Wastewater disinfection for reclaimed water production and distribution – a lab study for assessing the chlorine reactivity

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Abstract

Water reuse is a progressively relevant issue, given rapid urbanisation, increasing water demand and concerns about climate change. Moreover, treated wastewater is now seen as a real renewable resource that could play a key role in the ecological transition to a circular economy, and in the achievement of the Sustainable Development Goals, by making treatment plants real recovery facilities.

Before treated wastewater is discharged into water bodies and all the more so when it is reused, it must undergo disinfection treatment to inactivate pathogenic microorganisms and prevent their spread by water, so that they do not pose a danger to human and environmental health, complying with the standards and limits imposed by law are met. Chlorine has always been one of the most widely used disinfectants, due to its low cost, effectiveness and residual disinfection capacity. However, the biggest issue with chlorine disinfection is the possible formation of toxic by-products, which are generated when natural organic matter, the main precursor of chlorination by-products, is present in the water. It is therefore important to monitor the composition of the water and in particular the presence of effluent organic matter.

This is the context for this thesis work: given the costs and time required for the laboratory analyses necessary to detect the presence of trihalomethanes, tests were carried out using absorption spectra and fluorescence spectroscopy techniques to identify rapid and effective methods of detecting and quantifying organic matter, and to verify whether these methods could be used as alternative means of detecting the presence of by-products in chlorinated water. The tests included chlorination of wastewater samples from a wastewater plant in Lisbon and in addition to the search for organic matter and by-products, the influence of nitrogen on the shape of the breakpoint curve and chlorine decay were studied.

Keywords

Water reuse; chlorination breakpoint; disinfection by-products; fluorescence spectroscopy; UV-Vis absorption spectroscopy

Resumo

A reutilização de águas residuais é uma questão cada vez mais atual, dada a rápida urbanização, o aumento do consumo da água e as preocupações com as alterações climáticas. Além disso, as águas residuais tratadas são agora vistas como um verdadeiro recurso renovável que pode desempenhar um papel fundamental na transição ecológica para uma economia circular, bem como na concretização dos Objetivos de Desenvolvimento Sustentável, ao tornar as estações de tratamento de água residuais em verdadeiras instalações de recuperação de água e outros subprodutos.

Antes que as águas residuais possam ser reutilizadas, no entanto, devem ser submetidas a tratamento de desinfeção para que os microrganismos patogénicos sejam inativados e não representem um perigo para a saúde humana e ambiental, cumprindo as normas e limites impostos por lei. O cloro tem sido sempre um dos desinfetantes mais utilizados, devido ao seu baixo custo, eficácia e capacidade residual de desinfeção. Contudo, a maior questão com a desinfeção com cloro é a possível formação de subprodutos tóxicos, que são gerados quando a matéria orgânica, o principal precursor dos subprodutos da cloração, está presente na água. Por conseguinte, é importante controlar a composição da água e, em particular, a presença de matéria orgânica no efluente.

Este é o contexto deste trabalho de tese: dados os custos e tempo necessários para as análises laboratoriais necessárias para detetar a presença de trihalometanos, foram efetuados testes utilizando espectros de absorção e técnicas de espectroscopia de fluorescência para identificar métodos rápidos e eficazes de deteção e quantificação da matéria orgânica, e para verificar se estes métodos poderiam ser utilizados como meios alternativos de deteção da presença de subprodutos em água clorada. Os testes incluíram a cloração de amostras de águas residuais de uma estação de tratamento de águas residuais em Lisboa e, para além da procura de matéria orgânica e subprodutos, foi estudada a influência do azoto na forma da curva do ponto crítico da cloragem e no decaimento do cloro.

Palavras chave

Reutilização de águas residuais; ponto crítico da cloragem; subprodutos da desinfeção; espectroscopia de fluorescência; espectroscopia de absorção UV-Vis

Abbreviations

Abs Absorbance

BOD Biochemical Oxygen Demand

CDOM Chromophoric Dissolved Organic Matter

DBPs Disinfection By-Products

DOM Dissolved Organic Matter

DOC Dissolved Organic Carbon

DPD Dietil-p-fenildiamine

DNA Deoxyribonucleic Acid

EEM Excitation Emission Matrix

EPA Environmental Protection Agency

EU European Union

FRI Fluorescence Regional Integration

GDWQ Guidelines for Drinking Water Quality

GHG Greenhouse Gases

GWEG Guidelines for the safe use of wastewater, excreta and greywater

IFE Internal Filter Effect

ISO International Organization for Standardization

LNEC National Laboratory for Civil Engineering

MW Molecular Weight

NOM Natural Organic Matter

OM Organic Matter

ORP Oxidation/Reduction Potential

RNA RiboNucleic Acid

RWQC Recreational Water Quality Criteria

SDGs Sustainable Development Goals

SMP Soluble Microbial Products

SUVA Specific Ultraviolet Absorbance

THMs Trihalomethanes

TOC Total Organic Carbon

TSS Total Suspended Solids

UV Ultraviolet radiation

Vis Visible radiation

WHO World Health Organization

WWTP Wastewater Treatment Plant

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1. Introduction

1.1. Context

Given the rapid urbanization, the increasing exploitation of water resources and climatic variations, which are leading to increasingly recurring periods of drought, the pressure on water supply is steadily increasing and the problem of water scarcity is becoming more central and urgent. There is a clear need to minimize the use of drinking water in activities where its use is not strictly necessary and that would maintain the same performance with water from other sources, such as treated wastewater. Currently, most wastewater treated in sewage treatment plants is discharged into water bodies after treatment, there are few examples of reuse and these involve only a small volume of water.

Through its recovery, wastewater could be a renewable resource, because it can be a source of matter and nutrients and it can produce energy through the anaerobic sludge digestion, at the same time. The reuse of treated wastewater would therefore make wastewater treatment plants real recovery plants. All this would then contribute to the achievement of a circular economy and to a more sustainable water management. It is therefore necessary to focus on the reuse of water wherever this is possible, so as to reduce the volumes extracted from conventional resources and to recover water and nutrients.

In order to be able to reuse water, it must undergo a disinfection treatment, so that pathogenic microorganisms are inactivated and do not pose a danger to human and environmental health, and so that the standards and limits imposed by the law are met. Chlorine and chlorine derivatives are currently the most popular disinfectants, due to their cost-effectiveness, efficiency and ease of use and their residual disinfection potential. However, the biggest issue with chlorine disinfection is the possible formation of toxic by-products, which are generated when natural organic matter, the main precursor of chlorination by-products, is present in the water. It is therefore important to monitor the composition of the water and in particular the presence of effluent organic matter, which could react with chlorine and form by-products that are a risk to the environment and human health.

Since organic matter is composed by compounds capable to absorb radiation or emit fluorescence at specific wavelengths, and given the advances in absorbance and fluorescence spectroscopy techniques, these two are increasingly being used to derive information on water composition and to characterize the nature of the natural organic matter present.

1.2. Objectives

The aim of this work was to study at laboratory conditions the chlorination of a treated wastewater effluent from a wastewater treatment plant in Lisbon, with the assessment of the changes on water composition promoted by chlorine reaction including the formation of trihalomethanes. For this purpose, chlorination breakpoint curves were determined and the effects the chlorine concentration dosing on chlorine decay trend and on trihalomethanes formation were evaluated. Other objective was the application of absorbance and fluorescence spectroscopy methods on chlorinated water samples characterization.

1.3. Thesis organization

This thesis work was structured with an initial chapter outlining the current state of the art in water reuse. The topic was initially framed, presenting the need for reuse of treated wastewater, listing its opportunities and potential. The areas of reuse and possible applications were then presented, explaining their risks and benefits, and describing the regulatory framework of wastewater for reuse, both in the European Union, and in Italy and Portugal. Conventional treatments usually present in a treatment plant were then illustrated, with a focus on water disinfection and in particular disinfection using chlorine. The main disinfection by-products and their precursors were then presented, as well as spectroscopic methods for wastewater characterization, namely absorbance and fluorescence spectrophotometry. The next chapter presents the laboratory experiments that were the case study of this work, which involved testing treated wastewater samples from the Beirolas wastewater treatment plant in Lisbon. The experiments focused on the evaluation of chlorination breakpoint curves, chlorine decay over time and the analysis of UV (ultraviolet) absorbance spectra and EEM (Excitation/Emission Matrix) matrices resulting from spectrofluorescence analyses. Finally, the results of the experiments and the resulting conclusions were presented.

2. State of the art

2.1. Water reuse

2.1.1. Need for water reuse

Wastewater, comprising domestic or the mixture of domestic and industrial wastewater, or storm water runoff, if properly recovered, can produce energy and be a source of materials and nutrients. It can therefore constitute a true renewable resource and it could play a key role in the ecological transition to a circular economy and in achieving the Sustainable Development Goals [1, 2]. Industrial and domestic effluents contain energy, water, organic substances, phosphates, nitrogen, cellulose, rare earths and other resources, and thanks to new technologies it is increasingly feasible to recover resources from these effluents. Biogas, fertilizers, paper, metals, plastic and water can be recovered [2] and the wastewater treatment plants themselves, in this way, can be not only treatment plants, but real water recovery plants [1].

Wastewater treatment is particularly important, because discharging untreated effluent directly into water bodies can lead to eutrophication of the water bodies, and it also can pose risks to human health and the climate, as it contributes to greenhouse gas (GHG) emissions in the form of nitrous oxide and methane [2]. Wastewater treatment also causes emissions, but these are three times lower than without treatment [2].

Wastewater is therefore a global problem and is in fact considered within the Sustainable Development Goals (SDGs) of the 2030 agenda, more specifically in goal 6.3, which calls on governments to halve the percentage of untreated wastewater and increase recycling and reuse by 2030 [3]. In fact, at present, about 80% of all wastewater in the world is discharged into watercourses without proper treatment, creating health and environmental risks [3]. Increasing urbanization, in turn, causes an increase in wastewater production, which contributes to the problem [2].

The growing demand for water by cities, industry and the agricultural sector, and the ongoing search for water sources, means that the water recycling and reuse market, which was worth around $12.2 billion in 2016, is set to expand, due in part to the increased focus on preventing source water pollution and reducing untreated wastewater being discharged, especially given the continued growth in population and urbanisation and the increased concern about climate change [2, 4]. For these reasons, the reuse of effluents from sewage treatment plants is an issue to which more and more attention is being paid today, especially if wastewaters are subjected to very stringent purification treatments.

Nowadays, water reuse is considered part of the hydrological cycle, an indication of the importance of water resource management and the growing demand in the social, industrial and agricultural spheres. The hydrological cycle refers to the transfer of different forms of water into the environment. It therefore considers surface and groundwater resources, their treatment, their use in agricultural, civil and industrial spheres, and finally the treatment and reuse of water [4].

As already mentioned, untreated wastewater causes emissions, which can make up a significant part of the city's total emissions. In order to meet the SDGs, the amount of untreated wastewater should be halved, in order also to ensure universal access to adequate sanitation [3]. As the population is expected to increase in the coming years, there will be increasing pressure on cities to make more and more use of treated wastewater. However, the focus will not have to stop at protecting the environment and human health, but new ways will have to be found to utilize the materials, energy and water in treated wastewater [2].

Currently, wastewater treatment capacity stands at around 70 % of the wastewater generated in high-income countries, while it stops at 8 % in low-income countries. Therefore, it is necessary for governments and the private sector to take the initiative for decisive and large-scale actions, investing in infrastructure that would enable the ecological transition to a circular economy, which would bring with it environmental, economic and social benefits [2].

2.1.2. Application and possibility of reuse

Treated wastewater is mainly reused in agriculture, industry, compatible urban uses, or to maintain the functionality of surface water bodies and for groundwater recharge. For the latter two applications, replenishment is achieved either indirectly, through the runoff and the infiltration of reused water in agriculture, or through direct infiltration. The reuse of water for agricultural purposes or for the irrigation of green areas can utilize water used in agriculture, but also in the urban sector. Water reuse in industry, on the other hand, is generally reused in thermal power plants, paper production plants or others that require large amounts of water for processes. It is often the case that there are closed-loop processes, i.e., processes that treat wastewater from a processing cycle to recirculate it within the same cycle [4].

Depending on the intended use of the treated wastewater, different levels of treatment will be required in order to protect public health, the environment, but also process reliability in the case of reuse for industrial purposes [2].

The most common type of reuse is certainly water reuse in agriculture, followed by water reuse for the irrigation of green areas, i.e. parks, landscaped areas and golf courses, roadside verges and residential green areas. Green area irrigation systems are often part of dual distribution systems, i.e. where there are two separate distribution networks, one for drinking water and one for recycled water. In the industrial sector, treated water can be used to cover the needs of process or cooling operations, the main industrial use of water and often the only form of reuse. However, in the industrial sector, there are many and various uses that can be made of recycled wastewater, which is why it is often necessary to use more stringent treatments than conventional ones to ensure adequate quality levels for the water to be used. As already mentioned, treated wastewater can also be reused to recharge the aquifer, either by means of spillage basins or by direct injection into underground aquifers, provided that the characteristics of the water are comparable in quality to those of the aquifer. Injection into the aquifer may serve not only to recharge it, but also to store the resource within the aquifer for later use or even to create a hydraulic barrier to counter salt intrusion in the case of coastal areas.

Another type of use can be for interventions with a recreational-environmental nature, i.e. the creation of lakes or wetlands to maintain the outflow in watercourses [4, 5].

2.1.3. Risks and benefits

Reusing treated wastewater can bring numerous environmental, economic and social benefits, but it can also present risks, which must, however, be addressed in the name of environmental and human health protection [5].

Only a small percentage of treated wastewater is reused in the European Union (EU), most of it for irrigation, although reuse is accepted in all Member States, especially those with water scarcity, such as Italy and Portugal. The use of reclaimed wastewater in agriculture is still a niche option, since it is perceived as risky, not profitable and a barrier to food products trade.

However, if reuse is intended for the restoration of watercourses and wetlands, it may lead to the revival of certain aquatic ecosystems, while if it is intended for the recharge of aquifers, it may help to prevent the deterioration of groundwater, provided that it can be ensured that the chemical and biological status of the latter is not altered.

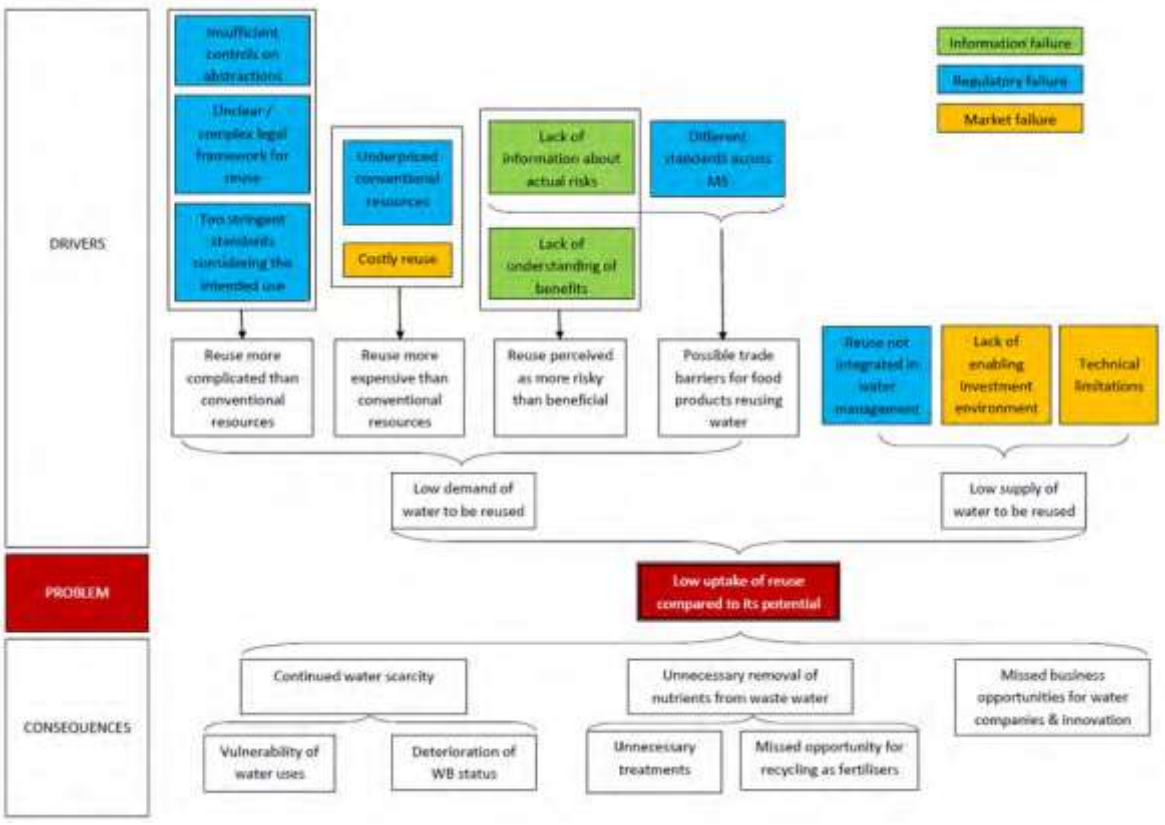

Figure 1 - Problems for water reuse [5]

The discharge of effluents from wastewater treatment plants into the sea represents a waste of water resources, especially in water scarce areas. The reuse of this effluent would reduce this waste, making it possible to cope with changes in water demand due to demographic and climate change. It would also provide an alternative drinking water, especially in water scarce areas, and improve the

stability of the water supply. If used for irrigation, the use of treated wastewater could reduce the use of artificial fertilizers, as it would provide nutrients.

However, the reuse of water is more challenging than the use of conventional water resources. One of the main problems is the appropriate choice of the treatment level and technology. It is crucial to find the right treatment, which meets quality standards, without overdoing unnecessary treatment that would only lead to an increase in carbon emissions. In addition, the quality of the incoming wastewater and the quality requirements, depending on the intended use of the reclaimed water, must be taken into account in the treatment design. It is also necessary to consider the difficulties related to the distribution of the wastewater, since the places for reuse of the treated water are hardly ever in the vicinity of the treatment plants, and to its storage, since there is a time lag between the production of the reclaimed water and the demand for its reuse.

Reusing reclaimed water is often more expensive than using conventional resources, due to the fact that, especially in agriculture, water is extracted cheaply and often illegally or without authorization. It is then necessary to consider the costs of treatment plants, and also of the distribution infrastructure, which must be separated from that for fresh water and will therefore cost a considerable amount of money; in addition, specific treatments are often required to meet the required quality standards. However, comparing with the costs of options such as desalination and water transport, reuse of treated wastewater is the most cost-effective option. It must be finally considered that, if there is a lack of water, its reuse is the only viable option. To keep the price low, subsidies and funding would be needed to cover the costs of the necessary infrastructure. However, the reuse of treated wastewater can also have economic benefits, as water is a resource and reusing it could stimulate new water pricing and also reduce the water demand. In addition, a new water reuse industry would be promoted, which could stimulate innovation and competitiveness. Finally, water reuse would lead to increased economic activity and consequently increased employment, improve food security as it would provide a regulated alternative source of irrigation and support local communities and businesses [5].

The perception of reclaimed wastewater as more risky than beneficial water source is also a limitation to its use. Public health risks, whether actual or perceived, are in fact another major obstacle to widespread reuse. Among the greatest concerns are the possible risks from water consumption or contact with food irrigated with treated water, especially in relation to exposure to pathogens, viruses, parasites and chemical contaminants. Nevertheless there are protective measures in order to reduce the health risks for consumers and workers, such as treating properly wastewater used for irrigation, limiting the crops that can be irrigated with this water, providing waiting periods to allow pathogens to be eliminated between applications, and the use of hygienic practices during harvesting and food preparation, such as washing, disinfecting and cooking [5].

In conclusion, it is necessary to convince stakeholders that agricultural products cultivated through irrigation with treated wastewater do not pose a danger to health and the environment. This can be achieved through standards that can demonstrate the effective control of treated wastewater before its reuse, but also through the adoption of good practices and through the proper information of the many

benefits that would be gained, such as increased water availability, mitigation of water shortages, energy savings, local economic development and reduced use of fertilizers, which would lead to a reduction in environmental impact [5].

