

Integrated Master in Bioengineering

Combination of Coagulation/Flocculation and Fenton processes in a WWTP Tertiary Treatment: Disinfection of Treated Urban Effluents for Reuse

Master Dissertation

by

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Abstract

Several concerns regarding water quality and scarcity are of utmost importance nowadays, since water is indispensable for human life and, thus, should definitely be cherished. Efforts have been made in order to improve wastewater treatment and promote a safe water reuse, including the development of European and country legislations. A combination of a coagulation/flocculation and Fenton processes was studied as a tertiary treatment to be implemented in Gaia Litoral WWTP, in order to generate a treated water that meets the Portuguese legislation requirements for its reuse.

Coagulation/flocculation assays using ferric chloride ($FeCl_3$) as coagulant were performed in a Jar Test, where a coagulant dosage of 120 mg/L and the natural pH of the effluent (7.14) provided the best results for removal of turbidity, TOC and enterobacteria. The following Fenton reaction was carried out in a batch stirred tank reactor and enterobacteria inactivation after 120 min at 25 °C was maximized with hydrogen peroxide and added iron ($FeSO_4 \cdot 7H_2O$) concentrations of 100 mg/L and 7 mg/L, respectively. The performance of the Fenton process without iron addition and with $FeCl_3$ as the iron source was also evaluated. The presence of enterobacteria with and without antibiotic resistance (amoxicillin and sulfamethoxazole), enterococci and total heterotrophs was evaluated before and after each step of the treatment. Values below detection limit (< 10 CFU/100 mL) were achieved for all enterobacteria immediately after treatment and after storage for 72 h, therefore meeting the strictest limit imposed by legislation for *E. coli*. Physico-chemical characteristics were also determined and met the established limits for most reuse applications. Implementation of this method at full scale in Gaia Litoral was studied and an economic analysis was performed.

The best conditions determined for this treatment provided the generation of treated water that meets the requirements imposed by European and Portuguese legislation for its reuse in irrigation and most of urban utilities. Its implementation at real scale in Gaia Litoral WWTP in batch operation mode is not fully viable in comparison with the current employed treatment, and further studies should be performed, namely in continuous mode.

Keywords: coagulation/flocculation, Fenton process, urban wastewater disinfection, wastewater reuse

Resumo

Diversas preocupações relativas à qualidade e escassez de água são de extrema importância nos dias de hoje, uma vez que a água é indispensável para a vida humana e, portanto, deve ser valorizada e estimada. Têm sido tomadas medidas com o intuito de melhorar o tratamento de águas residuais e promover uma reutilização segura da água, nas quais se inclui o desenvolvimento de legislações europeias e nacionais. Uma combinação dos processos de coagulação/floculação e Fenton foi estudada como tratamento terciário a implementar na ETAR de Gaia Litoral, a fim de gerar uma água que cumpra os requisitos da legislação portuguesa para a sua reutilização.

Os ensaios de coagulação/floculação utilizando cloreto de ferro ($FeCl_3$) como coagulante foram realizados num *Jar Test*, onde uma dosagem de 120 mg/L e o pH natural do efluente (7,14) providenciaram os melhores resultados para a remoção de turbidez, TOC e enterobactérias. O subsequente processo Fenton foi realizado num reator descontínuo perfeitamente agitado e as melhores condições para inativação de enterobactérias foram obtidas após 120 min a 25 °C, com adição de 100 mg/L e 7 mg/L de peróxido de hidrogénio e ferro ($FeSO_4 \cdot 7H_2O$), respetivamente. O desempenho do processo Fenton sem adição de ferro e com adição de $FeCl_3$ também foi avaliada. A presença de enterobactérias com e sem resistência a antibióticos (amoxicilina e sulfametoxazol), enterococos e heterotróficos totais foi determinada antes e após cada etapa do tratamento. Valores abaixo do limite de deteção (< 10 UFC/100 mL) foram alcançados para todas as enterobactérias imediatamente após o tratamento e após armazenamento por 72 h, atendendo ao limite mais rigoroso imposto pela legislação para *E. coli*. As características físico-químicas também foram determinadas e cumpriram os limites estabelecidos para a maioria das aplicações de reutilização. Foi estudada a implementação deste método em escala real na ETAR de Gaia Litoral, com o auxílio de uma análise económica.

O tratamento implementado nas melhores condições determinadas produziu uma água tratada que cumpre os requisitos impostos pela legislação europeia e portuguesa para a sua reutilização na rega e na maior parte das utilidades urbanas. A sua implementação em escala real na ETAR de Gaia Litoral em modo descontínuo não é totalmente viável em comparação com o tratamento atualmente empregado, remetendo para a realização de novos estudos, nomeadamente em modo contínuo.

Palavras-chave: coagulação/floculação, processo Fenton, desinfecção de águas residuais, reutilização de água residual

Declaration

I declare, on my word of honour, that this work is original and that all non-original contributions were duly referenced with identification of the source.

João Venâncio

João Pedro Ferraz Venâncio, July 5, 2021

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Abbreviations

AMX - Amoxicillin

AOP - Advanced Oxidation Process

ARB - Antibiotic Resistant Bacteria

ARG - Antibiotic Resistance Gene

BOD - Biochemical Oxygen Demand

CTWW - Coagulation Treated Wastewater

DL - Detection Limit

E. coli - *Escherichia coli*

Ent. faecalis - *Enterococcus faecalis*

FTWW - Fenton Treated Wastewater

HRT - Hydraulic Retention Time

STWW - Secondary Treated Wastewater

SUL - Sulfamethoxazole

TOC - Total Organic Carbon

TSS - Total Suspended Solids

UV - Ultraviolet

WWTP - Wastewater Treatment Plant

1. Introduction

Water is undoubtedly one of society's most precious resources, so improving its quality remains as one of the top priorities of health research programs [1,2]. The increasing generation of toxic waste and domestic sewage resulting from the lack of control in human activities, combined with population growth and climate change, has been leading to the contamination of aquatic environments. This contamination includes not only chemical micropollutants but also various opportunistic microorganisms, from bacteria and fungi to viruses [3–5]. Thus, it has been noticeable a decrease in water quantity and quality, a fact confirmed by the increase in the incidence of waterborne diseases, even in countries with high hygiene standards [3,5].

Another major water concern nowadays surrounds antibiotics. Due to their increasing consumption and the fact that they are not completely absorbed and metabolized after ingestion, their presence has been increasingly common in urine and faeces and consequently in urban effluents, contributing to the acquisition and dissemination of ARG and ARB [6–10]. In fact, WWTPs are recognized as sources of contamination by ARB, since they meet the conditions that promote horizontal transfer of ARGs between them [10,11]. ARB and ARGs are disposed into the environment in WWTPs discharges, eventually contaminating the food chain and even drinking water, which will lead to the proliferation of antibiotic resistance and consequently a decrease of these antibiotics efficiency in humans and animals [12,13]. Hence, the monitoring and inactivation of these hazardous microorganisms is critical.

Microbiological assessment of water quality is generally performed by measuring the concentration of indicator microorganisms [10,14,15]. These microorganisms are a constant presence in human intestines and consequently in faeces and in urban effluents, thus being an indication of fecal contamination. In this group of microorganisms, enterobacteria and enterococci, such as *Escherichia coli* (*E. coli*) and *Enterococcus faecalis* (*Ent. faecalis*), respectively, stand out [4,14].

E. coli is a Gram-negative bacterium, of the *Enterobacteriaceae* family, which resides in the intestinal tract of endothermic organisms, as is the case of humans [16]. In fact, with rare exceptions, this bacterium does not survive well outside of this environment, so its presence in environmental samples, food or water generally indicates recent fecal contamination or poor sanitation practices in food processing [15]. Its presence in a

sample does not directly indicate that there are pathogenic microorganisms in the sample, but it does indicate a greater risk of presence of other bacteria and viruses transmitted by faeces, such as *Salmonella* spp. or the hepatitis A virus [17]. Furthermore, *E. coli* is considered the third most important pathogen responsible for childhood diarrhoea [18]. This microorganism is also widely used in antibiotic-resistance monitoring, due to its presence in a wide range of hosts and its ability to carry out horizontal genetic transfer and thus acquire resistance [12]. *E. coli* is therefore a great indicator of water quality, allowing to not only detect unacceptable levels of faecal contamination in a sample, but also to evaluate the success of treatments applied in it.

Enterococci are Gram positive bacteria, from the *Enterococcaceae* family. They are facultative anaerobes and are capable of resisting adverse conditions, such as alkaline pH values or high salt concentrations [19]. This group includes intestinal enterococci, where *Ent. faecalis* stands out. This species is present in the faeces of warm-blooded animals and consequently in wastewater. On the other hand, its presence is not noticed in clean waters or in sources that have had no contact with animal or human life. Thus, the World Health Organization recommends monitoring *Ent. faecalis* as an indicator of faecal contamination in wastewater [4]. These microorganisms can cause urinary infections, bacteraemia and meningitis, among others, and are generally more resistant than *E. coli* to disinfection processes [3,19–21].

Besides microorganisms, organic pollutants such as organic matter, consumer products, dyes, pharmaceutical and industrial compounds have been detected in lakes, rivers and oceans, exhibiting a genuine threat to human health and ecosystems [22,23]. Therefore, wastewater treatment and its safe reuse are becoming crucial, as the likelihood of water stress problems in the near future rises [2].

Urban wastewater treatment's goals include the removal of readily biodegradable organic matter, as well as nitrogen and phosphorus compounds (main cause of eutrophication of receiving watercourses), degradation of toxic organic contaminants into more biodegradable and less harmful species, and microorganism inactivation, so that the water meets the requirements for its discharge and/or reuse [5,24,25]. Agricultural and urban green spaces irrigation, as well as other urban, recreational and industrial uses, are examples of treated wastewater reuse. However, water reuse can pose risks to health and environment due to pathogenic microorganisms, disinfection by-products and compounds of emerging concern that resist conventional treatment [26]. Several countries developed

legislations to regulate that activity, and in 2020 the European Parliament and the Council of the European Union created a regulation on minimum requirements for water reuse, where parameters like pH, BOD₅, TSS, turbidity, *E. coli* and others must be assessed and fulfil the established values [27]. Portugal also developed its own legislation [28].

According to WWAP, over 80% of wastewater globally is released to the environment without an adequate treatment [29]. As previously stated, the secondary treatment technologies implemented in the WWTPs are not sufficiently efficient and secondary effluents do not meet the imposed standards for water reuse, mainly in regard to microorganism inactivation. This is the case for Gaia Litoral WWTP, where a tertiary treatment is necessary.

1.1 Objectives

In this work, a combination of coagulation/flocculation and Fenton processes was applied with the goal of improving the tertiary treatment in Gaia Litoral WWTP and generate a treated water that meets the requirements established by legislation for its reuse. A parametric study was carried out to assess the conditions that favour the most the disinfection process of enterobacteria, in which *E. coli* is included. Physico-chemical characteristics of the treated effluent and microorganism regrowth were also evaluated, as well as the inactivation of other microorganisms, including antibiotic resistant bacteria, to assess compliance with the limits imposed by legislation. Another goal was to study the viability of implementation of this method in the WWTP, namely recurring to an economic analysis to establish a comparison with the currently employed treatment.

1.2 Company presentation

The internship was developed in “SimDouro – Saneamento do Grande Porto, S.A”. It is based in Gaia Litoral WWTP, located at “Rua Alto dos Chaquedas, s/nº0, Canidelo”, zip-code 4400 356, Vila Nova de Gaia [30]. This is a public limited company with exclusively public capital, created by Decree-Law No. 16/2017 from February 1st, responsible for the construction, management and concession of the multi-municipal sanitation system of Porto, on an exclusive basis and for a period of 50 years [30].

SimDouro has the responsibility of operating 22 WWTPs, 30 wastewater pumping stations and 2 laboratories for analytical process control. The company aims at the collection, treatment and final rejection of urban wastewater from about 519 000 equivalent inhabitants, covering an area of 1300 km². This corresponds to the totality of the municipalities of Arouca, Baião, Castelo de Paiva, Cinfães, Paredes, Vila Nova de Gaia and a part of the municipality of Penafiel (Sousa river basin). According to the concession contract, the company consists of “Águas de Portugal, SGPS, SA”, which holds 58.52% of the share capital and the previously stated municipalities, which hold the remaining 41.48% [30]. Since its birth and till the end of 2019, SimDouro invested 155 million euros to improve the intercity wastewater system, not only in the construction of new infrastructures, but also in the integration and rehabilitation of existing facilities.

SimDouro's mission is to perform a good management of the sanitation system, ensuring efficiency, reliability, quality of service and respect for the highest social and environmental value, and therefore achieving recognition for their competence, sustainability and value creation for the region. It assumes the commitment to actively contribute to the sustainable development of wastewater sanitation services and to the achievement of the national goals established for the sector. It puts its commitment in the fulfilment of social obligations and responsibilities towards shareholders, customers, employees, grantor, suppliers, community and other interested parties [30].

1.3 Work contributions

The experiments carried out in this work allowed to verify the effectiveness of a combination of a coagulation/flocculation and Fenton processes in the disinfection of a real secondary treated wastewater, namely from Gaia Litoral WWTP, as well as the ability to generate a treated water capable of meeting the requirements imposed by legislation for its reuse. The viability of its application at real scale at Gaia Litoral WWTP was also provided through an economic analysis. A clear understanding of the influence of operational parameters in a Fenton process applied in a final stage of disinfection of real urban wastewaters was also achieved, which is up to the author's knowledge a novelty in this area.

1.4 Road Map

This master thesis is divided by chapters. The first one (**Introduction**) exposes nowadays problems with water use and treatment, as well as the main goals of this work. Chapter 2 (**State of the Art**) includes what has been done to contain the previously exposed problematic, namely regarding tertiary treatment techniques in WWTPs. Gaia Litoral WWTP treatment is presented, and the processes implemented in this work are further described. In Chapter 3 (**Materials and Methods**) are presented the experimental procedure and the techniques employed in this work. All results obtained are presented in chapter 4 (**Results and Discussion**). Firstly, the determination of the best conditions for the coagulation and Fenton process is discussed. Then, microbiological analysis, including microorganism regrowth, and physico-chemical characteristics of all samples are broken down. Lastly, an economic analysis was developed in order to assess the viability of a future implementation at real scale in Gaia Litoral WWTP. Chapter 5 highlights the main outcomes of this work, as well as future work suggestions.

2. State of the Art

2.1 Gaia Litoral WWTP

Gaia Litoral WWTP started operating in May 2003 and is scaled for a population of 300,000 equivalent inhabitants in the year of the project horizon. The average treatment flow is 66,700 m³/day and the peak flow is 1.177 m³/s. The treatment process is based on an activated sludge treatment system in a conventional aeration regime [30] and is schematized in Figure 1.

Firstly, the effluent is subjected to a preliminary treatment where a screening occurs, removing larger suspended and floating solids that are always present in urban wastewater and are likely to cause disruption in the following operations and physical damage to equipment, such as tree branches, plastic fragments and other debris. The solids are stored in a container and then collected by entities capable of treating them. Due to space contingencies, the primary treatment is done in the same equipment as the preliminary treatment: a single compact part conceived and patented by Degremont: Sedipac 3D. This way it is possible to achieve a more compact solution that can be explored in an easier way and also much more balanced in terms of landscape integration, while causing less impact in terms of deodorization. In this equipment, sand is removed by compressed air diffusion, grease is removed by flotation and suspended matter is separated by accelerated lamellar settling. Sand and grease are also stored for further collection and treatment by external companies, while the subproducts formed in the settling process, named primary sludge, are forwarded to a thickener.

