pretreatment, AOPs would be useful to enhance the biodegradability of the pollutants, or alternatively, as a post treatment finishing step, they could completely oxidize all the organic matter [9, 14].

Cefalexin is one of the most prescribed antibiotics and is produced in great quantities [11-16]. It has a wide spectrum of antibacterial activity and high water solubility [13, 16]. In Europe, the second most prescribed antibiotic class is cephalosporins, and among them Cefalexin (CLX) is the most prescribed [15]. Since CLX has a biotransformation rate of only 10%, the remaining 90% is excreted unchanged in the urine [14, 18]. It has been ubiquitously found in the aquatic environment, with one report indicating concentrations from 151 to 182ng/L in the coastal waters around Hong Kong [15, 19], and has also been shown to be present in municipal wastewater (from 339.4 to 375 ng/L) [16, 20]. Reports have also shown CLX in the sewage of Shenzhen (>1μg/L) [21], New York City Watershed (80 to 502 ng/L) [22], and in India where the effluent concentration
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from a cephalosporin factory after treatment is approximately 29 mg/L [23]. These examples are evidence of one of the biggest hurdles of dealing with CLX in wastewater management: its high resistance to biodegradation.

The investigation of Michael Dodd [24] using ozone and a hydroxyl radical in attempt to eliminate the bacterial activity of CLX, showed that with only one method no change in the activity was noted, however, when both methods were used, the bacterial activity was effectively eliminated. In another study, a sonochemical degradation technique was used to significantly enhance the biodegradability (BOD$_5$/COD) of CLX, and then effectively remove it [16].

The biodegradability of CLX must be assessed at various stages of the AOPs in order to know the optimal time to introduce the biological process. Most of the biodegradability tests are based on activated sludge method. In the standard biological oxygen demand (BOD) test [25], the biodegradability is expressed by the BOD$_5$/COD ratio, where the COD is the chemical oxygen demand. This ratio provides information with regard to the portion of the organic materials present that can be aerobically degraded within 5d. There are four categories depending on the BOD$_5$/COD ratio value. When the ratio is greater than 0.45 the wastewater is easily biodegraded, in the range of 0.3 to 0.45 it is considered biodegradable, and when it is 0.2 to 0.3 it is difficult to biodegrade. At a ratio lower than 0.2 the material is deemed non-biodegradable [26].

In this study, a comparison of three AOPs, Anodic oxidation (AO), electro-generated hydrogen peroxide (AO-H$_2$O$_2$) and electro-Fenton (EF)) to degrade CLX were analyzed and its final biodegradability was assessed. The EF process was carried out using an ACF felt cathode. The effect of different operating conditions, such as pH, current density (I), ferrous ion (Fe$^{2+}$) concentration and CLX concentration were investigated. The biodegradability enhancement of CLX in the EF was evaluated at different reaction time.

3.2. Materials and methods

3.2.1 Materials

Analytical grade reagents were used in the experiments. Cefalexin was purchased from Wako (99%), its chemical structure and physical properties are given in Table 3.1 The pH values of solutions were adjusted with H$_2$SO$_4$, whenever required. Ferrous sulphate heptahydrate (99%, Wako) was used as the catalytic Fe$^{2+}$ species. The methanol, acetonitrile, formic acid, and distilled water used in the mobile phase in the liquid chromatography were HPLC grade, and obtained from Wako. All the experiments were prepared with Ultra-pure water obtained from a Millipore AutoPure WT101UV system (Millipore S. A., Molsheim, France) with resistivity of 18.2 MΩ cm at 25°C. Pressurized oxygen gas (99.9%) was used to saturate the solutions. The activated carbon fiber (ACF) felt was purchased from Xuesheng Technology Co. Ltd. (Shandong, China). The ACF size
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was 6 x 9 cm.

Table 3.1. Cefalexin (CLX) properties

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Molecular weight</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefalexin, Cephalexin, CLX</td>
<td><img src="structure.png" alt="Structure" /></td>
<td>365.40 u</td>
<td>1790 mg/L</td>
</tr>
</tbody>
</table>

3.2.2 Batch oxidation experiments

All experiments were performed in batch mode at room temperature and were conducted in an open undivided cylindrical cell containing 500 mL of Na$_2$SO$_4$ 0.05M solution as the supporting electrolyte; the batch was stirred with a magnetic bar to ensure homogeneity and the transport of reactants toward/away from the electrodes. The electrode was connected to a DC power supply AD-8735 (Tokyo, Japan) operated at the desired electric current intensities displayed throughout the experiment.

The working electrode for the Anodic Oxidation was made from 54cm$^2$ of RuO$_2$/Ti mesh for both the anode as well of the cathode. In the cases of AO with electro generated H$_2$O$_2$ and electro-Fenton processes, the used cathode was made of activated carbon felt (ACF) with the same size as the anode. It was saturated with a 200 mg/L of CLX solution for 12h three times before the experiments to avoid any TOC decrease due to the absorption.

A stock solution of cefalexin at a concentration of 1000 mg/L was prepared, and the aliquots taken from it were diluted in the reaction cell to achieve the initial concentrations from 50, 100, 200 to 300mg/L in the reacting solution. Quantities of ferrous sulphate (FeSO$_4$ •7H$_2$O) from 0.5mM to 1mM, were then added to the solution as needed. The reaction pH, which was determined with a (HM-30V TOA Instruments) pH-meter, was adjusted to a range from 2 to 5 ± 0.01 by adding H$_2$SO$_4$ 0.1M.

Prior to electrolysis, the solution was aerated for 30 min with pure oxygen at 0.1L/min rate and gas was input throughout the reaction time.

For each experiment, samples were collected at regular time intervals (0, 30, 60, 90, 120, 180, 270, 360 and 480 min). Each of them was filtered through hydrophilic PTFE filters with a porosity of
0.45 μm, purchased from Millipore.

### 3.2.3 H$_2$O$_2$ determination

Hydrogen peroxide concentrations were determined spectrophotometrically by the iodide method (detection limit of ≈ $10^{-6}$ M). Aliquots (1.5mL) of samples were added in 10 mm quartz cuvettes, along with 0.75 mL of 0.1 M potassium biphthalate and 0.75 mL of iodide reagent (0.4 M potassium iodide, 0.06 M NaOH, ≈ $10^{-4}$ M ammonium molybdate). The absorbance of the treated solution was then measured with a UV-visible spectrophotometer HACH DR4000 (HACH Co. USA) at \( \lambda = 352 \text{ nm} \). \[ \varepsilon = 26400 \text{ M}^{-1} \text{cm}^{-1} \] [27].

### 3.2.4 Chemical Oxygen Demand (COD)

The chemical oxygen demand (COD) was measured, according to the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA and WPCF, 1995), using the colorimetric method at 600 nm with a Hach-spectrophotometer (Hach DR/2000).

### 3.2.5 Biological Oxygen Demand. (BOD)

The biological oxygen demand was measured by an OxiTop® BOD controller model 1020 (WTW, Welhilheim, Germany) thermostated at 20˚ C. The pH of the samples was adjusted to a value between 6.8 and 7.5 before the addition of the bacterial seed and the necessary nutrients for bacterial growth. Both BOD$_5$ and BOD$_{14}$ were measured by this equipment.

### 3.2.6 HPLC measurement

The CLX concentration during the experiments was quantified by high pressure liquid chromatography, (HPLC) equipped with UV detector (262nm) (Waters 510 pump equipped with 712 WISP autosampler, Water Associates, USA), and a Spherisorb ODS-2.25cm x 4.6 cm column was used as the stationary phase. The mobile phase was a mixture of 0.01 M NaH$_2$PO$_4$; methanol (75:25). The flow rate was adjusted to 0.5 - 1 mL/min with, the temperature was maintained in the range of 30-40 °C. Evaluation and quantification were made using a chromatography data system [28].

### 3.2.7 Total Organic Carbon (TOC)

The TOCs of the initial and electrolyzed samples were determined with a TOC-analyzer (Shimadzu 5000A). Platinum catalysis was used in the combustion reaction. The carrier gas was oxygen with a flow rate of 150 mL min$^{-1}$. The detector of the TOC system was a non-dispersive infra-red detector, NDIR.

2.8 Mineralization current efficiency (MCE)
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TOC data allow the percentage of MCE to be determined for each treated solution at any given time by the equation

\[
\text{MCE} = \frac{nFV_s \Delta (\text{TOC})_{\text{exp}}}{4.32 \times 10^7 \text{ mlt}} \times 100
\]  

(3.1)

Where \( F \) is the Faraday constant (96487 C mol\(^{-1}\)), \( V_s \) is the solution volume (L), \( \Delta (\text{TOC})_{\text{exp}} \) is the solution decay (mgL\(^{-1}\)), \( 4.32 \times 10^7 \) is a conversion factor to homogenize units (3600 s h\(^{-1}\) x 1200 mg mol\(^{-1}\)), \( m \) is the number of carbon atoms of cefalexin, and \( I \) is the applied current (A) [29]. The number of electrons (\( n \)) consumed per cefalexin was taken as 70e\(^{-1}\), assuming the complete mineralization of CLX to CO\(_2\) with the release of SO\(_4^{2-}\), and NH\(_4^+\) as the main primary ions, after measuring the nitrogen species. The reaction describing this process is:

\[
\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_4\text{S} + 32 \text{H}_2\text{O} \rightarrow 16\text{CO}_2 + 3\text{NH}_4^+ + \text{SO}_4^{2-} + 69 \text{H}^+ + 70e^- 
\]  

(3.2)

An Ampere express the amount of charge transported at determined time, in other words is the amount of electrons transported per second in determined point when the current density is of 1 Ampere. Thus:

\[
I = \frac{Q}{t} = \frac{\text{Coulombs}}{\text{seconds}} = \frac{6.25 \times 10^{18} \text{ electrons}}{\text{seconds}}
\]

If

\[
I = \frac{n \ q_e}{t} \quad n = \frac{I \times t}{q_e} = \frac{1 \text{ A} \times \text{s}}{-1.6 \times 10^{-19} \text{ Coulombs}} = 6.25 \times 10^{18} \text{ electrons}
\]

Where \( q_e \) is the charge of an electron: \( q_e = -1.6 \times 10^{-19} \text{ Coulombs} \)

and \( n \) is the number of electrons

Then an Ampere is equivalent to next equation

\[
\text{Ampere} = \frac{\text{electrons} \times \text{Coulombs}}{\text{seconds}}
\]
3.3 Results and discussions

3.3.1 Comparative behavior of cefalexin in AO, AO-H$_2$O$_2$ and EF processes

![Graph showing COD decay as a function of electrolysis time for different processes.]

**Fig 3.1** COD decay as a function of electrolysis time for the ACF cathode adsorption, Anodic Oxidation, AO with electrogenerated H$_2$O$_2$ and Electro-Fenton processes, for 500mL of 200 mgL$^{-1}$ CLX solution.

Fig. 3.1 shows the COD decay of 200 mgL$^{-1}$ cefalexin solutions at pH 3.00 produced by the ACF cathode adsorption and three different AOP processes. At the beginning of the experiment, the ACF used as the cathode was saturated with CLX to assure the decay of CLX was due to the reaction of the processes and not to absorption by the ACF; however, some adsorption and desorption events may occur but their effect could be considered as negligible. The oxidation ability of the anodic oxidation (AO), the AO with electro-generated hydrogen peroxide (AO-H$_2$O$_2$), and the electro-Fenton (EF) processes were evaluated. After 480 mins of treatment, the COD removal rates were very low in the AO process and the AO-H$_2$O$_2$ process at 4% and 8%, respectively. In the EF process, however, 72% of COD removal was achieved.

In the AO process, only one reaction takes place on the RuO$_2$/Ti anode surface, the oxidation of water (Eq. (3.3)), which produces a low concentration of hydroxyl radicals [30]. The low oxidation ability is attributable to this low production of •OH.

In both AO-H$_2$O$_2$ and EF processes, the H$_2$O$_2$ was produced on the cathode (Eq. (3.4)). However, in the AO-H$_2$O$_2$ process, the oxidation of the H$_2$O$_2$ into O$_2$ at the anode is remarkable because of the absence of a catalyst. This is possible via the hydroperoxyl radical (HO•$_2$), which is weaker oxidant than •OH (Eq. (3.5-3.6)) [9,31].

\[
\text{H}_2\text{O} \rightarrow \cdot \text{OH} + \text{H}^+ + e^- \quad (3.3)
\]
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\[
O_2 + 2 \ H^+ + 2e^- \rightarrow H_2O_2 \quad (3.4)
\]

\[
H_2O_2 \rightarrow HO^* + H^+ + e^- \quad (3.5)
\]

\[
HO^* \rightarrow O_2 + H^+ + e^- \quad (3.6)
\]

\[
Fe^{2+} + H_2O_2 + H^+ \rightarrow Fe^{3+} + H_2O + ^\cdot OH \quad (3.7)
\]

The higher removal in the EF can be attributed to the ferrous catalyst due to a considerable increment of the \(^\cdot OH\) concentration in the solution bulk derivable from the catalytic decomposition of H\(_2\)O\(_2\) (Eq. (7)) \([32-34]\). The large quantities of \(^\cdot OH\) generated homogenously attack the CLX. These attacks to CLX could be dehydrogenation or hydroxylation, and the formed byproducts can further react with oxygen until its total mineralization is completed.

**Fig 3.2** Decay of CLX concentrate measured by HPLC on during the oxidation processes as a function of electrolysis time for Anodic Oxidation, AO with electrogenerated H\(_2\)O\(_2\) and electro-Fenton processes, for 500mL of 200 mgL\(^{-1}\) CLX solution

Fig. 3.2 shows variations of the CLX concentration during 480 mins of treatment. The decay of CLX concentration in every process was as follows. In the case of the AO and the AO-H\(_2\)O\(_2\) processes, the removal was 21% and 54% respectively; however, after 270 min of the EF process 100% removal was reached. It should be noted that for every process, the COD showed only a slight decay despite the significant decrement in CLX concentration. The difference in CLX concentration, shown in Fig. 3.1 and 3.2 is most probably not due to the complete oxidation CLX to CO\(_2\). Instead, since there was no significant COD removal, it is more likely attributable to the partial breakdown of the CLX molecule.
**Fig. 3.3** Mineralization current efficiency calculated from Eq. 1 for each oxidation process as a function of electrolysis time for the Anodic Oxidation, AO with electrogenerated H$_2$O$_2$ and electro-Fenton processes, for 500mL of 200 mgL$^{-1}$ CLX solution.

Fig. 3.3 depicts the mineralization current efficiency calculated for each process. Both the AO and AO-H$_2$O$_2$ processes had low efficiency. The MCE for AO-H$_2$O$_2$ is only marginally higher than the corresponding value for AO, which may explain the difference in the COD removal efficiency in the two processes. (4 and 8% respectively). In the EF process, the MCE is much higher than in the two other processes. The peaks in the beginning, at 60 and 180 mins of process, suggest the quick destruction of products more easily oxidized than the CLX intermediates produced during the process. The fall in the efficiency as the EF process proceeds indicates that the intermediates are more difficult to oxidize.

**Fig. 3.4** Biodegradability change on the CLX solutions after 8 hrs of oxidation processes compared to the biodegradability of CLX.
Biodegradability was evaluated in terms of the BOD5/COD ratio. Fig. 3.4 shows the biodegradability for the three AOPs and CLX without treatment. The values of the BOD5/COD ratio reflect the cefalexin bio-recalcitrance, and they were as follows: 0.015 for AO, 0.038 for AO-H2O2, 0.26 for EF and zero for CEX without treatment. These results clearly indicate that among the AOPs tested only the EF is capable of enhancing the biodegradability of CLX for further biological treatment.