To reduce the risk associated with water reuse in recent applications, the standard practice used, and indicated by WHO (World Health Organization) guidelines, is the multi-barrier approach, to ensure effluent quality and meet reuse requirements, with public health protection as the main objective. By using multi-barrier approaches, contamination risks associated with the reuse of treated water can be significantly reduced by combining the advantages of different processes and precautions. In fact, multi-barrier systems include the combination of different technologies, conventional and advanced, and different practices to increase protection from pollutants, depending on the objective of reuse [6].

What makes this approach valid is the fact that no single treatment used alone can be a barrier against all chemicals and pollutants. Therefore, it is necessary to act on several fronts, using different treatments to remove different contaminants, based on their physic-chemical properties, and also making use of administrative barriers and good practices. Treatment barriers may include a primary, or secondary treatment and sometimes a more specific tertiary disinfection treatment to reduce pathogen load or regulate salinity, depending on reuse needs [7].

A multi-barrier approach, however, does not only include technical barriers related to treatment, to be effective it must also include non-treatment barriers, such as the separation of wastewater sources, separating industrial and urban wastewater, or the mixing of treated water with other sources before its reuse. Any precautions taken during reuse, can also be considered in a multi-barrier system as a barrier equivalent, since they may correspond to a pathogen log reduction credits from a treatment barrier, For example drip irrigation of crops is equivalent to a 2 log reduction units achieved by a treatment barrier [6, 7].

Also not to be underestimated is the importance of monitoring, both at source and after treatment, as a good multi-barrier approach has to follow the quality of the effluent from the sewer to its final reuse. Finally, administrative barriers, such as limitations in permitted wastewater applications and imposed standards, are also part of the multi-barrier system [6].

2.1.4. Reuse in Europe, Italy and Portugal

In 2006, the reuse of treated wastewater in the EU was estimated to be 964 million m^3/year, about 2.4% of treated urban wastewater and less than 0.5% of the annual freshwater extraction in Europe. About 60 % of reclaimed water volume produced in EU was used by Spain and Italy, the other countries, especially those in the north, have much lower reuse rates. In Italy, wastewater reclamation is between 5 and 12% of the total effluent from wastewater treatment plants [5]. In 2015, treated wastewater reuse was estimated to have risen to 1,100 million m^3/year in the EU, about 0.4% of annual freshwater abstractions.

The reuse of treated wastewater is encouraged and regulated throughout the European Union through the Urban Wastewater Directive (91/271/EEC). In addition, specific legislation was recently passed in 2020 for the recovery of treated wastewater in agriculture [1].

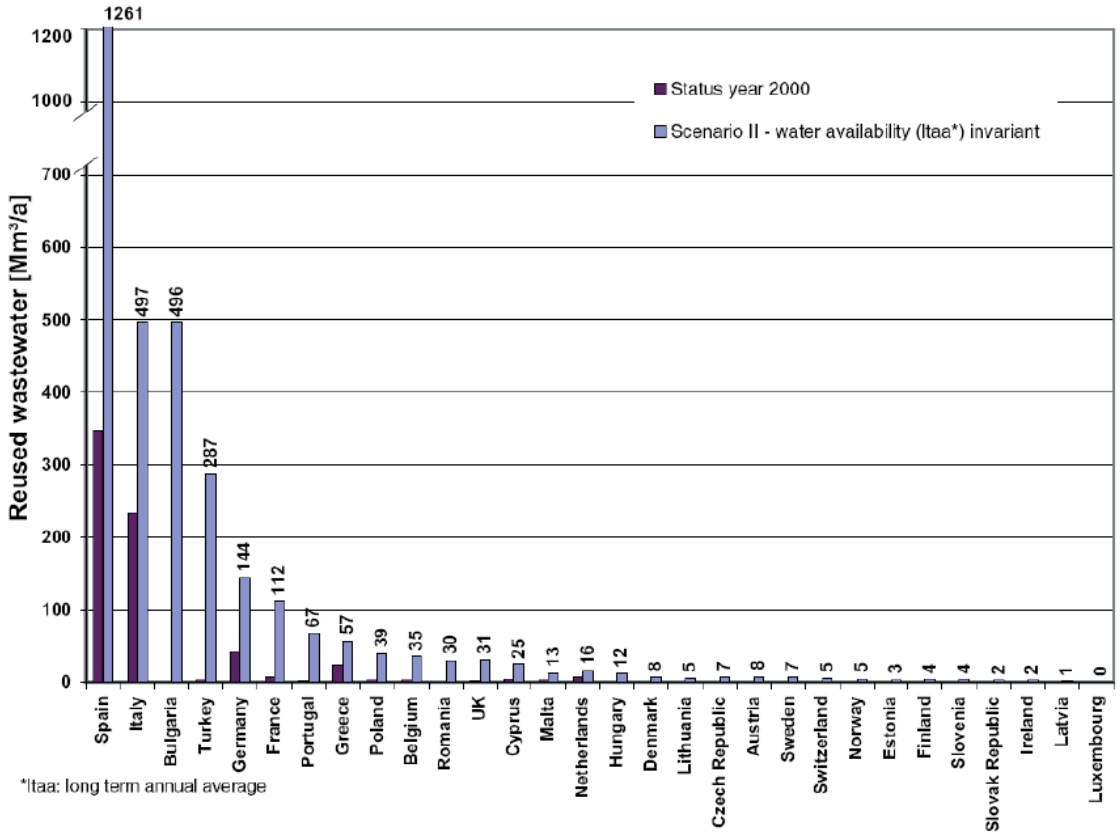

Figure 2 - AQUAREC model output for water reuse potential of European countries on a projection horizon of 2025 (Scenario II presents projection to 2025) [5]

This directive deals with the collection, treatment and discharge of urban wastewater (domestic and industrial wastewater and storm water runoff) and aims to ensure the protection of the water environment from the negative effects of wastewater discharges. According to the directive, European countries should reuse treated wastewater whenever appropriate and with minimal environmental impact, so that the withdrawal of surface water and groundwater from natural resources is reduced. Reutilizing water is seen as an alternative water source, especially in water scarce regions, and if done under safe and cost-effective conditions, it is a safe, but under-utilized way to increase water supply by reducing pressure on the others over-exploited resources. In May 2020, the European Parliament and Council adopted the regulation with the minimum requirements for the reuse of water for irrigation purposes in agriculture. This will enter into force in 2023 and ensures the safety of treated water in order to protect environment, human and animal health, so as to remove obstacles for a wider dissemination of reuse [8, 9, 10].

Irrigation use in agriculture may be carried out on crops intended for human and non-human consumption or for transformation processes. Reuse is also recommended in the regulations for industrial, civil and environmental purposes [8]. The regulations indicate the permitted uses and irrigation techniques and the minimum quality requirements. Treated wastewater is considered to

comply with the regulations if, in measurements, the values for *E.coli*, *Legionella* and intestinal nematodes, for BOD5 (Biochemical Oxygen Demand), TSS (Total Suspended Solids) and turbidity are met and if the concentrations detected do not endanger human health and the preservation of the ecosystem [8].

In Italy, the Environmental Law implementing the European Directive on urban wastewater treatment is the Legislative Decree n° 152/2006 and it requires individual regions to adopt regulations and incentives for water recycling and reuse of treated wastewater, leaving incentive measures in this sector to the regional authorities. The legislative decree also regulates the emission limits for water discharges, while the regulation with the technical standards for the reuse of treated wastewater is regulated by the Technical Regulation on the Reuse of Treated Wastewater (Ministerial Decree 2/05/2006, n°. 108), which replaces the similar Ministerial Decree n°. 185/2003) [11, 12].

These regulations set emission limits for treated wastewater and effluents, regulate their management in order to prevent water pollution, and set technical standards for the reuse of urban and industrial wastewater. Treated wastewater, according to the decrees, must be reused, but only if the methods of reuse are safe for the environment and public health and if ecosystems, crops and soil are not affected and there are no hygienic risks [11, 12].

Permitted uses for treated wastewater are: irrigation use of crops for food or non-food purposes, urban use for street washing or to feed cooling and heating systems, industrial use, as washing water or for processes, provided that the water does not come into contact with foodstuffs, pharmaceuticals or cosmetics [11]. Treated wastewater intended for reuse, be it irrigation or civil, at the output of the treatment plant must possess certain chemical, physical and microbiological quality at least equal to those indicated by the regulations [13]. As far as industrial reuse is concerned, on the other hand, the limits are agreed on the basis of the requirements of the production cycles to which the treated wastewater is destined, but always in compliance with the values laid down for discharge into surface waters [13].

The reuse of treated wastewater is therefore particularly recommended and favored by Italian legislation, since through its reuse it is possible to reduce water withdrawals from natural sources. However, many chemical parameters need to be monitored to comply with stringent microbiological limits [9].

The emission limits for wastewater depend on the various regions, which define the limits and parameters to be monitored, according to the characteristics of each region. In addition, they are also responsible for supporting and promoting the reuse of water and the creation of infrastructures for the adaptation and refinement of sewage treatment [9].

In view of climate change intensifying adverse weather conditions and droughts, Portugal is also paying increasing attention to the possibilities of water reuse, even identifying treated wastewater as a good and possible alternative for water supply [14, 15]. However, there are still few cases of water reuse in Portugal, due to the absence of adequate legislation and the lack of sufficient infrastructure for water treatment and distribution, as well as cost and energy requirements. There are several

projects for the reuse of treated wastewater already started in Portugal, especially in the Algarve region in the south of Portugal, which included the irrigation of golf courses or agricultural crops, such as citrus fruits, or the support of ecosystems. Until 2019, the only regulation in force concerning the reuse of water was a non-binding national standard, NP 4434:2005 [15, 14].

In August 2019, Decree-Law No. 119/2019 was approved and came into force on 21/08/2019. It regulates the production of water for reuse and it contains quality standards that treated wastewater must comply with in order to be reused and it aims to promote water reuse in Portugal [16]**.** The standards are defined by the Portuguese Environment Agency based on the assessment of the risk for health and the environment. So, depending on the results of the risk and the possible presence of barriers or preventive measures, the quality standards could be different from those defined, in terms of values or parameters, but the important thing is that the applicable quality is guaranteed in the intended end use [16]. According to the decree-law, the production of water for reuse can have different sources: urban, domestic, industrial, agricultural and runoff; and reuse can take place for a variety of non-drinking purposes, such as agricultural irrigation, or of green areas, use for street cleaning, as fire-fighting water or for recreational uses. Depending on the intended use, the treated wastewater will have to meet different quality standards [16].

To promote water reuse, Portugal aims to integrate the latest advances on water reuse at European level and the international initiatives developed by the International Organization for Standardization (ISO), to favor non-drinking uses for reuse, i.e. agriculture or public green or landscape [15, 14]. And to ensure that the best technologies are applied, Portugal relies on designing systems with a risk management framework and quality standards based on ISO 16075. In order to be reused, treated wastewater must be of a quality that meets the needs of the end users. Therefore, during authorization procedures, risk assessment on re-use projects will have to be carried out following guidelines drawn up by the Portuguese Environment Agency. These guidelines provide guidance on authorization procedures and technical support to assess risks to health and the environment. The risk assessment will allow the definition of quality standards to be applied to any project involving the reuse of treated wastewater. Furthermore, this will allow the optimal conditions to be chosen to have an associated risk that is the minimum [14, 16].

In 2011, reuse of treated municipal wastewater amounted to 0.59% of total water use, which corresponds to approximately 7km^3 of treated wastewater. But the reuse market is expanding and is set to overtake that of desalination, according to an estimate by Global Water Intelligence. It is estimated that by 2030 water reuse will reach 1.66% of the total water used, i.e. about 26 km^3 per year. Most of the reused treated wastewater, about 32%, is reused in agriculture, for irrigation, about 20% is used for landscape irrigation and 19% for industrial purposes. Only 2% is reused as groundwater recharge [5].

There are also guidelines for the safe use of treated wastewater, including for reuse in irrigation, published by WHO and ISO. The guidelines developed by the WHO and ISO standards have had a profound influence on the standards developed by the EU member states, which have then adapted the standards according to the specific national characteristics. Therefore, the standards of individual

EU countries are not homogenous [5]. These differences might prevent EU-wide companies, that provide re-use technologies, from having a clear framework and action at European level would therefore be necessary to provide incentives and encourage investments in research and re-use technologies [5].

2.2. Wastewater treatment

2.2.1. Conventional treatment

The objectives of wastewater treatment are to treat the effluent in order to allow compliance with the limits imposed by law in relation to the final destination of the effluent: disposal in a receiving body of water (river, sea), discharge on land or reuse.

Conventional treatments used in wastewater treatment (illustrated in Figure 3) are usually distinguished into pre-treatments and actual treatments.

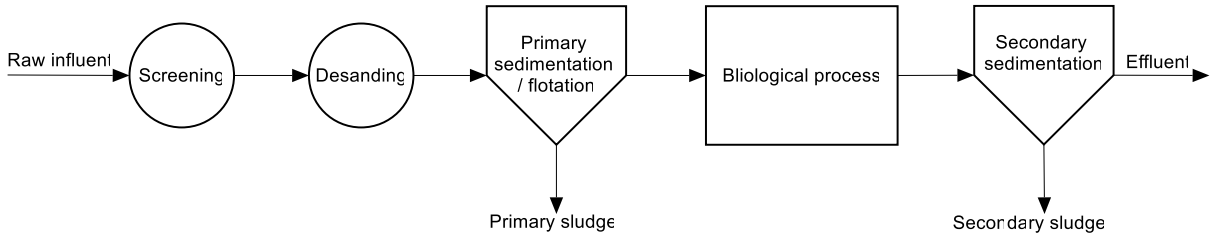

Figure 3 - Conventional treatments in wastewater treatment plants

Screening is the first operation in the pre-treatment of water, both waste and drinking water; it is a unitary operation with physical nature, which aims to remove suspended solids contained in the water, that could damage the plant's electromechanical equipment or produce blockages in downstream channels and pipes [4, 17].

Screening is performed with a device equipped with openings of uniform size and smaller than the average size of the materials to be removed. If a more thorough removal of solids is required by the process, a coarse screening can be followed by fine screening, to better protect the mechanical devices of the downstream units and to remove materials that make the reuse of processed sludge problematic [4].

Screening may be followed by desanding, a unitary physical operation to remove sand and inert material characterized by a higher specific weight than putrescible organic solids. They are generally inserted downstream of the bar screens and upstream of the primary settling. They make it possible to: protect moving mechanical parts from abrasion; reduce the formation of deposits inside the pipes; limit the frequency of cleaning downstream compartments, due to the uncontrolled deposit of solids [4, 17].

A fundamental operation after desanding is the primary settling, that has the purpose of removing settleable solids and floating material and produce a clarified effluent. The removals achieved are of the order of 50-70% of suspended solids and 25-40% of BOD.

Primary settlers are also used as tanks for the accumulation and treatment of excess rainwater: rainwater flows from mixed sewers, when they exceed the maximum capacity of the plant, are by-passed by treatment and sent directly to disinfection; their high content of both settleable and floatable solids, however, does not allow disinfection to operate efficiently; therefore, the preventive passage into primary settling can allow an effective pre-treatment [4, 17].

Another separation operation is the flotation, which allows solid or liquid floating material to be removed by exploiting its lower density compared to the density of the fluid in which it is immersed, thus not exploiting the force of gravity, but the speed of ascent. It can also be carried out together with settling, so that materials with a lower density than that of the fluid are removed by flotation, while those with a higher density are removed by settling [4, 17].

Some materials, due to their low density or small size, have such low settling rates that their removal by settling is not technically viable. On the contrary, precisely because of their low density and the possibility of agglomerating small-diameter particles into flocs, separation units can be created that facilitate the ascent of solids towards the free surface of the liquid and thus their flotation [4, 17].

The main advantage of flotation, compared to settling, lies in the high removal efficiency of extremely small particles and short hydraulic residence times. This results in rather small treatment unit sizes and therefore lower construction costs. At the same time, however, operating costs are significantly higher, again compared to those required for settling units.

Biological processes are used to remove soluble and particulate biodegradable constituents, which are transformed through the activity of specific microorganisms into stable, environmentally compatible end products. They also serve to remove non-settling colloidal material, which is incorporated within the biological flocs or biofilm (suspended biomass processes and attached biomass processes). Another objective is to remove or transform nutrients into less toxic and environmentally hazardous forms (nitrogen and phosphorus). Finally, they can also be used to remove specific, biodegradable organic compounds present in low percentages [4, 17].

In general, the biological process is suitable for removing all biodegradable fractions, because it exploits the natural activity of microorganisms (bacterial metabolism that uses these compounds as a source of energy and carbon for their growth).

Normally in domestic wastewater, it is sufficient for the secondary treatment to consist of a biological process followed by clarification. If the wastewater is industrial or mixed domestic-industrial in nature, there are components that are not biodegradable or that are very slowly biodegradable and would therefore be difficult for the microorganisms to remove, the biological process must be coupled to a chemical or chemical-physical process, which can be placed either upstream, as pre-treatment, if there are components in the effluent that could be inhibiting or toxic to the activity of the microorganisms, or downstream, as post-treatment, if there is bio-refractory DOC (Dissolved Organic Carbon) in the effluent [4, 17].

Depending on the type of metabolism carried out by the bacteria, one component or another can be removed: the process can be aerobic, in the presence of oxygen, anaerobic, without the presence of oxygen, anoxic, if oxygen is only present in the form of nitrites or nitrates, facultative, if the biomass can operate both in the presence and absence of oxygen [4, 17].

Another distinction is made in relation to the type of biomass. There are two broad classes:

- suspended biomass process in which the biomass is kept in suspension through mixing or aeration;
- suspended biomass process in which biomass grows on supports, which may be fixed or moving and designed to have a high specific surface area.

Biological treatment can achieve nitrogen and phosphorous removal by bacterial synthesis, as they are used by the biomass to build new cells. If, on the other hand, more intensive phosphorous removal is desired, phosphorous-accumulating microorganisms can be used, which accumulate a high amount of phosphorous within the cell, which is removed by purging the excess sludge. BOD removal, nitrification and denitrification can also be achieved [4, 17].

Finally, secondary settling can be used to produce a clarified effluent with a low solids content and a thickened sludge with a high concentration, to be recirculated and purged.

2.2.2. Disinfection of wastewater for water reuse

Disinfection consists of the partial elimination of pathogenic organisms [18] and differs from sterilization in that it is a selective elimination of microorganisms, i.e. it does not involve the elimination of all organisms present [4]. It aims to remove or inactivate pathogenic microorganisms to prevent the spread of infectious diseases.

Considering the wastewater treatment sector, the types of microorganisms that can cause serious consequences due to their pathogenic effects,and if they are spread in the water, include bacteria, bacteria spores, protozoan or oocysts, helminths and viruses.

Depending on the type of disinfectant agent used, pathogen elimination can occur as a result of: damage to the cell wall with lysis and death of the microorganisms, alteration of the permeability of the cell membrane with release of nutrients from the cell, alteration of the organisms' DNA and RNA, inhibition of the activity of enzymes that catalyze the biochemical reactions of bacterial metabolism [19]. In the case of sodium hipochlorite, HOCl and ClO- lead to conformational changes in proteins and destroy the native structureof the enzymes because of direct reaction and/or forming stable N-Cl bonds with them. Due to strong oxidizing capacity, HOCl could oxidize certain enzymes of the cells such as dehydrogenases and enzymes responsible for respiration [19].

Due to the increasing concern about pathogenic diseases that could be spread through water, standards for wastewater effluents are increasingly stringent. Some traditional treatments, such as settling or activated sludge, are known to remove up to 90-99% of certain microorganisms [20]. Nevertheless, they are not sufficient to achieve the existing discharge requirements for wastewater,

the requirements for the protection of bathing areas and the reuse of water, which is why it is necessary to include a disinfection phase within the treatment plant.

Each country and region has different standards and recommendations, but in general there is a trend towards progressive restrictions to ensure the protection of public health and the environment.