The effluent will then proceed to a secondary treatment, composed of a biological treatment with activated sludge and a secondary settling. In the first one, the microorganisms present in the biological reactor biodegrade organic matter and other organic and/or inorganic compounds still present in the primary effluent under aerobic conditions (secured by bottom diffusers), forming biological flocs for solid separation in the subsequent settling unit [31]. Thus, they are able to eliminate colloidal and carbonaceous pollution. The biological reactor is subdivided into four identical reactors operating in parallel and designed for normal operation at medium load. This division was conceived in order to provide flexibility to the plant, optimize energy consumption and avoid nitrification, the latter guaranteed by a low oxygen supply. There is also the option of a bypass to the primary treatment for operation at low load (prolonged aeration).

Secondary settling is carried out through the gravitational separation between the activated sludge and the treated wastewater provided by four rectangular clarifiers. The sludge extracted from the bottom of the secondary settlers is pumped into a sludge pit, from where they are recirculated to the aeration tanks or removed, as excess sludge, to the flotation tank. The final effluent is discharged into the Atlantic Ocean, 30 meters deep, through a submarine outfall under environmentally friendly conditions. A smaller portion of the secondary effluent is submitted to a tertiary treatment and the resulting water is used in internal applications such as equipment, vehicles and pavement washing, as well as irrigation. This step will be further described in the section 2.2.

Meanwhile, primary and secondary sludge formed in the previously described process are concentrated in a static (gravitational) thickener and in a flotation thickener, respectively, and then blend in a mixing tank. The homogeneous sludge is then forwarded to an anaerobic digester, in which the elimination of volatile matter produces biogas that acts as fuel for the cogeneration of electricity for internal consumption. The digested sludge is dehydrated by mechanical centrifugation and then stored for future transport to an environmentally friendly destination. The entire treatment process is schematized in Figure 1.

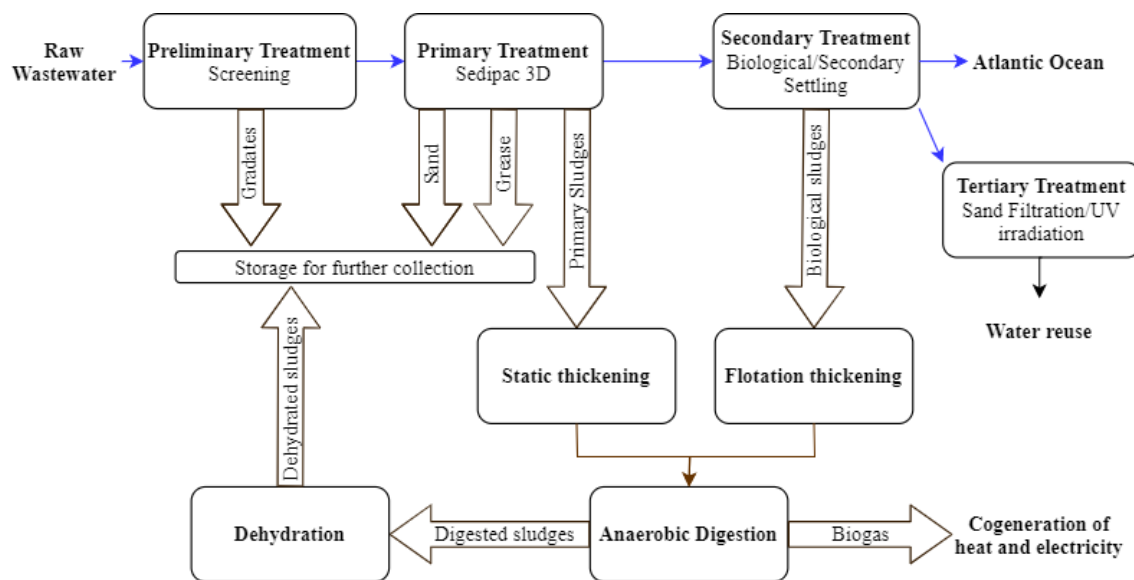


Figure 1 - Schematic diagram of Gaia Litoral WWT

2.2 Tertiary treatment

Tertiary treatment is the step that follows secondary treatment in wastewater treatment and acts as a polishing step. Its goal is to remove recalcitrant contaminants that the previous steps were not able to, and thus more advanced treatments systems are implemented [32]. Last traces of suspended solids and some nitrogen or phosphorus nutrients that would promote unwanted algae growth are some of the main stubborn contaminants still present in the water, which makes physical separation technologies such as filtration the main tertiary treatment process [33], using either a sand bed [34,35] or a membrane process [36,37]. If the nitrogen and phosphorus content is still high after the secondary treatment, an additional biological process (different from the activated sludge process) is implemented (e.g. constructed wetlands and membrane bioreactor) [11,33]. Chemical based approaches are also usually performed, such as coagulation/flocculation and disinfection methods, namely when a reduction in microbial load and/or stubborn micropollutants is required [11,32,38].

With regard to the reduction of the load of ARBs and ARGs, wastewater treatment processes are usually split into two types [39]: processes that capture and intercept those contaminants, such as coagulation/flocculation [40,41], constructed wetlands [42,43] and membrane separation [44]; processes in which the focus is the attack of cell membrane with reactive oxygen species to destroy cell integrity, such as disinfection methods and Advanced Oxidation Processes (AOPs) [38,45,46].

A combination of coagulation/flocculation, sedimentation and filtration is widely applied in wastewater treatment plants, often followed by a step of disinfection [41,47,48]. In this combination, the first steps are able to remove organic suspended matter and floating compounds, therefore reducing turbidity [16,33,49]. Some microorganisms adhere to the flocs formed in the coagulation/flocculation process and are removed as the aggregated compounds sediment or are retained in the filter, contributing to a reduction in the microbial load [47]. However, a considerable part of the microorganisms stays in the effluent, meaning that this combination of processes cannot guarantee a satisfactory reduction [16,24]. A final stage of disinfection is therefore very often applied to destroy viruses, bacteria and other harmful microorganisms present in the wastewater [24,47]. This step also contributes to a further reduction of the load of organic pollutants, whose stability allows them to resist the already mentioned conventional methods [2].

Gaia Litoral WWTP's tertiary treatment has the capacity to treat 100 m³/h, combining a step of sand filtration, followed by a disinfection process with UV irradiation. The secondary effluent leaves the settler and feeds two serial sand filters with a capacity of 100 m³/h each. The filtration process reduces the TSS content and turbidity, significantly improving the effluent's transmittance in comparison with the non-filtered one (from 50 to 60%). This improvement enables a more efficient UV irradiation treatment and therefore a cost reduction, as less UV modules are needed. The goal of this disinfection process is to remove microorganisms present in the effluent, as the UV radiation wavelength (253 nm) is able to target and damage DNA strands. Five UV modules, made up of a rigid structure supporting 8 overlapping lamps each, are installed horizontally in an open channel in which the effluent from the filters flows towards a cistern, where it is stored until further use. The equipment is dimensioned to have a UV irradiation of 31 mWs/cm² and guarantee a fecal coliform concentration lower than 100 CFU/100 mL.

Microbiological quality has been the main concern to be taken into account when sizing water treatment systems for reuse, as controlling aquatic microbial populations is considered crucial for the protection of public health and therefore has a strict regulation. UV irradiation is considered an effective method against a wide variety of pathogens, including chlorine-resistant organisms [50]. This method has a short contact time compared with other ones and there are scarce concerns on disinfection byproducts or other chemical residues formation [51,52]. The application of this method in wastewater disinfection of real secondary effluents has achieved *E. coli* inactivation of 5.1 log UFC/100 mL in 30 minutes [53] and 6.5 log UFC/100 mL in 80 min [54]. Rizzo et. al. achieved total *E. coli* inactivation after 60 minutes [55]. However, SimDouro states that several years of experience in multiple installations show that UV systems are not satisfactory for the purpose of microorganism disinfection. Its high energy consumption and high operational, equipment and maintenance costs are also major drawbacks, as well as having no residual effect [21,56], so other disinfection methods are required. Besides UV irradiation, chlorination and ozonation are the most commonly employed methods [4,53,56].

Chlorination is the most widely used method due to its availability, simple and low cost application and its effectiveness [51,57,58]. Chlorine destroys target organisms by oxidation of cellular materials and can be applied as hypochlorite solutions, gas or other chlorine compounds in liquid or solid form, like chloramine or chlorine dioxide [51].

Studies using this method in the disinfection of secondary wastewater from WWTPs have been developed [53,55]. This technique's main drawback is the formation of harmful disinfection byproducts and the toxic presence of residual chlorine in water for aquatic life [51,56]. The implementation of this method at Gaia Litoral WWTP to the detriment of UV irradiation was already studied in order to improve microorganism disinfection, but the results obtained were not satisfactory since the limits imposed by regulation for *E. coli* were not met.

Ozonation is an attractive method because of ozone's high redox potential (2.07 eV) and its effectiveness in disinfecting pathogens in drinking water and urban wastewater [51,59,60]. The disinfection of secondary wastewater from WWTPs by this method has been studied [46,53,61]. However, this is an energy and cost intensive process, with no residual effect, that also raises some safety concerns, since ozone may be toxic and cause irritation and therefore off-gases from the contactor must be destroyed in order to prevent worker exposure [1,51,53,56].

Overall, the deeper application of this processes is limited by the previously stated disadvantages, and studies affirm they can even contribute to an increase in the risk of antibiotic resistance dissemination in environments [11]. The need for alternative techniques promoted the exploration of more sustainable and cost-effective ones and in this context another advanced oxidation processes (AOPs) arose [1,21,56]. They have proven to be effective in reducing the load of organic pollutants and eliminating pathogens in the treatment and purification of wastewater, while producing nontoxic by-products like carbon dioxide and water [2,14,62,63].

In this work, a coagulation/flocculation process followed by a disinfection step with an AOP, namely Fenton reaction, was performed, so these processes are further analysed in the following sections.

2.3 Coagulation/Flocculation process

The material in waters and wastewaters may include suspended and/or dissolved organic and inorganic matter, and various biological forms such as algae, bacteria and viruses [48,64]. These components are usually in the microscopic to submicroscopic size range, being referred as colloids [64]. Due to their small size, the attractive body forces between particles are less intense than the repelling forces of the electrical charges, promoting their stability and thus maintaining a dispersed state [48,64,65]. Coagulation and flocculation in water treatment is based in the addition of chemical substances (coagulants and/or flocculants) that interfere with the physical state of those dissolved and suspended solids to promote its sedimentation and subsequently an easier removal [65]. Although the terms coagulation and flocculation are often used interchangeably and ambiguously, as they are intimately connected, their definition is not the same. While coagulation is the process where the destabilization of a given system occurs, giving rise to aggregations, flocculation refers to the process where the destabilized particles are induced to come together and form large(r) agglomerates [64,65].

Coagulants are divided into two types: metal coagulants and polymers. The first ones are the most commonly used and consist of aluminium and iron salts, such as aluminium sulfate and ferric chloride, while the second ones refer to a large variety of natural or synthetic water soluble macromolecular compounds capable of destabilizing or enhance flocculation of the constituents of a body of water [40,64,65]. The popularity of iron and aluminium coagulants is due not only to their effectiveness, but also to their relatively low cost, wide range of pH and availability [39,64]. However, high residual concentrations of aluminium have been regularly associated as a potential risk factor in Alzheimer's and other diseases [66–68]. In this work, the focus will be on the metal coagulants.

There are four types of mechanisms that promote particle destabilization: double layer compression, charge neutralization, colloid entrapment or sweep flocculation, and intraparticle bridging [65]. The main mechanism to act in the coagulation process with metal coagulants is pH dependent. At pH values below 6, charge neutralization is the predominant one [64,69]. When the coagulant is added to the water, aluminium and ferric salts dissociate to form hydrated species, such as $Al(H_2O)_6^{3+}$ or $Fe(H_2O)_6^{3+}$ [64]. This positively charged species are often represented as the Al^{3+} and Fe^{3+} ions, and they

interact with the negatively charged colloids to form neutral compounds [65,70]. This binding of particles makes them larger and heavier, promoting a quick settling to the bottom of the water under the action of gravity (sedimentation) [39,47].

When $\text{pH} > 6$, the increasing amount of hydroxides (OH^-) will react with the metal coagulant (if added at a high enough concentration) to form metal hydroxide precipitates, namely $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$ [48,64]. If the added dosage is higher than the solubility of those amorphous precipitates, colloids may provide the nucleation site for precipitation to occur, which leads to entrapment of the particles as the precipitate forms (colloid entrapment mechanism). The settling hydrous precipitates then enmesh with other colloidal particles in water causing destabilization by a sweep action and assist their removal [48,64,71,72]. This mechanism promotes the formation of larger and stronger flocs compared to charge neutralization and thus enables a faster aggregation [48]. Colloid entrapment is an important mechanism when the particle concentration is low, such as effluents from secondary treatment, where collision rates are low and hence flocculation is naturally slow [72]. It also has the advantage of having minimal dependence on the nature of the impurity particles to be removed, whether it comprises bacteria, clays, oxides or others [48].

Flocculation, i.e., the interaction of destabilized particles to form larger aggregates (flocs), starts the moment the coagulant is added, due to thermal agitation (Brownian movement). This process (perikinetic flocculation) occurs naturally but is not sufficiently rapid for water treatment standards, so a velocity gradient is often induced in the liquid, commonly by mechanical agitation or by creating turbulence in the water flow (ortokinetic flocculation) [64]. The coagulated/flocculated water is usually moved to a settling tank or filtered directly through a medium filter (sand or gravel), a microfiltration or a ultrafiltration membrane [47].

A priori selection of a coagulant for any wastewater is not possible on the basis of theoretical analysis, since it largely depends on its matrix (e.g. composition, turbidity and colloid concentration), so an empirical approach under different processing conditions is necessary in order to optimize the process and therefore maximize the removal of pollutants from wastewater [73,74]. Besides water's matrix and mixing speed and time, water's pH and coagulant dosage are the most relevant parameters to have an influence on the removal efficiency, being further described below [69,74,75].

2.3.1 pH

As mentioned before, hydrated reaction products are formed in the coagulation/flocculation process with aluminium and iron coagulants. As there are other ligands in solution, substitutions in the complexes may occur. One of those ligands is OH^- , and the extent of H_2O substitution for this ligand depends on its concentration in solution, i.e. solution's pH. For a particular colloidal suspension, it is expected that some hydrolysis species perform more effective destabilizations than others, as they have different charges and adsorptivities [64]. Furthermore, at closer to neutral pH values there is usually a decrease in metal solubility, contributing to a higher precipitation of the previously mentioned metal hydroxides and therefore a possible higher removal of impurity particles [70]. Hence, pH optimization is an important factor in this process.

2.3.2 Coagulant dosage

The effect of coagulant dosage in the coagulation process depends on the destabilization mechanism [64]. In the charge neutralization mechanism previously described for metal coagulants, the cationic species neutralize the negative charges on pollutant species in a process that requires a just-sufficient dose, which corresponds to the quantity of colloids and surface charge. Any dose above or below this optimum concentration will again promote the stabilization of the solution, hindering the removal of pollutants from wastewaters. On the other hand, in a double layer compression, there is little or no dependence on coagulant dosage after the minimum/optimum dosage is obtained [64,73]. As previously mentioned, high enough coagulant dosages can lead to a greater formation of metal hydroxide precipitates and enhance destabilisation following the colloid entrapment mechanism [64].