3.2 Optimization of the experimental conditions for the EF process

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

Ferrous ion concentration was shown to have a significant effect on TOC removal in the EF process. Fig. 3.5 shows the effect of different ferrous ion concentrations ranging from 0 to 3.5 mM under EF conditions for the electrolysis of 200 mg/L of cefalexin with an applied current of 6.66 mA/cm$^2$, and a pH of 3 for 480 mins. The ferrous ion catalyzes the decomposition of H$_2$O$_2$ into the reactive species ($^\bullet$OH) that oxidize the CEX molecules [32-36].

![Graph showing TOC removal with different Fe$^{2+}$ concentrations](image)

Fig. 3.5 Effect of different Fe$^{2+}$ concentrations on the TOC removal of 500 mL of 200mg/L CLX solutions with 0.05 M Na$_2$SO$_4$, pH 3.0 at 0.36 A, and 0.1LO$_2$/min. for 480 min. by electro-Fenton process.

After 8 hours of treatment, the TOC removals were 8%, 22%, and 49% for 0, 0.5 and 1 mM of Fe$^{2+}$ concentrations, respectively. When the initial concentration of Fe$^{2+}$ ions in the solution raised from 0 to 1mM, a remarkable enhancement in the TOC decay was observed. However, when the Fe$^{2+}$ concentrations was in the range of 2.5 to 3.5 mM, the TOC removal decreased to 38% and
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As expected, the Fe$^{2+}$ ion plays an important role in the initial decomposition of H$_2$O$_2$. It should be noted; however, that only small amount of Fe$^{2+}$ is required, due to the ion regeneration on the ACF cathode [33,34]. The reaction proceeds according to the (Eq. (3.8)) and slightly from the Fenton-like reaction (Eq. (3.9)) [29,32,36-38]. In the Fenton-like reaction, the Fe$^{3+}$ reacts with H$_2$O$_2$ and then it is reduced to Fe$^{2+}$, in a two step transformation where the hydroperoxyl radicals are produced instead of the hydroxyl radicals thus the TOC removal is affected. When the Fe$^{2+}$ ion concentration is high, competitive reactions can take place between Fe$^{2+}$ and •OH species which could cause the Fe$^{2+}$ ions to change into Fe$^{3+}$ ions (Eq. (3.10)). If this were to happen, the hydroxyl radical concentration would withdraw and there would be insufficient amounts to react with the pollutant [9,39].

\[
\text{Fe}^{3+} + \text{e}^- \rightarrow \text{Fe}^{2+} \quad (3.8)
\]

\[
\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{2+} + \text{HO}^* + \text{H}^+ \quad (3.9)
\]

\[
\text{Fe}^{2+} + \text{OH}^* \rightarrow \text{Fe}^{3+} + \text{HO}^- \quad (3.10)
\]

The high concentration of the ferrous ion generates an increasing formation of yellow precipitate of Fe(OH)$_3$ as a function of time during the electrolysis, which is deposited at the bottom of the reactor as well on the ACF surface. Once it has been deposited, this Fe$^{3+}$ cannot be reconverted to Fe$^{2+}$, and the result is less Fe$^{2+}$ available to react with the H$_2$O$_2$ in the media. This explains the decreased efficiency observed with an incremental change in the ferrous ion concentration will decrease the efficiency. It is important to establish the suitable amount of ferrous ion needed for the electrolysis since the reaction needs to be catalyzed in order to achieve higher efficiency. As shown in Fig. 3.5, the highest efficiency was achieved in the oxidation of 200 mg/L of CLX when the ferrous ion concentration was 1.0 mM.

### 3.2.2 Effect of initial pH solution

The effect of pH on the degradation of CLX in the EF process is shown in Fig 3.6. The experiment was conducted with 200 mg/L of CLX solution for 480 min with 1mM of Fe$^{2+}$ ion as the catalyst.
The electro-Fenton reaction is pH sensitive since the pH can affect the iron solubility, complexation, and redox cycling between 2+ and 3+ states of iron [9]. Thus the viability of the ferrous ion depends on the pH of the solution. A low concentration of ferrous ions will result in a decrease in efficiency since Fe$^{2+}$ acts as catalyst to enhance the conversion of H$_2$O$_2$ into $\cdot$OH (Eq. (3.7)). As shown in Fig. 3.6, the experimental TOC removals after 480 min were 7%, 23%, 49%, and 39% for pH 5, 4, 3 and 2 respectively, clearly indicating that efficiency is considerably influenced by the pH of the solution. These results are in agreement with what many authors have revealed: the technique becomes operative if the treated solution is at the optimum pH of 2.8 – 3.0, where the catalytic behavior of the Fe$^{2+}$/Fe$^{3+}$ coupling can be kept [9,34,37-41].

The lowest TOC removals at higher pH can be explained by the instability of the ferrous ion and the increment in the formation of oxides and hydroxides. In pH > 5, Fe$^{3+}$ species start to form precipitates like Fe(OH)$_3$, resulting in fewer free Fe$^{2+}$ ions which can react with H$_2$O$_2$ and consequently causing a reduction in the $\cdot$OH generation rate. At pH 4.0, the ferric ion disappears because it turns into hydroxyl, such as [Fe(OH)]$^{2+}$, [Fe(OH)$_2$]$^+$, and [Fe(OH)]$^{4+}$, or hydroperoxy complexes like [Fe(OH$_2$)$_{2+}$] and [Fe(OH)(HO$_2$)$_2$]$^+$ [9].

At lower pH values, the low TOC removal rate can be explained by the inability of the hydrogen peroxide to be decomposed into $\cdot$OH by Fe$^{2+}$ since H$_2$O$_2$ captures a proton (H$^+$) from the media and turns into H$_3$O$_2^+$. This electrophilic species slows down the reaction rate between hydrogen
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peroxide and the ferrous ion \[9,40,41]\.

Among the four acidic conditions used in this investigation, the highest \(^\cdot\)OH yield was observed at pH 3.0. As can be seen in Fig. 3.6 there is a clear relationship between pH control at the beginning and the TOC removal. This clearly indicates that strict pH control at the beginning of the process is needed for maximum CLX removal.

**3.2.3 Effect of applied current density.**

The electro-Fenton process is based on the continuous electrogeneration of H\(_2\)O\(_2\) at the cathode (Eq.4). The amount of the H\(_2\)O\(_2\) produced on the cathode depends on the applied current density \[9,42,43]\). Therefore, current density is an important parameter that exerts a large influence on the degradation rate of recalcitrant pollutants which undergo galvanostatic electrolysis. To examine its effect, a series of electrolysis reaction were investigated with varying applied current densities from 2.272 to 8.88 mA/cm\(^2\) to solutions containing 200 mg L\(^{-1}\) at pH of 3.00 for 8 h with 1mM of Fe\(^{2+}\) ion.
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**Fig 3.7(a).** Accumulation of H$_2$O$_2$ electrogenerated with an ACF cathode during the electrolysis of Na$_2$SO$_4$ solution at initial pH 3.0, aerated at 0.1 L O$_2$/min without the catalyst Fe$^{2+}$, using different current densities. 2.27 mA/cm$^2$ (◊), 4.44 mA/cm$^2$ (□), 6.66 mA/cm$^2$ (○) and 8.88 mA/cm$^2$ (Δ). For 180 min. **Fig 3.7(b)** Effect of different applied current densities, from 2.27 to 8.88 mA/cm$^2$, on TOC removal as a function of electrolysis time for 500 mL of 200mg L$^{-1}$ CLX under electro-Fenton conditions.

Fig. 3.7a shows the electrogeneration of H$_2$O$_2$ produced with the same current densities as those used in the EF process. When applied current was intensified, the hydrogen peroxide concentration increased. The rate of change is remarkable during the first 60 mins, and then the H$_2$O$_2$ accumulation becomes steady, indicating that the relationship between H$_2$O$_2$ generation and
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time is not proportional. The experiment was carried out without the addition of Fe\(^{2+}\), even though the H\(_2\)O\(_2\) either did not increase over time or otherwise remained constant because it was decomposed into O\(_2\) by parasitic reactions (Eq. (3.5-3.6)) [9,32,35].

\[
\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{H}^+ + \frac{1}{2}\text{O}_2
\]  

(3.11)

As can be seen in Fig. 3.7b, an increase in the applied current improved the mineralization rate. The TOC removal were 33%, 35%, 49%, and 45% for 2.272, 4.44, 6.66, 8.88 mA/cm\(^2\) respectively. This behavior is attributable to the huge amount of H\(_2\)O\(_2\) generated by the ACF cathode, which can then further react with Fe\(^{2+}\) ions (Eq. 3.7) increasing the concentration of hydroxyl radicals in the medium, that means an increase in the possibilities for the pollutant to react with them. Even so, when the applied current was increased to 8.88 mA/cm\(^2\), the TOC removal rate slightly decreased to 45%. This can be explained by the consumption of excess current in parasitic reactions of hydroxyl radicals, mainly involving the anodic oxidation of hydrogen peroxide which yields the intermediate HO\(_2\)^• radical, according to the (Eq. (3.5)). This phenomena decreases the •OH concentration in the media since it translates into fewer organic oxidation events [6,44].

The above results highlight the significant effect of electric current on the removal efficiency. Our results showed that for 200mgL\(^{-1}\) of CLX, the optimal applied current is 6.66 mA/cm\(^2\), since it facilitates the highest efficiency of TOC removal.

### 3.2.4 Effect of Cefalexin concentration

The oxidation ability of the EF process was evaluated using different drug concentrations under the optimal conditions found in this research. A pH of 3.0, a current density of 6.66 mA/cm\(^2\), with 1.0 mM of FeSO\(_4\) for 480 min. Fig 3.7a shows the effect of different initial CLX concentrations: 50, 100, 200 and 300 mg/L. In all cases, the CLX decreased as a function of time. The TOC removals were 62%, 63%, 49% and 43% respectively for 50, 100, 200 and 300 mg/L of CLX solutions.
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**Fig 3.8 (a)** Effect of different CLX concentrations, from 50 to 300 mgL⁻¹, solutions under the electro-Fenton conditions. **Fig. 3.8 (b)** Mineralization current efficiency change along the electro-Fenton process at different cefalexin initial concentrations.

In all of the concentrations investigated, the EF process was unable to completely mineralize the cefalexin; however, the TOC removal were higher at lower CLX concentrations, for instance at 50 and 100 mg/L. Further increase on CLX concentration causes retardation of reaction due to the increment in the number of collisions between the antibiotic molecules with themselves whereas the probability of collisions between CLX and •OH radicals decrease.

Nevertheless, the oxidative ability of the process changed as a function of cefalexin concentration and electrolysis time. In Fig. 3.8b, the mineralization current efficiencies for each concentration are shown, and it is clear that as the CLX concentration is raised, the MCE increases. A faster reaction rate most likely occurred in the media between the pollutant and the •OH radicals because of the high availability of CLX molecules. Consequently, the competitive non-oxidizing
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reactions became slower and more \(^{\cdot}\)OH radicals can react with CLX molecules. The MCE is considerably lower at the beginning of the oxidation process when the concentration of CLX is small, and does not change much when the concentration is raised from 50 to 100 mg/L. At higher CLX a higher MCEs was observed. The maximum efficiencies were during the first 180 min in all cases, and after this period the MCE slowly drops, suggesting that the byproducts formed during that period are more easily oxidized by \(^{\cdot}\)OH radicals than the intermediate formed during that time [11, 42]. A progressive reduction in the MCE after 360 min occurred due to the decrease in oxidation events, as can be seen in Fig. 3.8 b.

3.3 Biodegradability

Even though it is possible to completely mineralize a substrate using an AOP, it is much more cost effective to use it only to enhance the biodegradability of such substrate before integrating with biological processes [14, 36].

To decide the optimal time to introduce the biological process to run along with the AOP, the biodegradability of the pretreated effluent needs to be assessed. During the electro-Fenton process, the CLX molecule is oxidized non-selectively by \(^{\cdot}\)OH radicals, and it is known that sometimes the intermediates can be either considerably more toxic or less biodegradable than the treated compound [45].
Fig 3.9 (a) COD concentration and CLX concentration measured by HPLC during 24h of electro-Fenton process of 500mL of 200 mgL⁻¹ of CLX (b) The four stages of CLX intermediates evolution along 24h of the electro-Fenton process. (c) Biodegradability change in terms of BOD₅/COD and BOD₁₄/COD along the 24h of the electro-Fenton process

In this study, an analysis of the BOD₅, BOD₁₄ and COD was carried out to determine the changes in the CLX biodegradability. These results showed that untreated CLX was non-biodegradable, with biodegradability in both the BOD₅/COD and BOD₁₄/COD ratios of zero. Also the EF process was analyzed for 24h in terms of COD concentration, CLX concentration and biodegradability changes.

Fig. 3.9a compares the drop of COD and CLX concentrations during the EF process. After 4.5h of the EF process there was no more CLX in the reactor, but the COD removal was only 47%. The COD
removal slowly continued along with the process, at 8h the removal was 72% and at 24h it had increased only 88%.

The COD is widely used for determining the strength of wastewater streams. The COD can be calculated based on the theoretical oxygen demand (ThOD), which is the stoichiometric amount of oxygen required to oxidize a compound into end products such as CO$_2$, NH$_3$, H$_2$PO$_4^-$, SO$_4^{2-}$, and H$_2$O [46]. Therefore, the COD of concentration of CLX can be calculated theoretically based on the (Eq. (3.12)).

$$C_nH_mO_eX_kN_jSiPh + bO_2 \rightarrow nCO_2 + \frac{m-k-3j-2i-3h}{2}H_2O + kHX + jNH_3 + hH_2SO_4 + hH_3PO_4$$

(3.12)

Where X represents the sum of all halogens and b is given by (Eq. (3.13))

$$b = n + \frac{m-k-3j-2i-3h}{4} \cdot \frac{e}{2} + 2i + 2h$$

(3.13)

From these equations 1g of CLX correspond to 1.7 g of COD ($\alpha$), so that the intermediates in the reaction bulk can be calculated by the (Eq. (3.14)).

$$\text{Intermediates COD} = \text{Total COD} - \alpha \cdot \text{CLX}$$

(3.14)

Where $\alpha$ is an empirical constant that correlates the theoretical COD of CLX with the empirical COD change during the oxidation of CLX in the EF process.

The evolution of the intermediates along the EF process showed in Fig. 3.9b, were estimated by the above equation (Eq. (3.14)) by means of COD change [46]. The concentration of intermediates increased quickly at the beginning of the process. After 2 h, the concentration of the intermediates peaked and then substantially decreased during the next 5 hours.

Fig. 3.9c shows the evolution of the biodegradability at different times long the 24h of the EF process. Both ratios of the BOD$_5$/COD and BOD$_{14}$/COD increased as a function of time, but this was particularly true of BOD$_{14}$/COD. The oxidation process could be divided into four stages as shown in Fig. 9. During the first stage, the CLX is quickly oxidized by $^*$OH radicals with that the concentration of the intermediates increasing considerably and reaching a maximum at 2hrs, but CLX was still present in the media. At this stage, the BOD$_5$/COD and BOD$_{14}$/COD were 0.05 and 0.08, still non-biodegradable.

The second stage is from 2h to 4.5h, when all the CLX had been oxidized, making it undetectable. At this stage, the concentration of intermediates dropped, suggesting that the produced intermediates had further oxidized. However, the biodegradability was still limited: it was 0.1 in the BOD$_5$/COD and 0.2 in the BOD$_{14}$/COD even though there was no more CLX.
Biodegradability Enhancement of Cefalexinterature

The third stage was from 4.5 to 8h, when the biodegradability increased to 0.26 in the BOD$_5$/COD and 0.43 in the BOD$_{14}$/COD. According to these results, it would be possible to switch the EF process for a biological process after 8h, even though the BOD$_5$/COD ratio is not optimal since the BOD$_{14}$/COD is high enough to consider the introduction of a biological treatment.