In order to assess water quality from a microbiological point of view and to quantitatively evaluate the microbial risk, the search for indicator organisms is used [18]. It would not be possible to consider all human pathogens, so organisms are selected. Usually, at least one bacterium, one virus and one protozoan is checked, so that the entire range of major pathogen groups is covered. The GDWQ (Guidelines for Drinking Water Quality) and the GWEG (Guidelines for the safe use of wastewater, excreta and greywater), both issued by the World Health Organization (WHO), refer to rotavirus, campylobacter and cryptosporidium, but also recommend to consider local conditions [18], such as: epidemiological information on the presence of outbreaks from local waterways, evidence of pathogen persistence and infectivity and severity of illness [21, 22].

By using indicators to determine the microbiological quality of water, it cannot be obtained a direct estimate of the presence of pathogenic microorganisms in the water, but only an assessment of the probability of their presence.

The EPA (Environmental Protection Agency) RWQC (Recreational Water Quality Criteria), considers *coliform bacteria*, such as *Enterococci* and *Escherichia coli* bacteria as indicators [18].

These are present in feces in greater numbers than pathogens and are therefore easier to isolate and measure: if these are present, pathogens will certainly also be present. However, these bacteria cannot be used as indicators of water disinfection efficiency, because microorganisms have different reactions to disinfection: bacteria are the most sensitive, followed by viruses, bacterial spores and finally protozoa, with greater resistance. Therefore, using *coliform* bacteria and *Escherichia coli* as indicators of disinfection efficiency may not guarantee that even the most resistant microorganisms have actually been inactivated.

The effectiveness of a disinfection process can be influenced by several factors: concentration and nature of the disinfectant, contact time, mechanical devices, temperature, type of organisms and nature/characteristics of the water to be treated [23].

The contact time can be considered the most important aspect of disinfection: for a given concentration of disinfectant, the number of microorganisms that are eliminated increases as the contact time increases [24]. This relationship is expressed by Chick's law in a differential form, as follows [25] [26]:

$$\frac{dN_t}{dt} = -kN_t \quad (1)$$

Where $\frac{dN_t}{dt}$ is the change in the number of microorganisms over time, k is the reaction rate constant, or inactivation constant, N_t the number of organisms present at time t.

The inactivation rate constant is also related to the concentration of disinfectant agent and their relationship is expressed by:

$$k = k'C^n \quad (2)$$

Where k is the inactivation rate constant, k' the microorganism disappearance constant, C the disinfectant concentration, n the dilution coefficient.

The two relationships can be combined, resulting in:

$$\frac{dN_t}{dt} = -k'C^nN_t \quad (3)$$

Which integrated provides [26]:

$$\frac{N_t}{N_0} = e^{-k'C^nt} \quad (4)$$

Therefore [23]:

$$ln\frac{N_t}{N_0} = --k'^{C^n}t \quad (5)$$

And linearizing:

$$\ln C = -\frac{1}{n}\ln t + \frac{1}{n}\ln[\frac{1}{k'}(-\ln\frac{N_t}{N_0})] \quad (6)$$

- for n = 1 concentration and time are factors of equal importance
- for n > 1 concentration has greater importance than time
- for n < 1 time has greater importance than concentration.

However, this relationship does not take into account the variability and heterogeneity of the wastewater characteristics, but assuming that C represents the residual chlorine concentration, i.e. the amount of measurable chlorine remaining in water after chlorination, this is a relationship often used for regulatory purposes to control the disinfection process.

2.3. Wastewater chlorination for water reuse

Chlorine has been the most widely used chemical disinfectant agent worldwide since the late 1940s [20, 1] and has always played a key role in preventing the spread of water-borne infectious diseases, as it fulfils most of the requirements for a good disinfectant [4].

In general, an ideal disinfectant should be available in large quantities and at a reasonable price, should have deodorizing as well as disinfecting capabilities, should have a homogeneous composition, and should be toxic to microorganisms, but not to humans and animals. Furthermore, it is important that a disinfectant does not interact with organic matter more than with bacterial cells, that it is non-corrosive and that it has good penetrative capacities; it must also be safe to transport, store and handle, it must be water-soluble, and it must be stable, i.e. it must lose its germicidal power slowly. Finally, an ideal disinfectant should also be effective at high dilutions and over a wide temperature range [4].

The disinfectant power of chlorine is very high and is achieved by blocking the vital activities of microorganisms, avoiding their development [23]. Chlorine, in fact, changes the chemical structure of enzymes, going to affect the mechanisms of nutrition of bacteria, which are thus inactivated [18].

To increase chlorination efficiency, mixing characteristics and process control can be improved. Generally, the doses of chlorine typically used for municipal wastewater disinfection are around 5-20 mg/L, with a contact time of 30-60 minutes, with which the imposed standards for bacterial indicators *E.coli and coliforms* are met [20].

Using chlorination generally succeeds in effectively removing bacteria, although the presence of any suspended solids may delay or influence the process. However, it is not fully effective in removing cysts and viruses, except at high concentrations of free chlorine [20].

Various chlorine compounds can be used in water treatment plants, such as chlorine gas (Cl_2), sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$) and chlorine dioxide (ClO_2) [18].

The use of chlorine gas is frequently replaced by the use of sodium hypochlorite, due to safety issues in handling and storing liquid chlorine. This solution is only available in liquid form and generally contains between 12.5 and 17% sodium [4]. It can be either purchased in batches or prepared on site at the wastewater treatment plant. The systems that allow it to be produced on site, however, are only used to a limited extent, because of their complexity and the necessary power, since they are systems that require the use of electricity. But, by using sodium hypochlorite, the problems of transporting, dosing and storing gaseous or liquid chlorine are no longer an issue and it also improves operator safety and reduces operating and maintenance costs [20], although, at high concentrations, it decomposes rapidly and it is also sensitive to light and heat. For these reasons, containers made of corrosion-resistant materials should be used for storage and they should be located in cool rooms. It is necessary to take these precautions because sodium hypochlorite is a very corrosive substance that can develop chlorine fumes [4, 18]. Moreover, this is a simple and inexpensive process that requires no particular technical expertise, the dosing system consisting only of a pump and a storage tank.

2.4. Chemistry of chlorine in treated wastewater

When sodium hypochlorite (NaOCl) is added to water, hydrolysis reactions take place and hypochlorous acid (HOCl) is formed [23, 27].

$$NaOCl + H_2O \rightarrow HOCl + NaOH \quad (7)$$

The hypochlorous acid then undergoes an ionization reaction into hypochlorite ion (OCl^-) [23, 27]

$$HOCl \leftrightarrow H^+ + OCl^- \quad (8)$$

Where the ionization constant of the reaction is:

$$K_i = \frac{[H^+][OCl^-]}{[HOCl]} = 3 \times 10^{-8} \frac{mol}{l} \; a \; 25°C \quad (9)$$

The sum of hypochlorous acid and hypochlorite ion in water is referred to as "free available chlorine". Since the disinfectant power of HOCl is about 40-80 times higher than that of OCl^-, it is very important to know the relative distribution of the two species.

The percentage distribution of HOCl at different temperatures can be calculated using the relationship [4]:

$$\frac{[HOCl]}{[HOCl]+[OCl^-]} = \frac{1}{1+{}^{[OCl^-]}/_{[HOCl]}} = \frac{1}{1+K_i[H^+]} = \frac{1}{1+K_i 10^{pH}} \quad (10)$$

And depending on pH, the percentages of HOCl- and OCl^- ions vary according to the distribution shown in Figure 4 [17].

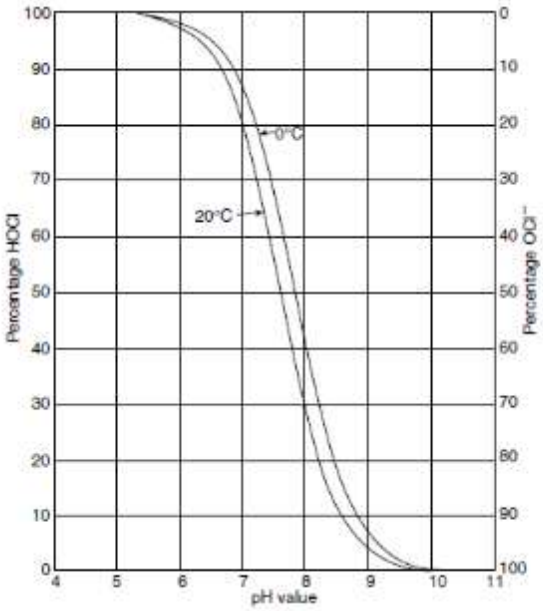

Figure 4 - HOCl and OCl distribution

HOCl is a strong oxidizing agent and reacts with reducing substances, resulting in the so-called 'chlorine demand' [23, 27]. These reactions can compete with each other, and the formation of disinfection by-products (DBPs) depends on their kinetic constants [4]. In addition, they lead to a reduction of oxidizing chlorine with disinfectant power, because they produce the chloride ion and chlorinated organic compounds, also called organic chlorine, without oxidizing or disinfectant properties. Substances that react with and reduce chlorine are inorganic Fe^+, Mn^+, NO_2^- and H_2S and organic material. Many organic compounds, in fact, can interfere with chlorination, and the interference depends on the functional groups they contain and on their chemical structure. Usually, the organic compounds with unsaturated bonds cause an immediate demand for chlorine. Substances containing polycyclic rings with hydroxyl groups or containing sulphur in their structure react very quickly with chlorine. Although the resulting chlorinated species had no disinfectant power, they can be quantified as residual chlorine by the DPD (Dietil-p-fenildiamine) method used for chlorine analyses

[4, 23]. On the other hand, saturated compounds and carbohydrates appear to have very little, if any, chlorine demand and would therefore not appear to interfere with chlorination. Hence, if oxidizable compounds such as humic acids or iron are present, the inactivation of bacteria will undergo a latency phase, as the chlorine will not be used immediately to inactivate the microorganisms, but will be used to oxidize these substances [4].

Therefore, for an effective wastewater disinfection the amount of chlorine supplied must be sufficient to meet the chlorine demand of these reactions and ensure a good disinfectant action. The fact that these reactions occur can therefore be seen as a disadvantage to the use of chlorine as a disinfectant agent, but these occur precisely because chlorine has the great property of having a high oxidizing power and therefore of being a powerful disinfectant [23, 27].

Wastewater may contain nitrogen, either in the form of ammonia, or bound to the various organic compounds present, and treatment plant effluents also contain significant concentrations of nitrogen, in the form of nitrates, especially if a nitrification unit is present. Hypochlorous acid, being a strong oxidant, reacts rapidly with the ammonia present, forming chloramines [23, 27], namely monochloramines, dichloramines and nitrogen trichloride, trough the following reactions

$$NH_3 + HOCl \rightarrow NH_2Cl\,(monochloramine) + H_2O \quad (11)$$

$$NH_2Cl + HOCl \rightarrow NHCl_2(dichloramine) + H_2O \quad (12)$$

$$NH_2Cl + HOCl \rightarrow NCl_3(nitrogen\ trichloride) + H_2O \quad (13)$$

These reactions depend on pH, temperature, contact time and the ratio of chlorine to ammonia [23, 27].

The chlorine contained in chloramines is known as combined chlorine, to distinguish it from free chlorine, compared to which it has less disinfectant power but-slower decay over time [4].

Since free chlorine is a strong oxidant, and it reacts with ammonia and other compounds, it is difficult to maintain a certain concentration of residual chlorine, free or combined, for disinfection purposes. This is why it is particularly important to study the so-called *breakpoint chlorination*, i.e. the chlorination process where enough chlorine is added to react with all oxidizable substances, so that by adding more chlorine, this remains as available chlorine. And it is by having a residual amount of chlorine as free or combined chlorine that effective disinfection is guaranteed.

In this regard, the chlorine demand of water can be defined as the total amount of chlorine that is consumed by substances in the water, other than microorganisms. These substances can be distinguished into classes, based on the speed at which they react with chlorine, as described in Figure 5.

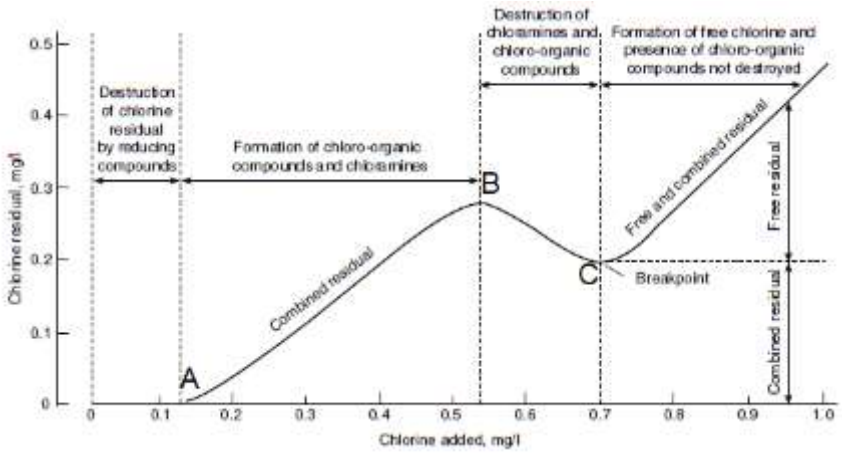

Figure 5 - Typical breakpoint curve with the chlorine demand that is imposed by readily oxidized compounds [17]

Considering a typical breakpoint curve (Figure 5), in the first section, from 0 to A, the first class can be identified, it consists of substances that react immediately with chlorine, which is transformed into the chloride ion. These substances are rapidly oxidizable species, such as Fe^{2+}, Mn^{2+}, H_2S and organic matter [23]. This first demand for chlorine is referred to as an immediate demand for chlorine, after which, if more chlorine is added, it will first react with the saturated organic compounds present, which exert a reduced demand for chlorine and do not interfere in the measurement of active chlorine, then it reacts with the unsaturated organic compounds, which form organic chlorine compounds, without disinfectant power, but which do interfere in the measurement of chlorine. At the end of this phase, again along the A - B section of the graph, chlorine reacts with ammoniacal nitrogen and organic nitrogen to form chloramines, in accordance with the reactions presented above. The distribution of monochloramines and dichloramines depends on the rate of formation, which depends on pH and temperature [23, 4, 17].

Increasing the added chlorine, between point B and point C, results in the oxidation of the chloramines and thus the disappearance of the combined active chlorine. A portion of the chloramines is converted to nitrogen trichloride, the remaining portion is oxidized to nitrogen gas N_2 and nitrous oxide N_2O, while the chlorine is reduced to chloride ion. The point C is the breakpoint, once passed, adding chlorine the amount of free chlorine available will proportionally increase, as hypochlorite [4, 17].

The disappearance of chloramines and the production of N_2 and N_2O during chlorination at the breakpoint can be expressed by the following reactions [28, 27, 23]:

$$NH_4^+ + HOCl \rightarrow NH_2Cl + H_2O + H^+ \quad (14)$$

$$NH_2Cl + HOCl \rightarrow NHCl_2 + H_2O \quad (15)$$

$$0.5NHCl_2 + 0.5H_2O \rightarrow 0.5\ NOH + H^+ + Cl^- \quad (16)$$

$$0.5NHCl_2 + 0.5NOH \rightarrow 0.5\ N_2 + 0.5HOCl + 0.5H^+ + 0.5Cl^- \quad (17)$$

The overall reaction is:

$$NH_4^+ + 1.5HOCl \rightarrow 0.5\,N_2 + 1.5H_2O + 2.5H^+ + 1.5Cl^- \quad (18)$$

The first reaction in the chain, reaction (14), is the main reaction that takes place in the first zone of the curve and involves chlorine and ammonia. In this zone, therefore, the residual chlorine will mainly consist of NH_2Cl [27].

Looking at Figure 6, it can be seen that in theory the maximum of total chlorine should occur for a chlorine to ammonia weight ratio of 5:1 [27]. Once this ratio is exceeded, the reaction (15) begins and $NHCl_2$ is formed. At the breakpoint, which is for a theoretical Cl_2/N-NH_4 ratio of 7:6 the ammonia is completely oxidized and also most forms of chloramine and there is the minimum of residual chlorine. Beyond the breakpoint there may still be $NHCl_2$ and NCl_3, but only in minute quantities [27].

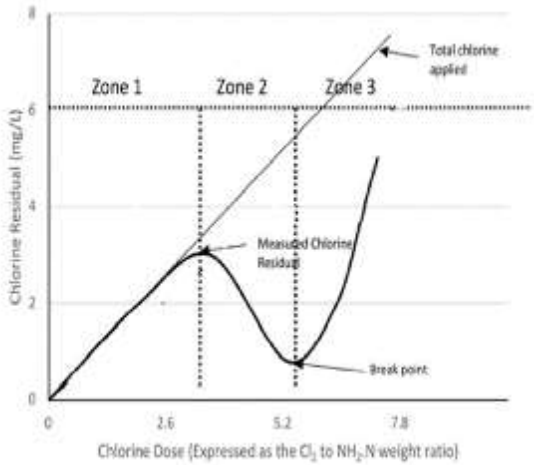

Figure 6 – Typical breakpoint curve with the chlorine dose expressed as Cl_2/N-NH_4

As seen from the reactions, hydrochloric acid is formed during chlorination. This, will react with the alkalinity of the effluent, causing a decrease in pH and thus a production of acidity. In addition, the chemical reagents used will produce an increase in the concentration of total dissolved solids, which should be controlled [23].

The amount of chlorine that must be added to achieve a specific residual chlorine concentration is referred to as the chlorine demand.

Chlorination at the breakpoint can produce negative impacts, such as the generation of bad odors due to the formation of nitrogen trichloride. In addition, the shape of the curve may vary significantly if other compounds that may react with chlorine, such as organic nitrogen, are present [4]. Therefore, disinfection in wastewater treatment plants is usually carried out with chloramines and not with free chlorine, in order to have a lasting disinfection residue and to reduce harmful disinfection by-products. Chloramine is in fact unreactive and has less odor than other forms of chlorine. However, if nitrites are present, this can lead to an accelerated loss of chloramines that cannot be reversed, requires more chlorine and forces the plant to switch to chlorination with free chlorine. This process, however, is particularly expensive in terms of money and cannot be maintained for a long time, so it is usually followed by a re-chlorination [27].

To report laboratory results concerning disinfection processes, the number of organisms and the concentration of residual chlorine in a certain time interval are usually used.

Disinfection efficiency, i.e. the disappearance of microorganisms, depends on the concentration of the disinfectant and on the contact time. In fact, to reach the disinfection standards set by the regulations, it must be ensured that the effluent remains in contact with the disinfectant for a certain period of time [29]. As an operational parameter and indicator of disinfection effectiveness, the CT value, product of the residual concentration of the disinfectant agent used and of the contact time between disinfectant and water, is usually used [18].

If enough chlorine cannot be added to reach breakpoint conditions, an adequate contact time must be maintained for disinfection to be adequate.

The efficiency of chlorination depends on many factors, such as: the initial mixing, the chemical characteristics of the effluent, the particles present in the effluent, the characteristics of the microorganisms.

Mixing plays a very important role in disinfection efficiency, however, it is not entirely clear what optimal levels of turbulence should be maintained in the system, as the optimal levels of turbulence within the reactors are not known.

The use of oxidizing agents for disinfection can lead to the formation of undesirable by-products, many of a carcinogenic nature. The formation of these compounds is due to several factors, such as free chlorine concentration, bromine concentration, pH, temperature. In order to avoid the formation of these by-products in the case of free chlorine disinfection, the chlorine dosage should be selected accordingly, as an alternative to the use of different chloramines or disinfectant agents.

To avoid the problem of the growth of microorganisms downstream of the disinfection treatment, it is necessary to provide for the addition of chlorine in locations at intermediate distances between the plant and users.

In order to ensure a sufficient residue of chlorine to guarantee a hygienic barrier, it is necessary to consider the phenomena of chlorine decay and the factors influencing them, to correctly manage the choice of chlorine concentration added, optimizing the dosage [30]. Chlorine decay can be described as the union of two groups of chlorine-consuming reactions: those occurring in the water, which represent the mass decay, and those occurring on the inner surface of pipes, which represent the wall decay [30]. The first is due to reactions between chlorine and dissolved compounds in the water, mainly consisting of natural organic matter, but also some inorganic compounds. The kinetic model used to represent this decay is of the first order and consists of the equation (19):

$$\frac{dC_{Cl}}{dt} = -K_b C_{Cl} \qquad (19)$$

Where C_{Cl} represents the chlorine concentration, t the time, K_b the mass decay coefficient, also called the mass reaction rate constant, which is determined experimentally.