2.3.3 Coagulation/flocculation application in wastewater treatment

This process is usually applied in the treatment of wastewater from several sources, such as dyes wastewater, municipal sewage, micro-polluted water, oily wastewater and paper industry wastewater [69]. It is used not only to separate suspended solids from water, but also to reduce colour, organic load and turbidity, to remove heavy metals, phosphates, substances with organoleptic characteristics such as taste and smell, algae and planktonic organisms, and to eliminate bacterial and viral populations and other microorganisms of pathogenic nature [69,76].

This process has also been widely used as a tertiary treatment in WWTPs, in which its focus is the removal of residual suspended particles, organic matter and phosphorus [39]. Taking into account nowadays need of water reuse, this process has also been studied with focus on the removal of microorganisms, including ARBs and ARGs [77]. When properly performed, coagulation/flocculation and sedimentation can result in 1-2 log removals of bacteria, viruses and protozoa [78].

Regarding secondary wastewater, Grehs et al. [40] studied the inactivation of total heterotrophs and faecal coliforms with and without antibiotic resistance using aluminium sulphate (220-350 mg/L) and tannin (50-80 mg/L) as coagulants at the natural pH of the effluent (7.3) and reported percentages of removal of 98.3-99.4% (1.9 to 2.6 log CFU/mL). Ghaneian et al. [74] noticed a reduction of fecal coliforms of 3 and 5 log MPN/100 mL at pH 7 with ferric chloride ($[FeCl_3] = 55$ mg/L) and polyaluminum chloride ($[PAC] = 45$ mg/L) coagulants, respectively. Rodríguez-Chueca et al. [16] reported a removal of enterococci and pseudomonads close to 1.50 log CFU/mL, while noticing an *E. coli* inactivation of 0.3 log CFU/mL ($[FeCl_3] = 15$ mg/L, pH = 5).

This process is also implemented in the final stages of drinking water treatment [79,80], namely by the US EPA surface water treatment, which uses a combination of coagulation/flocculation, sedimentation and filtration to enhance microorganism removal [64].

2.4 Advanced Oxidation Processes (AOPs)

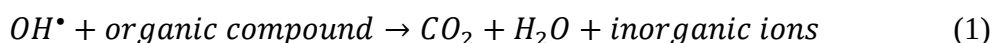
AOPs are a group of chemical treatment procedures designed to remove organic (or sometimes inorganic) materials present in water. Research and development of AOPs in wastewater treatment has grown over the years, given the potential that these technologies have to overcome the main disadvantages of conventional techniques, such as toxic disinfection byproducts in chlorination and no residual effect or high operational and maintenance costs with UV irradiation [1,56,81].

These processes are based on chemical oxidation, a high rate reaction in which one or more electrons are transferred from a species (reducing agent) to another (oxidizing agent) [82]. The greater the redox potential of the oxidizing agent, the greater its tendency to acquire electrons from another specie and therefore the more extent the oxidation reaction. Having that in mind, oxidative processes use oxidizing agents capable of forming radical species, benefiting from their high redox potentials [83]. The most popular one is the hydroxyl radical, which is the most reactive oxidizing agent in water treatment, with an oxidation potential of 2.8 eV, very close to fluorine potential (Table 1) [5,62,84]. Being a reactive electrophile, this radical reacts nonselectively and at nearly diffusion-limited rates with roughly all electron-rich organic compounds present in urban effluents, promoting a high oxidation capacity of those contaminants and a greater efficiency in applied treatments [83,85,86].

Table 1 - Oxidation potential of common oxidants [5,86].

Oxidant	Oxidation Potential (eV)
Fluorine (F_2)	3.0
Hydroxyl radical ($OH^\bullet$)	2.8
Sulfate radical ($SO_4^{\bullet-}$)	2.5-3.1
Ozone (O_3)	2.1
Persulfate ($S_2O_8^{2-}$)	2.1
Hydrogen peroxide (H_2O_2)	1.8
PerHydroxyl radical ($HO_2^\bullet$)	1.7
Permanganate (MnO_4^-)	1.7
Chlorine dioxide (ClO_2)	1.5
Chlorine (Cl_2)	1.4

When used in wastewater treatment, the hydroxyl radical contributes both to the degradation of organic recalcitrant contaminants (such as aromatic, phenolic and chlorinated compounds) and to the elimination of microorganisms. The reaction of $OH^\bullet$ with organic compounds leads to the formation of carbon-centered radicals, mainly by hydrogen abstraction from C-H, N-H and O-H bonds, adding to C-C bonds or adding to aromatic rings [83,84,87]. These compounds further react with $OH^\bullet$ and may be completely mineralized, generating water, carbon dioxide and sometimes inorganic ions as reaction products (Eq. 1) [62,87]. Inorganic ions formation depends on the precursor, usually being chlorides and nitrates. Since their concentration in the treated effluent is usually low, their presence at an environmental level is acceptable [63]. This ability to degrade the pollutants partially or even completely mineralize them is one of AOPs main advantage over traditional tertiary treatment processes like membrane filtrations and adsorption, which rather just concentrate or transfer them for another phase, respectively [2,14,62,63,88]. Generation and disposal of secondary waste material is thus prevented [2].



Regarding microorganism inactivation, as a prominent oxidant, the hydroxyl radical has the potential to cause permanent damage to the microorganism's cell structure, namely cell membrane, and microbial functions by attacking DNA either at the sugar or the base. This attack may lead to sugar fragmentation, base loss, strand breaks with a terminal fragmented sugar residue and eventually to cell lysis [14,85].

There are several types of AOPs, the main ones being represented in Figure 2 [62,89]. In addition to the use of an oxidizing agent (Table 1), radiation and/or catalysts are regularly used in order to speed up the oxidation reaction and thus contribute to a more effective treatment [83,84,90]. If the catalyst is in the same phase as the oxidant, the process is considered to be homogeneous. On the other hand, the process is heterogeneous when the catalyst is supported on a solid matrix and the reaction occurs on its surface [88].

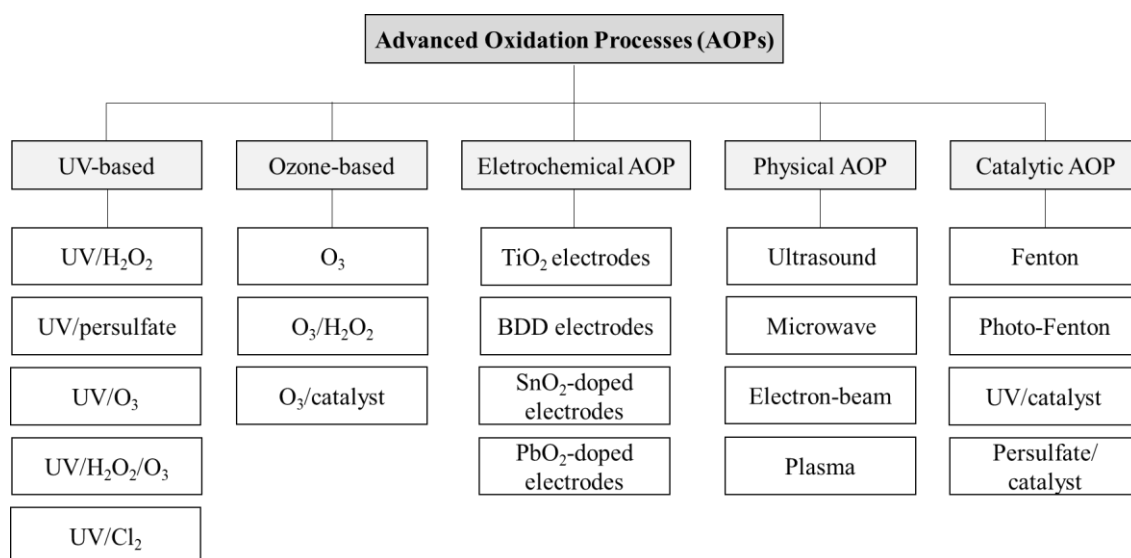


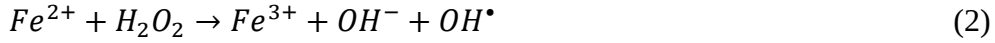
Figure 2 - Schematic representation and classification of different AOPs [62,89].

One of the most common ways of generating hydroxyl radicals is from hydrogen peroxide. This chemical compound is an oxidant on its own (Table 1) and its application has proven to be very effective in the removal of several organic and inorganic pollutants from wastewater, such as sulphites, hypochlorites, nitrites, cyanides, and chlorine [91]. The capacity of this uncharged molecule to diffuse into cells via their membranes has been documented, leading to cell damage and death, thus constituting a threat to microorganisms like *E. coli* [92,93]. However, oxidation by H_2O_2 alone is not effective for high concentrations of certain refractory contaminants, such as highly chlorinated aromatic compounds and some inorganic compounds (e.g. cyanides), as its reaction rates are low even at reasonable H_2O_2 concentrations. Hydrogen peroxide may be activated by ozone, UV radiation and transition metals, in order to generate more reactive species: hydroxyl radicals [84,86,91]. Iron is the most frequently used metal for this purpose, reacting with H_2O_2 in the so-called Fenton process [84,94].

2.4.1 Fenton Process

The possibility of hydrogen peroxide activation by iron salts in the oxidation of an organic compound was first reported in 1894 by Henry J. Fenton, whose name was given to the reaction [95]. In 1934, Haber and Weiss [96] established that the hydroxyl radical formed by the combination of those two compounds was responsible for the oxidation process. Since then, Fenton and related reactions have sparked interest due to their relevance to

biological chemistry, synthesis, the chemistry of natural waters and the treatment of hazardous waste [87]. Its mechanism is described in the following equations [87,97]:



In simple terms, the Fenton process consists in the combination of iron (II) and hydrogen peroxide to generate the hydroxyl radical, as described in Eq. 2. As this process is carried out in the dark, the regeneration of iron occurs at a very slow rate, following Eq. 3 and Eq. 4 [97,98]. While Eq.2 has a constant rate of $63 \text{ M}^{-1} \text{ s}^{-1}$, Eq. 3 has a constant rate several orders of magnitude lower ($0.002\text{-}0.01 \text{ M}^{-1} \text{ s}^{-1}$) [99].

Being a simple, flexible, low cost and high performance system that can operate efficiently at room temperature and atmospheric pressure are some of the advantages of the Fenton process [100,101]. There is no need for energy input and both chemicals used are easy to handle, inexpensive, harmless and cyclic in nature, so that a less concentration is needed [100,101]. While iron excels for its cost-effectiveness, environmental compatibility and low toxicity, hydrogen peroxide has the ability to be converted to molecular oxygen and water in the absence of any other oxidizable substances, two non-toxic products (Eq. 5) [5,87,101]. Furthermore, the mere presence of H_2O_2 may raise the permeability of cell membrane, reacting with iron ions to induce Fenton and Haber-Weiss reactions and thus promoting cell damage and even death [92].



The Fenton process is affected by several parameters. The main ones are the concentrations of oxidant (H_2O_2) and catalyst (Fe^{2+}), and the characteristics of the reaction, such a solution's pH, temperature, reaction time, initial concentration of pollutants and the presence of other ions [11,91,98]. This factors will control the rate of oxidation of organic and inorganic constituents and the regeneration capacity of iron during the process, making it important to understand the interactions and relationships between these parameters [91,100].

High concentrations of the Fenton oxidant and catalyst can act as scavengers of $OH^\bullet$, as described in Eq. 6 and Eq. 7 [87,91]. A scavenger is a chemical species that reacts with a free radical, generating stable products. The occurrence of this phenomenon prevents the

availability of hydroxyl radical for other reactions, such as the organic compounds degradation or the disinfection one. Even though the scavenging reaction performed by H_2O_2 generates another radical specie ($HO_2^\bullet$), it presents a lower oxidation potential than $OH^\bullet$ (Table 1). Minimizing this effect involves determining an optimal molar ratio of this species experimentally [84]. Other approaches, such as using iron catalytically, might also help reducing scavenging effects [87]. $[H_2O_2]$ and $[Fe^{2+}]$ needed for an effective treatment are influenced by the wastewater composition, as the amount and type of pollutants and other compounds present in the matrix might promote undesired secondary reactions [102].



In general, an increasing temperature will lead to an increase in the kinetic constants according to the Arrhenius law. However, thermal decomposition of hydrogen peroxide occurs at higher temperatures, possibly around 40-50°C, decreasing the extent of Fenton reaction [103,104].

Given the potential of Fenton treatments and to overcome some of its limitations, modified Fenton processes have been developed, such as electro-Fenton and photo-Fenton [88,105]. Photo-Fenton's main difference is the coupling of UV irradiation, which enhances catalyst regeneration and provides a higher radical generation, but also increases operating and maintenance costs [21,84]. Electro-Fenton takes advantage of electrochemical reduction of O_2 to continuously generate H_2O_2 *in situ*, but this generation is generally not very high and its implementation for integration to WWTPs needs further development to make it more sustainable and cost effective [105–107].

2.4.2 Fenton process application in wastewater treatment

Numerous AOPs have been applied in wastewater treatment, but Fenton and Fenton-like processes have shown to be the most cost effective, energetically efficient and less tedious method, whether used alone or coupled with conventional and biological methods [2]. It has been employed in the treatment of several types of wastewater at lab scale, such as olive-oil mill [108], pesticide [109], laboratory [110], cosmetic [111], dye [112], pharmaceutical [113], cork cooking [114], textile [115], phenolic [116], fermentation brine from green olives [117] and pulp mill effluents [118].

Fenton process in urban wastewater treatment is mostly used to degrade emerging chemical pollutants [97,119–121]. Regarding microorganism disinfection (ARBs and ARGs included), which is the focus of this work, modified Fenton processes are the most employed, namely photo-Fenton, which has been achieving promising results [38,122–126]. These studies favour the implementation of Fenton and Fenton-like processes in urban WWTP at neutral pH values, since it would be beneficial in terms of reducing operational costs with acidification and neutralization of substantial volumes of wastewater [21,122].

Regarding real urban effluents, inactivation values equal to or less than 1 log CFU/mL of total heterotrophs, total coliforms, *E. coli* and enterococci have been reported in continuous [122] (HRT = 60 min) and batch operation mode after 120 min [123] and 180 min [124] ($[H_2O_2] = 50$ mg/L and $[Fe^{2+}] = 20$ mg/L, pH = 7.0–7.1). 2.8–5.3 log removal of several ARGs have also been noted ($[H_2O_2] = 0.68$ mg/L and $[Fe^{2+}] = 0.34$ mg/L, pH = 7.0) [127]. Although some of the reported results may not seem encouraging, the conditions in which they were obtained were not optimized. If that was the case, better inactivation values should be obtained.