Finally, the fourth stage was from 8 to 24 h. During this stage, the intermediates kept reacting with the •OH radicals, in effect significantly enhancing the biodegradability, at 0.71 for BOD$_5$/COD and 1.0 for the BOD$_{14}$/COD. The above results suggest that even though the biodegradability increases as a function of treatment time, the time required to completely mineralize CLX (more than 24h) make it unfeasible. However, the EF does enhance the biodegradability sufficiently for use in combination with a biological treatment process [47,48].

3.5 Conclusions

This study demonstrated that the electro-Fenton process is the most effective oxidizing process for the enhancement of cefalexin biodegradability among three AOPs: Anodic oxidation (AO); AO-H$_2$O$_2$ electro generated hydrogen peroxide (AO-H$_2$O$_2$), and electro-Fenton (EF) processes.

Changes in the parameters used, including the ferrous ion catalyst concentration, the pH, the current density, and cefalexin concentration, resulted in significant differences in the efficiency of the electro-Fenton process. The results support the combination of the electro-Fenton process as a pretreatment for further biological treatment process since the biodegradability is sufficiently enhanced.
3.6 References


Biodegradability Enhancement of Cefalexinterature


Biodegradability Enhancement of Cefalexinterature

Conference EEE. 1-4, Henan.


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Biodegradability Enhancement of Cefalexinterature


Variations during the Electro-Fenton process: cathode changes and different transition metals as catalyst
Variations During the EF process

4.1 Introduction

The electro-Fenton process is an indirect electrochemical oxidation, where a strong oxidizing agent is electro-generated in our case is the hydroxyl radical. The free radicals electrogeneration is done by steps. First, the hydrogen peroxide is electro-generated at the cathode, and then the produced \( \text{H}_2\text{O}_2 \) reacts with a metallic catalytic mediator, which can be any transition metal, to produce hydroxyl radicals. The choice of the electrode material is a decisive election that will affect the selectivity and the efficiency of the process. The electrode material must have the following properties:

- High physical and chemical stability; resistance to erosion, corrosion and formation of passive layers.
- High electrical conductivity
- Catalytic activity and selectivity
- Low cost/life ratio (inexpensive and durable) [1]

In this experiment the anode is a titanium mesh coated with Ruthenium oxide (RuO\(_2\)). RuO\(_2\) electrodes have a high stability, but they are too expensive. So, to overcome this problem those electrodes were improved by using a Ti-mesh coated with RuO\(_2\). At the anode, the oxidation of water takes place, it leads the evolution to \( \text{O}_2 \) and a proton is released. This proton promotes the reduction of metallic catalyst \( \text{M}^{n+1} \), and the catalyst behavior can be kept for longer time [2].

In this research, the used cathode is an activated carbon felt (ACF) attached to a Ti-mesh coated with RuO\(_2\) which acts as support and as electricity conductor. ACF cathode has been reported to exhibit a high yield concentration of hydrogen peroxide working under its optimal operational conditions: pH of 3.00 and a current density of 6.66 mA cm\(^{-2}\) [3].

Activated carbon fiber has been used as a cathode because it poses various advantages. It has a high surface area, in other words in a small volume is possible to have bigger contact area. The ACF is composed by microcrystallites that consist of fused hexagonal rings of carbon atoms, the spaces between the individual microcrystallites are called pores and most of the adsorption processes take place there. The adsorption process occurs as a result of van der Waals forces and chemical bonding.

The fundamental property of the surface area is its additive condition, thus the surface area is the sum of the whole areas in a geometric figure. This characteristic of the ACF is due to its great porosity of 900-2500 m\(^2\) g\(^{-1}\), these micropores are directly open to the outer surface. In addition, it has excellent adsorption capabilities, good mechanical strength, and high conductivity, which may lead to high capacitance at a dense current and low inner resistance due to the easy ion transfer [1]. Moreover, it has a low cost and is not toxic. Finally, activated carbon felt are chosen over another forms of activated carbon because it can be use directly as electrode [2].
Variations During the EF process

The adsorption process is influenced by different factors, such as the activated carbon surface which has polar nature. Therefore, activated carbon fiber adsorbs non-polar molecules better than polar molecules. Also larger molecules are better absorbed as well if they have more functional groups such as double bonds or halogens [4]. The solubility of the adsorbate in the solvent where the adsorption occurs should have an inverse relationship [5].

The adsorption process could be also a reversible process known as desorption. However the dynamic equilibrium could be reached after sufficiently time.

The pH is also important because it can change the charge of the ACF as well of the adsorbant.

Finally, the nature of the adsorbant is also important. In this case the cefalexin antibiotic posses in their molecule two ionizable groups; it is a zwitterion. The zwitterions according to the pH where they are will have a different charge; the cefalexin ionic forms are given in Table 4.1.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Isoelectric</th>
<th>Anion</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Cation Structure" /></td>
<td><img src="image" alt="Isoelectric Structure" /></td>
<td><img src="image" alt="Anion Structure" /></td>
</tr>
</tbody>
</table>

It has been reported that the sorption is favored in acidic conditions (pH 2.5), and that the 80% of the adsorption occurred rapidly within 2h, followed by a gradually slow process until 60h when the equilibrium is achieved [6].

The Fenton reaction was first studied by H. Fenton with iron as a catalyst; however this reaction became important in the biological systems because it is involve with the oxidation process within the cells. At that time it was discovered that the Fenton reaction can be catalyzed by other transition metal ions.

The transition metals have partly filled d subshells either in the neutral atom or in ions in common oxidation states, when this orbital is incomplete the element may have different oxidation states formed by the unpaired electrons. The unpaired electrons make them more reactive, thus they can easily form bonds with other elements or molecules, which made them a good catalyst [7].

In previous analysis of the Fenton reaction in the biological systems it was reported that the reaction was either catalyzed by Fe, Mn, Co, Ni, or Cu ions [8]. As a consequence, some researchers have started to use them as catalyst for the Fenton reaction in other applications rather than biological systems, such as wastewater treatments. So the classical electro-Fenton process can be
Variations During the EF process

carried out with any appropriate homogenous redox couple \( M^{(n+1)/M^n} \) according to the general reaction:

\[
\text{H}_2\text{O}_2 + M^{n+} \rightarrow M^{(n+1)} + \text{OH}^- + \cdot\text{OH}
\]  

(4.1)

Several metal cations have been tested as catalyst. In this case the electro-Fenton process is influenced by the standard potential of the \( M^{(n+1)/M^n} \) redox couple used and by scavenging catalyst effect [9]. Table 4.2 shows the standard potential for each catalyst redox couple.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Redox couple</th>
<th>Potential (( E^\circ ))</th>
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</thead>
<tbody>
<tr>
<td>(Fe(^{2+})) Iron</td>
<td>Fe(^{3+}/Fe^{2+})</td>
<td>0.77 V</td>
</tr>
<tr>
<td>(Mn(^{2+})) Manganese</td>
<td>Mn(^{3+}/Mn^{2+})</td>
<td>1.50 V</td>
</tr>
<tr>
<td>(Co(^{2+})) Cobalt</td>
<td>Co(^{3+}/Co^{2+})</td>
<td>1.92 V</td>
</tr>
<tr>
<td>(Cu(^{2+})) Copper</td>
<td>Cu(^{2+}/Cu^{+})</td>
<td>0.16 V</td>
</tr>
</tbody>
</table>

In this chapter the Activated Carbon Fiber cathode was analyzed by SEM in order to understand and compare the changes that suffer before and during the electro-Fenton process. Also the effect of different metal ions used as a catalyst was investigated in the electro-Fenton degradation of the antibiotic cefalexin.

4.1 Materials and methods

4.1.1 Chemical and analysis

Analytical grade reagents were used in the experiments. Cefalexin was purchased from Wako (99%). The pH values of solutions were adjusted with \( \text{H}_2\text{SO}_4 \) whenever it required. Ferrous sulphate heptahydrate (99%, Wako) was used as the catalytic Fe\(^{2+}\) species, Copper (II) sulfate pentahydrate (99%, Wako) was used as the catalytic Cu\(^{2+}\), Manganese (II) sulfate pentahydrate (99%, Wako) was used as the catalytic Mn\(^{3+}\), Cobalt (II) sulfate heptahydrate (99%, Wako) was used as the catalytic Co\(^{2+}\). All the experiments were prepared with Ultra-pure water obtained from a Millipore AutoPure WT101UV system (Millipore S. A., Molsheim, France) with resistivity of 18.2 M\( \Omega \) cm at 25°C. Pressurized oxygen gas (99.9%) was used to saturate the solutions. The activated carbon fiber (ACF) felt was purchased from Xuesheng Technology Co. Ltd. (Shandong, China). The ACF size was 6 x 9 cm.
4.2.2 Batch oxidation experiments

All experiments were performed in batch mode at room temperature and were conducted in an open undivided cylindrical cell containing 500 mL of Na$_2$SO$_4$ 0.05M solution as the supporting electrolyte; the batch was stirred with a magnetic bar to ensure homogeneity and the transport of reactants toward/away from the electrodes. The electrode was connected to a DC power supply AD-8735 (Tokyo, Japan) operated at the desired electric current intensities displayed throughout the experiment.

The working electrode was compromised of, an anode made from 54cm$^2$ of RuO$_2$/Ti mesh and a cathode of activated carbon felt (ACF) with the same size. It was saturated with a 200 mg/L of CLX solution for 12h three times before the experiments to avoid any TOC decrease due to the absorption. The Anodic Oxidation was carried out without H$_2$O$_2$ in the solution, and with the same RuO$_2$/Ti mesh used as both anode and cathode. The scheme of the reactor as well of a picture of the cathode are showed in Fig. 4.1

The three processes were carried through the same operational conditions, at pH of 3.00, with a current density of 0.36 A, an oxygen input flow rate of 0.1 L/h and an operational volume of 500 mL. Furthermore the experiments were accomplished in 480 minutes.

Prior to electrolysis, the solutions were aerated for 30 min with pure oxygen at 0.1 L/min rate and gas was input throughout the reaction time.

For each experiment, samples were collected at regular time intervals (0, 30, 60, 90, 120, 180, 270, 360 and 480 min). Each of them was filtered through hydrophilic PTFE filters with a porosity of 0.45 µm, purchased from Millipore.

**Fig 4.1** Scheme of the reactor, showing the cathode. Next to the scheme is placed the picture of the ACF used as cathode after saturated with a CLX solution of 200 mg/L
4.2.4 Biological Oxygen Demand. (BOD)

The biological oxygen demand was measured by an OxiTop® BOD controller model 1020 (WTW, Welhilheim, Germany) thermostated at 20°C. The pH of the samples was adjusted to a value between 6.8 and 7.5 before the addition of the bacterial seed and the necessary nutrients for bacterial growth. The BOD$_5$ was measured by this equipment.

4.2.5 HPLC measurement

The CLX concentration during the experiments was quantified by high pressure liquid chromatography, (HPLC) equipped with UV detector (262nm) (Waters 510 pump equipped with 712 WISP autosampler, Water Associates, USA), and a Spherisorb ODS-2.25cm x 4.6 cm column was used as the stationary phase. The mobile phase was a mixture of 0.01 M NaH$_2$PO$_4$; methanol (75:25). The flow rate was adjusted to 0.5 - 1 mL/min with, the temperature was maintained in the range of 30-40 °C. Evaluation and quantification were made using a chromatography data system [28].

4.2.6 Total Organic Carbon (TOC)

The TOCs of the initial and electrolyzed samples were determined with a TOC-analyzer (Shimadzu 5000A). Platinum catalysis was used in the combustion reaction. The carrier gas was oxygen with a flow rate of 150 mL min$^{-1}$. The detector of the TOC system was a non-dispersive infra-red detector, NDIR.

4.2.7 Mineralization current efficiency (MCE)

TOC data allow the percentage of MCE to be determined for each treated solution at any given time by the equation

$$\text{MCE} = \frac{nFV_s \Delta \text{(TOC)exp}}{4.32 \times 10^7 \text{ ml}} \times 100$$

Where $F$ is the Faraday constant (96487 C mol$^{-1}$), $V_s$ is the solution volume (L), $\Delta \text{(TOC)exp}$ is the solution decay (mgL$^{-1}$), 4.32 X 107 is a conversion factor to homogenize units (3600 s h$^{-1}$ x 1200 mg mol$^{-1}$), $m$ is the number of carbon atoms of cefalexin, and $I$ is the applied current (A) [29]. The number of electrons (n) consumed per cefalexin was taken as 70e$^{-}$, assuming the complete mineralization of CLX to CO$_2$ with the release of SO$_4^{2-}$, and NH$_4^+$ as the main primary ions, after measuring the nitrogen species. The reaction describing this process is:

$$C_{16}H_{17}N_3O_4S + 32 H_2O \rightarrow 16CO_2 + 3NH_4^+ + SO_4^{2-} + 69 H^+ + 70e^-$$
4.2.8 Nitrogen species measurement

Ammonium (NH$_4^+$) concentration in treated solutions was determined from the standard colorimetric automated phenate method with above HACH DR/4000U Spectrophotometer. The formation of NO$_3^-$ and NO$_2^-$ in the photo-electro-Fenton (PEF) process was measured colorimetrically by an autoanalyser (QUAATRO; BLTEC, Tokyo, Japan).

4.2.9 Scanning Electron Microscopy

The images of the ACF cathode were taken with a JSM-6500F. JEOL Scanning Electron Microscopy. The images were captured as SEI (secondary electron imaging) and EDX (energy dispersive X ray). The samples of the ACF were dry at the analysis moment. They were prepared by gold coating before the analysis.

4.3 Results and discussions

4.3.1 Changes on the surface of the ACF cathode

The Activated Carbon Fiber has been used as a cathode because it is a good material with high conductivity, high surface area and low price. ACF is considered a three-dimensional electrode where hydrogen peroxide can be produced.

The cathodic synthesis of the H$_2$O$_2$ begins with the water oxidation on the anode:

\[ 2\text{H}_2\text{O} \longrightarrow \text{O}_2+4\text{H}^++4\text{e}^- \]  \hspace{1cm} (4.3)

Then the mechanism continues with the cathodic reduction of oxygen.

\[ \text{H}_2\text{O}_2+2\text{H}^++2\text{e}^- \longrightarrow 2\text{H}_2\text{O} \]  \hspace{1cm} (4.4)

Reaction 4.4 takes place in the porous of the ACF, as a consequence the ACF cathode surface will change along with the used time. Therefore the efficiency will be deteriorated.

Fig. 4.4 presents the comparison of the changes on the surface of the ACF. The ACF surface was analyzed by SEM-SEI microimages at different times, before and after the saturation process with the antibiotic, and lastly after 8 electro-Fenton processes.
Variations During the EF process

Fig. 4.2.a SEM micrograph of the ACF new, before any process.

Fig. 4.2.b SEM image of the ACF saturated with CLX.

Fig. 4.2.c SEM image of the ACF after been used in the EF process for 8-10 times.

Fig. 4.2.a shows the image of the original ACF before saturated. It is possible to see the fibers of the ACF surface looks homogeneous with some small free fibers. Even the micrograph is not colored the color of the fibers is the same, in other words no different material than carbon from the ACF was detected.

The saturation process of the ACF was done in three batches of 12h each one. The first one was with a cefalexin water solution without pH adjustment, in the next two saturations the pH was adjusted to 3.00. The pH of 3.00 was chosen because is the same pH at which the EF process is carried out, coincidentally at this pH the adsorption of CLX by the ACF is enhanced [Liu].

Fig. 4.2.b presents the image of the ACF surface after it is completely saturated. As can be seen the surface become smooth, no free small fibers can be observed because it is covered by a membrane-like substance. Some depositions of the antibiotic can be observed, they look like round pats over the fiber. The depositions in the ACF would block the pores, thus the adsorption capability of the ACF decreases. Even though some desorption process may occurred but will be as neglected. The complete saturation of the ACF is important in order to assure that the decrease on the CLX
Variations During the EF process

concentration was due to the oxidation process and not for the adsorption in the ACF.