Wall decay becomes important especially in the case of systems with metal pipes, because chlorine is involved in corrosion processes. Other factors that may intervene and influence wall decay are the presence of biofilm or sediment. However, unless there are corroded pipes, mass decay is predominantly responsible for chlorine consumption and wall decay only contributes in a small part. Wall decay can also be expressed with a first-order kinetic model via the equation (20):

$$\frac{dC_{Cl}}{dt} = -\frac{4k_f k_w C_{Cl}}{D(k_f + k_w)} \quad (20)$$

Where k_f represents the mass transfer coefficient, which depends on the velocity, k_w the wall decay coefficient, also called wall reaction rate constant, which is calibrated adapting it to the measured chlorine concentration, D the pipe diameter [30].

The operating conditions influencing chlorine decay rates are water temperature, initial chlorine concentration, the type and concentration of natural organic matter and flow velocity.

2.5. Disinfection byproducts and their precursors

The organic matter (OM) present in biologically treated wastewater comprises not only natural organic matter (NOM), derived from plants and animals decomposing existing in drinking water sources, but also soluble microbial products (SMPs) that are formed during the biological wastewater treatment, trace organic compounds from domestic and transformation products resulting from the various biotic and abiotic processes that can take place during treatment. Hence OM comprises a mixture of compounds, such as humic acids, fulvic acids, low molecular weight organic acids, carbohydrates, proteins, etc. [31] with heterogenous structures and different chemical properties [32].

OMs can be divided into two fractions: humic-like substances (e.g., fulvic and humic acids) and non-humic substances (carbohydrates, hydrocarbons, lipids and amino acids) [33] and contain both hydrophobic and hydrophilic compounds [34]. The hydrophobic part comprises higher molecular weight compounds with aromatic rings, or phenolic hydroxyl groups with double bonds. Hydrophilic compounds, on the other hand, generally have lower molecular weights and contain aliphatic ketones and alcohols [34].

The hydrophobic part of the OMs generally has a higher absorbance at 254 nm (UV254) and specific ultraviolet absorbance and reacts faster with chlorine than with bromine. In contrast, the hydrophilic part of the OMs has lower UV254 and SUVA (Specific Ultraviolet Absorbance) values [34].

It is important to monitor the presence of dissolved natural organic matter in reclaimed water, as this can be a source of nutrients and energy for bacteria regrowth, but also because it can react with disinfectants during disinfection and form by-products [35, 36].

One of the main disadvantages of chlorine disinfection is precisely the generation of toxic by-products (DBPs). Reactions between organic substances in some water sources and chlorine can lead to the formation of trihalomethanes (THMs) and other compounds that, besides being potentially carcinogenic to humans, can be very harmful to the environment, even at low concentrations [20, 37].

Given the heterogeneity of OMs their presence is usually measured indirectly with parameters such as TOC (Total Organic Carbon), DOC, ultraviolet absorbance at 254 nm (UV254), or specific ultraviolet absorbance (SUVA) [33, 35]. The latter is usually the most suitable, as it is a good indicator of the presence of humic compounds in water, those with a greater tendency to form DBPs than the non-humic fraction; whereas TOC is not particularly accurate for estimating DBPs formation, as its measurement includes a large number of different compounds [33].

Since concentrations of chloroform and other THMs were first detected in chlorinated drinking water in the 1970s, attention to disinfection by-products has been on the rise, also in view of stricter regulatory standards and the results of epidemiological investigations, which linked chloroform to cancer in laboratory animals [33]. However, although some 700 different DBP species are distinguished in the literature, the studies about quantitative and human health effects only cover a small fraction of them [33]. Numerous investigations have shown that the majority of DBPs in treated water are trihalomethanes (THMs), about 50% of the total organic halogenated DBPs [33, 38], while the remainder is found in larger molecules of natural organic matter [35].

THMs are halogen-substituted single-carbon compounds. They are formed by reactions between organic compounds with chlorine or bromine, and their general formula is CHX3, where X represents atoms of chlorine, bromine, fluorine, iodine or a combination thereof [33]. The most commonly present THMs are chloroform ($CHCl_3$), chlorodibromomethane ($CHClBr_2$), bromodichloromethane ($CHBrCl_2$) and bromoform ($CHBr_3$) [33, 39, 38]. The THM most commonly present in water systems is usually chloroform, although high levels of brominated THMs can be found at high concentrations of Br [33, 39, 34].

After tests for toxicity, neurotoxicity, and carcinogenicity studies of THMs, the World Health Organization (WHO) established guidelines for the health implications of DBPs in drinking water. Currently, the concentration of total THMs accepted by the European Union is 100 µg/l (EU directive 2020/2184 on the quality of water intended for human consumption), but member states may set their own standards, provided they are no less stringent than those set by the directive [40].

The concentration of THMs depends on the source of the water, the processes and treatment used for the effluent and the distribution system. Parameters that can influence the formation of THMs are pH, organic matrix, concentration of bromide, free chlorine and contact time [33, 34, 38]. In chlorination at the breakpoint, THM formation depends on both the bromine content and the ammonia concentration [38].

There is a great lack in literature concerning the formation of trihalomethanes in wastewater: most of the available studies concern drinking water, which, however, has a very different organic matter content. The organic matter contained in wastewater is in fact much more complex, hence the trihalomethane formation mechanims may be different.

In drinking water it has been noted that there is an increase in THM formation with higher pH, although the increase in THMs is balanced by a decrease in other DBPs [41, 34]. Temperature also influences the formation of by-products and it has in fact been noted that an increase in temperature increases

the formation of THMs, which is therefore higher in summer and lower in winter [33]. With regard to the presence of bromides, as already mentioned, if there are low concentrations of available bromide, but an excess of chlorine, then the dominant THM species will be chloroform, whereas if bromine is found in higher concentrations than organic matter and chlorine, brominated THMs will form [39, 34]. Chlorinated THMs, therefore, will form in waters with low bromine, due to reactions between hypochlorous acid and the hydrophobic fraction of OM, whereas brominated THMs will form in waters with bromine due to reactions of the hypobromous acid with the hydrophilic part of OM [34].

The formation of THMs always occurs in the presence of free chlorine and, in general, the reaction can be divided into three parts: initially, chlorine meets the chlorine demand of the inorganic matter and very few THMs are formed; subsequently, chlorine reacts with 'fast-reacting' organic matter and the formation of THMs increases; finally, chlorine reacts with 'slow-reacting' organic matter and THMs continue to form, but at a lower rate [33]. Generally, the formation of THMs increases as the chlorine dose increases, however, this only happens up to the point where the reaction is no longer limited by chlorine, i.e. beyond a certain amount of chlorine its addition has been found to have minimal impact on the increase in THMs [33]. Finally, it should be emphasized that organic matter, are also indispensable for the formation of THMs [33, 41].

2.6. Spectroscopic methods for wastewater characterization

It is therefore necessary to know the properties and environmental behavior of OM [35, 36]. However, due to their heterogeneous nature, their complexity and the large number of functional groups they can possess, there is not only one single analytical method capable of providing information on the structure or functionality of OMs [20]. In fact, depending on the characteristic of each OM fraction, a different technique should be used.

Certain characteristics of OM can be studied by optical detection, as it is composed of fractions capable of absorbing UV-Vis (Ultraviolet and Visible) radiation and emitting fluorescence at specific wavelengths [32]. The part of organic matter that can absorb light is called chromophore and this influences the optical properties, intensity and spectral distribution of light within the water column [36].

UV-Vis spectroscopy can be used to obtain information on the chromophore fraction of organic matter, the aromaticity and reactivity of OM, and fluorescence spectroscopy can give a quantification of the OM content [32, 36].Spectroscopy is among the most widely used methods to characterize natural dissolved organic matter (DOM) in wastewater [31, 36] and derived parameters such as absorption coefficients, absorption ratios or differential spectra are used to study it [36].

Fluorescence techniques have also been successfully used to characterize or detect humic substances and other organic matter fractions, using EEMs as true fingerprints of the single compound or a mixture of fluorescent compounds [31].With fluorescence spectroscopy, only a small part of chromophore organic matter, known as fluorescent organic matter, can be studied, as this can emit fluorescence when excited by sources with a certain energy. [36]. Therefore, it is possible to study organic matter using UV-Vis spectroscopy and fluorescence spectroscopy, obtaining information on chromophore and fluorescent organic matter, their aromaticity, sources and reactivity, respectively.

In addition, the use of spectroscopic instruments such as UV-Vis spectroscopy and fluorescence spectroscopy is convenient and widely used, especially when it is necessary to characterize large numbers of samples, due to their ease of use, low cost, high sensitivity and rapid response [36].

2.6.1. UV-Vis absorption spectroscopy

One of the tools used in the study of OM is UV-Vis absorption spectroscopy and is particularly useful for identifying the abundance and quality of OMs, tracing their source and verifying their reactivity and variation. It is an easy-to-use, highly sensitive, fast-responding and relatively inexpensive method [36].

Given the variety of light-absorbing OM fractions, UV-Vis absorption spectra do not usually have any particular characteristics. Usually, specific indices or parameters obtained by processing spectral data are used, which can provide additional information [36].

These parameters are generally absorption coefficients or absorption ratios.

Absorbance at 254 nm (UV 254) and SUVA 254, i.e. the ratio of UV 254 to dissolved organic carbon (DOC) can be used as parameters to indicate the content of aromatic compounds in humic substances [32]. SUVA 280 is used to identify compounds with double bonds, while SUVA 365 and SUVA 436 give information on the size of molecules and chromophoric groups in OMs [42].

Absorption coefficients consist of the absorbance normalized for the optical path and these absorption coefficients at certain wavelengths can be used to detect hydrophobic or hydrophilic fractions in OM [32], or they can be related to the attenuation of organic compounds and thus used to monitor the removal of organic pollutants in wastewater treatment processes [43].

Absorbance coefficients at different wavelengths reveal the presence of different fractions of OM, therefore, the absorption ratio, i.e. the ratio of absorption coefficients at two different wavelengths can reveal the sources and chemical compositions of OM [32].

To investigate the sources and chemical composition of dissolved organic matter in water samples, absorption ratios, i.e. the ratios of absorption coefficients at two different wavelengths, are often used. Indeed, the absorption coefficient at different wavelengths may represent different concentrations of chromophoric dissolved organic matter (CDOM) fractions, so the ratios may represent different characteristics of the organic matter, such as molecular weight, aromaticity, humification and hydrophobicity, as illustrated in Table 1 [36].

Absorbance ratios, since organic molecules with different molecular weights absorb different wavelengths of light, can be related to the molecular weight of DOMs. For example, the absorption ratio between the absorbance at 254 nm and 365 nm can be used to indicate the molecular weight of CDOMs, as it is negatively correlated with the MW (Molecular Weight) of DOMs. This same ratio can also be used to indicate the aromaticity of the DOM, as it is negatively correlated with this characteristic as well. The state of humification can also be derived from absorption ratios, such as A300/A400, A300/A401 or A300/A402. These ratios can in fact be used to indicate the state of humification of humic acids in the soil. A logarithmic form of the ratio A400/A600 has also been used

to estimate the humification. However, this ratio, being associated with long wavelengths, is not to be considered reliable due to the poor signal-to-noise ratio associated with these wavelengths [36].

Some absorption ratios can also be used to research the composition of DOMs, such as the ratios A254/A436 and A254/A437, which can estimate the relative composition of native DOMs versus terrestrial DOMs, and the ratio A340/A254, which can be used to predict the concentration of DOC [36].

Other commonly used absorption ratios are E2/E3 (250/365nm), E2/E4 (265/465nm), as they can show changes in molecular weight, aromaticity, polarity, humification and hydrophobicity [32].

Table 1 – Absorbance ratios and different characteristics of OM [36]

Types	Absorbance ratio	Target characteristics	Range	Relationships	Sources
E2/E3	A254/A365	Aromaticity, MW	-	Negatively correlate with aromaticity and MW	Plant and manure DOM
E2/E4	A254/A436	Sources, aromaticity	4.37-11.34	Estimate the relative composition of autochthonous versus terrestrial DOM	River
E2/E5	A254/A437	Sources	19.52-27.45	Estimate the relative composition of autochthonous versus terrestrial DOM	Estuary (seawater)
E3/E4	A340/A254	DOC prediction	0.20-0.38	To predict DOC concentration	River
E4/E6	logA400/A600	Humification aromaticity	0.512-0.963	Correlate negatively with humification and aromacity	Soil humic acids
E3/E4	A300/A400	Humification	2.67-3.10	Correlate negatively with humification	Soil humic acids
E3/E5	A300/A401	Humification	5.14-7.03	Correlate negatively with humification	Soil fulvic acids
E3/E6	A300/A402	Humification	3.4-8.4	Correlate negatively with humification	Lake

In fact, the detection of increases in the E2/E3 ratio may indicate a decrease in the aromaticity and molecular weight of the compound.

However, it must be made clear that in order to be able to apply spectral ratios in the study of OM, a correct measurement of UV-Vis absorption spectra is required, as there are many environmental factors that can interfere with the measurement and calculation of the parameters, such as turbidity, temperature and pH. Hence, it is necessary to eliminate all interference when acquiring, calculating and interpreting spectral data [32].

2.6.2. Fluorescence spectroscopy

Fluorescence spectroscopy is a highly sensitive optical technique used to quickly investigate the chemical composition of OM [44].

The fluorescence spectroscope uses UV-Vis light to illuminate the molecules of the sample, which jump from the ground state to an excited state and then return to the ground state by emitting light at longer wavelengths [32].

With fluorescence spectroscopy, a fluorescence excitation-emission matrix (EEM) is obtained, it consists of three vectors along the x, y and z coordinates: excitation wavelength, emission wavelength and fluorescence intensity. This matrix is usually represented as a contour map.

Fluorescence spectroscopy is particularly well suited to the study of OM, as they have fluorescent structures with characteristic excitation/emission (EX/EM) peaks, which therefore allows the fluorophores present to be identified and quantified [44].

Fluorescence spectroscopy provides much information on the chemical nature of OM, as fluorescence is a characteristic of the structure and functional groups of molecules [45]. Fluorescence, however, is very sensitive and can be influenced by several factors, such as solution type, pH, ionic strength, temperature, interaction with organic substances [31]. The fluorescence intensity is also very sensitive to the molecular structure of the OM and decreases as the size of the molecules increases. However, it is difficult to identify individual fluorophores with fluorescence, due to the complex chemical structure and spectral overlap [45].

There are several methods for interpreting data obtained by fluorescence spectroscopy and for interpreting information on the structure of OMs. Some of these are peak-picking and fluorescence regional integration (FRI) [32].

Generally, six pairs of fluorescence peaks EX/EM can be identified in the OM, which can be associated, by peak-picking, with as many fluorescent components [46]:

- B peak, at ~230/~300 nm, referable to tyrosine-like structures,
- two T peaks, at ~230 and ~280/~340 nm, typical of tryptophan-like structures, with at least one aromatic ring,
- peak A, at ~250/400-450 nm, with two aromatic rings and a humic-like structure,
- C peak at 300-350/400-460 nm, with humic-like structure with two or more aromatic rings,
- C +, at ~380/~480 nm, with allochthonous structure with two or more aromatic rings or microbially produced substances.

However, this correlation is not entirely accurate and can sometimes be uncertain due to shifting or overlapping peaks, the presence of more than one peak or due to interferences. It must also be considered that some fluorophores have more than one EX/EM peak [32].

Another method is to decompose the EEM matrices into five peak regions (FRI method), identified along excitation and emission wavelengths (Table 3), and to obtain an analysis of the configuration and heterogeneity of the OM [32].

Table 2 – EX/EM peaks and OM associated

Peaks	EX/EM	Fluorescent compounds
Peak B	230/300 nm	Tyrosine-like structures
Peaks T	230 e 280/340 nm	Tryptophan-like structures with at least one aromatic ring
Peak A	250/400-450 nm	Humic-type structure with two aromatic rings
Peak C	300-350/400-460 nm	Humic-type structure with two or more aromatic rings
Peak C+	380/480 nm	Allochthonous structure with two or more aromatic rings or microbially produced substances

Table 3 - Fluorophores in OM at different EX/EM regions

Region	EX regions	EM regions	Fluorophore
I	< 250 nm	< 330 nm	Aromatic proteins I, tyrosine
II	< 250 nm	330 – 380 nm	Aromatic proteins II, tryptophan
III	< 250 nm	> 380 nm	Fulvic acid-like substances, hydrophobic acids
IV	> 250 nm	250 – 380 nm	Tyrosine/tryptophan-like and SMP-like species
V	> 250 nm	> 380 nm	Humic acid-like organics, hydrophobic acids

Fluorescence peaks corresponding to excitation wavelengths shorter than 250 nm and emission wavelengths shorter than 330 nm can be referred to aromatic proteins I, such as tyrosine, if the emission wavelengths are between 330 and 380 nm can be referred to aromatic proteins II, such as tryptophan and if the emission wavelengths are greater than 380 nm can be referred to fulvic acid-like substances [32].

Instead, peaks at longer excitation wavelengths, greater than 250 nm, can be attributed to protein-like compounds and if the emission wavelengths are between 250 and 380 nm to soluble microbial

products (SMPs), while they are assigned to humic acid-like organic compounds, if the emission wavelengths are greater than 380 nm [32].

This identifies groups of substances with similar structure and negligible distinction. In addition, this type of analysis can be used to keep track of DBP precursors generated by OM. In fact, neutral and acidic hydrophobic compounds are the main substances that form DBPs [32].

The use of this technique to estimate the composition of OM, however, is not yet fully accepted as component concentration and fluorescence intensity are not always linearly dependent [44].

Moreover, fluorophores are not fully representative of the structure of OM. This is because only a fraction, less than 1%, of the constituents of OM are fluorescent and fluorescence spectroscopy is a technique that cannot detect non-fluorescent components [32].

3. Materials and methods

3.1. General remarks

The present work entailed the execution of laboratory experiments to assess the chlorination breakpoint curves in treated wastewaters and the decay of chlorine over time. It involved the collection of treated wastewater samples from Beirolas wastewater treatment plant (WWTP) in Lisbon and their transportation to LNEC (National Civil Engineering Laboratory) lab facilities, the design and execution of the chlorination experiments, and the characterization of the water samples before and after chlorination. The total-, the free- and the combined chlorine concentrations were determined in these assays. The ammonium concentration was also assessed in some cases. To evaluate the change in water composition after sample chlorination, the chlorinated samples were also analyzed by excitation-emission fluorescence spectrophotometry, UV absorption spectra and UV absorption at specific wavelengths. The quantification of disinfection by-products, namely trihalomethanes, were also performed in some chlorinated samples and their presence were tentatively correlated with the spectroscopic characteristics of the samples. In addition, and to complement the lab work, the sizing of a chlorination unit was carried out for a chlorination unit based on the experimental results obtained. The materials and methods details are described in the following sections

3.2. Beirolas wastewater treatment plant

The Beirolas wastewater treatment plant has an area of approximately 1660 hectares, is located in the eastern part of Lisbon and treats wastewater from the municipalities of Lisbon and Loures. It is capable of treating, with secondary level treatment, a daily flow of 54,000 m^3 of water and serves a population of approximately 214,000 population equivalent [47].

Figure 7 - Aerial view of the Beirolas Wastewater Treatment Plant and Parque Tejo Gardens [48]

The wastewater undergoes pre-treatment consisting of screening and sieving to remove coarse and finer solids, desanding and degreaser to remove sand and floating materials. The effluent then goes to primary treatment where the sludge is separated by primary settling and then sent for thickening. There is then biological treatment in two reactors, comprising an anaerobic, an anoxic and an aerobic phase [44]. Currently, part of the treated wastewater undergoes a tertiary treatment consisting of an ultrafiltration step followed by a chlorination step to produce reclaimed water for irrigation purposes.