Fenton and Fenton-like processes may also be coupled with other processes in tertiary treatment of urban wastewater, such as coagulation. This kind of combination has been studied once by Rodríguez-Chueca et al. [16]. A photo-Fenton process applied on its own to the secondary wastewater resulted in a total inactivation of *E. coli*, and enterococci and pseudomonads removal of 4.04 and 3.50 logs, respectively, after 210 min ($[Fe^{3+}] = 5$ mg/L, $H_2O_2 = 25$ mg/L, pH = 5, irradiance = 500 W/m²). Meanwhile, this same process performed after a coagulation/flocculation process achieved better results. The coagulation/flocculation process allowed a removal of enterococci and pseudomonads close to 1.50 log, while noticing an *E. coli* inactivation of 0.3 log ($FeCl_3 = 15$ mg/L, secondary urban wastewater). The photo-Fenton process under the same conditions but using only the residual iron present in the sample after the coagulation/flocculation process as catalyst (0.4 mg/L) resulted in a total inactivation of all the microorganisms under study (4.61, 5.64 and 5.30 logs of *E. coli*, enterococci and pseudomonads, respectively). The combination of these two processes in the opposite order provided worse results.

3. Materials and Methods

3.1 Sampling and experimental procedure

The wastewater used in this study (herein named STWW) was collected after the secondary settling process from Gaia Litoral WWTP, located in North of Portugal. The samples were subjected to a combination of coagulation/flocculation followed by the Fenton process, and the effect of various operating conditions was evaluated in order to determine the best ones that maximize the disinfection. The wastewater obtained after coagulation/flocculation is referred to as CTWW, while the one obtained after the Fenton process is named FTWW. Three experimental replicates were assayed with independent STWW samples collected on different days, where microbial load was assessed in the three types of samples (STWW, CTWW and FTWW), as well as microorganism regrowth after storage in the dark at room temperature for 72 h. Physico-chemical characteristics were evaluated in one of the replicates.

Coagulation/flocculation and Fenton assays were performed in the day or the day after STWW collection (refrigeration at 5 °C, for a maximum period of 24 h), in order to ensure that the characteristics of the sample did not undergo changes that could influence the experimental results.

3.2 Chemicals

Hydrogen Peroxide (H_2O_2) 30% (w:v) was purchased from VWR, ferric chloride ($FeCl_3$) was acquired from Quimitécnica with a purity of 40% and ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) was obtained from Panreac with a purity of 99%. The sodium hydroxide ($NaOH$, 99%) and sulphuric acid (H_2SO_4 , 98%) used for pH adjustment were from EKA and VWR, respectively. Amoxicillin and sulfamethoxazole were both purchased from Sigma-Aldrich, with a purity of 99%.

3.3 Coagulation/Flocculation

Coagulation/flocculation assays were carried out in a Jar Test (VELP P4) apparatus with four stirred beakers of 600 mL capacity, with controlled mixing speed and at room temperature. The beakers were filled with 400 mL of STWW and, when needed, pH was adjusted with $NaOH$ (1 N) and/or H_2SO_4 (1 N). The samples were immediately stirred at 200 rpm for 3 min upon coagulant addition ($FeCl_3$, 40-120 mg/L) to promote homogenization, and then at 15-20 rpm for 20 min, before settling for at least 30 min.

3.4 Fenton reaction

Volumes of 250 mL or 1 L of CTWW were fed to a glass batch reactor previously sterilized, with a capacity equal to the sample volume. The reactor was equipped with a water recirculation jacket connected to a thermostatic bath (Huber Polystat) to control the temperature inside. After reaching the target temperature, the desired amounts of iron ($FeSO_4 \cdot 7H_2O$ or $FeCl_3$) and hydrogen peroxide were added (when necessary). The addition of hydrogen peroxide marked the initial instant of the reaction. Throughout the runs, the wastewater inside the reactor was stirred at 200 rpm; for this purpose, a magnetic bar and stir plate (VWR, model VS-C7) were used. Samples were taken at specific reaction times to perform microbiological and/or physico-chemical analysis.

3.5 Enumeration of cultivable microorganisms

Enterobacteria with and without resistance to amoxicillin or sulfamethoxazole, enterococci and total heterotrophs were the microbial groups monitored in this work.

The filtration method was used to enumerate the target bacterial groups in the water samples. Samples were serially diluted (1:10, up to 10^{-4}) in sterile saline solution (0.85% NaCl) and 1 mL of the diluted samples, or up to 10 mL sample, was filtered through a cellulose nitrate membrane with a pore size of 0.2 μm (Sartorius Stedium Biotech GmbH). The membranes were placed onto the surface of culture medium in Petri dishes. Difco Membrane Fecal Coliform (mFC) Agar with and without amoxicillin (32 mg/L) and sulfamethoxazole (350 mg/L), Slanetz and Bartley Agar and Plate Counting Agar (PCA) were the culture media used to enumerate enterobacteria, enterococci and total heterotrophs, respectively. Enterobacteria and enterococci cultures incubated at 37 °C for 24 h and 48 h, respectively, while total heterotrophs incubated at 30 °C for 72 h.

Colony Forming Units (CFU) were enumerated in the respective culture medium and expressed as CFU per 100 mL of sample, with a detection limit (DL) of 10 CFU/100 mL, since 10 mL was the maximum volume filtered. Microorganism inactivation was calculated by Eq. 8, where $Inactivation_t$, CFU_t and CFU_0 stand for the microorganism inactivation at time t , and the Colony Forming Units per 100 mL at time t and at the initial instant, respectively.

$$Inactivation_t (\%) = \left(1 - \frac{CFU_t}{CFU_0}\right) \times 100 \quad (8)$$

All determinations were performed in duplicate, and the coefficient of variation is less than 16% for all parameters measured.

3.6 Physico-chemical characteristics

Total organic carbon (TOC) was determined according to Method 5310D [128] on a Shimadzu TOC-L apparatus. In this technique, the sample is catalytically oxidized at 720 °C, generating carbon dioxide which is measured in an infrared detector.

Biological oxygen demand (BOD₅) was determined according to Method 5210D [128]; it quantifies the oxygen consumed, for 5 days at 20 °C, by the microorganisms to degrade organic matter susceptible to biological degradation. For this purpose, an OxiTOP (Velp Scientifica) apparatus was used.

Total phosphorus (P_{total}) was quantified by colorimetry by the ascorbic acid method (Method 4500P – E [128]), after digestion with ammonium persulfate. The intensity of the blue color resulting from the reaction between phosphorus and ascorbic acid was measured by absorbance at 800 nm. For this purpose, a Thermo Electron Corporation model Helios γ spectrophotometer was used.

Total nitrogen (N_{total}) was quantified by potassium persulfate digestion (Method 4500 N. C [128]), which converts organic nitrogen, ammonia and nitrite into nitrate, with successive determination of nitrate by molecular absorption spectrophotometry after reaction with brucine (Method D992-71 [129]).

Ammonia nitrogen - NH₃ - was measured by ammonia ion selective electrode (from ThermoScientific, model Orion 9512HPBNWP) according to Method 4500-NH₃ D [128]. Total suspended solids (TSS) were quantified by gravimetric analysis – Method 2540 B [128], respectively.

Turbidity was determined using a turbidimeter HI88703 from Hanna Instruments, following the Method 2130 B [128].

Iron present in solution was measured by flame atomic absorption spectrometry (Method 3111 B [128]), using an AAS UNICAM spectrophotometer (model 939/959). The samples were filtered through nylon filters with 0.45 µm of porosity and then acidified with concentrated nitric acid till pH ~1.0 to keep iron dissolved.

All determinations were performed in duplicate except for BOD₅, and the coefficient of variation is less than 1.1% for TOC, 31% for P_{total}, 1.3% for N_{total}, 7.7% for NH₃, 18% for TSS, 5.9% for turbidity and 10% for iron.

4. Results and Discussion

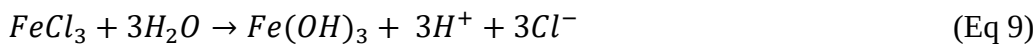
The secondary treated wastewater (STWW) was subjected to a treatment consisting of a combination of coagulation/flocculation and Fenton processes. The operating conditions of both treatment processes were studied in order to maximize the disinfection efficiency, i.e., microbial inactivation. The goal was to generate a treated wastewater that meets the requirements imposed by legislation for its reuse, namely the amount of *E. coli*. Since this microorganism belongs to the family *Enterobacteriaceae*, ensuring the inactivation of this group of microorganisms also guarantees *E. coli* inactivation. The strictest limit imposed by legislation for *E. coli* is < 10 CFU/100 mL [27,28], which corresponds to the highest quality water class (class A, Table 2) for reuse in irrigation and some urban utilities. Hence, this was the target value in the following experiments.

Table 2 - Limits of *E. coli* (CFU/100 mL) imposed by European and Portuguese legislation [27,28] for water reuse in irrigation

Water class	<i>E. coli</i> (CFU/100 mL)
A	< 10
B	$< 10^2$
C	$< 10^3$
D	$< 10^4$
E	$< 10^5$

4.1 Coagulation/Flocculation process

Ferric chloride ($FeCl_3$) was the coagulant used in this work, due to its effectiveness and easy obtainment by the company. In addition, the use of this coagulant contributes to the presence of a residual amount of iron in the coagulated wastewater (CTWW), further enhancing the following Fenton process. Its hydrolysis results in the formation of insoluble ferric hydroxide flocs. According to the iron speciation diagram, $Fe(OH)_3$ and $Fe(OH)^{2+}$ are the predominant species for pH 3-9 (Eq, 9-11) [105,130].



A Jar Test was carried out to determine the best conditions for the coagulation treatment. Different process parameters of the coagulation process are studied in batch mode to find out the ones who get the most suitable performance [73]. Coagulant dosage (40-140 mg/L, at the natural pH of the STWW = 7.14) and pH (3-9) were the two parameters tested. Results are shown in Figures 3 to 7.

4.1.1 Effect of the coagulant dosage

It is possible to observe in Figure 3a that an increase in the coagulant dosage resulted in a higher decrease in water's pH after the coagulation process. This was due to the successive increase of iron in solution, which is known to contribute to the formation of H^+ and therefore lower solution's pH (Eq. 9-11) [130]. Sludge volume was also affected by coagulant dosage: the higher the amount of iron added, the greater the volume of sludge formed, as expected (Figure 3b).

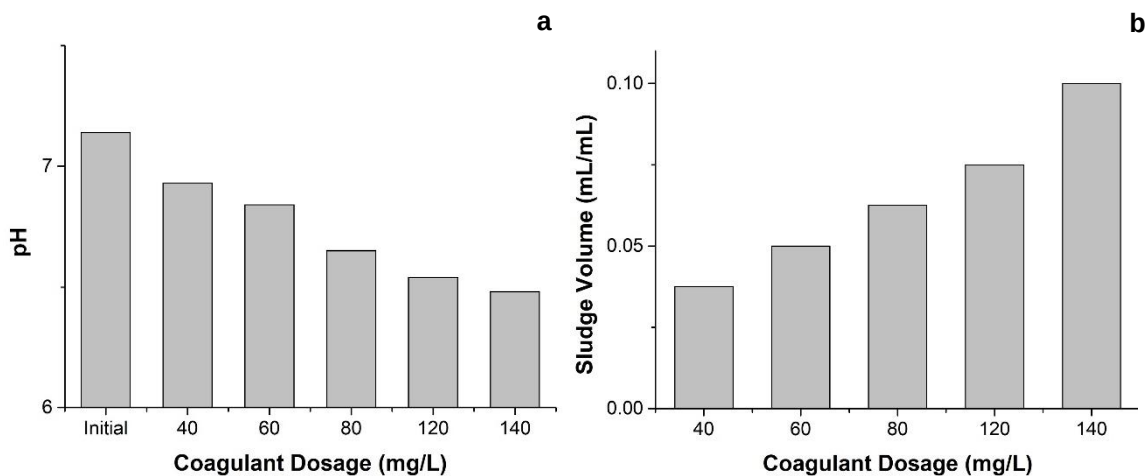


Figure 3 - Variation of pH (a) and sludge volume (b) with coagulant dosage in the coagulation process (initial pH of the STWW - 7.14).

Removal of turbidity and TOC, as well as enterobacteria inactivation, are displayed in Figure 4. The coagulant dosage of 120 mg/L achieved the highest removal of turbidity – 93% (Figure 4a), while the best results for TOC removal were obtained for the coagulant dosages of 120 and 140 mg/L – 46% (Figure 4b). In what concerns enterobacteria removal, coagulant dosages of 80, 120 and 140 mg/L were able to provide inactivation values of 99% and higher (Figure 4c). While increasing coagulant dosage may favour the formation of metal hydroxide precipitates and enhance destabilisation due to the colloid

entrapment mechanism, it can also reverse the charge neutralisation effect and promote the re-stabilization of the suspension [64]. This combination of effects might explain the non-improvement of the process for coagulant doses of 80 mg/L and higher.

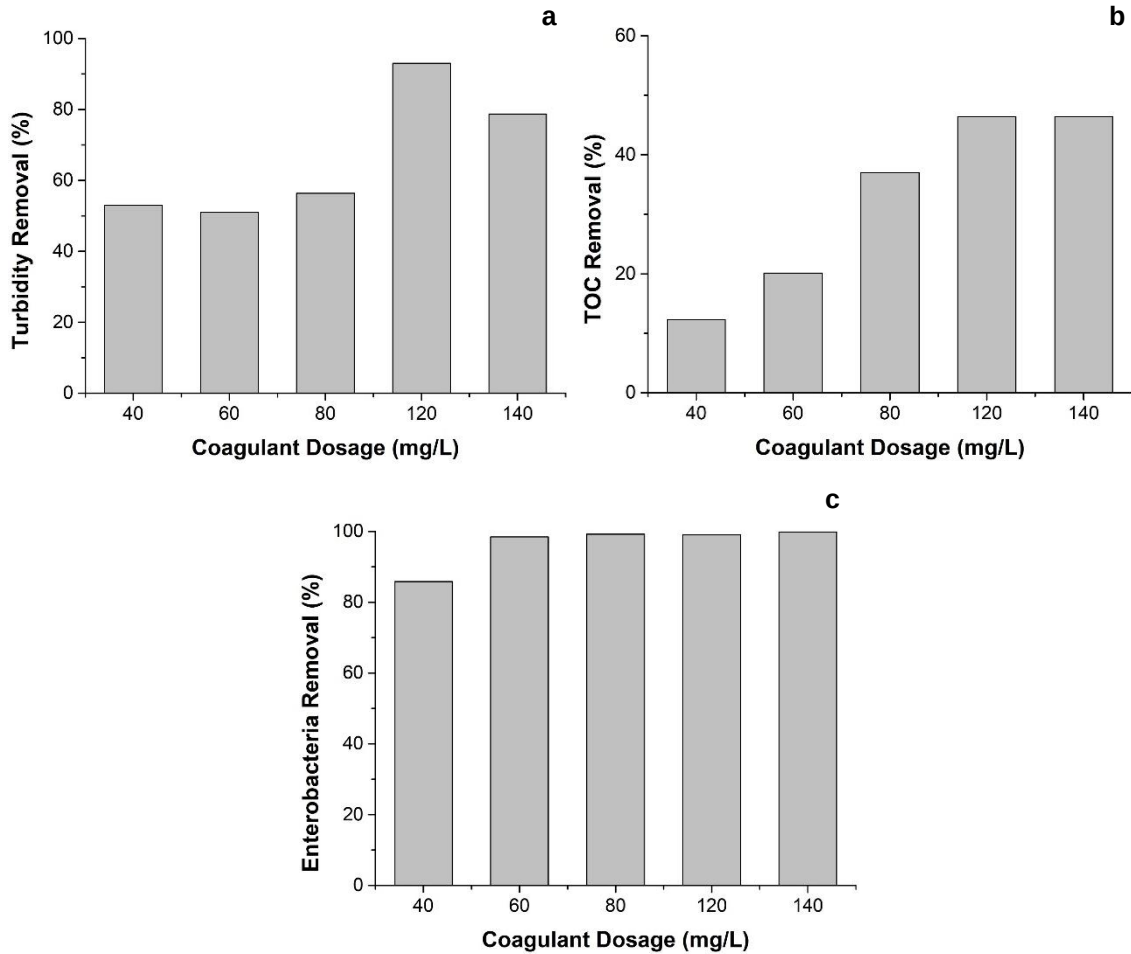


Figure 4 - Effect of coagulant dosage on turbidity (a), TOC (b) and enterobacteria removal (c) after the coagulation process (initial pH of the STWW = 7.14).