The ACF is expected to change after used as a cathode in the electro-Fenton process, Fig. 4.2.c shows the change of ACF surface after used in 8 electro-Fenton processes. In this image is possible to notice the presence of white spots due to material with higher density detected by X rays, may be that material is the iron catalyst. The surface also presents some small damages, the fiber looks like tear in the upper part. It could be possible done by the iron in the media bulk.

![SEM-EDX image of the ACF after been used in the EF process for 8·10 times](image)

With the Scanning electron microscopy is possible to identify metals and some other compounds [4] in a technique referred as Energy-dispersive X-ray spectroscopy (SEM-EDS or SEM-EDX). This analytical technique relies on the principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray spectrum [5]. Fig. 4.3 shows the SEM-EDX of the ACF after the EF process, in that image two parts on the same fiber were compared. One belongs to a white spot in the fiber; it was expected to be a metal part. As seen in the analytical diagram (a) in Fig. 4.3, it was confirmed that in the white part iron is present as well of some other elements. The other elements detected in the diagram (a) were: the peak \( \alpha \) belongs to carbon, the peak \( \beta \) is due to oxygen, the peak \( \gamma \) to sulfur, and the peak \( \delta \) to iron. There are two peaks belonging to iron, it means that it can be detected at different retention times. On the other hand, the second analyzed point was allegedly to be one part of the ACF. The results showed in the analytical diagram (b) confirm that it was part of the ACF. The main composition is carbon as showed in peak \( \alpha \), then the second element was sulfur (peak \( \gamma \)) from the electrolyte \( \text{Na}_2\text{SO}_4 \), the third was oxygen, but iron was not detected.

The above findings suggest that the ACF cathode change a long of the process and thus it is
Variations During the EF process

It is recommendable to change it in order to keep its efficiency.

4.3.2 Different metal ions as catalyst for the electro-Fenton process

4.3.2.1 Comparative removal efficiency of each catalyst.

The experiments were carried out under the same operational conditions using different transition metal as catalyst in each experiment. Fig. 4.4 depicts the TOC abatement along with each electro-Fenton process. The TOC removals were found as 49% for the ferrous ion (Fe$^{2+}$), 40% for the manganous ion (Mn$^{2+}$), 60% for the cupric ion (Cu$^{2+}$) and 81% for cobaltus ion (Co$^{2+}$). The highest TOC removal was reached with the Co$^{2+}$ ion, this result agrees with Pimentel [9], Co$^{2+}$ is a better catalyst than Mn$^{2+}$ and Cu$^{2+}$ ions, yet it is also better than Fe$^{2+}$ ion. In our experiment Fe$^{2+}$ and Mn$^{2+}$ ions showed similar degrade behaviors. Between these two ions, Fe$^{2+}$ and Mn$^{2+}$, ferrous ion was a better catalyst since the TOC removal was higher but the difference was only of 9%. The low efficiency with the manganous ion could be ascribed to the electrode deposition reported in several electrochemical studies in acidic solutions according to the reaction Eq. 4.5:

\[
\text{Mn}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{MnO}_2(s) + 4\text{H}^+ + 2\text{e}^- \quad (4.5)
\]

In the molecule MnSO$_4$, the sulfate anion is changed for oxygen because the manganese is oxidized from 2+ to 4+, this reaction is favored in acidic media.

Ferrous ion was deeply studied in our previous research; it was found that a concentration of 1mM yields the best catalytic behavior in the electro-Fenton process. However, ferrous ion was not the best catalyst, since higher removals were achieved with cupric and cobaltous ions. The lower
Variations During the EF process

The efficiency of Fe$^{2+}$ could be attributed to the scavenging reactions between the ferrous ion with the hydroxyl radicals, as shown in Eq. 4.6.

$$\text{Fe}^{2+} + \cdot\text{OH} \rightarrow \text{FeOH}^{2+} \quad k = 4.3 \times 10^8 \text{ M}^{-1} \text{s}^{-1} \quad (4.6)$$

In the electro-Fenton process copper is the second most used catalyst after iron. However, their efficiency is different; sometimes one is more efficient than the other [12,13]. In our experiment, we found that the cupric ion has better catalyst action than ferrous ion and its removal trend is more alike with cobaltous ion. At the beginning of the process the removal is faster in both cases Cu$^{2+}$ and Co$^{2+}$, but with the Cu$^{2+}$ ion after 270 min the removal rate decreased. It could be explained by the cathodic deposition of copper on the Ti/RuO$_2$ mesh, thus less copper ions were available to participate in the Fenton reaction. The deposition of copper in the cathode can be justified by the values of the standard reduction potential of Cu (II) (E$^{°}_{\text{Cu}^{2+}/\text{Cu(s)}}$ = 0.34V/SHE) and Cu (I) (E$^{°}_{\text{Cu}^{+}/\text{Cu(s)}}$ = 0.52 V/SHE) ions, which favor its easy reduction [9]. The highest removal over the ferrous and manganese ions can be explained by the two steps of the reaction: (i) oxidation of the Cu$^{2+}$ complexes with the intermediates and (ii) the production of small amounts of $\cdot$OH in the medium form the Cu$^{2+}$/Cu$^+$ catalytic system involving the reduction of Cu$^{2+}$ to Cu$^+$ with HO$_2^*$ radicals Eq. 4.7 and/or with organic radicals R$^*$ by reaction Eq. 4.8

$$\text{Cu}^{2+} + \text{HO}_2^* \rightarrow \text{Cu}^+ + \text{H}^+ + \text{O}_2 \quad k = 5 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{s}^{-1} \quad (4.7)$$
$$\text{Cu}^+ + \text{R}^* \rightarrow \text{Cu}^{2+} + \text{R}^* \quad (4.8)$$

Followed by the regeneration of Cu$^{2+}$ by oxidation of Cu$^+$ with H$_2$O$_2$ from the Fenton-like reaction Eq. 4.9

$$\text{Cu}^+ + \text{H}_2\text{O}_2 \rightarrow \text{Cu}^{2+} + \cdot\text{OH} + \text{OH}^- \quad k = 5 \times 10^4 \text{ dm}^3 \text{ mol}^{-1} \text{s}^{-1} \quad (4.9)$$

The highest removal was achieved when using cobaltus ion as the catalyst. The higher catalytic action over Mn$^{2+}$ and Cu$^{2+}$ could be attributed to the available amount in the reaction bulk, since cobaltus and the ferrous ions cannot be deposited in the cathode because of the cathode potential that is (-0.25 V/SHE). And the higher efficiency over the ferrous is because the scavenging reaction between cobalt and hydroxyl radicals is lower than the one for iron.

$$\text{Co}^{2+} + \cdot\text{OH} \rightarrow \text{CoOH}^{2+} \quad k = 8 \times 10^5 \text{ M}^{-1} \text{s}^{-1} \quad (4.10)$$

Finally from Fig. 4.4 is clear that any of the four tested transition metals could be used as catalyst in the electro-Fenton process, since the removals are bigger than the removal done just by electrogennated hydrogen peroxide (previously reported in Chapter 3).
Variations During the EF process

4.3.2.2 The Mineral Current Efficiency.

![Fig. 4.5 Mineral current efficiencies for the electron-Fenton process using different catalyst.](chart)

The Mineral Current Efficiency is used in the electrochemical processes as an indicator of how efficiently the electrical current is been used. The MCE was evaluated as function of time for each of the electrolysis. As showed in Fig.6, every metal has different MCE. The highest MCE of 30% was for the EF process catalyzed with Cu²⁺ ion in the minute 90. In this process the MCE was low at the beginning but it fast enhanced to from 17% to 30%, then it sharply decreased to the half at minute 180 and kept almost constant with a slightly decrease at the end of the electrolysis. The behavior could be ascribed to the copper deposition in the cathode. At the beginning of the process the cupric ion was free in the media bulk, thus it can reacts with the electrogenerated hydrogen peroxide which coincidently reached the highest concentration at 90 minutes (previously reported in Chapter 4) at the same time when copper has the higher MCE. Although, this tendency cannot be continued due to the cathodic deposition of copper either as Cu²⁺ or Cu⁺, as consequence less cupric ion are available to react with the electrogenerated hydrogen peroxide.

In the case of iron as the catalyst, the higher MCE is at the beginning of the process. It suggests a quick destruction of CLX during the first 60 minutes. Then the MCE sharply decreased within the next 60 minutes and kept slowly decreasing along of the process. The decrease could be due to the formation of some coordinated organic compounds with iron, either as Fe²⁺ or mostly as Fe³⁺ ions. Thus the recovery of the catalyst ion Fe²⁺ is not possible, therefore the catalytic decomposition of hydrogen peroxide into hydroxyl radicals is limited. As a result less oxidation events takes place, besides may be the formed byproducts are more difficult to degrade. [14]

The MCE for manganese as catalyst as seen in Fig. 4.5 has the lowest values in comparison with
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other metals. At beginning of the process the MCE was a little higher than in the rest of the process and it kept almost constant until the eight hours of the process. The low efficiency of the manganese ion could be explained by its deposition on the cathode which occurs from the beginning of the process. During the deposition the oxidation state changed form Mn$^{2+}$ to Mn$^{3+}$ to produce MnO$_2$. Eq. 4.5. It was evidenced by the color change of the solution and the cathode to orange color [15].

On the other hand, when cobaltous is used as catalyst the MCE showed a different behavior than those presented by the other metals. At the beginning of the process, after 60 minutes, it has a drop in the efficiency. Later on the efficiency increased and remained almost constant during the whole experiment. Actually, the efficiency slightly increased in the last half of the experiment. The MCE for the last two hours was almost the same as the MCE of the cupric ion.

The MCE values showed in Fig. 4.5 reveals that the metals have different behavior to decompose the electrogenerated hydrogen peroxide and the best metals to be used as catalyst are cupric as well of cobaltous ions.

4.3.2.3 Inorganic intermediates.

![Ammonia accumulation during the electro-Fenton process using different catalyst ions.](image)

Fig. 4.6 Ammonia accumulation during the electro-Fenton process using different catalyst ions.

During the electro-Fenton process not only the organic matter is oxidized also the inorganic substances are susceptible of been attacked by the hydroxyl radicals. In this case the inorganic substances refer to the heteroatoms inside the cefalexin molecule. Cefalexin poses two heteroatoms, sulfur and nitrogen. Sulfur is supposed to be oxidized to sulfate, however it was not
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measured because it is not possible in our experiment since the electrolyte is Na₂SO₄. On the other hand, the nitrogen evolution was analyzed in terms of ammonia, nitrite and nitrate accumulation.

Fig. 4.6 shows the behavior of the ammonia concentration during the 8h of the electro-Fenton process for each metal. As could be noticed, the trend on the accumulation was quiet similar for all the metals. It suggests that may be the oxidation mechanism is closely similar to each other.

The ammonia generation in the electro-Fenton process can be explained by the reaction between the hydroxyl radicals and the nitrogen moieties in the molecule. In the cefalexin structure, there are three nitrogen atoms. Two are in the structure forming amino moieties, as secondary and ternary. The other one is included in the heterocycle of the β-lactamic ring. The total amount of nitrogen in 200 mg of cefalexin is 23 mg; it has been stated that the stoichiometric amount of nitrogen is not achieved usually the highest achievement is about 50%. In all the process the accumulated amount was similar around the 30%.

The ammonia accumulated by the electro-Fenton process carried out with Mn²⁺ ion was the lowest, the accumulation after 8h of treatment was 6.3 mg/L. In the case of Fe²⁺ ion, the final accumulation was 7.0 mg/L. The next accumulated concentration was for Co²⁺ ion where the ammonia reached a concentration of 7.4 mg/L. Finally, the highest accumulation was in the EF process carried out with Cu²⁺ ion with 8.0 mg/L. The ammonia formation happens when the oxidation state of nitrogen in the moiety is -3. Also form nitrogen inside of a heterocycle that could be either transformed to NH₄⁺ or NO₃⁻ ions. In addition, secondary, ternary and quaternary nitrogen atoms are pronominally converted to ammonium ion [16].

Through the electro-Fenton process carried out with the cupric ion, the ammonia accumulation was different from the other catalyst. In this case the main accumulation of ammonia was in the first 270 minutes of the process. Then, the concentration remained almost the same until the end of the process. It suggests that copper is maybe reacting with the amino groups since this reaction is favored in acidic conditions. In this case the amino group acts as Bronsted-Lowry base that can react with the ion formed by copper [Cu(H₂O)₆]²⁺ (Eq. 4.11) for primary amines and (Eq 4.12) for amines with more organic radicals. The formation of the copper ion, [Cu(H₂O)₆]²⁺, has been reported in sulfate acidic solutions. So, it is also possible that cupric ion form coordinated compounds with amines [17,18]. The reaction is in equilibrium so its effect is low but could be enough to make the difference on the ammonia behavior when cupric ion is used as catalyst.

\[
\begin{align*}
[Cu(H₂O)₆]²⁺ + 2NH₃ & \rightleftharpoons [Cu(H₂O)₆(OH)]₂⁺ + NH₄⁺ \quad (4.11) \\
[Cu(H₂O)₆]²⁺ + R₂-NH & \rightleftharpoons [Cu(R₂-NH)(OH)]₂⁺ + NH₄⁺ \quad (4.12)
\end{align*}
\]

To sum up, during the four processes carried out with different catalyst the ammonia accumulation showed very similar behavior, just when copper was used as catalyst the accumulation trend was
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different. However in all cases the amount of ammonia was very similar.

4.3.2.4 Nitrite and nitrate ions en each experiment.

The nitrogen moieties could be also oxidized into nitrite and/or nitrate ions. Usually the main byproduct in the electro-Fenton process is ammonia rather than the before mentioned ions. Moreover, in the photocatalysis experiments also has been reported that if the ratio between $\text{NH}_4^+/\text{NO}_3^-$ is analyzed and measured, ammonia quickly appears and kept higher than nitrate ion. Regarding to the nitrite ions it accumulation tends to be lower than the other two nitrogen byproducts.

**Fig. 4.8a** Accumulation of nitrate and nitrite species during the degradation of a 200 mg/L of CLX solution using cupric ion as the catalyst for the electro-Fenton process. (b) Comparison
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of the accumulation of nitrite and nitrate ions in the electro-Fenton process carried out with different catalysts.

Fig. 4.8a shows the accumulation of the nitrate and nitrite ions in the electro-Fenton process using cupric ion as catalyst. During the process nitrate ion was highly accumulated to a concentration of 0.035 mg/L while the nitrite ion accumulation was only of 0.002 mg/L. Actually the nitrite accumulation could be neglected since the concentration is too low. In this process the oxidation of nitrogen moieties into nitrate is higher than in the other EF process catalyzed by different metal ions.

The formation of nitrate ion is mainly produce by the direct attack of the hydroxyl radicals to the nitrogen instead of the adjacent (α) carbon atoms. Another explanation is that if the amine has more radical groups the transformation into ammonia will take longer time than the time to be converted into nitrate ion.

In general, the behavior of the nitrogen inorganic byproducts in the process carried out with cupric ion was different as can be noticed in Fig. 4.8b. The nitrogen groups were oxidized since the beginning of the process not only at the end. Finally, they were mainly oxidized as NH$_4^+$ and NO$_3^-$, the accumulation of the NO$_2^-$ was not significant.
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**Fig. 4.9a** Accumulation of nitrate and nitrite species during the degradation of a 200 mg/L of CLX solution using manganese ion as the catalyst for the electro-Fenton process. (b) Comparison of the accumulation of nitrite and nitrate ions in the electro-Fenton process carried out with different catalysts.

Fig. 4.9a depicts the accumulation of the nitrate and nitrite ions in the electro-Fenton process when the ion Mn$^{2+}$ is used as catalyst. During the process nitrate ion is slowly accumulated in the first 180 minutes, later on the accumulation became faster. However the final accumulation is only of 0.013 mg/L. The behavior of the accumulation of the nitrite ion is similar to the one presented by the cupric ion, in other words it can be consider as insignificant. It was only of 0.005 mg/L. As shown in Fig. 4.9b the manganese ion presented the lower final accumulation of nitrate ion during the electrolysis.