3.3. Wastewater sample collection and characterization

The wastewater samples were collected downstream the ultrafiltration unit (permeate flow) in pre-rinsed polypropylene flasks (5L), at two different days (6 April 2022 and 31 May 2022) and were used in tree experimental trials.

Figure 8 - Pictures of the ultrafiltration and chlorination unit and of the ultrafiltrated not chlorinated wastewater sample

The samples were transported to the laboratory as soon as possible, and the volume collected was divided for chlorination assays and for water characterization analyses. There was no need to refrigerate the sample during transportation due to small distance between the WWTP and LNEC lab.

Prior to be used in the chlorination assays, the wastewater samples were characterized. Analyses were carried out on all samples. Table 4 summarizes the chemical parameters analysed and their relevance to this study.

Table 4 - Chemical Parameter used for wastewater samples characterization

Parameter	Relevance of the parameter
pH	Disinfection efficiency and chlorine reactions decay depends on pH
Electrical conductivity at 25°C, µS/cm	Indicator of inorganics content
ORP (Oxidation/Reduction Potential), mV	Gives an indication of the ability of the wastewater sample to be oxidized or reduced by chemicals
Turbidity, NTU	Gives an indication of the presence of suspended particles which can interfere in the chlorine demand
Total phosphorus, mg P/L	Chlorine demand tends to be higher with high phosphate levels
Ammonia, mg NH_4/L	Reacts with free chlorine producing combined chlorine and nitrogen disinfection by-products
Ammoniacal nitrogen, mg N-NH_4/L	Reacts with free chlorine producing combined chlorine and nitrogen disinfection by-products
Total organic carbon (TOC), mg C/L	To assess the presence and the removal of organic compounds
Dissolved organic carbon (DOC), mg C/L	To assess the presence and the removal of dissolved organic compounds
UV absorbance at 254 nm (A254), cm^{-1}	To assess the presence and the removal of specific organic contaminants (with aromatic and/or C=C bonds)
Absorbance at 436 nm (A436),,cm^{-1}	To assess the presence and the removal of compounds that confers colour to the water
Transmittance at 254 nm (T254), %	Gives an indication of the presence of suspended particles which can interfere in the chlorine demand
Alkalinity, mg $CaCO_3$/L	Disinfection efficiency and chlorine decay depends on pH; the alkalinity provides an indication on the buffering capacity of the wastewater
Hardness, mg $CaCO_3$/L	Gives an insite of the scalling potential of the wastewater

3.4. Chlorination tests

The chlorination tests comprised three experiments. The purpose of the first experiment was to reconstruct the breakpoint curve, and it was intended to be a preliminary investigation to obtain data aiming at design properly the second experiment, namely the definition of initial chlorine concentrations and the contact times.

The second experiment aimed the study of the breakpoint curve in more detail and included the analyses of the oxidation by-products. Analyses of UV spectra, EEM and THM analyses were carried

out in order to study the wastewater changes due to the chlorination process, identify the type of by-products formed and link them to the various stages of the breakpoint curve.

The third experiment comprised the assessment of the decay curve of the chlorine concentration over time for different chlorination doses.

All the experiments were performed by the bottle test method [49] using ambar glass Winkler bottles (ca. 100 mL) treated with 10 mg/L free chlorine in ultrapure water and rinsed thoroughly with ultrapure water, to avoid any chlorine demand contamination. Before testing, it was checked that no residual chlorine was present in the bootles. Several bottles were filled with the water samples to which different concentrations of chlorine were added, the chlorinated samples were then placed in an incubator (Leec) at constant temperature and after a predetermined contact time, the chlorine species present in solution were determined.

In order to determine the amount of chlorine to be added into each bottle sample, it was necessary to know previously the ammonia-nitrogen concentration of the wastewater sample. To do this, the ammonia concentration was measured as described in section 3.4.5.

A 5% sodium hypochlorite (NaOCl) solution (Panreac) was used for chlorination, diluting it each time before each experiment in ultrapure water to prepare a working solution to be added to the wastewater to be chlorinated. The actual concentration of each working solution was always checked. To do this, the NaOCl working solution was properly diluted in 100 mL of ultrapure water and then the free chlorine was measured as described in section 3.4.1.

3.4.1. Breakpoint Chlorination

The first chlorination breakpoint chlorination experiment was performed using the wastewater sample collected WWTP on 6 April 2022. This water was chlorinated with 15 different concentrations of chlorine to achieve stablished initial chlorine to ammonium-nitrogen mass concentration ratios (Cl/N-$NH4^+$). For that, a working solution of NaOCl was prepared (0.85 g/L, as Cl_2) and the proper volumes were added to the wastewater (Table 5) previously distributed into 42 Winkler glass bottles.

The contact times chosen were 1 hour, 2 hours and 24 hours, to also look into changes in the breakpoint curve over time. The Cl/N-$NH4^+$ratios used were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 20, 30 for the 1 hour and 24 hour contact times, and 1, 4, 6, 7, 8, 10, 20 for the 2 hour contact time.

After the defined contact time the respective sample bottle was withdrew and the total chlorine, free chlorine, monochloramines and dichloramines were immediately measured following the procedure described in Section 3.4.1.

The second chlorination breakpoint experiment was performed with wastewater collected on 31 May 2022. In this experiment, instead of adding the chlorine solution in individual 100 mL bottle, for each chlorine concentration, it was decided to add the chlorine to 500 mL of wastewater sample in a volumetric glass and then fill three Winkler bottles, one for each contact time to be investigated. The

volumes of NaOCl working solution (13.7g/L as Cl_2) used are described in Table 5. Twenty-nine 100 mL bottles were needed, each filled to the neck, making sure that there was no headspace.

The contact times were extended in the second experiment, choosing to conduct the tests at 2 hours, 4 hours and 24 hours. With regard to the concentrations of chlorine to be added, chlorine/nitrogen ratios were chosen more widely spaced, but in such a way that they were equally evenly distributed over the breakpoint curve. For the 2 hour and 24 hour tests Cl/N-$NH4^+$ (m/m) ratios of 0.5, 1, 3, 6, 9, 10, 11, 12, 14, 16, 18, 20 were chosen, while for the 4 hour test, Cl/N-$NH4^+$of 1, 6, 10, 14, 20 were used. Total chlorine, free chlorine, monochloramines and dichloramines were measured for all samples. Again, for the measurement of chlorine, the samples were diluted in 100 or 50 mL volumetric flasks, using ultrapure water, while for the other analyses, the samples were used as such.

Table 5 indicates the concentration of NaOCl dosed to achieve the desired Cl/N-$NH4^+$ and the correspondent volume of NaOCl working solution used at each assay. The ammonium content of the wastewater samples, the actual chlorine concentration of the NaOCl working solutions, and the sample volume to be chlorinated is also presented in Table 5.

It should be noted that for the first experiment, given the low nitrogen value measured, it was not necessary to add an high concentrated solution nor high volumes of chlorine to achieve even high chlorine/nitrogen ratios. For the second and third experiments, given the high nitrogen values measured, the chlorine concentrations to be achieved in order to have the same chlorine/nitrogen ratio values used in the first experiment were correspondingly high.

Table 5 - Concentration of the NaOCl (mg/L, as Cl 2) dosed in each sample and correspondent volume of NaOCl working solution added in each experiment

	06/04/2022		31/05/2022		14/07/2022	
Cl/N-NH4+	Dosed NaOCl (mg/L Cl_2)	NaOCl working solution (mL)	Dosed NaOCl (mg/L Cl_2)	NaOCl working solution (mL)	Dosed NaOCl (mg/L Cl_2)	NaOCl working solution (mL)
0.25					4.3	0.20
0.5			8.4	0.31	8.6	0.39
1	1.8	0.21	16.7	0.61	17.3	0.78
2	3.6	0.42				
3	5.4	0.63	50.2	1.83		
4	7.2	0.84				
5	8.9	1.05				
6	10.7	1.26	100.3	3.66		
7	12.5	1.47				
8	14.3	1.68				
9	16.1	1.89	150.5	5.49		
10	17.9	2.10	167.2	6.10		
11			183.9	6.71		
12	21.5	2.53	200.7	7.32		
14		0.00	234.1	8.54		
15	26.8	3.16				
16			267.6	9.76	276.3	12.56
18			301.0	10.99		
20	35.8	4.21	334.4	12.21		
30	53.7	6.31				
N-NH4 in WW sample (mg /L)	1.8		16.7		17.3	
NaOCl working solution (g/L as Cl_2)	0.85		13.7		11.0	
WW sample volume (mL)	100		500		500	

3.4.2. Chlorine decay

For the third experiment, the wastewater collected on 31 May 2022 was used, but the tests were carried out on 14 July 2022. It was therefore necessary to repeat the measurements of certain parameters in order to verify that the water characteristics had not changed significantly. Conductivity, pH, TOC, ammoniacal nitrogen and nitrogen in the form of ammoniacal nitrogen and intensity spectra at wavelengths of 254 nm and 436 nm were measured. In this experiment, the initial chlorine concentration were selected to reach Cl/N-NH4+ ratios of 0.25, 0.5, 1 and 16. The contact times considered were 10 minutes, 30 minutes, 1 hour, 4 hours, 24 hours and 96 hours. Four 500 mL bottles were filled, one for each chlorination condition, then chlorine was added and each bottle was divided into smaller bottles of 100 mL. During the third experiment, the samples were left at a room temperature of approximately 26°C. After the stablished contact time, total chlorine, free chlorine,

monochloramine and dichloramine were measured. Ammonia nitrogen content was measured in the samples with contact times of 4 and 24 hours and TOC was determined in the samples corresponding to contact time of 24 hours.

To characterize time 0 (0 minutes) ultrapure water in 50 and 100 mL volumetric flasks, chlorinated at the four different concentrations was used and free chlorine was measured.

3.4.3. Characterization wastewater samples after chlorination

Analyses of the UV-vis spectra and EEM and THM measurements were carried out on samples with Cl/N-NH4+ ratios of 1, 9, 10, 12, 16, 18, 20 and on non-chlorinated wastewater, from the second breakpoint chlorination tests. The samples were diluted in ultrapure water for THM analyses, using the same dilution factor as used for chlorine analyses, while for the other analyses, the samples were used with no dilution.

3.5. Analytical methods

In order to obtain reliable experimental results, an attempt was made to use good laboratory practices and standardized analytical methods. For instance, the first two experiments were carried out trying to pass as little time as possible between the collection of the wastewater and the start of the experiment, so that the results would not be affected by possible changes in the water. The bottles to be used for chlorination were always properly washed and treated to avoid chlorine demand contamination and then washed with the sample. The cuvette used in the spectrofluorimeter was also washed three times with ultrapure water before each reading and then once with the sample to be analyzed, before being filled to take the measurements. Gloves were used during all the experiments.

The analyses reported in sections 3.5.3 to 3.5.8 were made by LNEC´s lab technician and the THM quantification (3.5.9) was made by an accredited laboratory for water analyses.

3.5.1. Chlorine quantification

The total chlorine, the free and combined chlorine were measured by DPD method, using a pocket colorimeter and respective test kit from HACH company. This procedure is the equivalent to standard method 4500-Cl G described elsewhere [50].

Given the reading limit of the chlorine pocket colorimeter (2 mg/L Cl_2), whenever needed, the samples were properly diluted with ultrapure water using a 100 mL volumetric flask before the measurements.

To measure total or free chlorine it was necessary to fill the sample cell of the instrument with 10 mL of sample and measure the blank. Afterwards, the content of one DPD total chlorine powder pillow, provided in the test kit, or of one DPD free chlorine powder pillow was added to the sample cell. The cell contents was gently shaked for 20 seconds, to mix the cell contents, and after 3 minutes (in the case of measuring total chlorine) or 1 minute (for free chlorine) of reaction time the developed pink color was measured by the colorimeter and the correspondent chlorine concentration was recorded.

After measuring free chlorine, using the same cuvette, proceed to measure combined chlorine by adding potassium iodide and measuring first monochloramines and then dichloramines.

For monochloramine quantification 2 drops of 0.1 g/L KI solution were added to the sample (10mL) and, after gently shaking, the reading was made immediately. To determine dichloramine, KI crystals (0.1 g) were afterwards added to the same sample, and after 2 min the readings were performed.

Figure 9 - Pictures of chlorinated samples and the chlorine measuring procedure

Two pocket colorimeters were used at chlorination breakpoint experiments (namely, Kit 1 for free and combined chlorine measurements and Kit 2 for total chlorine readings). Therefore, it was necessary to apply a correction factor, presented in Figure 10, in order to obtain consistent and comparable results between total chlorine readings, free chlorine and combined chlorine.

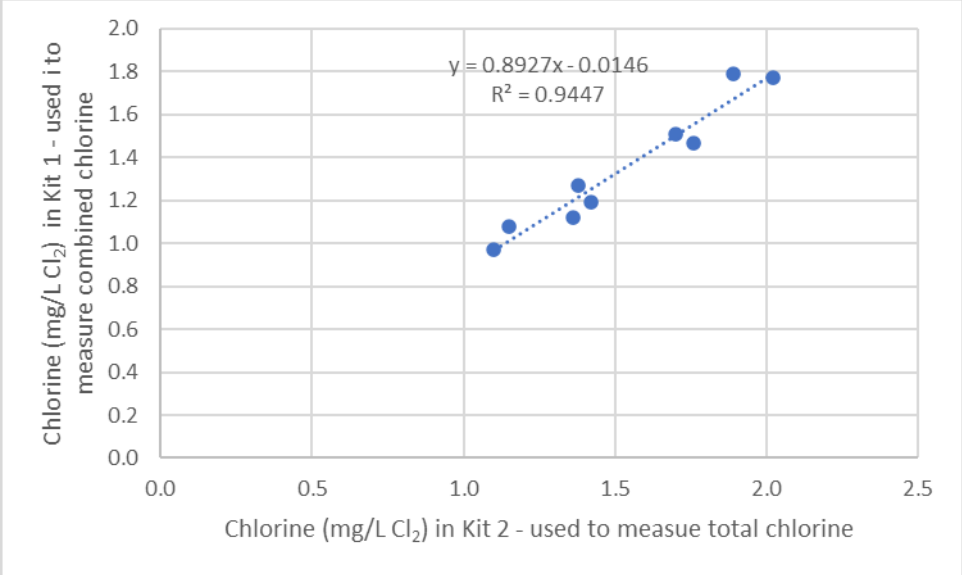

Figure 10 - Model used to correct the values

3.5.2. EEM and UV absorbance spectra

To study the dissolved organic matter, it was used a spectrofluorimeter AquaLog® from HORIBA Jobin Yvon to record excitation-emission fluorescence matrices and absorbance spectra. This equipment uses the technique *Absorbance-Transmission Excitation Emission Matrix, or A-TEEM™*, to measure in water samples the absorbance spectrum and to give simultaneously a fluorescence excitation emission map[1].

[1] https://www.horiba.com/int/scientific/products/detail/action/show/Product/aqualog-environmental-water-research-analyzer-3497/

From the instrument, a three-dimensional excitation-emission fluorescence matrix is obtained, in which the excitation wavelength is recorded on the x-axis, the emission wavelength is represented on the y-axis and the absolute intensity values on the z-axis. In this way, measuring the fluorescence intensity that correspond to a specific excitation/emission pair, a footprint of the wastewater is obtained with which the dissolved organic matter inside can be studied.

The samples analyzed in the spectrofluorometer were picked from the second and third experiments. During the second experiment, the chlorinated samples were stored in clean bottles placed in an incubator at 25° C and were subsequently analyzed with the spectrofluorometer after 24 hours, while during the third experiment the chlorinated samples were left at room temperature of approximately 26° C, and then analyzed after 24 hours and after 96 hours. Before each fluorescence reading, the cuvette was washed twice with ultrapure water and once with the sample itself.

Excitation-emission fluorescence maps (EEMs) were obtained using an integration time of 0.1s, for an excitation range of 240-600 nm and an emission range of 148.76-694.49 nm, with an increment of 3 nm in the second experiment and 3.54 nm in the third.

Thanks to the excitation-emission matrix, it is possible to identify the fluorescence characteristics of organic matter in water samples. However, this technique has problems associated with fluorescence measurements, such as the internal filter effect (IFE) and interference from Rayleigh and Raman scattering.

With the AquaLog® software, both of these effects can be removed. By also measuring the blank with the spectrofluorometer, the software is able to automatically remove the Raman scatter line by subtracting the EEM of the blank from the EEM of the sample [51].

To remove IFE and Rayleigh scattering, two algorithms are used. During data processing, first the algorithm to remove the inner filter effect was used. This measures the sample absorbance spectrum in the overlapping range of the excitation and emission spectra to correct for primary and secondary IFE. The algorithm involves applying the following equation (21) to each excitation and emission wavelength coordinate of the EEM [51, 52]:

$$F_{ideal} = F_{obs}^{10^{\frac{Abs_{EX}+Abs_{EM}}{2}}} \qquad (21)$$

Where:

- F_{ideal} represents the ideal fluorescence signal spectrum expected in the absence of the IFE;
- F_{obs} is the observed fluorescence signal;
- $Abs_{EX}$and Abs_{EM} are the measured absorbance values at the excitation and emission wavelength coordinates, respectively.

Subsequently, the Rayleigh masking algorithm was applied. This algorithm is based on the excitation and emission spectral bandwidth and it cancels the signal intensities for both first- and second-order Rayleigh lines [51, 52].

To analyze and interpret the EEMs, the presence of peaks in the I, II, III, IV, V areas were investigated, as illustrated in Figure 11, with illustrative purposes only.

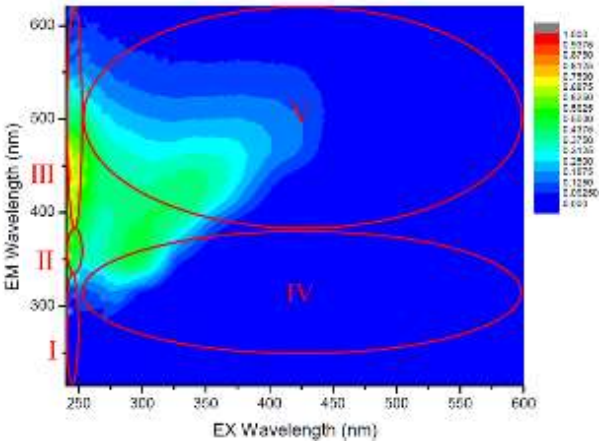

Figure 11 - Example of EEM spectra

In order to better identify the peaks within the contour plots, the results were also normalised and rescaled.

Thanks to the Aqualog software, it was also possible to automatically obtain the UV spectra of each sample.

3.5.3. Total and dissolved organic carbon quantification

The organic matter content of the RW samples were also assessed measuring total organic carbon (TOC) and dissolved organic carbon (DOC) (the latter in filtered samples, through 0.45 µm, polypropylene membrane, GH Polypro Pall Corporation) using the UV/persulphate chemical oxidation according to the standard method EN 1748, with a TOC analyzer Fusion (Teledyne Tekmar USA) [53].

3.5.4. UV-Vis absorbance at 254 nm and 436 nm

OM was also characterized by the UV–Vis absorbance at 254 nm (A254) and 436 nm (A436), respectively, representing organic compounds with aromatic rings and color, measured in 0.45 µm filtered samples using a UV–Vis spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA) with 10 mm optical path length quartz cells.

3.5.5. Ammonia quantification

To design the chlorination break point experiments it was necessary to know the concentration of nitrogen within the wastewater in order to decide on the dose of chlorine to be dosed, i.e. the concentration of chlorine within the sample solution.

To determine the dose of chlorine to be added, the concentration of N-NH4 was measured using a LCK303 ready-to-use cuvette test from Hach, with pre-dosed reagents, with the following characteristics[2] (Table 6).

[2] https://it.hach.com/lck

Table 6 - Characteristics of the cuvette test for measuring N-NH4

Type	**LCK303**
Measuring range	2 - 47 mg/L NH4 -N
Method	Indophenol Blue
According to standard	ISO 7150-1, DIN 38406 E5-1, UNI 11669:2017
Quality control	LCA703
N tests	25
GHS hazard code	GHS05; GHS07; GHS09

With a Spektral photometer CADAS50 from DR LANGE, the NH4 concentration was measured and then the corresponding N-NH4 concentration was calculated, as shown in the Table 5.