Overall, the coagulant dosage of 120 mg/L provided the best results and was therefore used in the following runs.

4.1.2 Effect of the pH

Figure 5a shows a similar effect to the one noticed in the previous runs in what regards the pH after the coagulation process, as a slight decrease is noticeable in all the runs due to H^+ formation by iron addition (Eq. 9-11). An increase in the volume of sludge formed was observed as the pH increased until the natural pH of the effluent (Figure 5b). From

that value on, the volume of sludge formed was constant. These results are explained by the fact that iron's solubility decreases as pH increases, which leads to a greater precipitation of this metal at neutral pH values, and therefore a greater sludge formation [87].

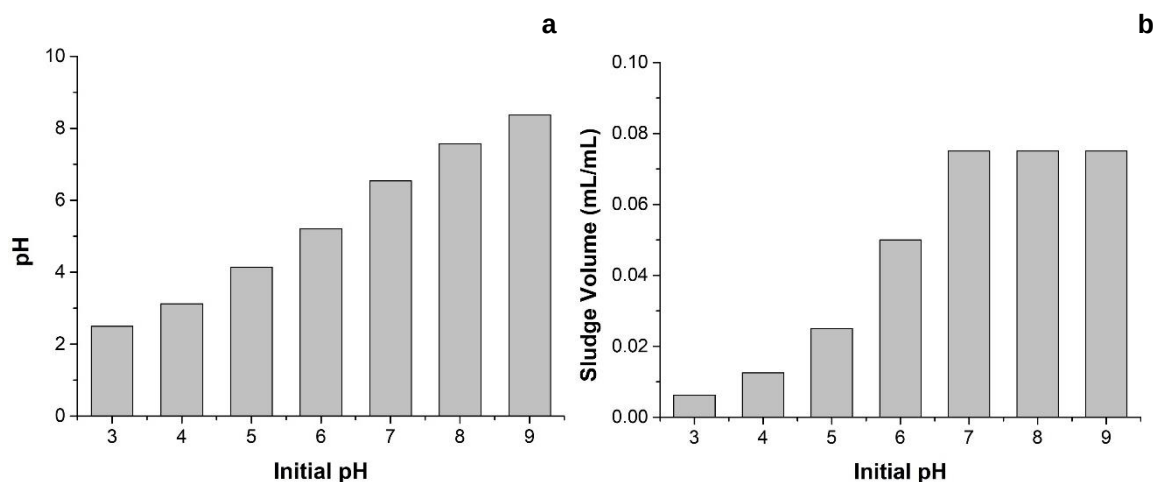


Figure 5 - Variation of pH (a) and sludge volume (b) with initial pH after the coagulation process for the CTWW ($[\text{FeCl}_3] = 120 \text{ mg/L}$). The run at initial pH 7 was performed at the natural pH of the STWW = 7.14.

The highest removal of turbidity (93%) was obtained when the sample was at its natural pH of 7.14 (Figure 6a), while the runs at that same pH and higher recorded the highest removals of TOC – 46% (Figure 6b). The observed turbidity removal of 93% is in order with the ones obtained at the same pH by Grehs et al. [40], who reported a 94% and 89% turbidity removal with aluminium sulphate and tannin coagulants, respectively, and higher than the ones obtained by Ghaneian et al. [74] (74% and 77% with ferric and polyaluminum chloride, respectively).

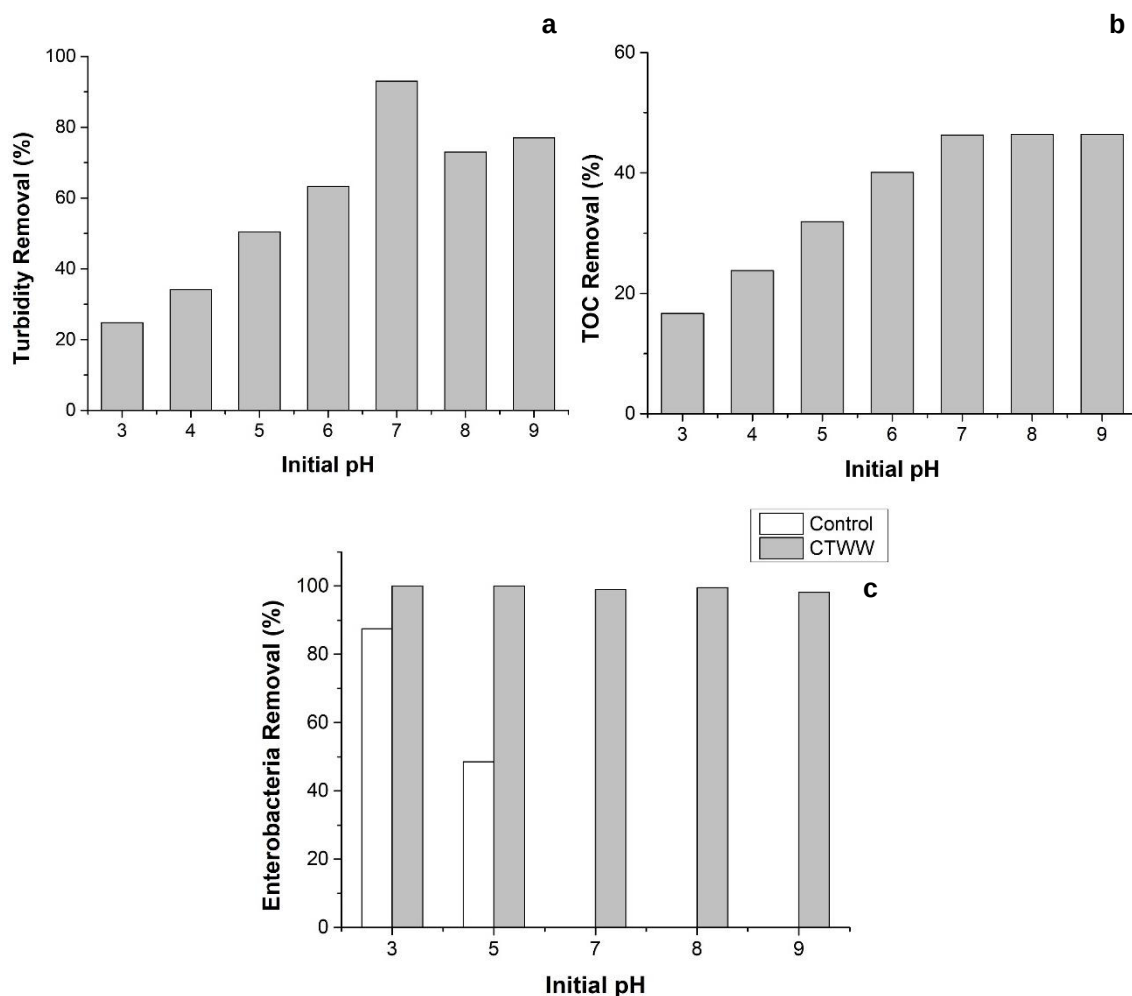


Figure 6 - Effect of initial pH on turbidity (a), TOC (b) and enterobacteria (c) removal after the coagulation process ($[\text{FeCl}_3] = 120 \text{ mg/L}$), where the white bars represent the control ($[\text{FeCl}_3] = 0 \text{ mg/L}$). The run at initial pH 7 was performed at the natural pH of the STWW = 7.14.

Enterobacteria removal was assessed for pH 3, 5, 7 (natural pH of the STWW = 7.14), 8 and 9 and it is possible to observe in Figure 6c that all the runs performed, with the exception of the one carried out at pH 9, obtained inactivation values above 99%. However, as it is known, pH plays an important role in microorganism growth and survival, so medium acidification might have had influence in the disinfection process at lower pH [131]. To evaluate its effect in the results obtained at pH 3 and 5, two control runs (without the addition of coagulant) were performed, in which pH was adjusted to 3 and 5 (white bars in Figure 6c). It is noticeable that lowering the pH to 3 and 5 resulted in an 88% and 49% enterobacteria inactivation, respectively. Thus, it is possible to conclude that a significant part of the inactivation obtained previously was due to the pH effect and not to the coagulation/flocculation process. Taking into account only the

inactivation due to the coagulation/flocculation process, the best results were obtained for pH 7.14 and 8.

Taking a look at these results, the pH with best overall results was the natural pH of the effluent - 7.14. Not only it provides the best turbidity removal, but it also allows to reduce acidification and neutralization costs.

The subsequent coagulation assays were performed in the best conditions obtained - $[FeCl_3] = 120$ mg/L and natural pH = 7.14. The mean values obtained for the enterobacteria load before and after the coagulation/flocculation process in the several coagulation runs made throughout the work with these conditions are represented in Figure 7, with their associated standard deviation.

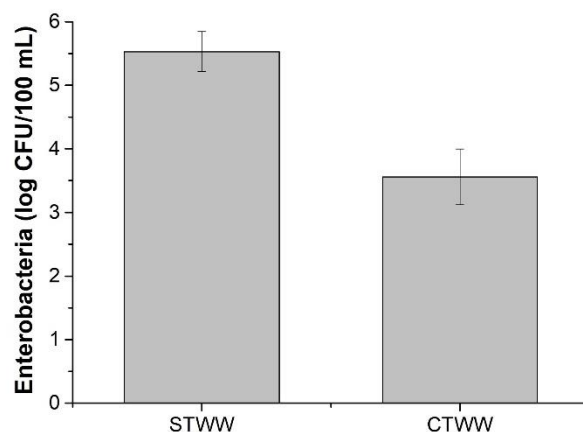


Figure 7 - Quantification of enterobacteria STWW and CTWW ($[FeCl_3] = 120$ mg/L and natural pH of the STWW = 7.14). Bars represent standard deviation.

It is possible to observe a near 2 log CFU/100 mL reduction with this process (5.53 to 3.56 log CFU/100 mL). These results are in order with the 1.9 to 2.6 log CFU/mL enterobacteria reduction (inactivation of 98.3-99.4%) obtained by Grehs et al. [40] with aluminium sulphate and tannin coagulants, and significantly surpass the 0.3 log CFU/mL *E. coli* reduction obtained by Rodríguez-Chueca [16] using the same coagulant that was used in this work but at a concentration approximately 10 times lower ($[FeCl_3] = 15$ mg/L, pH = 5).

4.2 Fenton process

The effluent sample obtained after the coagulation process (CTWW) was subjected to a Fenton treatment. The combination of these two processes was performed having in mind that residual iron from the coagulation/flocculation process would be found in the CTWW and used as catalyst of the Fenton process. Oxidant and catalyst added concentrations, as well as temperature, were the operational parameters of the oxidative process whose effects were assessed in the determination of the conditions that maximize the disinfection process.

4.2.1 Influence of reaction time

A preliminary run was performed to evaluate the disinfection process performance along reaction time, measured by inactivation of enterobacteria. The reaction was carried out at a temperature of 25 °C, at the natural pH of the CTWW and with added concentrations of oxidant (H_2O_2) and catalyst (Fe^{2+} from $FeSO_4$) of 50 mg/L and 4 mg/L, respectively. While hydrogen peroxide concentration used is similar to the one reported in recent bibliography [122–124], iron was added in a smaller amount (5 times less), as one of the goals was to use residual iron left from the coagulation process to catalyse hydrogen peroxide decomposition. That amount was measured in the CTWW and a mean value of 0.51 mg/L was obtained. Thus, the total iron concentration to act in the Fenton process was 4.51 mg/L. Samples were taken 5, 10, 15, 30, 60, 90 and 120 min after the start of reaction and the results obtained are shown in Figure 8.

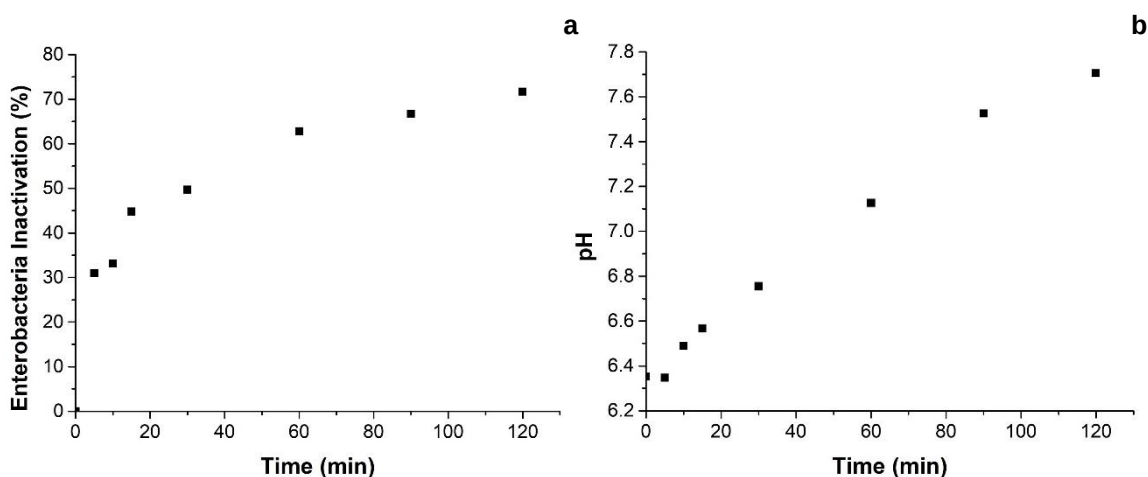


Figure 8 - Evolution of enterobacteria inactivation (a) and pH (b) during the Fenton process ($[H_2O_2] = 50$ mg/L, $[Fe^{2+}]_{add} = 4$ mg/L, initial CTWW pH = 6.35 and $T = 25$ °C).

Inactivation of enterobacteria increased with time, as expected, since the oxidation reaction generates hydroxyl radicals that act on microorganisms present in the wastewater (Figure 8a). It was noticeable that there was always an increase in disinfection over time, although gradually less accentuated, which indicates that the reaction was still occurring after 90 to 120 min, even though in a lower rate.

Figure 8b shows the pH evolution over reaction time. In the first 5 min, it is possible to observe a slight decrease in the pH, followed by an increase matching the bacterial inactivation profile. This pH behavior was observed in most of the runs performed in this work. The initial decrease is probably due to the simple addition of iron salt, whose hydrolysis contributes to the acidification of the medium [130]. The first assumption for the following rise of pH was the generation of OH^- by the Fenton main reaction (Eq. 2). However, further analysis reported an increasing amount of ammonia after the reaction, which should be the main explanation for the pH rise (section 4.4) [132].