These results were expected because the oxidation efficiency of the manganese ion was the
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lowest among the other metal ions. Even though is important to remark that the oxidation up to nitrate ion was possible since no nitrite ion was accumulated. In this process the accumulation of nitrogen byproducts was the lowest. The total nitrogen oxidation attained by the EF process catalyzed by the Mn2+ ion was of 28%.

**Fig. 4.10a** Accumulation of nitrate and nitrite species during the degradation of a 200 mg/L of CLX solution using cobaltus ion as the catalyst for the electro-Fenton process. (b) Comparison of the accumulation of nitrite and nitrate ions in the electro-Fenton process carried out with different catalysts.

Fig. 4.10a presents the accumulation of the nitrate and nitrite ions during the electro-Fenton catalyzed by the ion Co2+ for 8h. The accumulation of the nitrate ion is quite different in this process in respect with the other three processes because since the beginning of the process until 180 minutes there was no generation of nitrate ion. On the other hand nitrite ion was accumulated until
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180 minutes when the nitrate ions reached the highest concentration in the process. It indicates that the nitrogen moieties were first oxidized to $\text{NO}_2^-$, and later this ion was oxidized into $\text{NO}_3^-$. As the process continued the nitrite ion kept decreasing while the nitrate ion significantly increased. It has been reported that the nitrate ion formation usually has an induction period, suggesting that several steps occurred before nitrates ions are formed. Only in this process the nitrate formation steps are clear. The final concentration of the nitrate ion was 0.017 mg/L.

The accumulation of the ammonia ion showed a tendency very similar to the other processes carried out with the $\text{Fe}^{2+}$ and $\text{Mn}^{2+}$ ions, as can be noticed in Fig. 4.10b.

Finally, the total oxidation of the nitrogen moieties by the EF process using cobaltous ion as catalyst was of 32%.

![Accumulation of nitrate and nitrite species during the degradation of a 200 mg/L of CLX solution using ferrous ion as the catalyst for the electro-Fenton process.](image1)

![Comparison of the accumulation of nitrite and nitrate ions in the electro-Fenton process carried out with different catalysts](image2)

**Fig. 4.11a** Accumulation of nitrate and nitrite species during the degradation of a 200 mg/L of CLX solution using ferrous ion as the catalyst for the electro-Fenton process. (b) Comparison of the accumulation of nitrite and nitrate ions in the electro-Fenton process carried out with different catalysts.

The accumulation of the nitrate and nitrite ions in the electro-Fenton process when ferrous ion was
used as catalyst is described in the Fig. 4.11a. In this process both ions were accumulated. The accumulation of the NO$_3^-$ was slowly during almost the half of the process, 270 minutes, then it fast increased to a final concentration of 0.06 mg/L. In the case of the nitrite ion its evolution was similar to the nitrate ion. The concentration of the nitrite ion at the end of the process was of 0.02 mg/L. The process catalyzed by the ferrous ion accumulated higher concentrations of NO$_2^-$ and NO$_3^-$ ions, during the whole process. It suggests that the CLX molecule is may be more attacked in its nitrogen moieties than in the other processes which have lower concentration of both ions. However the total oxidation of nitrogen moieties was only 31% since most of the oxidation is due to the ammonia conversion rather than the nitrate and nitrite ions formation.

In conclusion, as can be seen in the fig. 4.11b, which compares the accumulation of the nitrate ion during the four processes. Its accumulation was low in all of them. On the contrary the final accumulation of nitrite ion was very low in all process, except for the EF catalyzed by Fe$^{2+}$ ion. Even though in all process the presence of the nitrate ion was detected this ion was no considered as the final oxidation state of the nitrogen moieties of the cefalexin molecule because the highest concentration represents barely the 0.15% of the total nitrogen.

4.3.2.4 Changes in the pH during the process

The pH during the processes is expected to decrease because of the formation of more oxidized species formed along of the process form the breakdown of CLX molecule into organic acids. It has been reported that when the molecule is broken smaller carbon chains are produced and they are oxidized until they convert into organic acids. Also oxaminic acid and ammonia had been reported. All of these compounds have an acidic nature, which are able to decrease the pH. Fig. 4.12 shows the change on the pH along of the electro-Fenton process carried out with different catalyst ions.
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Fig. 4.12 pH change along of the electro-Fenton processes carried out with different catalyst ions

The bigger change of the pH was register for the process catalyzed by cupric ion. It dropped from 3.02 to 2.7. The difference respected to the other process could be ascribed to the ammonia accumulation that was higher since the beginning of the process as well of the generation of more acidic species. Then the Mn$^{2+}$ catalyzed process presented also a higher drop in pH, this result is surprised because its oxidation efficiency was the lower but maybe the generated by products had more acidic nature. The process catalyzed by Co$^{2+}$ has a smaller drop in the pH in comparison with the processes catalysed by Cu$^{2+}$ and Mn$^{2+}$. Finally, the lowest drop in the pH was for the process catalyzed by the ferrous ion even though it didn’t have the lowest removal rate.

The change on the pH could also affect the availability of the metal ions. For example, the optimal pH for the ferrous ion is between 2.8 and 3.0 [9]. As mentioned before in acidic conditions cupric ion form complex ions that reacts a little different than the cupric ion standing alone. Also it can affects the viability of the manganese ion, since manganese can have more oxidation states than the other used metals, it is more pH sensitive.

4.3.2.5 Biodegradability

The electro-Fenton process is mainly used as a pre-treatment of toxic wastewater, in order to enhance the biodegradability. Usually the EF process is not used to completely mineralize the pollutants because it represents a high cost treatment. So the suitable application is as pre-treatment, thus it is necessary to know at what reaction time is convenient to stop the electrolysis process and switch it to a biological process. The decision is based on the biodegradability enhancement that depends on the BOD value, since the biodegradability is expressed as $\text{BOD}_5/\text{COD}$. In this case the BOD for the Fe$^{2+}$ and Co$^{2+}$ were assed.
Fig. 4.13 BOD change after the electro-Fenton process using Co²⁺ as the catalyst, and (b) using Fe²⁺ as the catalyst for more than 5 days.

The biodegradability was enhanced in both of the processes the highest enhancement was achieved by the process carried out with cobalt as catalyst. However the biodegradability in terms of BOD₅/COD was not strongly changed. The BOD₅/COD of the process catalyzed by ferrous ion was of 0.26, while the BOD₅/COD for cobaltus ion was of 0.35. It reflects the recalcitrancy of the cefalexin antibiotic.

4.4 Conclusions:

The activated carbon fiber is a good option to be used for its characteristics, such as big surface area, high adsorption and high current conductivity. An important step is to completely saturated the ACF before the electro-Fenton process.

The optimal conditions to saturate the ACF were in acidic conditions during 36h with a cefalexin solution of 200 mg/L.
The ACF is wearing away after been used for long time, because its pores are oxidized so is important to change it every 8 – 10 electrolysis process.

Besides the ferrous ion other transition metals could be used as catalyst, such as Mn$^{2+}$, Cu$^{2+}$ and Co$^{2+}$. In this experiment the cobaltous ion showed the higher efficiency in a concentration of 1mM at pH of 3.00. However, it is necessary to do more research about the optimal conditions for the other two metals Mn$^{2+}$ and Cu$^{2+}$. 
Variations During the EF process

4.5 References

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between Manganese (III) and hydrogen peroxide in acid perchlorate solution. Inorganic chemistry.


CHAPTER
5

Mineralization of antibiotic sulfamethoxazole by photoelectro-Fenton treatment using activated carbon fiber cathode and under UVA irradiation
5.1 Introduction

Sulfamethoxazole (SMX, Table 5.1), one of the most consumed pharmaceuticals, is a synthetic antimicrobial frequently used in human medicine to treat bronchitis and urinary tract infections and also in veterinary medicine, for prevention and treatment of infections, as well as to promote growth of food-producing animals[1]. After use, the substance is metabolized in the liver and its unmetabolized form (about 15% of an administrated dose [2,3]) is excreted with urine or feces, then enters wastewater and ultimately discharged into conventional wastewater treatment plants (WWTPs). Its presence can promote bacterial antibiotic resistance which have some implications in treating infections, with a resulting effect on morbidity, mortality, and economic cost [1,4, 5]. So that is important to remove antibacterial agents completely from the environment [4]. Although past studies demonstrate that sulfonamides are biodegraded in sewage, this process is quite limited. As a result, SMX has been frequently detected in municipal wastewater and surface water bodies in recent years [6]. Research efforts are underway to develop powerful oxidation techniques for achieving their overall destruction from wastewater for avoiding the dangerous accumulation of SMX in the aquatic environment. Several authors have described quick removal of SMX from waters by advanced oxidation processes (AOPs), which can produce hydroxyl radicals (•OH) and are powerful alternatives designed to eliminate SMX or transform them into less-toxic more biodegradable intermediates, such as ferrate[7,8], O₃[9-14], O₃/H₂O₂[11], TiO₂ photocatalysis[14-17], UV photolysis [18-20], UV/H₂O₂ [2,21], photo-Fenton[22,23], as well as solar photo-Fenton [24,27]. During SMX oxidation, the formed metabolites may also be harmful compounds and should be removed from water. Therefore, complete mineralization should be the main aim of these processes.

<table>
<thead>
<tr>
<th>Table 5.1 Sulfamethoxazole (SMX)</th>
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<tbody>
<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
</tr>
<tr>
<td>(4-amino-N-(5-methyl-3-isoxzol yl)benzenesulfonamide)</td>
</tr>
</tbody>
</table>

Electrochemical advanced oxidation processes (EAOPs) like anodic oxidation and indirect oxidation based on H₂O₂ electrogeneration are very attractive for wastewater decontamination due to their low cost and high effectiveness without producing dangerous wastes. The anodic oxidation is a popular EAOPs, which need an active anode, such as boron-doped diamond (BDD) anode. The anodic oxidation of SMX at BDD anode has been investigated by several authors[28,29]. The usual
Mineralization of sulfamethoxazole by PEF

Indirect electrooxidation methods is electro-Fenton (EF), which is based on the continuous supply of \( \text{H}_2\text{O}_2 \) generated from two electron reduction of oxygen on the catalytic cathode, such as carbon felt [30,31], \( \text{O}_2\)-PTFE cathode [32-37] and activated carbon fiber (ACF) [38,39] to a contaminated acid solution containing \( \text{Fe}^{2+} \) or \( \text{Fe}^{3+} \) as catalyst has shown that the EF process can degrade antibiotic SMX efficiently [40].

\[
\text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}_2 \quad E^\circ = 0.695 \text{ V/NHE} \quad (5.1)
\]
\[
\text{Fe}^{2+} + \text{H}_2\text{O}_2 + \text{H}^+ \rightarrow \text{Fe}^{3+} + \text{H}_2\text{O} + \cdot\text{OH} \quad (5.2)
\]
\[
\text{Fe}^{3+} + e^- \leftrightarrow \text{Fe}^{2+} \quad E^\circ = 0.77 \text{ V/NHE} \quad (5.3)
\]
\[
\text{H}_2\text{O} \leftrightarrow 1/2\text{O}_2 + 2\text{H}^+ + 2e^- \quad E^\circ = 1.23 \text{ V/NHE} \quad (5.4)
\]

The photoelectro-Fenton (PEF) process is a modification of the EF process in which the solution treated under EF conditions is simultaneously irradiated with UVA (\( \lambda_{\text{max}} = 360 \text{ nm} \)) or UVC (\( \lambda_{\text{max}} = 254 \text{ nm} \)) light [39]. The action of this UV irradiation is complex and can be accounted for by [40]: (i) the production of greater amount of \( \cdot\text{OH} \) from photoreduction of Fe(OH)\(^{2+}\), which is the predominant Fe\(^{3+}\) species at pH 3.0, via photo-Fenton by reaction (5.5) and (ii) the photolysis of complexes of Fe(III) with generated carboxylic acids, such as oxalic acid, which is produced during the oxidation of most organics, and the fast photolysis of Fe(III)-oxalate complexes (\( \text{Fe(C}_2\text{O}_4\text{)}^n\text{)}^{3-} \), \( \text{Fe(C}_2\text{O}_4\text{)}^{n-}\text{)}^{2-} \), \( \text{Fe(C}_2\text{O}_4\text{)}^{2n-}\text{)}^{3-} \) shown as reaction (5.6). When UVC was used as light source, \( \text{H}_2\text{O}_2 \) absorbs radiation with a molar absorption coefficient of 18.6 L M\(^{-1}\)s\(^{-1}\), it decomposes with a quantum yield of 0.98, generating \( \cdot\text{OH} \) radical [Eq. (5.7)] [39]. The PEF process has been well proven to be a very efficient method for complete mineralization of a lot of refractory organic pollutants in the wastewaters [42-45].

\[
\text{Fe(OH)}^{2+} + \text{hv} \rightarrow \text{Fe}^{2+} + \cdot\text{OH} \quad (5.5)
\]
\[
2\text{Fe(C}_2\text{O}_4\text{)}^{(3-2n)} + \text{hv} \rightarrow 2\text{Fe}^{2+} + (2n-1)\text{C}_2\text{O}_4^{2-} + 2\text{CO}_2 \quad (5.6)
\]
\[
\text{H}_2\text{O}_2 + \text{hv} \rightarrow 2\cdot\text{OH} \quad (6.7)
\]

The aim of this study was to determine the potential of PEF for the complete mineralization of 200 mg L\(^{-1}\) SMX aqueous solutions. Anodic oxidation (AO), AO in presence of electro-generated \( \text{H}_2\text{O}_2 \) (AO-EH\(_2\text{O}_2\)), AO-EH\(_2\text{O}_2\) under UVA irradiation (AO-EH\(_2\text{O}_2\)-UVA), EF and PEF processes for degrading SMX were also compared. The influence of current density, initial SMX concentration and initial Fe\(^{2+}\) concentration on the total organic carbon (TOC) reduction rate and mineralization current efficiency (MCE) on the SMX degradation in the PEF process was explored. The antibiotic decay was followed by high performance liquid chromatography (HPLC), and its aromatics intermediates was identified.
Mineralization of sulfamethoxazole by PEF

by HPLC-mass spectrometry (HPLC-MS), while short-chain organic acids was detected by ion-exclusion chromatography. NO$_3^-$ and NH$_4^+$ were also determined to evaluate the extent of the corresponding mineralization process.

5.2 Materials and methods

5.2.1 Chemicals and analyses

Sulfamethoxazole (98%) was obtained from Wako. The physicochemical properties of SMX are shown in Table 5.1. FeSO$_4$ • 7 H$_2$O (99%, Wako) and Na$_2$SO$_4$ (anhydrous, 99%, Wako) were used as catalyst and supporting electrolyte, respectively. All solutions were prepared with ultra-pure water obtained from a Milipore AutoPure WT101UV system (Millipore S. A. S., Molsheim, France) with resistivity 18.2 MΩ cm at 25 °C. Acetonitrile and distilled water used as solvents for liquid chromatography mobile phase were HPLC grade from Wako. The pressurized oxygen gas (99.9%) was used to saturate the solutions.