3.5.6. Electrical conductivity, pH and redox potential

Conductivity, pH, redox potential (ORP) were measured using a potentiometer (C863, CONSORT, Bruxelles, Belgium) and specific electrodes.

3.5.7. Alkaninity and hardness

Alkalinity and hardness were determined by volumetry following standard methods [50].

3.5.8. Turbidity

Turbidity was measured by nephelometry using a turbidimeter Turb 550 IR (Xylem, New York, NY, USA).

3.5.9. Trihalomethanes quantification

The trihalomethanes (THM) quantified were chloroform, dichlorobromomethane, dibromochloromethane and bromoform by gas chromatography-mass spectroscopy technique. Total trihalomethaes were assessed by summing the concentration of the four mentioned species.

4. Results discussion

4.1. General remarks

The chlorination assays were performed in batch conditions, with no stirring, in closed bottles, aiming to understand the chlorine reactivity with water components and chlorine concentration decay. The reclaimed water disinfection and chlorine decay in distribution systems are out of the scope of this section/thesis hence neither the microbiological attenuation issues, nor hydraulic and pipe wall effects on chlorine reaction and decay were assessed.

4.2. Physical- chemical characterization of treated effluents

In order to characterize the effluents used for the experiments, some physical and chemical characteristics of the water samples were analyzed. The water quality parameters were selected based on their relevance on the chlorine reaction and chlorine decay, as explained in Table 4. The results are shown in Table 7.

Table 7 - Chemical characterization of Wastewater samples collected on 6th April 2022 (BEI-UF 06-04-22) and on 31st May 2022 (BEI-UF 31/05/22)

	BEI-UF 06/04/22 Sample 1	BEI-UF 31/05/22 Sample 2	BEI-UF 31/05/22 [a] Sample 3
pH	7.0 at 19.7°C	7.3 at 22.7°C	8.0 at 25.7°C
Electrical conductivity at 25°C, µS/cm	1325	1453	1428
ORP, mV	195	249	n.d.
Turbidity, NTU	0.03	0.10	n.d.
Total phosphorus, mg P/L	n.d.	<2	n.d.
Ammonia, mg NH_4/L	2.3	21.5	22.2
Ammoniacal nitrogen, mg N-NH_4/L	1.8	16.7	17.3
TOC, mg C/L	3.3	4.4	4.7
DOC, mg C/L	3.2	4.4	n.d
A_{254}, cm^{-1}	0.1427	0.1569	0.168
A_{436}, cm^{-1}	0.0128	0.0147	0.0295
Transmittance at 254 nm, %	70.6	67.9	n.d.
SUVA, L/mg-C/m	4.5	3.6	3.5[b]
Alkalinity, mg $CaCO_3$/L	100	211	n.d.
Hardness, mg $CaCO_3$/L	203	206	n.d.

n.d. – not determined; [a] after 44 days of storage in refrigerated conditions (8 ºC); [b] calculated with TOC value, since DOC and TOC were almost the same,

The water collected on the two different days showed different characteristics, and, as expected, some of the water quality parameters of the sample collected on 31 May changed after the storage of 44 days (even in refrigerated conditions) and hence is considered a different water sample (sample 3).

Concerning the pH, the water collected on 6 April (sample 1) and 31 May (sample 2) had neutral pH, and sample 3 was more alkaline presenting pH 8.0. Normally, the pH values of water intended for irrigation are between 5.5 and 8.5, and water with a pH below 4.5 is avoided, as it could have an acidifying effect and be potentially toxic to crops, but also because at this value the availability of absorption of heavy metals is greater *[54]*. Moreover the reactivity of chlorine and its disinfection power depends on water pH, since, as mentioned in Section 1.4, the percentage of HOCl and OCl ions present will be different depending on the pH. It must therefore be considered that following the addition of chlorine, there will necessarily be a decrease in pH, however it can be controlled by the buffering capacity of the water, expressed by its alkalinity. Which in this case turned out to be much higher in the water collected on 31 May (sample 2).

The electrical conductivity was quite similar in the three samples, while the ORP was found to be slightly higher in the sample 2, and hence theoretically this water sample is less oxidisable than sample 1.

Although the turbidity of sample 2 were three times higher than sample 1, both water samples were highly clarified waters, with almost no suspended particles. The transmittance values at 254 nm corroborates these results, in fact it was higher for sample 1, which showed lower turbidity than sample 2.

Total phosphorus was only measured in the sample 2, collected on 31 May, and was below the instrument's quantitative limit (2 mg P/L).

With regard to ammonia, this is the parameter that differs the most between the water samples. The concentration of ammonia at sample 1 was 10 times lower, and consequently also of ammonia nitrogen, than that measured in the sample 2 (collected on 31 May). In sample 3 (water collected on 31 May and stored for 44 days in refrigerated conditions) the measured ammonium had increased, but not appreciably.

The organic matter of water samples was practically in dissolved material, since TOC and DOC values were nearly the same. The organic matter content was higher in sample 2 comparing to sample 1, as shown by the respective TOC, absorbance at 254 nm (A_{254}, an indicator of compounds with aromatic rings) and absorbance at 436 nm (A_{436}, which correlates with color) values. Sample 3, although presenting TOC similar to sample 2, had higher values of A254 and A436, denoting changes in the organic matter composition of water sample.

The specific ultraviolet absorbance (SUVA), was slightly higher in the water collected on 6 April (sample 1) than in the water collected on 31 May (sample 2), whereas it did not change during the 44 days it was kept in the fridge. This parameter, determined at 254 nm, correlates with the aromaticity percentage and is therefore useful for characterizing natural organic matter and estimating the predominance of dissolved aromatic carbon content. SUVA254nm have been used to estimate the potential for the formation of disinfection byproducts. A high SUVA value would have indicated a large portion of humic matter in the water and a greater tendency to react with disinfectants to form DBP [55, 56]. In addition, it can provide information on the hydrophobicity of the compounds in the water;

since in all three cases the measured SUVA is greater than 3, the wastewater collected contains predominantly hydrophobic compounds and should have similar disinfection by products formation potential.

Finally, the water samples hardness was around 200 mg $CaCO_3$/L. Therefore, in both cases the water can be defined as very hard [57, 58] and this could be an issue for the distribution system, as it could cause limescale and scaling.

4.3. Chlorination breakpoint tests

4.3.1. Breakpoint in wastewater sample 1 – low NNH4 content

The main purpose of Assay 1 was to obtain primary data of breakpoint chlorination curve with a treated wastewater sample, at different contact times, in order define adequate experimental conditions to be applied in the Assay 2. In fact, the results allowed to check whether the Cl_2/N-NH_4 ratios chosen were sufficiently well distributed over the breakpoint curve, so that it could be studied in its entirety.

In this Assay 1, chlorine was only measured in the form of total chlorine, without investigating its various components, namely combined and free chlorine. The total residual chlorine measured in the samples chlorinated with different chlorine concentrations, for contact times of 1 hour, 2 hours and 24 hours, are shown in Figure 12. For a better reading and interpretation of the results, a representation of the data with dots (experimental data) and lines (to guide the eye, for 1h and 24 h contact time samples) was used.

Chlorine reaction times are very fast and in fact the times used in chlorination chambers in treatment plants are in the order of 15-20 minutes. In this case, however, the aim was not to focus on the chlorine reaction, but to study its decay and changes within the chlorinated water over a longer period of time, which is why the times chosen for the tests are in the order of hours.

It is evident from the figure that for all contact times, there was an initial offset phase, in which there was no residual chlorine, but all added chlorine was immediately consumed by reducing matter present in the water. Immediately thereafter, as the added chlorine dose increases, the total residual chlorine raised, albeit slowly, reaching a maximum around a Cl_2/N-NH_4 ratio of 9-10, which corresponds to the maximum available residual chlorine. In these chlorination conditions, the reaction of the added chlorine with the ammonia and the organic compounds present takes place, leading to the formation of chloramines, which are detected in water as total residual chlorine, causing the concentration rise observed in the curve. In the next phase ($10 < Cl_2$/N-$NH_4 < 12$), the residual chlorine concentration dropped rapidly and reached its minimum, i.e. the breakpoint, at a Cl_2/N-NH_4 ratio between12 and 15 for 1 h of contact time, and around 15 for the contact time of 24 h. At higher Cl_2/N-NH_4 ratios there was a new increase in total residual chlorine, which was more pronounced in the case of 1h contact time compared with 24h contact time.

Comparing the chlorine residual concentration at the different contact times, for the same Cl_2/N-NH_4 ratios, the decay of chlorine over time was noticed, there was a slight decay of total chlorine from 1

hour to 2 hours contact time, and a significant decay was observed at 24 hours over the whole curve, except for the point corresponding to the Cl_2/N-NH_4 ratio of 12, which corresponds to, or is near, the breakpoint (and theoretically to the water demand for chlorine), in which the amount of total residual chlorine was almost the same. In quantitative terms, comparing the results at 1h vs 24h, a maximum total residual chlorine decay of 57% was observed prior the breakpoint at the Cl_2/N-NH_4 ratio of 7, whereas after the breakpoint a 75% decay was observed at the Cl_2/N-NH_4 ratio of 20.

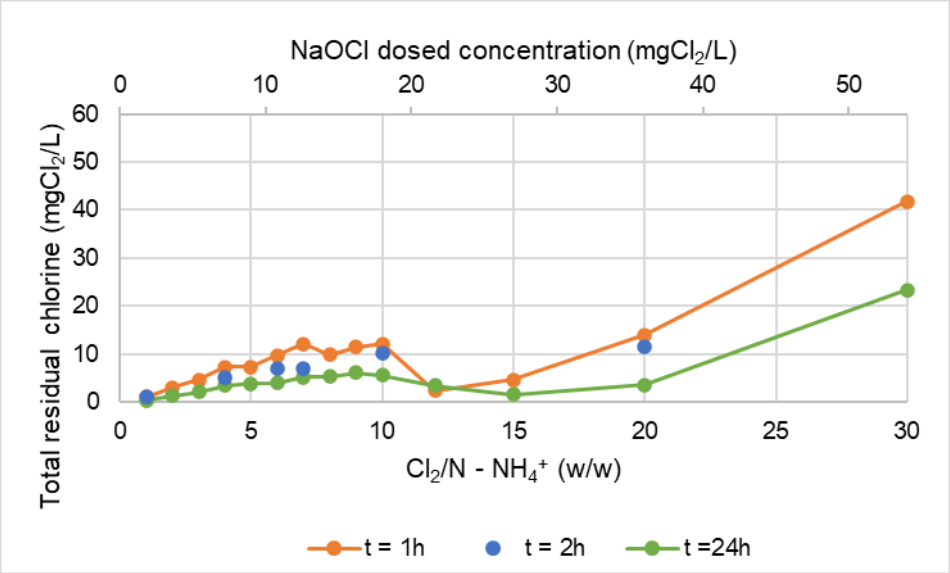

Figure 12 - Breakpoint chlorination curve from Assay 1

4.3.2. Breakpoint win wastewater sample 2 – high N-NH4 content

The second assay aimed to study the chlorination breakpoint curve of the treated wastewater in more detail. Residual concentrations of total, free and combined chlorine were measured after three different contact times and at different dosed chlorine concentrations, illustrated in Figure 13. In this assay the N-$NH4^+$ concentration was also measured, as illustrated in Figure 14. The Cl_2/N-NH_4 ratios were selected based on the results of Assay 1, and contact times of 2 hours, 4 hours and 24 hours were chosen.

Figure 13 shows the residual concentrations of the chlorine species, i.e. total chlorine (Figure (a)), combined (Figure (c) and (d)) and free chlorine (Figure (b)), at different contact times, for the different dosed chlorine concentrations. Comparing graph (a) and graph (c), it can be seen that in the first phase of the curve, for Cl_2/N-NH_4 ratios from 1 to 12, chlorine was present almost entirely as combined chlorine. In the initial phase, chlorine reacts with ammoniacal nitrogen, leading to the formation of chlorine compounds (chloramines), which were detected in water as combined residual chlorine. Most of the chloramines produced were monochloramines, detected from Cl_2/N-NH_4 ratios of 1 to 10 and reaching a maximum in their production for Cl_2/N-NH_4 ratios of 6-7, after which chloramines concentration begin to drop, as the ammonia nitrogen in the water is consumed and the further increase in the dose of chlorine oxidizes the combined residual chlorine.

Dichloramines were detected at very low concentrations and only begin to have substantial concentrations when Cl_2/N-NH_4 ratios around 9 were reached, with concentration peaks around ratios of 12, at 1-hour contact time and ratios of 11 at 24-hour contact time.

The breakpoint was reached for Cl_2/N-NH_4 ratios around 12-13 for all contact times tested.

After the breakpoint the total residual chlorine detected is entirely free residual chlorine. However, although free residual chlorine was not expected to be detected before the breakpoint, measurements showed it to be present for all three contact times, albeit in very low concentrations. This can be explained by the fact that organic chlorine compounds formed together with chloramines before the breakpoint are usually quantified as free chlorine. Residual free chlorine concentrations measured before this point are therefore to be considered as organic chlorine compounds and not as free chlorine.

Although little data is available for the breakpoint curve with a contact time of 4 hours, it can be seen that this has a very similar behavior to the curve with a contact time of 2 hours. While the residual chlorine concentration values measured after a contact time of 24 hours are lower, indicating a decay with time.

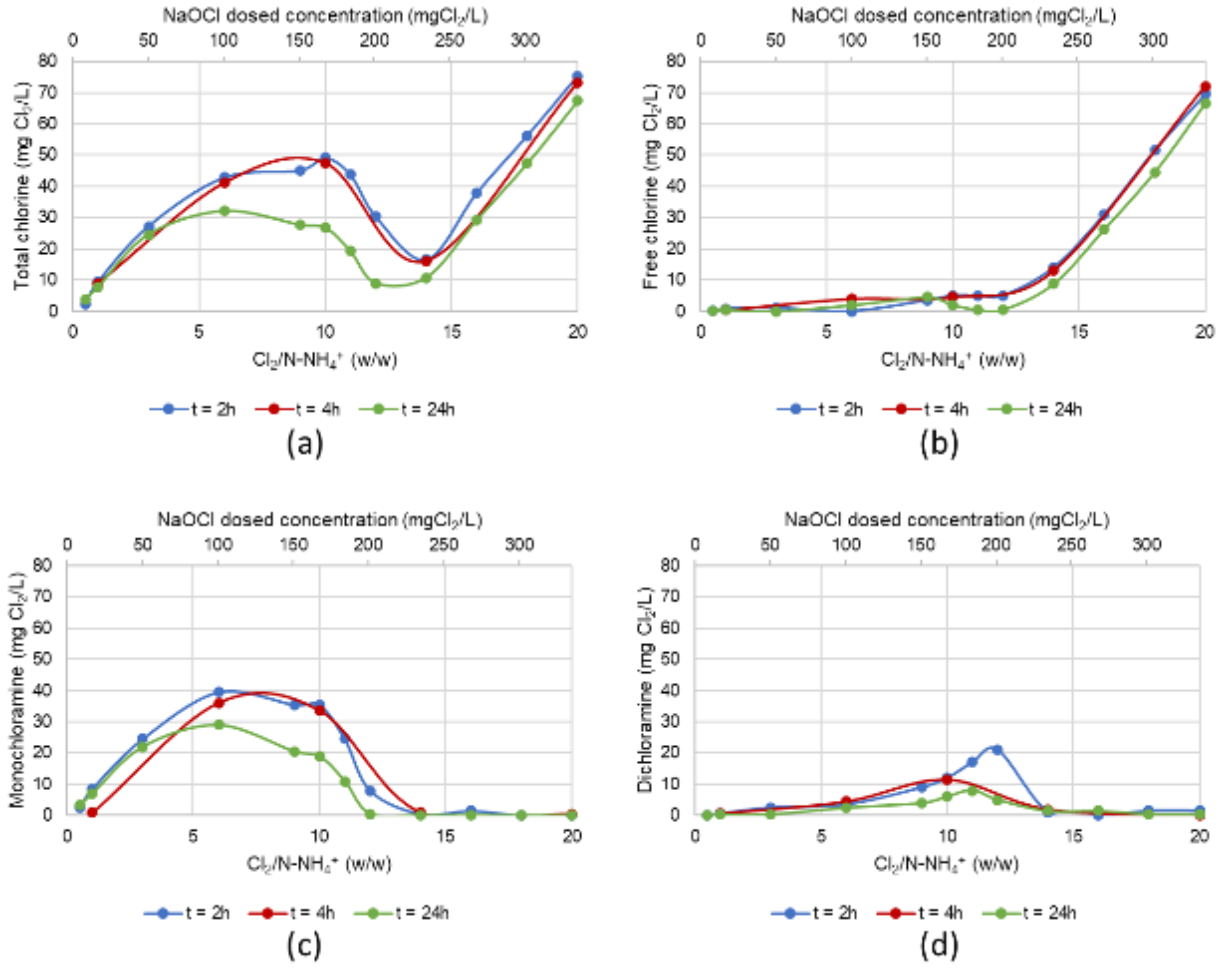

Figure 13 - Breakpoint chlorination curve from Assay 2

Since the effluent wastewater used at Assay 2 had an initial ammonia concentration 10 times higher than those used for Assay 1, the results of these assays allowed the assessment of the influence of ammonium on the chlorination breakpoint curve:. Considering the curve (a) in Figure 13, representing the residual total chlorine concentration, and comparing it with the breakpoint curve in Figure 12, it can be seen that the breakpoint shifted to the right for contact times of 2 and 4 hours, occurring at a Cl_2/N-NH_4 ratio of 14, whereas it shifted to the left, at a Cl_2/N-NH_4 ratio of 12, in the case of a contact time of 24 hours. Similarly, the maxima in combined residual chlorine occurred in different Cl_2/N-NH_4 ratios. In Assay 1 the maximum was reached for ratio values of 9-10 for all contact times, whereas in Assay 2 the maxima were shifted to the left and occurred at ratios of 6-10.

Figure 14 shows the measured ammoniacal nitrogen concentrations in the samples, at different dosed chlorine concentrations and at two different contact times, 2 hours and 24 hours.

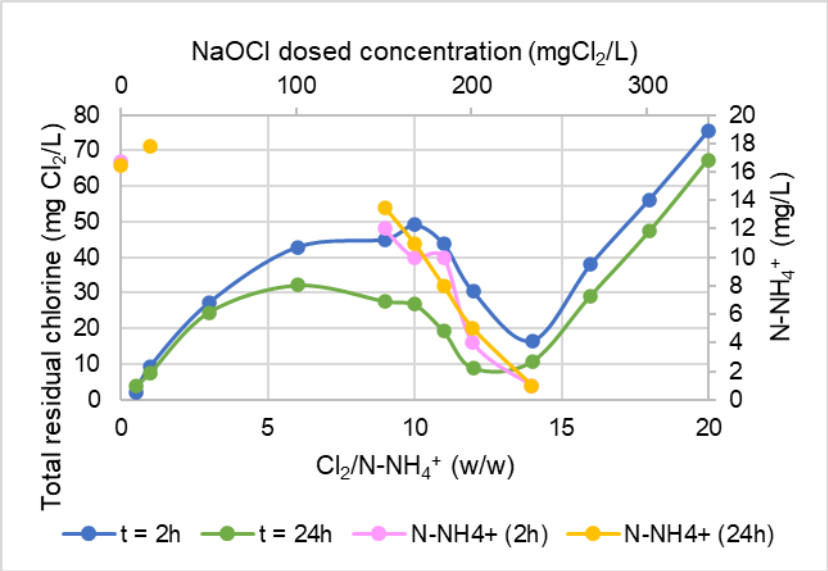

Figure 14 - Concentration of N-NH4 over breakpoint chlorination curves, at 1 h and 24 h contact time

The measurements, for both contact times, gave the expected results. In fact, points belonging to the descending part of the breakpoint curve were determined, where the added chlorine reacts with the combined residual chlorine and oxidizes it, resulting in a decrease of ammoniacal nitrogen in the water. Ammoniacal nitrogen did in fact progressively decrease from concentration values of 16 mg/L to values of 1 mg/L for Cl_2/N-NH_4 ratios of 14 (chlorination breakpoint).