For sampling in the following experiments, two reaction times beyond the initial instant were selected: 5 and 120 min after the start of the reaction. The shorter time was chosen due to the fact that sometimes it is not possible to observe and compare the effect of some parameters on the process efficiency after long reaction times and the longer one was selected since it was noted that the oxidative process and adjacent disinfection was still occurring at that time.

4.2.2 Influence of added iron concentration

The dose of catalyst was the first parameter to be optimized, as it plays a critical role in the oxidative process. Five experiments were carried out, where the added iron concentration varied in range 0 to 10 mg/L, which corresponded to a total catalyst dose of 0.51 to 10.51 mg/L. The remaining conditions were kept constant ($[H_2O_2] = 50$ mg/L, initial natural pH = 6.33-7.03 and $T = 25$ °C) and results obtained are shown in Figure 9.

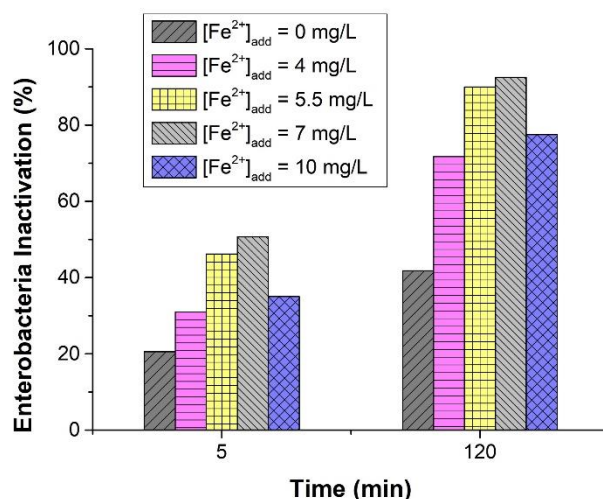


Figure 9 - Effect of added iron concentration on enterobacteria inactivation after 5 and 120 min of the Fenton process ($[H_2O_2] = 50$ mg/L, initial natural pH = 6.33-7.03 and $T = 25$ °C).

The run without addition of iron ($[Fe^{2+}]_{add} = 0$ mg/L) demonstrated a small but still significant enterobacteria inactivation - 41.8% after 120 min. These results are due to the fact that not only hydrogen peroxide by its own is an oxidant (Table 1), but also due to the leftover iron in solution from the coagulation/flocculation process (0.51 mg/L), which contributes to the Fenton process. Nevertheless, it is possible to conclude by these results that the Fenton process with $[H_2O_2] = 50$ mg/L and using only the leftover iron as catalyst does not provide a satisfactory disinfection process.

An increasing iron concentration should contribute to a higher generation of hydroxyl radicals (Eq. 2) and lead to a more efficient disinfection. However, results show a more effective enterobacteria inactivation with an added iron concentration of 7 mg/L compared to a higher one of 10 mg/L. This is probably due to scavenging reactions of the hydroxyl radical, in which the excess iron in solution reacts with the hydroxyl radical (Eq. 6), decreasing the amount of these radicals available to inactivate microorganisms, as is desired in the disinfection process.

Regarding pH (Table 3), the use of a real urban effluent contributed to slightly different values for the initial instant in the several runs (6.33 to 7.03). The greater the amount of iron added, the greater the decrease in pH after 5 min of reaction, as expected since the simple addition of iron contributes to the acidification of the solution [130].

Table 3 - Evolution of pH during the Fenton process for each tested iron concentration ($[H_2O_2] = 50$ mg/L, initial pH = 6.33-7.03 and $T = 25$ °C).

Time (min)	pH				
	$[Fe^{2+}]_{add} = 0$ mg/L	$[Fe^{2+}]_{add} = 4$ mg/L	$[Fe^{2+}]_{add} = 5.5$ mg/L	$[Fe^{2+}]_{add} = 7$ mg/L	$[Fe^{2+}]_{add} = 10$ mg/L
0	7.03	6.35	6.79	6.90	6.33
5	7.07	6.34	6.76	6.83	6.11
120	7.82	7.71	7.65	7.67	7.73

According to these results, the best value for the added iron concentration was the one of 7 mg/L; thus, this concentration was used in the subsequent experiences.

4.2.3 Influence of peroxide hydrogen concentration

This set of assays aimed at determining the best dose of oxidant. Aside from a run without addition of hydrogen peroxide ($[H_2O_2] = 0$ mg/L), concentration values of 40, 50, 75, 100 and 125 mg/L were tested and results are shown in Figure 10 (with $[Fe^{2+}]_{add} = 7$ mg/L, initial natural pH = 6.72-7.01 and $T = 25$ °C).

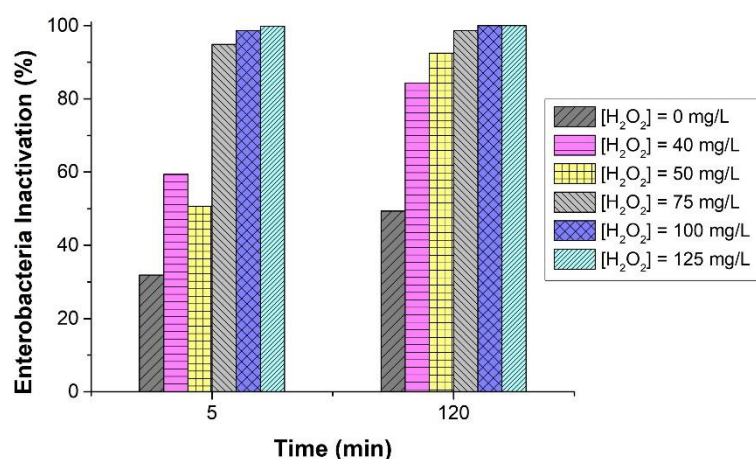


Figure 10 - Effect of hydrogen peroxide concentration on enterobacteria inactivation after 5 and 120 min of the Fenton process ($[Fe^{2+}]_{add} = 7$ mg/L, initial natural pH = 6.72-7.01 and $T = 25$ °C).

In the run without addition of hydrogen peroxide ($[H_2O_2] = 0$ mg/L) it was observed an enterobacteria inactivation of 49.4% after 120 min. Since the oxidizing agent is not

present, these results should be due to the simple addition of iron. The positively charged iron ions are able to establish interactions not only with the natural organic matter present in the wastewater but also with microorganisms, namely enterobacteria, leading to the formation of iron-enterobacteria and iron-natural organic matter complexes. These interactions may be due to uneven distribution of charges or by binding with the lipopolysaccharides which constitute the outer surface of bacterial membranes [94,133]. Furthermore, microorganisms themselves might also adsorb to particles present in the wastewater matrix [134]. Since the run occurs at neutral pH, some iron complexes will precipitate and deposit, dragging the enterobacteria attached to them or attached to the naturally organic matter co-precipitating with iron, thus leading to their removal. The interaction between iron oxides in the proper conditions might also induce a strong oxidative damage on bacterial surface [94]. In addition, added iron may also activate some other compounds present in the wastewater that eventually may generate radicals and therefore contribute to disinfection.

Once again it was expected that a higher concentration of a Fenton reagent, in this case hydrogen peroxide, would result in hydroxyl radicals being formed at a higher rate, and in a more effective disinfection (Eq. 2). Results are according to this premise, as it is possible to observe that the increase in H_2O_2 concentration led to higher enterobacteria inactivation. In fact, concentrations of 100 and 125 mg/L provided values below DL (< 10 CFU/100 mL), which corresponds to the strictest imposed value for *E. coli* in the legislation [27,28].

The best results were obtained for the higher hydrogen peroxide concentrations used ($[H_2O_2] = 100$ mg/L and $[H_2O_2] = 125$ mg/L). Having in mind the goal of a future implementation of this method on an industrial scale and therefore a lower reagent cost, the concentration of 100 mg/L was selected as the best.

4.2.4 Influence of temperature

Lastly, the effect of temperature in the disinfection process was assessed. A rising temperature causes an increase in reaction kinetics according to Arrhenius law, leading to an increased generation of hydroxyl radical if values where thermal decomposition of hydrogen peroxide occurs are not achieved (around 40-50°C) [135]. On the other hand, temperature is also a critical environmental factor when it comes to microbial

development, being therefore widely used in disinfection and sterilization processes [136]. In Gaia Litoral WWTP, wastewater temperature values during treatment are usually around 25 °C in the summer and around 15 °C in the winter. Therefore, three runs were performed at 15 °C, 20 °C and 25 °C. Results are shown in Figure 11 (with $[H_2O_2] = 100 \text{ mg/L}$, $[Fe^{2+}]_{add} = 7 \text{ mg/L}$ and initial natural pH = 6.47-6.79).

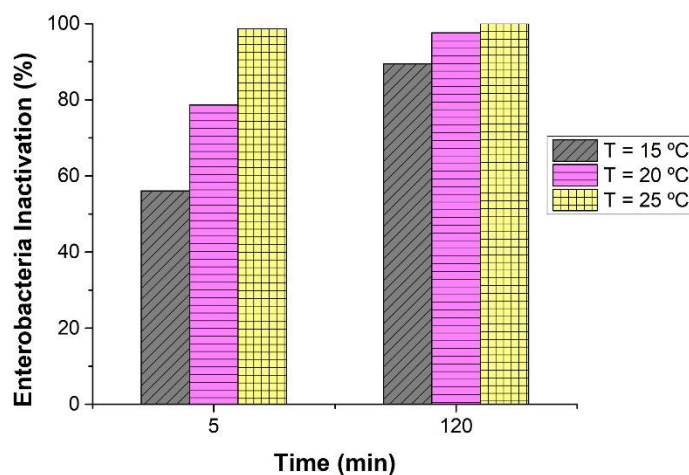


Figure 11 - Effect of temperature on enterobacteria inactivation after 5 and 120 min of the Fenton process ($[H_2O_2] = 100 \text{ mg/L}$, $[Fe^{2+}]_{add} = 7 \text{ mg/L}$, initial natural pH = 6.47-6.79).

As expected, the temperature that led to better results was 25 °C. Indeed, higher temperatures contribute to a more efficient disinfection, since they favor the hydrogen peroxide oxidation reaction and therefore lead to a greater generation of hydroxyl radicals [135]. Enterobacteria optimal growth temperature, including *E. coli*, is usually around 37 °C, so the tested temperatures did not contribute to thermal inactivation of this microorganisms [137].

Looking at these results, it is possible to conclude that this method provides a more efficient disinfection in summer time. Inactivation percentages obtained for lower temperatures (89.4% and 97.7% for 15 °C and 20 °C, respectively) are high but not high enough to meet the strictest limit imposed by the legislation (Table 4) [27,28]. However, this FTWW could still be reused in irrigation (class C and lower, Table 2) and in some urban utilities, such as street cleaning.

Table 4 - Enterobacteria quantification during the Fenton process for each tested temperature ($[\text{H}_2\text{O}_2] = 100 \text{ mg/L}$, $[\text{Fe}^{2+}]_{\text{add}} = 7 \text{ mg/L}$, initial natural pH = 6.47-6.79).

Time (min)	Enterobacteria (CFU/100 mL)		
	T = 15 °C	T = 20 °C	T = 25 °C
0	1.7×10^4	1.5×10^3	8.5×10^3
5	7.3×10^3	3.1×10^2	1.2×10^2
120	1.8×10^3	3.4×10^2	< 10

Values higher than 25 °C could favor to a greater extent the above mentioned reactions and consequently the disinfection process. Other authors also reported a higher microorganism inactivation (*E. coli* and *Ent. faecalis*) at higher temperatures in Fenton and Fenton related processes, including ones above 25 °C [135,138]. However, higher temperatures require higher energy costs, which might not be desirable.

4.2.5 Influence of iron addition in disinfection

In section 4.2.3 it was noticed an increase in enterobacteria inactivation when hydrogen peroxide concentration was also increased. To assess if that improvement in the disinfection process was due to an enhanced Fenton reaction and not by the effect of the increase in hydrogen peroxide by itself, two control runs were performed. Results obtained in the two controls and in the previously determined best conditions are presented in Figure 12 and Table 5.

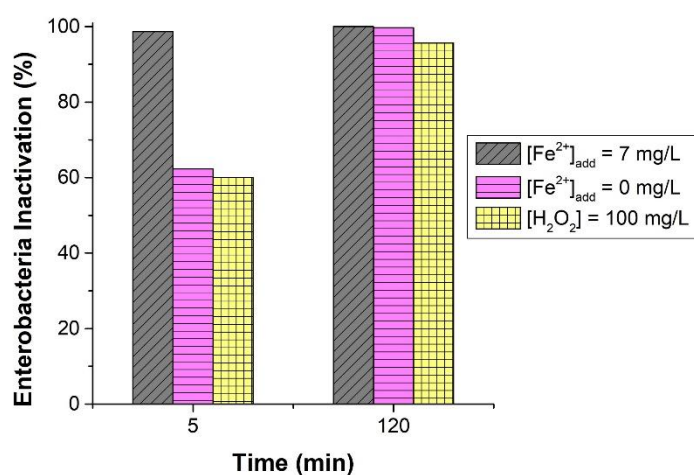


Figure 12 - Enterobacteria inactivation during Fenton oxidation of CTWW in the best determined conditions ($[\text{Fe}^{2+}]_{\text{add}} = 7 \text{ mg/L}$, grey bars) and without iron addition ($[\text{Fe}^{2+}]_{\text{add}} = 0 \text{ mg/L}$, pink bars), and with only hydrogen peroxide addition in a 1:100 diluted STWW ($[\text{H}_2\text{O}_2] = 100 \text{ mg/L}$, yellow bars) ($[\text{H}_2\text{O}_2] = 100 \text{ mg/L}$, initial natural pH = 6.63-6.79 and T = 25°C).

Table 5 - Enterobacteria quantification during Fenton oxidation of CTWW in the best determined conditions ($[Fe^{2+}]_{add} = 7 \text{ mg/L}$) and without iron addition ($[Fe^{2+}]_{add} = 0 \text{ mg/L}$), and with only hydrogen peroxide addition in a 1:100 diluted STWW ($[H_2O_2] = 100 \text{ mg/L}$) ($[H_2O_2] = 100 \text{ mg/L}$, initial natural pH = 6.63-6.79 and $T = 25^\circ\text{C}$).

Time (min)	Enterobacteria CFU/100 mL		
	$[Fe^{2+}]_{add} = 7 \text{ mg/L}$	$[Fe^{2+}]_{add} = 0 \text{ mg/L}$	$[H_2O_2] = 100 \text{ mg/L}$
0	8.5×10^3	6.5×10^3	1.3×10^3
5	1.2×10^2	2.5×10^2	5.0×10^2
120	< 10	2.0×10^1	5.5×10^1

The first control was performed in the CTWW and done in the same conditions as the run with the best results but without the addition of iron ($[H_2O_2] = 100 \text{ mg/L}$, initial natural pH of CTWW = 6.63 and $T = 25^\circ\text{C}$), being from this point forward referred to as control without iron addition ($[Fe^{2+}]_{add} = 0 \text{ mg/L}$) (Figure 12, pink bars). In this case, the Fenton process was catalyzed by the residual iron left from the coagulation/flocculation process (0.51 mg/L).