5.2.2. Procedures and equipment

Experiments were conducted at room temperature in an undivided, two-electrode quartz cell containing a 125-mL solution stirred with a magnetic bar, which is shown in Figure 5.1. The anode is a 4 cm × 4 cm RuO$_2$/Ti mesh. The cathode is a 16 cm$^2$ ACF felt, which is pre-saturated with SMX solution for 12 h to preclude the TOC decrease due to SMX adsorption on ACF felt. Pure O$_2$ is fed into the solution at 100 mL min$^{-1}$, and prior to the electrolysis, O$_2$ was bubbled through the aqueous solutions for 30 min. AO degradations (without H$_2$O$_2$ in solution) were performed using either a 16 cm$^2$ RuO$_2$/Ti mesh as anode and a 16 cm$^2$ RuO$_2$/Ti mesh as cathode. AO-H$_2$O$_2$, EF, and PEF treatments were then carried out using a 16 cm$^2$ activated carbon fiber cloth cathode, fed with pure O$_2$ at 100 mL min$^{-1}$. PEF trials became operative when the solution was irradiated with ultraviolet lamps (Handy UV Lamp SLUV-8, 254/365 nm, As One Co. Ltd.). The lamp is placed parallel at the side of the cell, at 5 cm from the solution, giving a photoionization energy input to the solution of 1407 μW cm$^2$ for the 365 nm lamp (this data is obtained from the Instruction Manual).

A catalytic quantity of ferrous ion was introduced into the solution before the beginning of electrolysis. The current passed through the solution were measured and displayed continuously throughout electrolysis by using a regulated digital DC power supply (AD-8735, A&D, Tokyo, Japan). Prior to the electrolysis, the pH of initial solutions was set at 3.0 by the addition of 0.1 M H$_2$SO$_4$. HM-30V model (TOA Instruments) pH meter was employed for pH measurements. The ionic strength was maintained constant (0.05 M) by addition of Na$_2$SO$_4$. The 200 mg L$^{-1}$ SMX simulated wastewater is degraded by the PEF process in an aqueous medium containing 0.05 M Na$_2$SO$_4$ and 1 mM Fe$^{2+}$. The current is maintained at a constant 0.36 A.
Mineralization of sulfamethoxazole by PEF

![Diagram of PEF reactor](image)

**Fig. 5.1** Experimental set-up of the photoelectron-Fenton (PEF) reactor. 1, RuO$_2$/Ti anode; 2, ACF cathode; 3, UVA lamp; 4, O$_2$ in pipe; 5, magnetic stirrer; and 6, dark box

### 5.2.3 Chemical Analysis

Before the analysis of the treated solutions, all samples were filtered through hydrophilic PTFE filters of 0.45 μm purchased from Millipore. Total organic carbon (TOC) was measured using a Shimadzu model TOC 5000A TOC analyzer, equipped with an autosampler (ASI 5000A). The measurement was done following the Standard Methods 5210D procedures. H$_2$O$_2$ concentrations were determined spectrophotometrically by the iodide method with a HACH DR4000U UV-visible spectrophotometer (HACH Co., USA) at $\lambda = 352$ nm ($\varepsilon = 26400$ M$^{-1}$ cm$^{-1}$, detection limit of $\approx 10^{-6}$ M [38]). UV-Vis spectrophotometric measurements were carried out with a HACH DR 5000 spectrophotometer (HACH Co., USA). Ammonium (NH$_4^+$) concentration in treated solutions was determined from the standard colorimetric automated phenate method with above HACH DR/4000U Spectrophotometer. The formation of NO$_3^-$ and NO$_2^-$ in the PEF process was measured colorimetrically by an autoanalyser (QUAATRO; BLTEC, Tokyo, Japan).

### 5.2.4. HPLC and LC-MS analysis

HPLC analysis of SMX decay and carboxylic acids formation. The decay of SMX concentration during the treatment was quantified using an Agilent HPLC 1200 Series system equipped with a diode array detector set at 270 nm and an Xterra® MS C18 5 μm 150 mm × 2.1 mm analytical column (Waters Co., USA). The mobile phase consisted of 80:20 water (adjusted to pH = 3.0 with phosphoric acid)/acetonitrile at a flow rate of 0.5 mL min$^{-1}$. The injection volume was 10 μL. SMX had a retention time of 3.0 min under these conditions. Generated short-chain carboxylic acids were identified and quantitatively followed by ion-exclusion chromatography using above HPLC fitted
Mineralization of sulfamethoxazole by PEF

with a Bio-Rad Aminex® HPX-87H column (300 mm × 7.8 mm (i.d.)) at 35 °C and selecting the photodiode detector at λ = 210 nm. The mobile phase was formed of 4 mM H₂SO₄ solution at 0.6 mL min⁻¹.

**LC/MS Analysis of Reaction Product Mixtures.** Organic intermediates were analyzed on a Waters Alliance 2695/ZQ4000 high performance liquid chromatography-mass spectrometer equipped with above Waters X Terra® MS C₁₈ column (150 mm ×2.1 mm i.d., 5 μm particle size), thermostat (30 °C). MS analyses were conducted using positive mode electrospray ionization (ESI+), over a mass scan range of 80-500 m/z under the following conditions: Capillary 3.5 kV, cone 25 V, extractor 3 V, RF lens 0.3 V, source temp. 120 °C, dissolution temp. 300 °C. Dissolvation and cone gas flow were set to 300 L hr⁻¹ and 50 L hr⁻¹, respectively. The volume of injection was 10 μL. A and B mobile phases were acetonitrile and water with 0.1% formic acid, respectively, at a flow rate of 0.2 mL min⁻¹. A linear gradient progressed from 10% A (initial conditions) constant for 5 min, followed by a linear gradient to 100% A in 50 min, after which the mobile-phase composition was maintained at 100% A for 3 min [41]. Under these conditions, the retention time of SMX was 16.7 min.

**5.2.5 Mineralization Current Efficiency (MCE)**

This change in the oxidative ability with electrolysis time can be better explained from its mineralization current efficiency (MCE)[34,36]:

\[ MCE = \frac{\Delta(TOC)_{exp}}{\Delta(TOC)_{theo}} \times 100 \]  

(6.8)

where \( \Delta(TOC)_{exp} \) is the experimental TOC decay ([TOC]₀-[TOC]ₜ) and \( \Delta(TOC)_{theo} \) is the theoretically calculated TOC decay assuming that the applied electrical charge (= current × time) is completely used for the following reaction.

TOC decay is related to the mineralization of SMX by electrochemically produced •OH radicals producing CO₂, H₂O NH₄⁺ and SO₄²⁻. As such, the mineralization reaction can be written as follows:

\[
C_{10}H_{11}N_{3}O_{3}S + 21H_2O \rightarrow 10CO_2 + SO_4^{2-} + 3NH_4^+ + 41H^+ + 42e^-
\]

(6.9)

which involves the consumption of 42 F mol⁻¹ of SMX.
5.3. Results and discussion

5.3.1. Hydrogen peroxide electrogeneration in the PEF process

![Graph showing electrogeneration of H₂O₂](image)

**Fig. 5.2** Electrogernation of H₂O₂ during the electrolysis of 0.05M Na₂SO₄ and initial pH of 3.0 solutions in the absence of Fe²⁺ and UVA with ACF cathode.

The ability of the electrolytic system to accumulate hydrogen peroxide supplied by the ACF felt cathode from reaction (5.1) was studied by determining the concentration of accumulated H₂O₂. Several electrolyses of 0.05 M Na₂SO₄ and initial pH 3.0 solutions were carried out in the absence of Fe²⁺ and UVA. These trials were performed with an ACF felt cathode at different current intensities, which is shown in Figure 5.2. A progressive increase of H₂O₂ concentration was detected during the electrolysis with the current increasing from 0.12 A to 0.50 A during the initial 120 min of electrolysis, in which the concentration of H₂O₂ in final solutions with ca.321, 609, 759 and 765 µM H₂O₂ were obtained at 0.12, 0.24, 0.36 A and 0.50A, respectively. The H₂O₂ concentration did not increase linearly with time. Rather, after approximately 30 min, it reached steady-state concentrations and remained almost constant. Even though no Fe²⁺ was deliberately added to the cell, a mechanism for the decomposition to O₂ either on the anode (heterogeneous process) or in the medium (homogeneous process) (Eq.5.10)[34,38]. As well as this, H₂O₂ was also able to anodically oxidized to yield intermediate HO₂⁻ radicals (Eq.5.11 and 5.12). In the steady state, H₂O₂...
was electrogenerated and simultaneously destroyed in the system at the same rate. It was found from Figure 5.2 that the stable H$_2$O$_2$ concentration increases with the increase of current density at the range of 0.12 A to 0.50 A, while the strong reaction of H$_2$O$_2$ anodically oxidizing at the anode at 0.50 A meant almost negligible increases in the H$_2$O$_2$ concentration. This can be explained by the following equations:

\[ H_2O_2 \rightarrow H_2O + \frac{1}{2} O_2 \]  
(5.10)

\[ H_2O_2 \rightarrow HO_2^- + H^+ + e^- \]  
(5.11)

\[ HO_2^- \rightarrow O_2 + H^+ + e^- \]  
(5.12)

5.3.2. Comparative degradation behavior

![Graph showing TOC reduction and mineralization current efficiency over time](image)

**Fig. 5.3** (a) TOC reduction as a function of treatment time for the ACF adsorption, AO, AO-H$_2$O$_2$, AO- H$_2$O$_2$-UVA, EF and PEF conditions. (b) Change of the mineralization current efficiency (MCE)
A first series of trials was carried out by electrolyzing 200 mg L\(^{-1}\) SMX at pH 3.0 and 0.36 A for 6 h to clarify the comparative oxidation power of EAOPs tested. The EF and PEF treatments were operative using 1.0 mM Fe\(^{2+}\) as catalyst. Figure 5.3(a) summarized the results of TOC decay corresponding to destruction of 200 mg L\(^{-1}\) SMX solutions at pH 3.0 by: (a) ACF adsorption; (b) AO; (c) AO-EH\(_2\)O\(_2\); (d) AO-EH\(_2\)O\(_2\)-UVB; (e) EF and (f) PEF conditions, respectively. The applied current was constant at 0.36 A. As could be seen, the ACF felt adsorption alone on SMX was limited to remove only the 5.3% after 360 min. The low adsorption ability was attributed to the pre-saturation of the ACF felt before every experiment. Similarly anodic oxidation alone could only reduce TOC less than 25%. In these experiments, we used a RuO\(_2\)/Ti anode, a low oxidation power anode characterized by strong interaction between the electrode and \(\cdot\)OH resulting in high electrochemical activity for the oxygen evolution reaction and a low chemical reactivity for organic oxidation. In this strong interaction, the absorbed \(\cdot\)OH interacts with the anode, resulting in a transition of the oxygen from \(\cdot\)OH to the anode surface, which forms a higher oxide which then selectively oxidizes organic pollutants via the surface redox couple [48]. When AO-H\(_2\)O\(_2\) was used, the TOC degradation was a little faster, with 28% of the TOC decayed. In this reaction, intermediates are slowly mineralized by the \(\cdot\)OH generated from the heterogeneous or homogeneous reactions between the ruthenium electrode (RuO\(_2\)/Ti) and H\(_2\)O\(_2\) in accordance with the classic interpretation of the Haber and Weiss cycle [48–50]. The decomposition rate of SMX in the AO-H\(_2\)O\(_2\)-UVA process was significantly better, with 36% of the TOC removed, and this can be attributed to the additional degradation from UVA irradiation. In the EF process, TOC was rapidly reduced by as much as 63% after 360 min of electrolysis. This can be explained by the fast homogeneous reaction of organics with the great amount of \(\cdot\)OH generated from reaction (5.2), which then non-selectively oxidized most of the products remaining in the solution. In contrast, a significant acceleration in the PEF method with 1mM Fe\(^{2+}\) had an even higher oxidative ability, with 80% of the TOC of solution reduced after 360 min of electrolysis. The faster TOC removal in the PEF process is thought to result in the generation of more \(\cdot\)OH due to the fast photodecomposition of Fe(III)–oxalate complexes by UV light and the additional photo-reduction of Fe(OH)\(^{2+}\) species according to reactions (5.5) and (5.6), and this would mean an additional supply of Fe\(^{2+}\) and \(\cdot\)OH for the mineralization of SMX [41].

In order to confirm the oxidation power of the different process, the MCE values for the above trials calculated from Eq. (5.8) are depicted in Fig. 5.3b. As expected, a maximum efficiency of about 10.1% for PEF, 7.2% for EF, 2.5% for AO-EH\(_2\)O\(_2\)-UVA, 2.0% for AO-EH\(_2\)O\(_2\) and 1% for AO is found at approximately 30 min. mark. The MCE follows the same trend as the relative oxidation power of methods tested, increasing in the sequence: AO < AO-EH\(_2\)O\(_2\) < AO-EH\(_2\)O\(_2\)-UVA < EF < PEF. The above findings suggest that the fastest decontamination of acidic SMX wastewaters is achieved in the PEF process.
5.3.3. Optimization of experimental conditions

![Graph showing the effect of applied current on TOC removal](image)

**Fig. 5.4** Effect of applied current during the PFE process. The applied currents were 0.12, 0.24, 0.36 and 0.5 A at pH 3, during 360 min

5.3.3.1. Effect of applied current

The influence of applied current on the degradative behavior was examined by electrolyzing a 200mgL⁻¹ SMX solution of pH 3.0 at 0.12 A, 0.24 A, 0.36 A and 0.50 A. As shown in **Fig. 5.4(a)**, 53% of TOC was removed at 0.12A after 360 min, while at 0.24, 0.36 and 0.50 A, TOC removal was 66%, 80% and 67%, respectively. It was found that increasing the current density in the range 0.12–0.36 A causes quicker mineralization rate in the PEF process. Increasing the current density means the SMX is removed more rapidly due to the concomitant increased production rate of H₂O₂ generated at the ACF cathode. This enhances the generation of •OH from Fenton reaction, because the production of •OH in the medium was proportional to the H₂O₂ concentration, as shown in the following equation (5.13) [51]:

\[
[^{1}{}\text{OH}] = \lambda (\frac{d[^{1}{}\text{OH}]}{dt}) = \lambda k_{1}[\text{Fe}^{2+}][\text{H}_2\text{O}_2] \tag{5.13}
\]

where \(k_{1}\) was the second-order rate constant (μM⁻¹ min⁻¹) of reaction 2; \(\lambda\) was the average life of the hydroxyl radical (min); [Fe²⁺] and [H₂O₂] were the concentration of ferrous ion (μM) and hydrogen peroxide (μM), respectively.

However, when the applied current density is 0.50 A, the mineralization decreases. This is an indicative of the low generation of the oxidant •OH due to three factors at first, as discussed above.
when the current density is high, the H₂O₂ might be anodically oxidized to yield the intermediate HO₂⁺ radical, which is a less powerful agent than ·OH. Secondly, at high current density the Fe²⁺ in the solution may also be anodically oxidized to Fe³⁺, it decreases the formation of ·OH as explained in Eq. 5.13; Finally, the other reason is that when the applied current is high, the dimerization of ·OH to H₂O₂ (reaction (5.14)) or its destruction with H₂O₂ (reactions (5.15)) may also reduce the efficiency of the PEF process [52].

\[ 2^\cdot \text{OH} \rightarrow \text{H}_2\text{O}_2 \quad (5.14) \]

\[ ^\cdot \text{OH} + \text{H}_2\text{O}_2 \rightarrow \text{HO}_2^\cdot + \text{H}_2\text{O} \quad (5.15) \]

5.3.3.2. Effect of Fe²⁺ concentration

Figure 5.5 shows the results of 200 mg l⁻¹ SMX solutions treated for 360 min by the PEF process as dosing Fe²⁺ from 0 mM to 2.0 mM at pH 3.0 and 0.36 A. An obvious increase of the rate of TOC decay was observed with adding Fe²⁺ ion into solution and raising initial Fe²⁺ concentrations from 0 mM to 1 mM might result in an augment of TOC removal. After 360 min of electrolysis the TOC removal 36%, 58%, 80% for 0, 0.5 and 1mMFe²⁺ solution, respectively. When the Fe²⁺ concentration was increased to 2mM, however, the TOC abated more slowly, with 74% of the TOC removed after 360 min of electrolysis. This is consistent with what was expected: the initial Fe²⁺ was rapidly transformed into Fe³⁺ from reaction (5.2), with the small catalytic amount of regenerated Fe²⁺ in the medium in effect regulating the continuous production of oxidizing ·OH. The influence of higher Fe²⁺ concentration upon the degradation behavior of SMX is likely due to the percentage of ·OH scavenged by Fe²⁺, as shown in Eq. (5.16) [38].
5.3.3.3. Effect of initial SMX concentration

![Graph showing the effect of initial SMX concentration](image)

**Fig. 5.6** Effect of the initial SMX concentration during the PEF process during 360 min, at pH of 3.0, 1mM of Fe^{2+} and a current density of 0.36A

The effect of the initial antibiotic concentration on mineralization was studied in four concentrations of SMX solutions in the presence of 1mM Fe^{2+} with a pH of 3.0 at 0.36A by the PEF process. Fig. 5.6 shows the results: 77% of the TOC was removed for up to 300mgL\(^{-1}\) of SMX within 360 min electrolysis, whereas the removal rate was 80%, 85% and 65% in 200, 100 and 50mgL\(^{-1}\) SMX solutions, respectively. This change in the oxidative ability can be better explained from its MCE. As shown in the inset of Fig. 5.6, the MCE value usually increases when the SMX concentration is increasing. This trend is associated with a faster destruction of all pollutants, since higher concentrations of them in the medium means that they can react more rapidly with generated oxidants, mainly the •OH produces from reactions (5.2), (5.6), (5.8) and (5.9). When the SMX concentration is 300mgL\(^{-1}\), however, the MCE falls to a minimum value then increases over time with electrolysis. This is thought to be attributed to the proportion between the concentration of SMX and the concentration of •OH generated from the PEF reaction [48,53].