It was difficult to find studies in the literature reporting breakpoint chlorination tests in wastewater, however, the study by Yang et al. (2005) concerning DBP formation in breakpoint chlorination of wastewater was found and was used to compare and confirm the results obtained.

The results of Assay 1 were compared with those obtained by Yang et al. (2005) [38] from experiments on the chlorination of wastewater samples with different characteristics. These authors used three different types of wastewater for their experiments, two of which had undergone secondary treatment with nitrification and a third for which secondary treatment was not provided, and which therefore had much higher ammonia and DOC values [38]. Furthermore, two of the three wastewater

samples contained approximately 25% seawater [38]. The three samples were initially characterised by measuring the concentrations of ammonia, organic carbon, UV254 and bromide and calculating the specific absorbance (SUVA). The sample characteristics are shown in Table 8, where they are compared with the water samples used in this thesis, already reported in Section 4.1.

In terms of nitrogen content, the waters samples 1 and 3 used at Yang et al. (2005) studies (the latter has seawater contribution and only received primary treatment), are comparable with sample 1, 2 and 3, respectively, used in this thesis,. The DOC contents, however, are quite different and are much higher in the water collected by Yang et al. (2005). On the other hand, the absorbance at 254 nm is comparable, but given the much higher DOC, the SUVA values measured by Yang et al are much lower that the water samples of this thesis.

Table 8 - Characteristics of the wastewater samples used at Yang et al (2005) and in this thesis

Treatment scheme	Yang et al. 1 secondary + nitrification	Yang et al. 2 secondary + nitrification	Yang et al. 3 enhanced primary	BEI-UF 06/04/22 Sample 1	BEI-UF 31/05/22 Sample 2	BEI-UF 31/05/22 Sample 3
Seawater blend-in	No	Circa25%	Circa 25%	No	No	No
NH4+ (mg-N/L)	2.5	2.8	21.2	1.8	16.7	17.3
DOC (mgC/L)	7.0	7.2	47.2	3.2	4.4	n.d
UV254 (cm-1)	0.175	0.112	0.174	0.1427	0.1569	0.168
SUVA (L/mg m)	2.48	1.56	0.37	4.5	3.6	3.5
Br- (mg/L)	0.96	22.0	31.5	n.d.	n.d.	n.d.

As for the breakpoint curves reconstructed by Yang et al. (2005) [38], the breakpoint detected after contact times of 2 and 24 hours, was for added chlorine concentrations of 9.5 and 71 mg Cl_2/L for sample 1 and 3, respectively. Whereas, in Assays 1 and 2 the breakpoint occurs for added chlorine concentrations of 26.8 mg Cl_2/L and 200-234 mg Cl_2/L, respectively, showing that this waters had higher chlorine demand than those used in Yang et al. (2005). Even if DOC concentration of this samples were lower than in Yangs study, the SUVA point out to the presence of higher predominance of hydrophobic compounds and this could justify the higher demand. Therefore, chlorine demand would seem to depend not only on the concentration of OM but also on the nature of organic matter.

4.4. Characterization of chlorinated samples

4.4.1. Assessing organic matter changes in the breakpoint chlorination tests through UV-Vis spectra and EEM

To assess the changes in water composition with chlorination the chlorinated samples were analyzed by spectrophotometry, measuring the UV-vis absorption spectra and UV absorption at specific wavelengths, and by fluorescence emission excitation spectra (EEM).

The Figure 15 shows the UV-Vis spectra of the 24h contact time water samples from Assay 2 for each chlorination dose, representing the overall variations of the organic matter comparing with the no chlorinated sample. For better comparison, the same spectra are also shown individually in Figure 16.

Examining the UV-Vis absorbance curves, it can be seen that for Cl_2/N-NH_4 ratios of 1 (Cl_2/N-NH_4 1), the absorbance curve is still quite similar to that of the curve for the non-chlorinated sample (Cl_2/N-NH_4 0), although an increase in absorbance is noted for wavelengths shorter than *ca.* 270 nm, while for longer wavelengths, a decrease in absorbance is noticed. The samples from Cl_2/N-NH_4 9 and 10 showed very similar absorbance curves comparing with sample Cl_2/N-NH_4 1, but much higher up to about 280 nm; for longer wavelengths the absorbance was lower than those observed for samples Cl_2/N-NH_4 0 and Cl_2/N-NH_4 1. These results suggest an increasing concentrations of new compounds that absorbs at 240-280 nm resulting from the addition of increasing doses of chlorine (probably organic chloramines), while the concentration of other compounds (that absorbs at wavelength > 280 nm) diminishes due to chlorine oxidation. Near the breakpoint conditions (Cl_2/N-NH_4 12) the chlorinated samples presented similar absorption spectra observed for samples Cl_2/N-NH_4 9 and 10 but with lower absorption intensity, suggesting the presence of the same compounds but in lower concentrations.

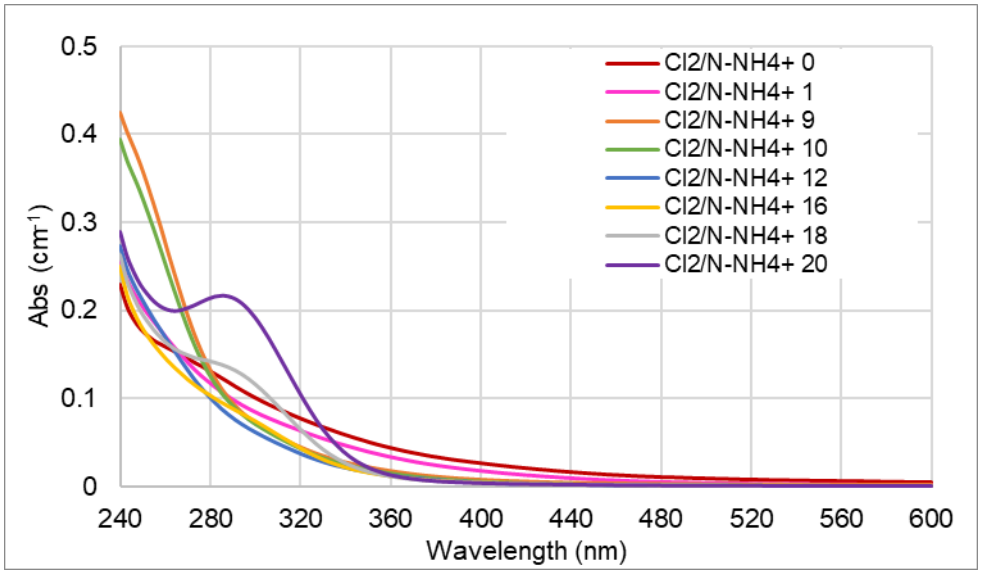

Figure 15 – UV-VIS spectra of non-chlorinated water and chlorinated water samples after 24h contact time - Assay 2

In Figure 16 it can be seen that between the absorption spectrum of Cl_2/N-NH_4 12 sample and that at ratio 16, there is an increase in absorbance at 280-300 nm range, which increases again at the ratios of 18 and 20, giving rise to a peak at ca. 290 nm. Again, this increase in absorbance at this wavelength could be due to the formation of new compounds at high doses of chlorine.

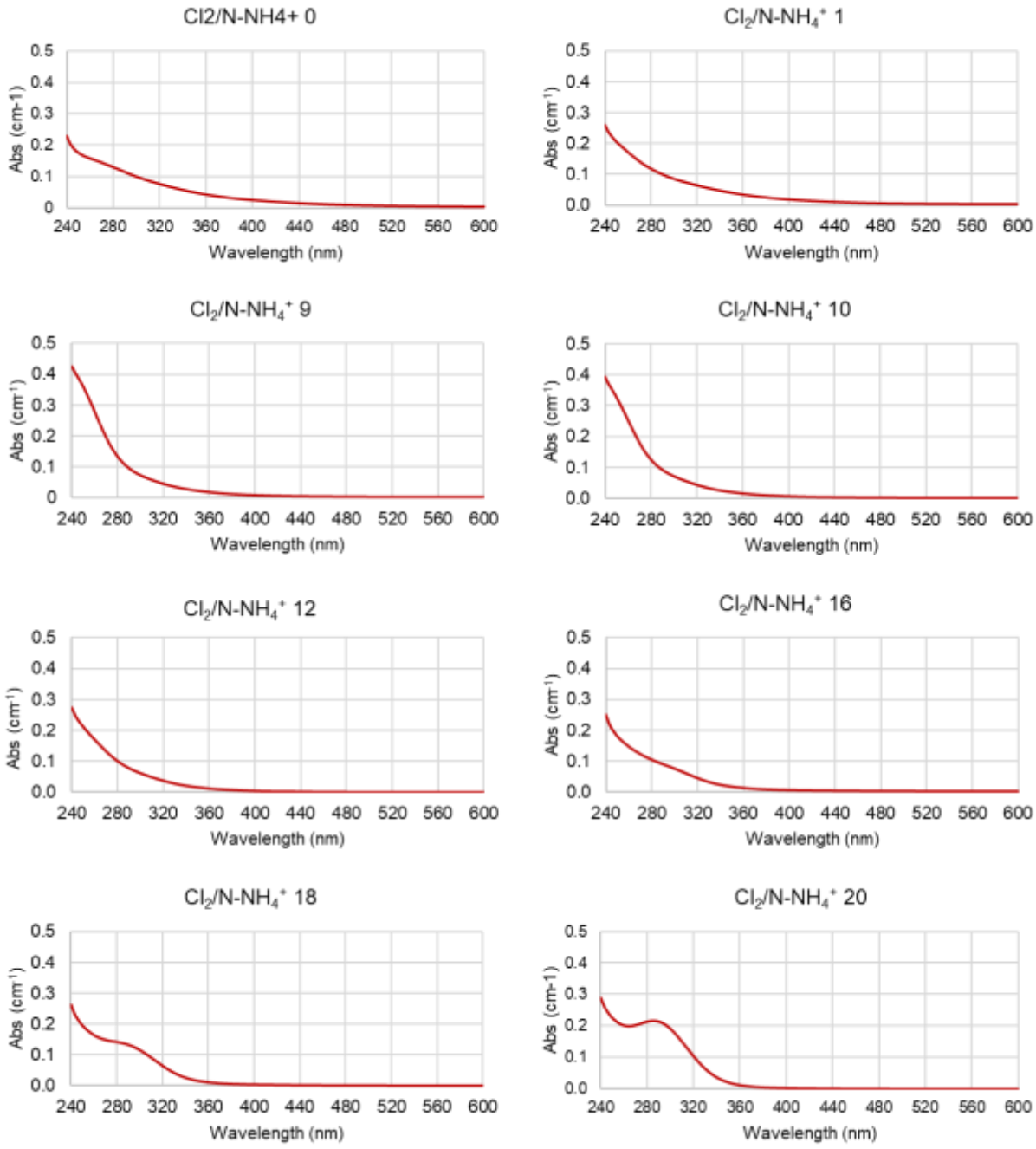

Figure 16 - UV-VIS spectra after 24h contact time - Assay 2

To assess the OM nature changes during chlorination tests, the excitation-emission fluorescence spectra of samples were measured. Figure 17 presents the EEM of non-chlorinated water sample and of each chlorinated water samples prior the chlorination breakpoint, with 24 h contact time. The EEMs

of the chlorinated water samples subsequently the chlorination breakpoint, are shown in Figure 18. To interpret the contour plots obtained with the Aqualog software, the information already reported in Section 2.6.2 was used, summarized in Table 9 [32].

Table 9 - Fluorophores in OM at different Ex/Em regions

Region	EX regions	EM regions	Fluorophore
I	< 250 nm	< 330 nm	Aromatic proteins I, tyrosine
II	< 250 nm	330 – 380 nm	Aromatic proteins II, tryptophan
III	< 250 nm	> 380 nm	Fulvic acid-like substances, hydrophobic acids
IV	> 250 nm	250 – 380 nm	Tyrosine/tryptophan-like and SMP-like species
V	> 250 nm	> 380 nm	Humic acid-like organics, hydrophobic acids

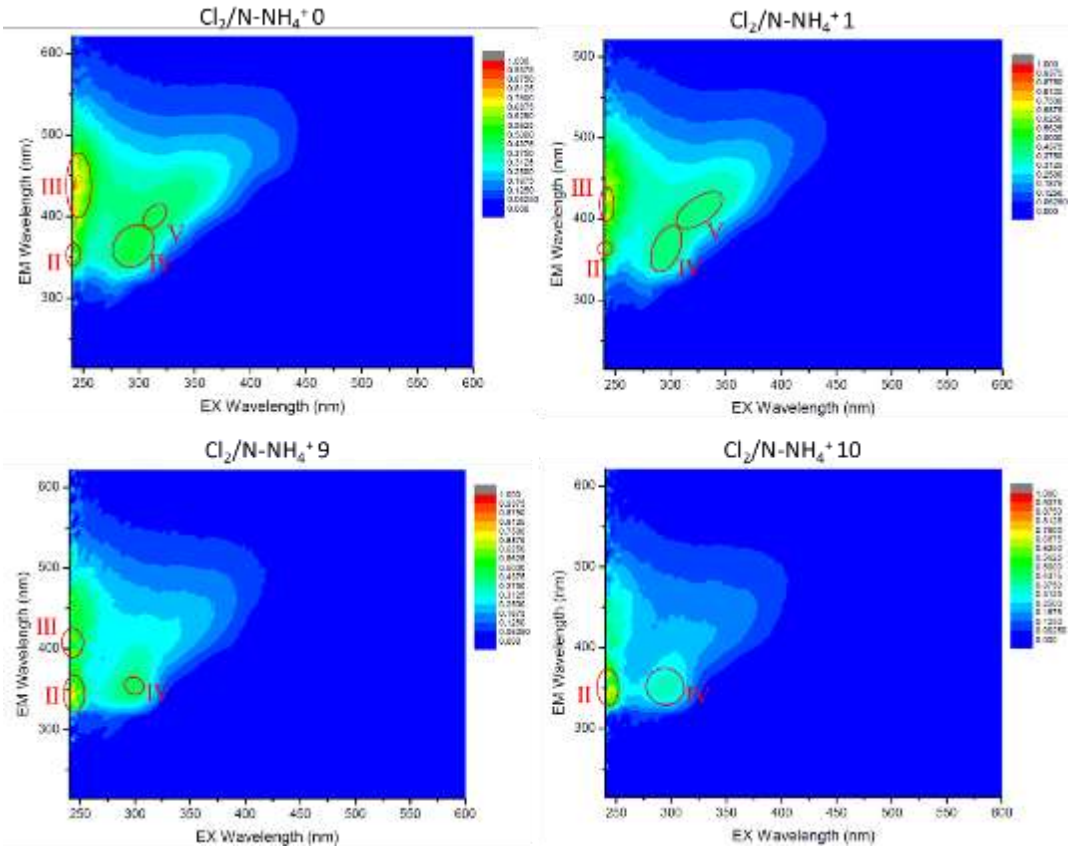

Figure 17 - EEM of non-chlorinated water sample (Cl_2/N-NH_4 0) and chlorinated samples prior breakpoint (Cl_2/N-NH_4 1, Cl_2/N-NH_4 9 and Cl_2/N-NH_4 10) after 24 h of contact time

To facilitate the reading of the EEMs obtained from the Aqualog software, and to highlight the peaks more clearly, the data were normalized and the contour plots rescaled. It must therefore be considered

that the intensities represented by the legends are specific to each contour plot and that it is not possible to compare them with each other. Please refer to Figure 19 for a quantitative comparison of the peaks and regions detected at different concentrations of added chlorine.

In the non-chlorinated sample, four regions were detected (II, III, IV and V), presenting the regions II and III, the highest fluorescence intensities. Prior to chlorination, compounds such as aromatic proteins, tryptophan-like substances, fulvic acid-like substances and hydrophobic acids, tyrosine and tryptophan-like and SMP-like species and humic acid-like organics, hydrophobic acids are therefore present in the water sample.

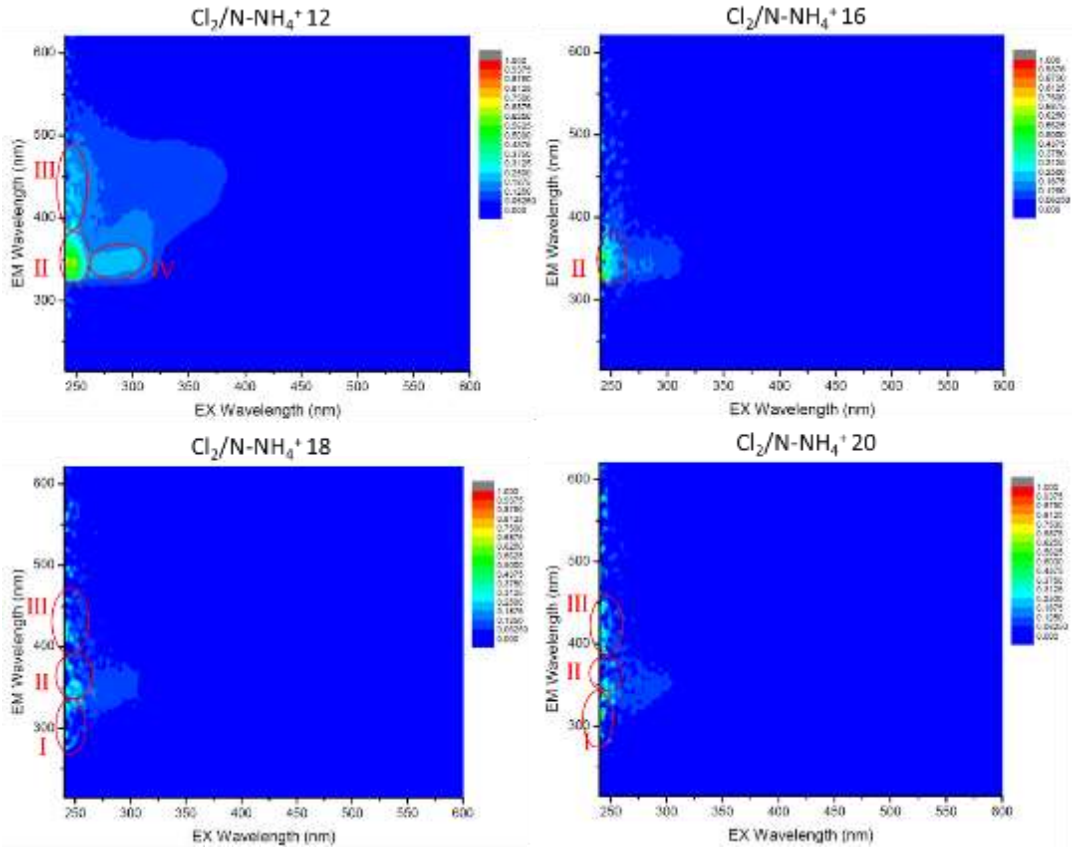

Figure 18 - EEM of chlorinated samples at and after breakpoint (Cl_2/N-NH_4 12, Cl_2/N-NH_4 16, Cl_2/N-NH_4 18, Cl_2/N-NH_4 20) after 24 h contact time

With the addition of chlorine, a decrease in the area of these regions was noted; the disappearance of the zone V was observed in Cl_2/N-NH_4 9 sample and the zone III was no longer observed at Cl_2/N-NH_4 10.

From the ratio Cl_2/N-NH_4 12 onwards, only zone II was detected, which disappears completely in the contour plot for the ratio Cl_2/N-NH_4 18, an indication that all the fluorescent organic matter were oxidized.

From Figure 17 and Figure 18, it can be seen that region I is never present, except at very high concentrations of added chlorine, i.e. for Cl_2/N-NH_4 18 and 20, where the low intensities detected for regions I, II and III are to be attributed not so much to the actual presence of the compounds

associated with these regions, but to instrument disturbances or interference. No samples therefore showed the presence of aromatic proteins or tyrosine-like compounds.

Figure 19 quantitatively depicts the fluorescence intensity maxima found in the five regions detected in each contour plot, for each added chlorine concentration.