The second one was performed in a diluted STWW, to ensure that the initial microbial load conditions were the same as after the coagulation process. Since the coagulation process was promoting an enterobacteria inactivation of about 2 log, a 1:100 dilution was performed. Then, this sample was also submitted to the Fenton process in the best conditions but without the addition of iron, so that there was no sufficient iron present in the wastewater matrix to activate hydrogen peroxide ($[H_2O_2] = 100 \text{ mg/L}$, initial natural pH of CTWW = 6.76 and $T = 25^\circ\text{C}$). This run is referred to as control with only hydrogen peroxide ($[H_2O_2] = 100 \text{ mg/L}$) (Figure 12, yellow bars).

Both control runs showed a near total enterobacteria inactivation after 120 min (99.7% for $[Fe^{2+}]_{add} = 0 \text{ mg/L}$ and 95.6% for $[H_2O_2] = 100 \text{ mg/L}$, Figure 12), which corresponded to remaining enterobacteria amounts of 20 and 55 CFU/100 mL for the control without iron addition and with only hydrogen peroxide, respectively (Table 5). Nevertheless, the strictest limit imposed by legislation was not met. These results are very similar to the ones obtained in the best conditions (with addition of iron). However, it is possible to notice that after a shorter time (5 min) the inactivation is significantly lower (62.3% for $[Fe^{2+}]_{add} = 0 \text{ mg/L}$ and 60.0% for $[H_2O_2] = 100 \text{ mg/L}$ vs 98.6%), which

points to the importance of iron in activating hydrogen peroxide and speeding up the disinfection process.

Table 6 shows pH values during the Fenton process for each run. While the control without iron addition ($[Fe^{2+}]_{add} = 0$ mg/L) revealed a similar behavior to the run at best conditions ($[Fe^{2+}]_{add} = 7$ mg/L) and the other Fenton runs performed during this work (slight decrease, followed by a constant increase), no pH change was observed in the control with only hydrogen peroxide ($[H_2O_2] = 100$ mg/L). Since this is the only run performed with no iron in solution, pH increase during the oxidation process should be associated with the presence of iron. A run in the best conditions previously obtained but in distilled water was performed to see if the OH^- generated in the Fenton process was the reason for pH increase. There was a decrease in pH after 5 min (6.87 to 3.71) and there was no further increase over 120 min of reaction time. These observations indicate that the Fenton process is not directly responsible for the pH increase. Instead, iron in solution should be reacting with something present in the wastewater matrix that will contribute to the increment in pH.

Table 6 - Evolution of pH during Fenton oxidation of CTWW in the best determined conditions ($[Fe^{2+}]_{add} = 7$ mg/L) and without iron addition ($[Fe^{2+}]_{add} = 0$ mg/L), and with only hydrogen peroxide addition in a 1:100 diluted STWW ($[H_2O_2] = 100$ mg/L) ($[H_2O_2] = 100$ mg/L, initial natural pH = 6.63-6.79 and T = 25°C).

Time (min)	pH		
	$[Fe^{2+}]_{add} = 7$ mg/L	$[Fe^{2+}]_{add} = 0$ mg/L	$[H_2O_2] = 100$ mg/L
0	6.79	6.63	6.76
5	6.75	6.70	6.75
120	7.65	7.71	6.78

The obtained results showed that the presence of residual iron from the coagulation process contributes to a slightly more efficient disinfection process in comparison with the control with only hydrogen peroxide. They also point to the need of iron addition in the Fenton process if a faster inactivation is required. If that is not the case, the FTWW without addition of iron can be reused for irrigation (class B and lower, Table 2) and urban utilities such as street washing and cooling waters [27,28].

4.2.6 Fenton vs Fenton-like process

With a view to reducing reagent costs, the possibility of using ferric chloride (reagent used in the coagulation process) instead of ferrous sulfate as iron source in the Fenton process was evaluated. The process was performed under the best conditions obtained previously for the ferrous sulfate ($[H_2O_2] = 100 \text{ mg/L}$, $[Fe^{3+}]_{add} = 7 \text{ mg/L}$, initial natural pH = 6.95, $T = 25 \text{ }^\circ\text{C}$) and results are shown in Figure 13.

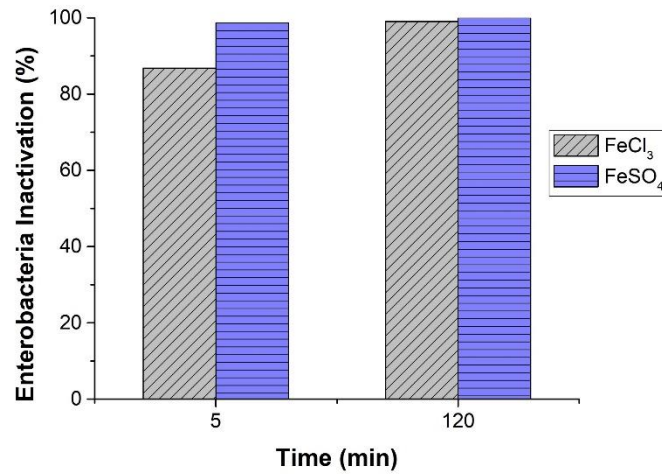


Figure 13 - Enterobacteria inactivation using $FeCl_3$ or $FeSO_4$ as iron source in the Fenton process ($[H_2O_2] = 100 \text{ mg/L}$, initial natural pH = 6.37-6.79, $T = 25^\circ\text{C}$ and $[Fe]_{add} = 7 \text{ mg/L}$).

It is possible to observe in Figure 13 a near total enterobacteria inactivation of 99.0% after 120 min when using ferric chloride as iron source. Although the obtained 125 CFU/100 mL surpasses the strictest value imposed by legislation, reuse in irrigation (class C and lower, Table 2) and some urban utilities (street washing and cooling waters) is still possible.

The dissociation of ferric chloride in solution generates Fe^{3+} and Cl^- ions, as opposed to the Fe^{2+} ions formed when using ferrous sulfate. First of all, the Fenton process with ferric ions starts with Eq. 3, where Fe^{3+} is reduced to Fe^{2+} by reacting with hydrogen peroxide, and then is followed by Eq. 2, where the hydroxyl radicals are formed. As previously mentioned, Eq. 3 has a constant rate several orders of magnitude lower than Eq. 2 ($0.002\text{-}0.01$ vs $63 \text{ M}^{-1} \text{ s}^{-1}$), so the generation of hydroxyl radicals is expected to be slower, as will be the disinfection process. Second of all, chloride ions can contribute to the inhibition of the Fenton oxidation, as they act as scavengers of $OH^\bullet$ [87]. Although these effects are not preponderant for the inactivation results obtained after 120 min, they

are noticeable in the ones for the sample taken at the shortest reaction time (5 min), where the run with ferric chloride demonstrated a lower enterobacteria inactivation than the one with ferrous sulfate (86.8% vs 98.6%).

Regarding pH (Table 7), it is noticeable a higher decrease in its value after 5 min of reaction with $FeCl_3$ than with $FeSO_4$. Once again, results can be explained resorting to the fact that Eq. 3 will be the first one occurring in the Fenton process with $FeCl_3$, leading to an initial formation of H^+ .

Table 7 - Evolution of pH during the Fenton reaction using $FeCl_3$ or $FeSO_4$ as iron source in the Fenton process ($[H_2O_2] = 100$ mg/L, initial natural pH = 6.37-6.79, $T = 25^\circ C$ and $[Fe]_{add} = 7$ mg/L).

Time (min)	pH	
	$FeCl_3$	$FeSO_4$
0	6.37	6.79
5	5.90	6.75
120	6.79	7.65

4.3. Microorganisms inactivation and regrowth

The previous experiments allowed the determination of the best conditions for the parameters that influence the wastewater disinfection processes: for the coagulation/flocculation process, a coagulant concentration of 120 mg/L and initial pH of the effluent of approximately 7.14; for the Fenton oxidation at natural pH of the CTWW, a hydrogen peroxide and iron concentrations of 100 mg/L and 7 mg/L, respectively, a temperature of 25 °C and a reaction time around 120 min. An experiment was carried out in those conditions (three replicates with independent STWW samples), where the inactivation and regrowth of total heterotrophs, enterococci and enterobacteria with and without antibiotic resistance was evaluated. The mean values obtained are shown in Figure 14, accompanied by the respective standard deviation.

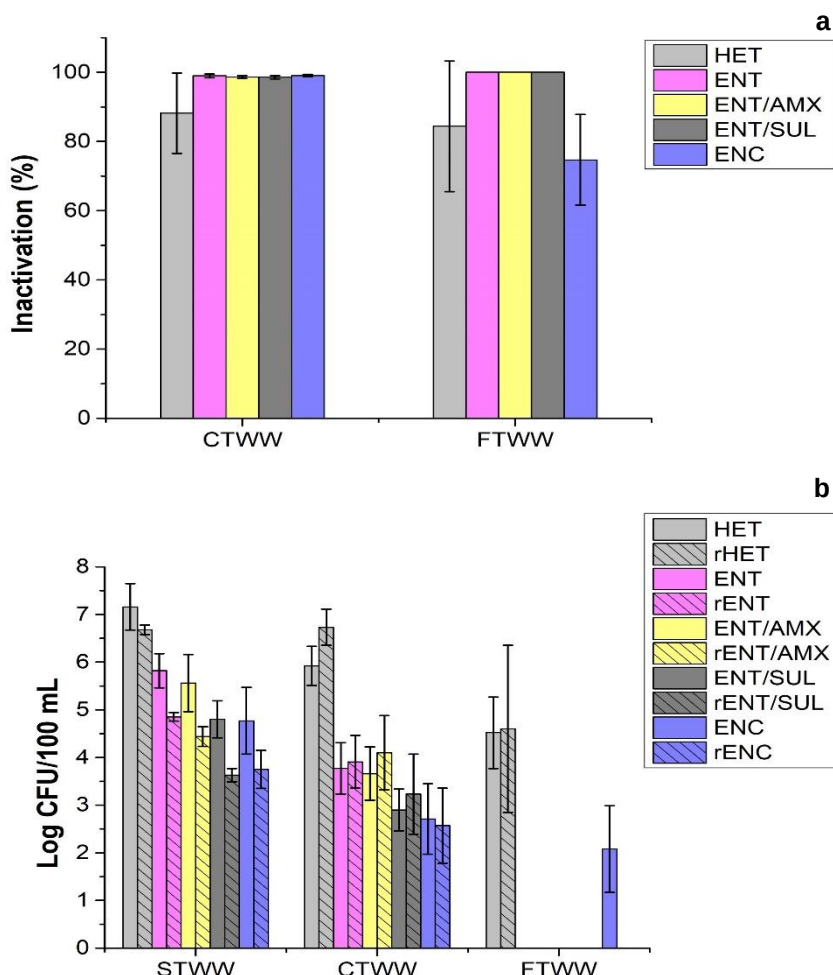


Figure 14 - Inactivation (a) and quantification (b) of total heterotrophs (HET), enterobacteria (ENT), enterobacteria with resistance to AMX (ENT/AMX) and SUL (ENT/SUL), and enterococci (ENC) in the STWW, CTWW and FTWW immediately after treatment and after storage for 72 h in the dark at room temperature (rHET, rENT, rENT/AMX, rENT/SUL and rENC). Bars represent standard deviation (N = 3 independent STWW samples).

Results obtained for enterobacteria with no antibiotic resistance were in agreement with the ones obtained previously in this work: an inactivation around 99.0% in the coagulation/flocculation process, corresponding to a 2.0 log CFU/100 mL reduction, and a reduction to values below DL, corresponding to a log reduction of 3.8 log CFU/100 mL, with the Fenton process. Total enterobacteria inactivation in real urban effluents has been reported with photo-Fenton treatments [16,38,122,123], but up to the author's knowledge never with a conventional Fenton process.

Regarding antibiotic resistant enterobacteria, resistance to two of the most commonly administrated antibiotics were tested: amoxicillin (AMX) and sulfamethoxazole (SUL).

AMX is the most commonly prescribed antibiotic in the outpatient setting in Europe, responsible for about 40% of total outpatient antibiotic use [139]. It is used in the treatment of a wide range of upper respiratory tract infections, such as otitis media, bronchitis, pneumonia, and others (e.g. typhoid, gonorrhea, and urinary tract infections) [140,141]. SUL is also a commonly employed antibiotic and is indicated to treat a variety of urinary tract, respiratory system, and gastrointestinal tract infections [142]. It is possible to notice in Figure 14b that the STWW contains 5.6 and 4.8 log CFU/100 mL of enterobacteria with AMX and SUL resistance, respectively. Comparing these values to the amount of enterobacteria without resistance present in the STWW (5.8 log CFU/100 mL), it is possible to verify that 64.9% of enterobacteria are resistant to AMX and 9.6% are resistant to SUL. These conclusions point to the growing antibiotic resistance problematic discussed in the introduction of this work, as well as the inability of wastewater treatment processes to inactivate these microorganisms.

The performed coagulation/flocculation process contributed to an inactivation of about 98.6% of both resistant enterobacteria, which corresponds to a 1.9 log CFU/100 mL reduction. This inactivation is in agreement with the ones obtained by Grehs et al. [40] (around 2 log removal of fecal coliforms with AMX resistance using aluminum sulfate and tannin coagulants). The ensuing Fenton process was able to reduce both these microorganisms to values below DL (3.0-3.7 log reduction).

An inactivation of 99.1% of enterococci was noticed after the coagulation/flocculation process (2.1 log reduction), while the following Fenton process contributed to a 74.7% inactivation (0.7 log reduction), leaving 2.1 log CFU/100 mL after treatment. A reduction of 1.5 log by a coagulation/flocculation process, followed by a total inactivation (5.6 log) by a photo-Fenton process has been reported [16]. Other studies with the photo-Fenton process achieved total enterococci inactivation [38,122,123], while one of them [38] stated that of all the tested disinfection processes, the ones with H_2O_2 provided the best results for enterococci inactivation, namely the photo-Fenton process (< 1 CFU/100 mL).

Regarding total heterotrophs, an 88.2% inactivation was observed with the coagulation/flocculation process (1.3 log CFU/100 mL reduction), followed by an 84.4% inactivation (1.5 log CFU/100 mL reduction) with the Fenton treatment, still being detected 4.5 log CFU/100 mL in the treated water.

Microbiological analysis of the STWW, CTWW and FTWW was repeated 72 h after the treatment, in order to assess microorganism regrowth (Figure 14b). This occurrence is influenced by parameters such as temperature, light and availability of carbon and energy sources [143–145]. The treated effluent was stored at room temperature in the dark, in order to simulate the conditions under which the water would be stored in the WWTP before its application.

The analysis performed on STWW revealed reductions of 0.5 log for total heterotrophs and from 1.0–1.2 log for the remaining microorganisms. Regarding CTWW, increases of 0.3, 0.4 and 0.8 log were noticed for enterobacteria resistant to SUL, AMX and for total heterotrophs, respectively, while no significant differences were registered for the remaining microorganisms. The FTWW exhibited an absence of regrowth of enterobacteria with and without antibiotic resistance. Values below DL were obtained for enterococci, pointing to the inactivation of the previously determined 2.1 log CFU/100 mL. Even though the mean value obtained for total heterotrophs after 72 h is equal to the one obtained immediately after treatment, a considerable reduction was noticed in two of the runs, while a significant increase was observed in the other one (explaining the high standard deviation obtained).