5.3.4. Degradation of SMX and products time-course

5.3.4.1. SMX decay and UV-Vis spectra change

Several trials were made by electrolysing 200mgL\(^{-1}\) SMX solutions at pH 3.0 by AO, AO-H\(_2\)O\(_2\), AO-H\(_2\)O\(_2\)-UVA, as well as under EF and PEF conditions at 0.36A to clarify the oxidizing ability of the
Mineralization of sulfamethoxazole by PEF

different processes. As can be seen in Fig. 5.7, SMX is degraded at the lowest initial rate only when AO was used, but after 180 min electrolysis, only about 53% SMX was removed. SMX decay in the AO-H₂O₂ experiment was extensive with 81% SMX removed within 180 min, and almost total degradation after 360 min electrolysis.

The increase in the degradation rate of SMX in AO-H₂O₂ is due to the homogeneous reaction with the •OH formed. With UVA irradiation, SMX degradation was at 93% after 180 min electrolysis in AO-H₂O₂-UVA, and total degradation happens after 270 min. SMX degraded remarkably quickly under both EF and PEF conditions, with total SMX removal possible after only 90 min of electrolysis. In the EF process, the fast abatement of SMX is largely attributable to the behavior of •OH on SMX from the Fenton reaction. In the PEF process, the SMX decays even faster than in EF process due to the production of additional •OH from the photoreduction of Fe(OH)²⁺ and the production of additional Fe²⁺ from the photolysis of Fe³⁺ complexes with carboxylic acids under UVA irradiation [41]. It is worth noting that the photodegradation of SMX may also take place in the PEF process, thus enhancing the SMX removal [47].
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To clarify the changes of molecular and structural characteristics of SMX as a result of electrolysis in the different processes, the decay in UV-Vis absorbance recorded for 360 min of treatment of SMX by AO, AO-EH$_2$O$_2$, AO-EH$_2$O$_2$-UVA, EF and PEF methods at 0.36 A and pH = 3.0 were illustrated in Figure 5.7(b). As could be observed from these spectra, before the treatment, the absorption...
Mineralization of sulfamethoxazole by PEF

Spectrum of SMX in water was displays a single broad band in the ultraviolet region located at 267 nm, which is ascribed to the construction from \( \pi \rightarrow \pi^* \) and \( n \rightarrow \pi^* \) electronic transitions [45]. The adsorption peak at \( \lambda = 267 \) nm disappeared completely under 360 min electrolysis in AO-EH\(_2\)O\(_2\)-UVA, EF and PEF processes, indicating a full destruction of SMX. This is much obvious from comparing the UV spectra change for AO, AO-EH\(_2\)O\(_2\) and PEF process. In PEF process, the UV absorption is removed more completely than that in AO and AO-EH\(_2\)O\(_2\) process, which confirms that PEF method was an effective treatment for degradation of refractory organic pollutant in the water. 3.4.2. Intermediate products in electrochemical experiments
5.3.4.2.1. Evolution of inorganic intermediates

**Fig. 5.8.** a Concentration of $\text{NH}_4^+$ ions accumulated in the medium vs. electrolysis time during the treatments of AO, AO-$\text{H}_2\text{O}_2$, AO-$\text{H}_2\text{O}_2$-UVA, EF and PFE processes. b. $\text{NO}_3^-$ ions accumulated in the EF and PFE process was also measured (pH 3.0 and 0.36 A).

Generally, total mineralization of an organic compound containing heteroatoms leads to the formation of carbon dioxide and inorganic ions. While sulfur atom is recovered as sulfate irrespective to its initial oxidation state, nitrogen moieties in organic compound can be transformed
to either NH₃ (NH₄⁺ in acidic media) and/or nitrite and nitrate ions. Although in this study sulfate ions were not monitored because of the supporting electrolyte Na₂SO₄ in the solution, many authors have confirmed SO₄²⁻ was the ultimate byproduct in SMX degradation [26-54]. Evolution of inorganic ions (NH₄⁺, NO₃⁻, NO₂⁻) during electrolyses of 200 mg L⁻¹ SMX solutions at 0.36 A under AO, AO-EH₂O₂, AO-EH₂O₂-UVA, EF and PEF conditions was quantified. As shown in Figure 6.8(a), a stationary NH₄⁺ concentration is detected in all cases, and a faster NH₄⁺ ion accumulation can be observed in EF and PEF process, after 6 h electrolysis, 20.1 mg L⁻¹ and 22.4 mg L⁻¹ NH₄⁺ generated in EF and PEF process, respectively, compared with 10.7 mg L⁻¹, 11.5 mg L⁻¹, 14.5 mg L⁻¹ NH₄⁺ ion accumulation in the AO, AO-EH₂O₂ and AO-EH₂O₂-UVA process, respectively, which indicating the more rapid SMX destruction by ·OH generated from Fenton reaction. On the other hand, nitrate is also detected in EF and PEF electrolysed solutions (no nitrite ions were detected) (Fig. 5.8 (b)). A NO₃⁻ concentration up to ca. 3.7 mg L⁻¹ was detected in solution treated 6 h by the PEF process, while about 1.9 mg L⁻¹ NO₃⁻ accumulated in the EF process. Comparing the evolution of NH₄⁺ with NO₃⁻ in the PEF process, it can be found that more NH₄⁺ formed, i.e., after 6 h electrolysis, 22.4 mg L⁻¹ for (67.5% of initial nitrogen) of the SMX-derived nitrogen is mineralized in the form of NH₄⁺ ions and, 67.5%, while only 3.7 mg L⁻¹ (11.2% of initial nitrogen) SMX-derived nitrogen is mineralized in the form of NO₃⁻ ions. The results also indicate the aromatic amino group of SMX is a primary site for ·OH attack, then the initial nitrogen of SMX is progressively transformed into converted into NH₄⁺ ion. Different quantities of nitrite, nitrate and ammonium ions may be linked to the different features of the N-containing structure. Nitrogroups are converted predominantly to NO₃⁻ ions, while the nitrogen in heterocyclic aromatic rings can be transformed to both NH₄⁺ and NO₃⁻ species, tertiary and quaternary nitrogen atoms are converted predominantly to NH₄⁺ ions [54]. Figure 5.8a and 5.8b also evidences that the evolution of both NH₄⁺ and NO₃⁻ ions depends on the EAOP applied, faster and higher concentration NH₄⁺ and NO₃⁻ accumulated during the PEF process, which is due to its higher oxidation ability might lead to more complete mineralization.
5.3.4.2.2. Evolution of carboxylic acids

Treated solutions were analyzed by ion-exclusion HPLC chromatography to detect and follow the evolution of short-chain carboxylic acids generated during the EF and PEF mineralization processes. These chromatograms exhibited peaks related to oxalic (tr = 6.21 min), maleic (tr = 7.91 min), oxamic (tr = 8.56 min), formic (tr = 12.86 min) and acetic (tr = 14.09 min) acids. Oxalic and oxamic acids were the most persistent carboxylic acids. Figure 5.9a shows a continuous accumulation of oxalic acid in the EF and PEF process. In the EF process, the concentration of oxalic acid increases up
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to 62 mg L\(^{-1}\) after 180 min of electrolysis at 0.36 A, then it was slowly destroyed, and it remains about 13.5 mg L\(^{-1}\) in the treated solution after 360 min of electrolysis. In the EF process, both uncomplexed oxalic and Fe\(^{3+}\)-oxalate complexes can be formed. Oxalic acid can be directly oxidized to CO\(_2\) by \(^{•}\)OH at slow rate (the absolute rate constant between \(^{•}\)OH and oxalic acid is \(1.6 \times 10^6 \text{ M}^{-1} \text{s}^{-1}\)\([55]\)). The oxidation of these complexes by \(^{•}\)OH can be proposed according to the following reaction:

\[
(\text{Fe(C}_2\text{O}_4)_n)^{(2-2n)} + 3^{•}\text{OH} \rightarrow 2\text{CO}_2 + (n-1)(\text{C}_2\text{O}_4)^{2-} + \text{Fe}^{3+} + 3\text{OH}^{-} \quad (5.18)
\]

where at pH 3.0, the predominant complex is given by \(n = 1\). Oxalate complexes are finally oxidized to CO\(_2\) with production of Fe\(^{3+}\), which is quickly reduced to Fe\(^{2+}\) again, thus being able to form more Fe\(^{2+}\)-oxalate complexes as well as \(^{•}\)OH from reaction (5.2)\([55]\). In contrast, in the PEF process, oxalic acid content raised until a maximum value of 37 mg L\(^{-1}\) at 120 min, then it was destroyed quickly, and after 360 min electrolysis, there is only 3.3 mg L\(^{-1}\) present in the treated solution. This is due to the photocatalytic action of UV leading to the rapid mineralization of the Fe(III)-oxalate complexes formed in the solution treated by the PEF process (Eq.5.6)\([32,34, 36, 37,41]\).

Fig. 5.9b evidences that in the PEF process oxamic acid concentration increases and attains a steady value near 20 mg L\(^{-1}\) from 180 to 270 min, before dropping slightly to ca. 18 mg L\(^{-1}\) at 360 min, while in the EF process oxamic acid was found a continuous increase up to 30 mg L\(^{-1}\) was obtained after 360 min electrolysis (see Fig. 5.9b). Much more oxamic acid is accumulated a much larger extent in the EF process than in the PEF process, which is due to oxamic acid is attacked poorly by \(^{•}\)OH in the EF process and UV irradiation can enhance its degradation in the PFE process. \([56-58]\).

### 5.3.4.2.3. Evolution of aromatic intermediates and SMX degradation pathway

**Table 5.2. Identification of the SMX degradation products by LC-MS**

<table>
<thead>
<tr>
<th>Compound</th>
<th>(t_r) (min)</th>
<th>([M-H]^+)</th>
<th>Identification</th>
<th>Chemical structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14.542</td>
<td>270</td>
<td>C(<em>{30})H(</em>{12})N(_3)O(_4)S</td>
<td>![Chemical Structure Image]</td>
</tr>
<tr>
<td>B</td>
<td>18.074</td>
<td>255</td>
<td>C(<em>{9})H(</em>{12})N(_2)O(_4)S</td>
<td>![Chemical Structure Image]</td>
</tr>
<tr>
<td>C</td>
<td>15.309</td>
<td>271</td>
<td>C(<em>{30})H(</em>{12})N(_3)O(_5)S</td>
<td>![Chemical Structure Image]</td>
</tr>
</tbody>
</table>
Mineralization of sulfamethoxazole by PEF

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>3.058</td>
<td>99</td>
<td>C₆H₈N₂O₂</td>
</tr>
<tr>
<td>E</td>
<td>2.836</td>
<td>173</td>
<td>C₆H₈N₂O₂S</td>
</tr>
<tr>
<td>F</td>
<td>3.239</td>
<td>256</td>
<td>C₆H₈N₂O₂S</td>
</tr>
<tr>
<td>SMX</td>
<td>16.379</td>
<td>254</td>
<td>C₂₀H₁₉N₃O₃S</td>
</tr>
</tbody>
</table>

In order to identify the intermediates in the solution and thus determine a probable reaction pathway, a mass spectroscopy study was carried out. The intermediates during SMX degradation in the PEF process were identified by LC–MS by the peaks formed, and the molecular structure of SMX is reported in Table 5.1. Six intermediate compounds were identified, as shown in Table 5.2. Product A, with an [M+H]+ of 270, can be attributed to the addition of 16 mass units to the parent peak, which is consistent with the addition an •OH to the SMX structure in different positions to yield monohydroxylated derivatives. The best-fit formula was C₁₀H₁₂N₃O₄ (a protonated molecule) [15,24,47]. The fragment m/z 156 was missing, and the ion fragment at m/z 172 [C₆H₆NO₃S], together with those atm/z 108 andm/z 99, appeared in the SMX spectrum as well as in most of the transformation products, indicating that the attack of •OH occurred on the benzene ring.

The intermediate B at [M+H]+ 255 mass units can be attributed to the substitution of the amine group by •OH radical attack on the aniline ring (β-cleavage, Table 5.1), forming a hydroxylated structure and releasing NH₄+. Intermediate C at [M+H]+ 271 is the hydroxylated compound of intermediate B, the best-fit formula for which was C₁₀H₁₂N₃O₅ (a protonated molecule), which results from the addition of •OH on the isoxazole ring of Intermediate B. Intermediate D was identified as 3-amino-5-methylisoxazole (AMI) with m/z = 99 for [M+H]+ [1,15,24,47] originated by the cleavage of the β-position. Intermediate E at [M+H]+ 173 mass units was identified as sulfanilamide (C₆H₈N₂O₂S) as a results of β-cleavage, which was an important intermediate in the photocatalysis [17], solar photo-Fenton [24], photolysis [46] and ozonation [59,60] processes during SMX degradation. Intermediate F at [M+H]+ 256 mass units can be attributed to the substitution of the methyl group by •OH radical attack on the isoxazole ring, resulting in the formation of a hydroxylated structure. This ion fragment m/z 156 suggests that the initial degradation of SMX was due to the attack of •OH radicals on the isoxazole ring.
Fig 5.10. Proposed degradation pathway for SMX mineralization in acid aqueous medium by hydroxyl radicals (•OH) and UVA irradiation in the PEF process with ACF cathode

As a result of these analyses, a reaction pathway for the complete mineralization of SMX with •OH as the main oxidant is proposed in Fig. 5.10. The first oxidation step of SMX is the attack by •OH nonselectively at different sites of the SMX molecule, resulting in the formation of hydroxylated derivatives, which are oxidized further by •OH as additional byproducts are released. The overall mineralization of SMX is accompanied by the release of SO\textsubscript{4}\textsuperscript{2-}, NH\textsubscript{4}\textsuperscript{+}, and NO\textsubscript{3}\textsuperscript{-} ions, with the uncomplexed oxalic acids formed being destroyed by •OH, and the complexed Fe\textsuperscript{3+}-oxalate being rapidly mineralized by the photocatalytic action of UVA. The release of NH\textsubscript{4}\textsuperscript{+} as a major nitrogenated product from SMX degradation is based on findings reported in some papers regarding the fate of nitrogen atoms contained in primary amines during the PEF treatment.