Bacterial reactivation amid storage of distinct types of treated wastewater has been broadly reported, regardless of the treatment process used [38,40,146–148]. In what concerns the ones with the same storage conditions as this study (72 h in the dark at room temperature), Moreira et al. [38] noticed an absence of regrowth of fecal coliforms and enterococci after treatment with H₂O₂-assisted processes, while in the case of treatment by heterogeneous photocatalysis without H₂O₂ (P25, TiO₂ and GO-TiO₂) the microbial loads of these groups were 2 to 3 log values lower than before treatment. Grehs et al. [40] observed a regrowth of all the analysed culturable bacterial groups, namely a roughly 3 log increase of total heterotrophs, after a coagulation treatment with a tannin coagulant. It was also noticed a slight decrease on microbial load after storage of a sample subjected to a coagulation process with aluminium sulfate. Moreira et al. [149] reported a near 4.5 log regrowth of total heterotrophs and a non-significant enterobacteria and enterococci regrowth after a photocatalytic ozonation process.

The obtained results show no enterobacteria regrowth in the FTWW, which means that the final treated water can be stored for at least 72 h in the cistern available in Gaia Litoral

WWTP for further reuse without risk of not meeting the limits imposed by legislation for *E. coli*.

4.4. Physico-chemical characterization

Physico-chemical characteristics of the three wastewater samples were evaluated. TOC, turbidity, TSS, total and ammonia nitrogen, total phosphorus, iron and biological oxygen demand were the parameters assessed. Table 7 displays the values for each of those parameters, the results obtained from the previously done microbiological analysis and the limits imposed by the European [27] and Portuguese [28] legislation for the reuse of treated water for irrigation (class A) and urban utilities (strictest limits).

It is noticeable that the applied treatment was able to fix some of the parameters of the STWW that did not fulfill the imposed requirements, namely enterobacteria, turbidity, TSS and BOD₅. Iron concentration after the Fenton process increased in comparison with CTWW (0.51 to 1.23 mg/L), as expected due to iron addition, but was still under the established value. This increase in dissolved iron should be the reason for the increase in turbidity in relation to CTWW (0.85 to 2.55 NTU). It was also observed an increase in ammonia nitrogen (NH_3), namely after the Fenton process. This behavior has been reported before in Fenton processes [132]. This inorganic substance is probably formed due to the mineralization of some nitrogen containing compounds present in the wastewater by the Fenton process, and should be the main responsible for the increase in pH noticed in the experiments performed along this work [94,132].

Almost all the studied parameters have values below the strictest limits established by legislation, with the exception of those referring to nitrogen. The limits imposed for total and ammonia nitrogen (N_{total} and NH_3 , respectively) for reuse in irrigation is recommended to minimize the risks of biofilm formation and obstruction of irrigation systems, but it is not mandatory. Furthermore, the limit displayed for ammonia nitrogen for urban utilities is only applied to some of them (recreational uses of landscape setting and cooling waters).

Thus, it is possible to conclude that the generated treated water meets the requirements imposed by European and Portuguese legislation for its reuse in irrigation and in urban utilities, with the exception of recreational uses of landscape setting and cooling waters.

Table 8 – Microbiological and physico-chemical characteristics of STWW, CTWW and FTWW immediately after treatment and after storage for 72 h (rFTWW). Limits imposed by European and Portuguese legislation for its reuse in irrigation and urban utilities.

Parameter	STWW	CTWW	FTWW	rFTWW	Limits imposed [27,28]	
					Irrigation ⁽¹⁾	Urban utilities
Total heterotrophs (CFU/100 mL)	1.5×10^7	8.4×10^5	3.4×10^4	4.0×10^4	-	-
Enterobacteria (CFU/100 mL)	6.7×10^5	5.9×10^3	< 10	< 10	< 10	< 10
Enterobacteria with AMX resistance (CFU/100 mL)	3.7×10^5	4.6×10^3	< 10	< 10	-	-
Enterobacteria with SUL resistance (CFU/100 mL)	6.3×10^4	7.9×10^2	< 10	< 10	-	-
Enterococci (CFU/100 mL)	5.9×10^4	5.1×10^2	1.2×10^2	< 10	-	-
TOC (mgC/L)	11.8	8.5	8.4	-	-	-
Turbidity (NTU)	6.7	0.9	2.6	-	< 5	< 5
TSS (mg/L)	13.5	8.0	9.5	-	< 10	-
N _{Total} (mgN/L)	73.6	70.1	68.6	-	< 15 ⁽²⁾	-
NH ₃ (mgNH ₄ ⁺ /L)	59.7	61.9	69.4	-	< 10 ⁽²⁾	< 5 ⁽³⁾
P _{Total} (mgP/L)	0.9	0.3	0.1	-	< 5	< 2
Fe (mgFe/L)	-	0.5	1.2	-	< 2	-
BOD ₅ (mgO ₂ /L)	11	6	5	-	< 10	< 25

(1) Limits for water class A.

(2) Optional parameter. Might be applicable to some irrigation projects to minimize the risks of biofilm formation and obstruction of irrigation systems.

(3) Only applicable for recreational uses of landscape setting and cooling waters.

4.5 Implementation at real scale

Once proven the effectiveness of the treatment methodology studied in this work, a possible way of implementing it at real scale in Gaia Litoral WWTP was studied. An economic analysis was performed, in order to establish a comparison between this method and the one currently used (UV irradiation).

As previously mentioned, Gaia Litoral WWTP already has a facility for tertiary treatment, in which part of the secondary treated water feeds two serial sand filters, followed by a passage in a UV channel and then storage in a cistern. This system can be adapted to a new configuration that fits the studied treatment methodology. Coagulation/flocculation would happen in the sand filters, where the coagulant would be added before the first filter. The turbulence in the water flow would promote homogenization and the passage through the filters would remove the formed flocs and enable the disinfection process. Then, the effluent would go to the first of two compartments of the cistern, where hydrogen peroxide and the iron source (ferric chloride) would be added in a Fenton process. Since in this work the Fenton process was studied in batch mode, this analysis will contemplate a batch Fenton process. The first compartment of the cistern has a capacity of 41 m³, so each cycle of the Fenton process would be performed with that amount of wastewater.

In this scenario, the secondary effluent is fed to the filters at a rate of 100 m³/h for approximately 25 min, to provide exactly the right volume to fill the improvised reactor (41 m³). To guarantee a $FeCl_3$ dosage of 120 mg/L, ferric chloride (40%) must be fed at a rate of 30 L/h (Sigma/ 1 Basic, capacity range of 17-144 L/h). A sludge production of 7.5 m³/h is estimated. When the first compartment of the cistern is full, stirring is initiated and both hydrogen peroxide (50%) and ferric chloride (40%) are added. An H_2O_2 volume of 8.20 L is pumped at a rate of 11 L/h (ProMinent concept plus, maximum capacity of 13.5 L/h) for 45 min, while ferric chloride is pumped at a rate of 62.5 L/h for 2 min (2.08 L). Although these conditions were not the ones studied at lab scale, a gradual addition of hydrogen peroxide could favour the disinfection process, since it might prevent scavenging effects in the first moments of reaction due to the presence of large amounts of H_2O_2 [150,151]. Reaction time was extended to 3 h due to the gradual addition of peroxide hydrogen. After that time, stirring stops and sedimentation of iron occurs for 30 min. Precipitated iron sludge is then removed through a purge and the treated water is

forwarded to the second compartment of the cistern, where it is stored until further application.

An economic analysis of the proposed treatment vs the current implemented method was carried out, and the costs are summed up in Table 9.

Table 9 - Reagent, operating, maintenance, equipment and total costs for the implementation at real scale of the coagulation and Fenton processes tested in this work, as well as for the currently implemented method (UV irradiation).

Process	Costs				
	Reagent	Operating	Maintenance	Equipment	Total
Coagulation	0.10 €/m ³	-	-	-	0.10 €/m ³
Fenton	0.11 €/m ³	0.01 €/m ³	-	2363 €	0.12 €/m ³
UV irradiation	-	8.82 €/day	8.37 €/day	-	17.19 €/day

Reagent costs were given by Rivaz Química S.A.: $FeCl_3$ (40% of purity, density at 25 °C = 1.42 kg/L) - 229 €/ton; H_2O_2 (50% (w:v), density at 25 °C = 1.2 kg/L) - 410 €/ton. Costs associated with energy are of 0.10 €/kW.h.

Since the coagulation process will be preceding both Fenton and UV irradiation treatments, its costs will not have influence in the comparison made. Thus, only reagent costs were calculated - 0.10 €/m³ (Eq. 12).

$$Cost_{FeCl_3} (\text{€/m}^3) = \frac{Price_{FeCl_3} (\text{€/ton}) \times [Fe^{3+}] (\text{ton/m}^3) \times \rho_{FeCl_3} (\text{kg/L})}{\frac{\%FeCl_3}{100} (\text{kg/L})} \quad (\text{Eq. 12})$$

Regarding the Fenton process, reagent, operating and impeller acquisition costs were taken into account. Reagent costs add up to a value of 0.11 €/m³ (Eqs. 13 and 14). Operating costs are mainly due to the mixing during the process, which requires a power of 0.92 kW per cycle, translating into a cost of 0.01 €/m³ (Eq. 15). The sum of these two variables result in an expenditure of 0.12 €/m³. Having in mind the low viscosity of wastewater and the small volume of the improvised reactor, the most appropriate impeller for mixing is a propeller one [152]. The proposed impeller has an acquisition cost of 2363 € (Sulzer, model ABS XRW 210), which is not accounted for the total costs of the process used in the comparison with the UV radiation treatment.

$$Cost_{H_2O_2} (\text{€/m}^3) = \frac{Price_{H_2O_2} (\text{€/ton}) \times [H_2O_2] (\text{ton/m}^3) \times \rho_{H_2O_2} (\text{kg/L})}{\frac{\%H_2O_2}{100} (\text{kg/L})} \quad (\text{Eq. 13})$$

$$Cost_{FeCl_3}(\text{€/m}^3) = \frac{Price_{FeCl_3}(\text{€/ton}) \times [Fe^{3+}] (\text{ton/m}^3) \times \rho_{FeCl_3} (\text{kg/L})}{\frac{\%FeCl_3}{100} (\text{kg/L})} \quad (\text{Eq. 14})$$

$$Cost_{energy}(\text{€/m}^3) = \frac{P (\text{kW}) \times t (\text{h}) \times Price_{energy}(\text{€/kW h})}{Volume_{treated\ effluent} (\text{m}^3)} \quad (\text{Eq. 15})$$

Regarding the currently employed treatment in Gaia Litoral WWTP, the costs are mostly due to maintenance and operation. Maintenance costs include UV lamps replacement every 2 years, having a total cost of 8.37 €/day. Operating costs boil down to the energy consumed by the lamps, which account for 8.82 €/year (Eq. 16). Overall, this process costs 17.19 €/day.

$$Cost_{energy}(\text{€/day}) = P (\text{kW}) \times Price_{energy}(\text{€/kW h}) \times 24 \text{ h/day} \quad (\text{Eq. 16})$$

Since UV lamps are always turned on, UV costs are fixed, as they do not depend on the volume of treated wastewater. Therefore, the most economical alternative between this and the Fenton process will depend on the needed volume of treated water. In 2020, around 726 m³/day were disinfected, and a growing demand is the tendency. The discontinuous Fenton process is incapable of generating this amount of daily volume, as one cycle takes up to 4-5 h and only treats 41 m³. Regarding costs, only a flow rate lower than 141 m³/day would make the Fenton process more economic than the UV irradiation one.

Continuing with the current process is not an option, since requirements for *E. coli* are not being met. One possible option is the study of the Fenton process in continuous mode, which would enable the generation of more volume per day. Other alternative is the coupling of an oxidant agent with the UV process to promote a more efficient disinfection.

5. Conclusions and Recommendations

5.1 Main conclusions

The influence of coagulation/flocculation and Fenton process parameters on the effectiveness of this treatments in the disinfection of an urban wastewater sample generated in Gaia Litoral WWTP was evaluated in this work. The best turbidity, TOC and enterobacteria removal were obtained when the process was performed at the natural pH of the effluent (7.14) and with a ferric chloride concentration of 120 mg/L. Regarding the Fenton process, enterobacteria inactivation was still occurring after 120 min and optimum values of hydrogen peroxide and added iron of 100 mg/L and 7 mg/L, respectively, were determined. A temperature of 25 °C provided better results than temperatures of 15 and 20 °C, demonstrating that the process is more efficient in summer.

In the best conditions, values below detection limit for enterobacteria were obtained, meeting the strictest limit imposed by regulation for water reuse. The Fenton process performed without addition of iron, i.e., using the residual iron in solution from the coagulation/flocculation process (0.51 mg/L), had a slower disinfection but achieved similar enterobacteria inactivation after 120 min (99.7%). Using $FeCl_3$ as the iron source instead of $FeSO_4$ also resulted in a near total inactivation (99.0%). Although the strictest requirement of legislation was not met in both cases (20 CFU/100 mL and 125 CFU/mL, respectively), the treated waters can still be reused for irrigation (class B and C, respectively) and in some urban utilities.

The coagulation/flocculation technique contributed to removals of 1.9-2.1 log (98.6-99.1%) of enterococci and enterobacteria with and without resistance to SUL and AMX, and to 1.3 log removal of total heterotrophs (88.2%). Values below DL of all enterobacteria were determined after the Fenton process, while a 0.7 and 1.5 log removal of enterococci and total heterotrophs, respectively, was achieved (74.7% and 84.4%, respectively). Microorganism regrowth analysis after 72 h of storage showed no enterobacteria regrowth for the FTWW, as well as values below detection limit for enterococci and no apparent pattern for total heterotrophs for that same sample.

The physico-chemical characteristics of the treated wastewater were determined and its combination with the microbiological results show that the generated water meets the

requirements imposed by European and Portuguese legislation for its reuse in irrigation and most of urban utilities.

The analysis of the implementation of this process at full scale shows that the Fenton process in batch mode is not capable of generating the currently produced flow of treated wastewater (726 m³/day) and that it is only economically more advantageous for values lower than 141 m³/day.

5.2 Recommendations for future work

For future work it is suggested the implementation of this process at real scale in Gaia Litoral WWTP, as described in the economic analysis, to further assess its ability of generating a treated water susceptible to reuse.

A study on the implementation of the Fenton process in continuous mode should also be carried out, in order to surpass the drawback of low volume generation of treated water per day. Determination of the best conditions should primarily be done at lab scale, namely regarding oxidant and catalyst concentrations, and then assessed at real scale in Gaia Litoral WWTP, if applicable.

The addition of an oxidant agent before the UV irradiation treatment could also be studied, in order to increase disinfection efficiency and provide microbiological values below the limits imposed by Portuguese legislation. If hydrogen peroxide is the chosen one, a Fenton process would occur due to the residual iron left from the coagulation/flocculation process with ferric chloride.

Regarding the coagulation/flocculation process, determining the best conditions for the process using ferrous sulfate as coagulant and establishing a comparison with the ones obtained for ferric chloride could be valuable. Since the first one contributes to a more effective Fenton process, the use of that reagent in both processes could achieve better results and lower costs.

It would be interesting to perform a further analysis on ARG presence, in order to assess its evolution over treatment and observe the effects of this methodology at a DNA level. Evaluating the bacterial community composition immediately after the treatment and after storage for a certain amount of time could also provide some valuable information on the community regrowth after such an extreme disinfection process.

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