5.4. Conclusion

The SMX mineralization in acidic solution of pH 3.0 has been comparatively studied by the AO, AO-EH\textsubscript{2}O\textsubscript{2}, AO-EH\textsubscript{2}O\textsubscript{2}-UVA, EF and PEF processes. The rate of TOC decay for AO method is very slow. The anodic oxidation of the substrate in presence of electrogenerated H\textsubscript{2}O\textsubscript{2} has also been considered, which the TOC removal rate is also slow, being notably enhanced in the presence of Fe\textsuperscript{2+} (the EF process) due to the homogeneous production of •OH from reaction (5.2). The process is even accelerated by UVA radiation (the PEF process), which mainly increases the photodecomposition rate of intermediates at long electrolysis time, which allows to remove 92% of TOC after 6 h of electrolysis of 200 mg L\textsuperscript{-1} SMX solutions at 0.36 A. HPLC analysis was performed for the detection and quantification of the generated carboxylic acid, being oxalic, oxamic, maleic, formic and acetic acids. Aromatic intermediates formed in the electrochemical experiments were
Mineralization of sulfamethoxazole by PEF detected using HPLC-MS analysis. Ammonium ions were also detected in all solution tested. The maximum NH$_4^+$ concentration produced corresponds to 80% of the initial nitrogen in solution. Lower concentrations of nitrate ions were also found in electrolysed solutions in EF and PEF process. A general pathway for SMX mineralization is proposed in which involves the hydroxyl radical as main oxidant species.
5.5 Reference


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Environ., 2009, 92, 262-270


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620-626.


Conclusions

CHAPTER

6

Conclusions
6.1 Conclusions

Along with the change in our life styles, a huge amount of substances like the PPCPs are used to enhance our lives. However, most of these substances represent a threat to the environment due to their persistence on it. For some of them the threat goes beyond the environment directly to human health, like antibiotics, which promote bacterial resistant to antibiotics. The Advanced oxidation processes are a good alternative to remove them. In this study, some of the AOPs were used to treat two different antibiotics commonly reported in water.

The results obtained in this study lead to the following conclusions:

1) The hydroxyl radical is the main oxidizing species in the Advanced Oxidation Processes used in this experiment. They were directly produced like in the Anodic oxidation process or indirectly generated by hydrogen peroxide decomposition.

2) The direct oxidizing process, Anodic oxidation, has lower efficiency than the other methods due to the low hydroxyl radical generation at the anode by water electrolysis. In both cases, with cefalexin and sulfamethoxazole, Anodic oxidation could not mineralize them. However, the difference between the degradation of the two antibiotics was significant; while only 21% of CLX was degraded SMX was in a 53%.

3) The Anodic oxidation process was improved with the concomitant in situ electrogeneration of hydrogen peroxide (AO-EH$_2$O$_2$). Hydrogen peroxide is another strong oxidizing agent. In the case of SMX oxidation the process was able to completely degraded it after 360 min. On the contrary, CLX was only degraded in a 54%.

4) The AO EH$_2$O$_2$ to oxidized SMX was improved with the irradiation of UV-light, the total degradation occurred in a shorter time. The UV-light act as a catalyst that decompose faster the hydrogen peroxide than the spontaneous reaction.

5) The addition of the ferrous ion to the AO-EH$_2$O$_2$ allow the reaction with the hydrogen peroxide in the media bulk, known as the Fenton’s reagent. Thus the electro-Fenton process takes place. In this process the amount of generated hydroxyl radical is bigger than in the other two processes. Both antibiotics were completely degraded, nonetheless SMX was quickly removed while CLX needed 270 min.

6) The generated byproducts of the electro-Fenton process for SMX, were spectrophotometrically analyzed. The results confirmed the destruction of SMX and also
Conclusions

evidence the formation of iron complexes.

7) The electro-Fenton process to oxidize SMX was enhanced with UV-light irradiation; by this means SMX was completely oxidized. The reason is that UV-light can break down the bonds of the small chain carboxylic acids as well of photolysis the iron complexes. It was confirmed by the elucidation of the degradation pathway of the SMX by the AOPs.

8) Photo electrochemical processes are more effective to mineralize the pollutants, since the UV-irradiation not only increased the hydroxyl radicals but disrupt the chemical bonds in small molecules as well in iron complexes.

9) The analysis of CLX by the BOD method demonstrated that CLX is a bio-recalcitrant compound. However, CLX biodegradability could be enhanced by the electro-Fenton process.

10) The effect of pH in the electro-Fenton process is crucial to keep the catalyst action of the ferrous ion in the media bulk. The optimal pH was found as 3.00. At pH higher than 3.00 the removal efficiency considerably decreases because of the iron complexes formation and lower pH affects the hydrogen peroxide molecule.

11) The current density exerts large influence because the hydrogen peroxide generation depends on it. As reported by other authors, the optimal current was of 6.66 mA/cm², even though higher densities will produce more hydrogen peroxide the efficiency will be affected by parasitic reactions between the hydroxyl radicals or hydrogen peroxide anodic decomposition.

12) The effect of the amount of the pollutant also affects the efficiency. The Mineral Current Efficiency showed that at higher pollutant concentrations it is increased.

13) The amount of the catalyst is very important. In this case the suitable amount of ferrous ion was found as 1mM. Lower concentrations are not enough to react with the hydrogen peroxide in the media bulk and higher concentrations will react with the organic byproducts and form complexes with them.

14) In this research different catalyst ions were used, the most effective was Cobalt (II) sulfate, then Copper (II) Sulfate. Those ions are good alternatives to iron because with them complete mineralization of the pollutant could be done in less time.
15) When the Manganese (II) sulfate was used as catalyst its efficiency was the lowest due to its accumulation on the cathode.

6.2 Recommendations

1) The degradation mechanism of cefalexin should be investigated. It will help to understand how the hydroxyl radicals attack the molecule. In addition, the low biodegradability enhancement after 480 min of electro-Fenton process would be able to be explained.

2) A deeply analysis on the biodegradability enhancement by electro-Fenton process catalyzed with different metal ions should be completed.

3) A deeply investigation of the optimal conditions for each of the other catalyst metals should be done in order to set up the suitable parameters such as concentration, pH, etc. All this analysis with a special regard on Cu$^{2+}$ and Mn$^{2+}$ ions since it has been reported that due to their cathodic deposition higher amount are need.

4) The amount of hydroxyl radicals produced in the reaction bulk by each of the catalyst under the optimal conditions for each one should be compared.
Appendix
**A.1 Electro-Fenton Reactor**

In this experiment the reactor was composed by two electrodes made of Titanium mesh coated with RuO$_2$. The electrodes were submerged into a glass container of 500 mL. The electrodes were fixed into an acrylic support to hang them, thus they will have the same separation distance in every experiment. The acrylic support also have two small orifices were two plastic tubes pass through; one is to introduce the oxygen into the solution and other one to take samples. When the cathode was the ACF, it was attached to the Ti/RuO$_2$ mesh with an insulating wire.

It was stirred by a magnetic bar introduced in the solution.

**A.2 Analytic procedures**

**A.2.1 Organic carbon quantification**

The basic principle for the quantification the organic carbon relies on the destruction of the organic matter present in the sample although there are a few non-destructive techniques identified in the literature that are currently under development. The destruction of the organic matter can be performed chemically or via heat at elevated temperatures. All carbon forms in the sample are converted to CO$_2$ which is then measured directly or indirectly and converted to total organic
carbon or total carbon content, based on the presence of inorganic carbonates. [1]

A.2.1.1 Total Organic Carbon

The TOC test is used to measure the organic matter present in water, which is especially applicable to small concentrations of organic matter. As a consequence, it is used as a non-specific indicator of water quality. With this technique is possible to detect below 1 part per million (ppm) down to 4 part per billion (ppb), this sensitivity allows to easily address the 0.500 ppm required by the drinking water and pharmaceutical industries [75].

A typical analysis for TOC measure both the total carbon present as well the inorganic carbon, the latter representing the content of dissolved carbon dioxide and carbonic acid salts, subtracting the inorganic carbon from the total carbon yields TOC. Some equipment removes the inorganic carbon portion by purging with diluted acid and air prior to the analysis, it eliminates the inorganic carbon. An exception for air use is if the sample contains VOC’s that can be stripped by the air. Then the organic carbon is oxidized in the presence of a catalyst into CO2, which is quantitatively measured by means of an infrared analyzer. The test is performed by injecting a known quantity of sample into high-temperature furnace or chemical oxidizing environment. The typical concentration of TOC in the domestic water is from 80 to 290 mg/L.

A.2.1.2 Chemical oxygen demand

The COD test is another mean to measure the content of the organic matter in the water. It represents the chemical oxygen demand (COD), i.e., the amount of oxygen consumed by such a wastewater during the general chemical oxidation of organic components to harmless inorganic product. It does not differentiate between biologically available and inert organic matter, and it is a measure of the total quantity of oxygen required to oxidize all organic material into carbon dioxide and water. COD values are always greater than BOD values, but COD measurements can be made in a few hours while BOD measurements take five days. It ranges from 5000 to 10 000 mg L⁻¹ [1]

The principal reaction uses dichromate as the oxidizing agent, it is represented by the general reaction, Eq. 2.52, the reaction is unbalanced:

\[
\text{Organic matter (C}_x\text{H}_y\text{O}_z) + \text{Cr}_2\text{O}_7^{2-} + \text{H}^+ \xrightarrow{\text{Catalyst}} \text{Cr}^{3+} + \text{CO}_2 + \text{H}_2\text{O} \quad (2.52)
\]

A.2.1.1 Mineral current efficiency

The extent of the mineralization as well of the efficiency of the process can be calculated from the abatement of the COD and/or TOC values of the treated solution [2]. There are different ways to calculate the efficiency according to the available data.

Mineral current efficiency:

When TOC data is determined for a single pollutant, the mineralization current efficiency for electrolyzed solutions at given time t (h) is calculated from equation:
The TOC is expressed as mg of carbon L$^{-1}$, $n$ is the number of electrons exchanged in the mineralization process of the organic compound, $m$ is the number of carbon atoms of the molecule under study, and $4.32 \times 10^7$ is the conversion factor for units homogenization ($= 3600 \text{ s h}^{-1} \times 12000 \text{ mg carbon mol}^{-1}$).

In the last two cases, the overall reactions regarding to completely mineralization of the pollutant until CO$_2$ must be calculated. This equation should also consider the oxidation of the heteroatoms present in the molecule, such as Nitrogen, Sulfur, Chlorine, etc., in order to know to what extent they have been oxidized they need to be measured. This general equation is to calculate the electron balance for the oxidation of the organic pollutant.

Higher values in the MCE indicates that a rapid conversion of the organic matter into more oxidized forms like CO$_2$ is taking place, also in that moment is when the process shows higher efficiencies. Generally, at the beginning of the electro-Fenton process is when the higher MCE values are reached [3].

**Apparent current efficiency:** It is the simplest equation that involves the TOC data.

\[
\text{ACE} = \frac{(\Delta \text{TOC})_{\text{exper.}}}{(\Delta \text{TOC})_{\text{theor.}}} \times 100
\]

The theoretical TOC corresponds to the theoretical calculated TOC removal assuming that the applied charge (=current x time) is consumed only to mineralize the pollutant.

**Instantaneous current efficiency:** It involves the use of the COD data and can be expressed and calculated by two different ways:

\[
\text{ICE} = 4FVR \frac{(\text{COD})_t - (\text{COD})_{t+\Delta t}}{i_A \Delta t} \times 100
\]

\[
\text{ICE} (%) = \frac{(\text{COD})_t - (\text{COD})_{t+\Delta t}}{8 / \Delta t} \times 100
\]

COD are the chemical oxygen demands at times $t$ and $\Delta t$ (in mol O$_2$ dm$^{-3}$) respectively, $i_A$ is the applied current density (A dm$^{-2}$), $F$ is the Faraday constant, $A$ is the anodic surface (dm$^2$) and $V_R$ is the electrolyte volume (dm$^3$).

**Average current efficiency at given time:**

\[
\text{CE} (%) = \frac{(\Delta \text{COD})_t}{8 / \Delta t} \times 100
\]

For this equation the experimental COD decay is expressed as $\text{O}_2$/L.
A.2.1.2 Biological oxygen demand

It is the most widely used parameter of organic parameter to estimate the degradation rate of a substance dissolved in water. This technique is a biological process where living microorganisms are involved. The biodegradation is a key step in the wastewater treatment plants because the removal of some high toxic pollutants takes place in this step, those pollutants could be the PPCPs.

Biodegradability refers to the biotransformation of the organic matter by bacteria, fungi, yeast or another microorganisms to more simple molecules through a biological activity, sometimes this oxidation process could mineralized the organic matter into inorganic constituents (H$_2$O, CO$_2$, NO$_3^-$, SO$_4^{2-}$, PO$_4^{3-}$, Cl$^-$).

The BOD measurement was established in London during the Industrial Revolution, as a consequence of the diseases emerged for the water pollution. In the term BOD$_5$, 5 refers to five days which was the time that took the polluted water to reach a natural water body where this water was discharged.

This parameter involves the measurement of the dissolved oxygen used by microorganisms to oxidize the organic matter. Despite the wide spread use of the BOD test, it has a number of limitations. The biodegradability cannot be an absolute value such as mol, weight, etc., but like the boiling point, it depends on the conditions under which the determination is made [78].

Even though BOD has limitations still is an important water quality parameter.

In this experiment the BOD was measured by OxiTop ® equipment.

OxiTop-C is a BOD self-measuring mercury free system. The principle is based on the pressure measurement in a closed bottle system, where the CO$_2$ produced by microbial activity is absorbed by NaOH creating vacuum. The pressure difference can be read by the operating unit and is presented as mg/L of BOD.

The total volume solution to be analyzed depends on the range of BOD value.

<table>
<thead>
<tr>
<th>Sample volume (mL)</th>
<th>Expected BOD (mg O$_2$/L)</th>
<th>Multiplication factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>432</td>
<td>0 - 40</td>
<td>1</td>
</tr>
<tr>
<td>365</td>
<td>0 - 80</td>
<td>2</td>
</tr>
<tr>
<td>250</td>
<td>0 – 200</td>
<td>5</td>
</tr>
<tr>
<td>164</td>
<td>0 – 400</td>
<td>10</td>
</tr>
<tr>
<td>97</td>
<td>0 – 800</td>
<td>20</td>
</tr>
<tr>
<td>43.5</td>
<td>0 – 2000</td>
<td>50</td>
</tr>
<tr>
<td>22.7</td>
<td>0 - 4000</td>
<td>100</td>
</tr>
</tbody>
</table>

In the present research the used volume was 432 mL because the expected BOD was lower than 40mgO$_2$/L.
Besides the wastewater some micronutrients must be added as well of a pH buffer.

Solution preparation:

- Buffer: Phosphate buffer, 1.5N: Dissolve 207g of NaH$_2$PO$_4$ in water, neutralize to pH of 7.2 with a 6N solution of KOH and dilute to 1L.
- Ammonium chloride solution, 0.71N: Dissolve 38.2g of NH$_4$Cl in water, neutralize to pH 7 and dilute to 1L.
- Magnesium sulfate solution, 0.41N: Dissolve 101g of MgSO$_4$ $\cdot$ 7H$_2$O in water and dilute to 1L.
- Ferric chloride solution: Dissolve 4.84g of FeCl$_3$ $\cdot$ 6H$_2$O in water and dilute to 1L.
- Calcium chloride solution, 0.25N: Dissolve 336g of CaCl$_2$ in water and dilute to 1L.

The used microorganisms were not acclimated organisms. They were directly used from the wastewater treatment plant in Sendai, Japan.

Each volume was filled with the following substances. 2.595 mL of phosphate buffer solution, 0.865 mL of NH$_4$Cl of each solution and 0.650 mL of biomass [5].

This equipment allows measuring not only for five days, but for more days.

Then the biodegradability was measured by the ratio BOD$_5$/COD.
A.3 References