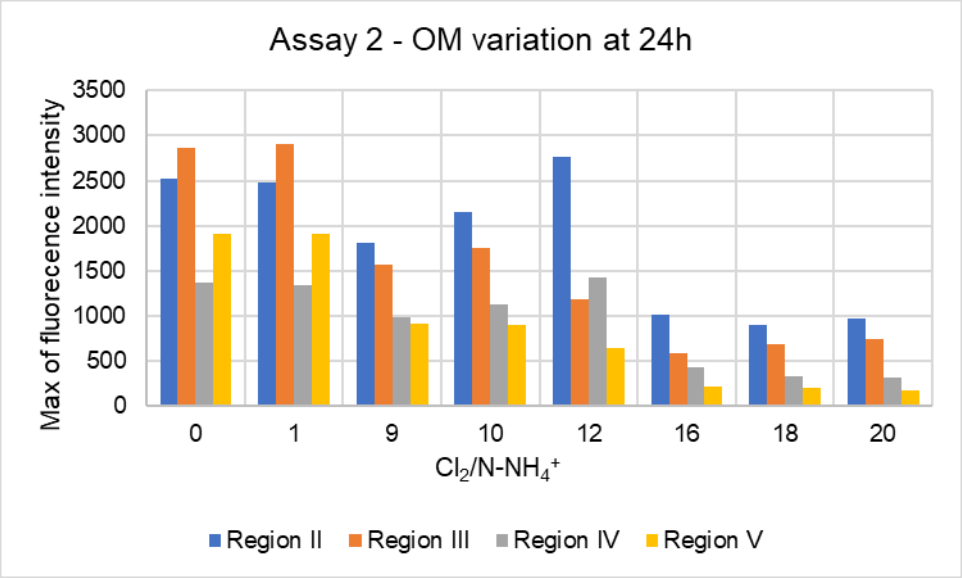

Figure 19 – Fluorescent organic matter (OM) content variation for nonchlorinated and chlorinated water samples, at 24h contact time - Assay 2

In the case of a Cl_2/N-NH_4 ratio of 1, no great differences were noted compared to the non-chlorinated sample (Cl_2/N-NH_4 0) since the maximum intensities detected were similar suggesting that the corresponding compounds did not react in great extent with chlorine. In those conditions the region with the highest signal was region III, followed by region II, region V and region IV. For the ratios of 9 and 10, the water samples had similar distribution of the maximum intensities of the regions (i.e., region II > region III > region IV > region V), and distinct from the non-chlorinated sample. A significant decrease in comparison to the values of the non-chlorinated sample and a slight increase in the intensities between the ratio of 9 and the ratio of 10 were noted. In the ratio Cl_2/N-NH_4 12, a noticeable increase in the maximum intensity of region II is noted, also in comparison with the non-chlorinated sample, which could indicate the formation of new compounds belonging to the class of aromatic proteins. With the further addition of chlorine, a drastic decrease in this peak is also noted, suggesting that these compounds were no longer produced or were oxidized in those conditions.

It can also be seen that, while in the non-chlorinated sample and in the sample with ratio Cl_2/N-NH_4 1 the region with the highest intensity is region III, followed by II and V, for samples 9 and 10 the region with the highest intensity becomes region II, followed by III, while region IV and V have comparable intensity peaks. In the sample with ratio 12, region II is far behind the others, reaching intensities of over 2500, while the other regions stop at intensities of 1200/1400. In the last three samples characterized by high Cl_2/N-NH_4 ratio values, region II remains the one with the highest intensity, but never above intensities of 1000.

4.4.2. Trihalomethanes formation in the breakpoint chlorination tests

The total trihalomethanes (THM) concentration as well as the content of each THM specie (chloroform $CHCl_3$, bromoform $CHBr_3$, dichlorobromomethane $CHBrCl_2$, dibromochloromethane $CHBr_2Cl$) was determined at 2 hours and 24 hours contact times, in some chlorinated water samples of the breakpoint chlorination assay 2.

The total trihalomethanes was estimated as the sum of the 4 THM species, so for the samples in which THM measured values were below the quantification limits of the analytical method, the total trihalomethanes should be overestimated.

Figure 20 shows the trihalomethanes species investigated and the total trihalomethanes, after a contact time of 2 hours. Chloroform was the predominant trihalomethane and its concentration increased from 10 µg/L (at $Cl_2/N\text{-}NH_4$ 1) to 100 µg/L after the break point (at $Cl_2/N\text{-}NH_4$ 16), reaching 225 µg/L at $Cl_2/N\text{-}NH_4$ 20. At $Cl_2/N\text{-}NH_4$ 20 chlorination condition, dichlorobromomethane was also detected. The trihalomethanes are not expected to de predominant as disinfection byproducts (DBP) in the first phase of the breakpoint curve since the major chloramine disinfection byproducts are nitrogenous DBPs, such as ammonium ions, organic chloramines or N-nitrosamines, if low molecular weight, hydrophilic dissolved organic matter is present [59, 60]; THM are produced especially after the breakpoint [33]. Nevertheless, at 24h of contact time (Figure 21) an increase of chloroform concentration was observed, raising from 10 µg/L (at 2 h contact time) to 70 µg/L when 3.8 mg/L of residual monochloramine were present in the water sample ($Cl_2/N\text{-}NH_4$ 1). When monochloramine concentration raised up to 20 mg/L ($Cl_2/N\text{-}NH_4$ 9 and 10), the chloroform concentration reached 100 µg/L. Close to breakpoint conditions ($Cl_2/N\text{-}NH_4$ 12), chloroform concentration increased 50% and after that, i.e. from a ratio of 16 onwards, the raising of residual free chlorine was accomplished by a huge increase on chloroform. The dichlorobromomethane was also detected in chlorination conditions succeeding breakpoint but in lower concentrations than chloroform however its concentration did not vary with increasing residual free chlorine concentration, probably due to bromine limitation in wastewater.

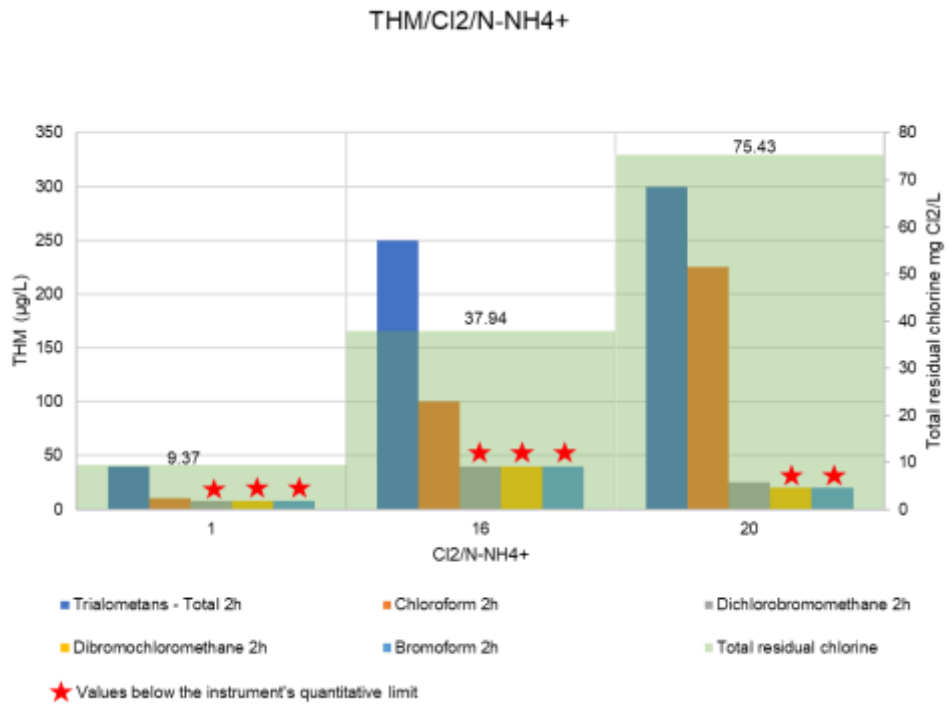

Figure 20 - Trihalomethanes content of chlorinated water samples, after a contact time of 2hours

These results evidences the increase of trihalomethane production over time for all three ratios considered, but especially for ratio 20, where chloroform production from 2 to 24 hours even doubles.

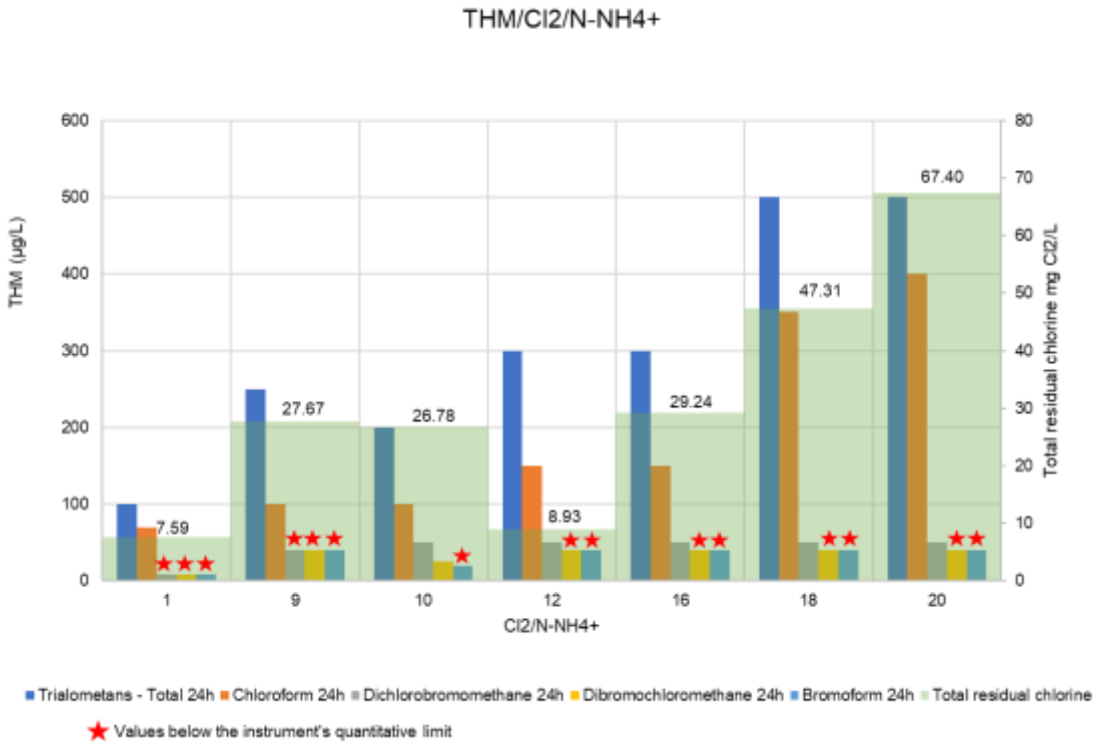

Figure 21 - Trihalomethanes content of chlorinated water samples, after a contact time of 24 hours

The THM formation during chlorination of wastewater was also studied by Yang et al. (2005). They showed that higher SUVA values produce a higher THM concentration, although the effect of bromide

on DBP formation is much stronger. The bromide effect on was not assessed in the present work, however some brominated THM were detected in for higher chlorination doses. The results on THMs obtained by Yang et al. confirm what was found during the THM analyses carried out in Assay 2, i.e. that before the breakpoint there is a slight increase in THM production with increasing chlorine dosage, once the breakpoint is reached and passed, there is instead a considerable increase in the production of these by-products.

As far as the formation of the different THM species is concerned, the results from the Yang et al 1 sample show a main presence of chloroform, the most abundant species that increases as the chlorine dose increases, and only after the breakpoint, a minimal presence of bromochloromethane is noticed, as in the results obtained from Assay2.

In Figure 22, Figure 23, Figure 24 and Figure 25 is presented the THM content and UV-Vis absorbance of water samples before and after the breakpoint, in an attempt to find any correlations between this parameters. With regard to wavelengths in the UV range, before the breakpoint (Figure 22) there appears to be an increasing linear correlation between THM formation and absorbance at 254 nm, suggesting the formation of compounds capable of absorbing this wavelength. While there appears to be a decreasing, albeit weak, linear correlation with absorbance at 291 nm, suggesting the degradation of THMs capable of absorbing this wavelength. On the other hand, no correlation is observed between THM formation and the other wavelengths considered. After the breakpoint (Figure 23), however, correlations between THM formation and wavelengths at 254 and 290 nm are also lost.

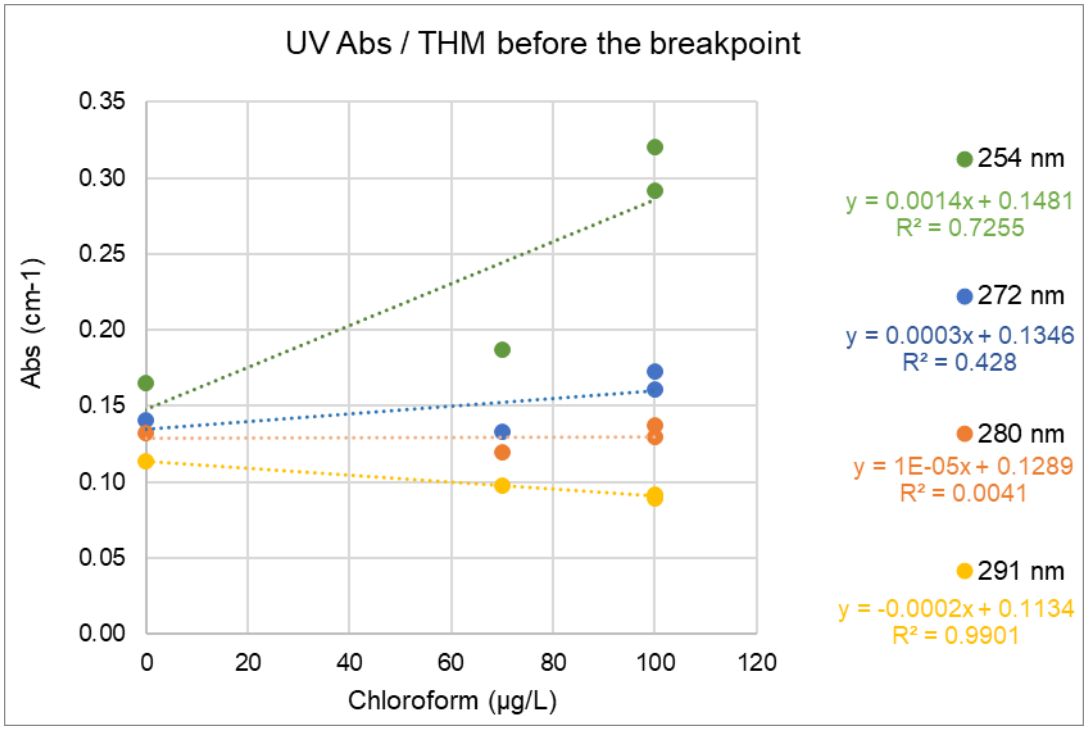

Figure 22 - Absorbance vs THM for UV wavelengths before the breakpoint

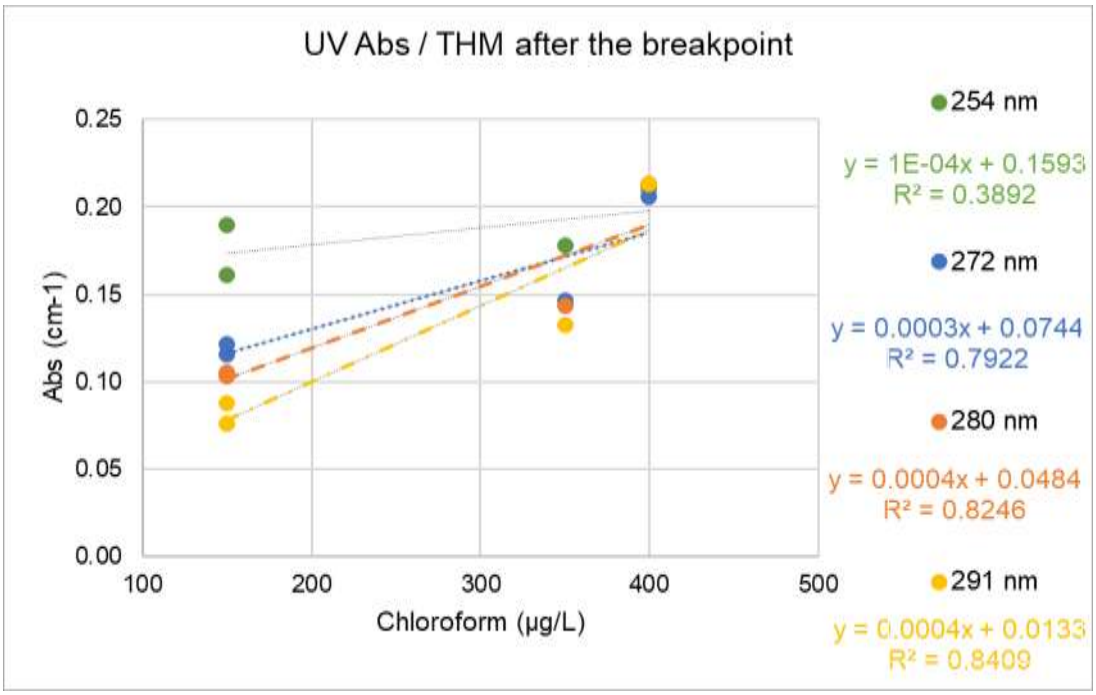

Figure 23 - Absorbance vs THM for UV wavelengths after the breakpoint

A correlation can also be seen for the visible only before the breakpoint (Figure 24). The correlation is linear decreasing and occurs for all wavelengths considered, thus indicating a degradation or decrease in THMs capable of absorbing those wavelengths. After the breakpoint (Figure 25), on the other hand, no correlation is apparent.

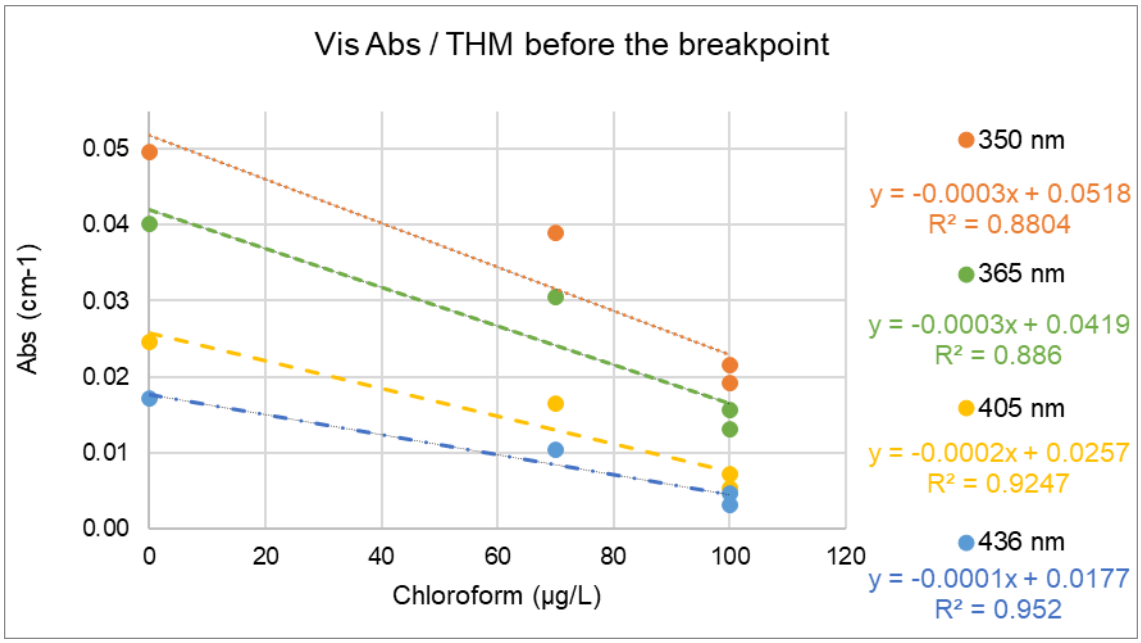

Figure 24 - Absorbance vs THM for visible wavelengths before the breakpoint

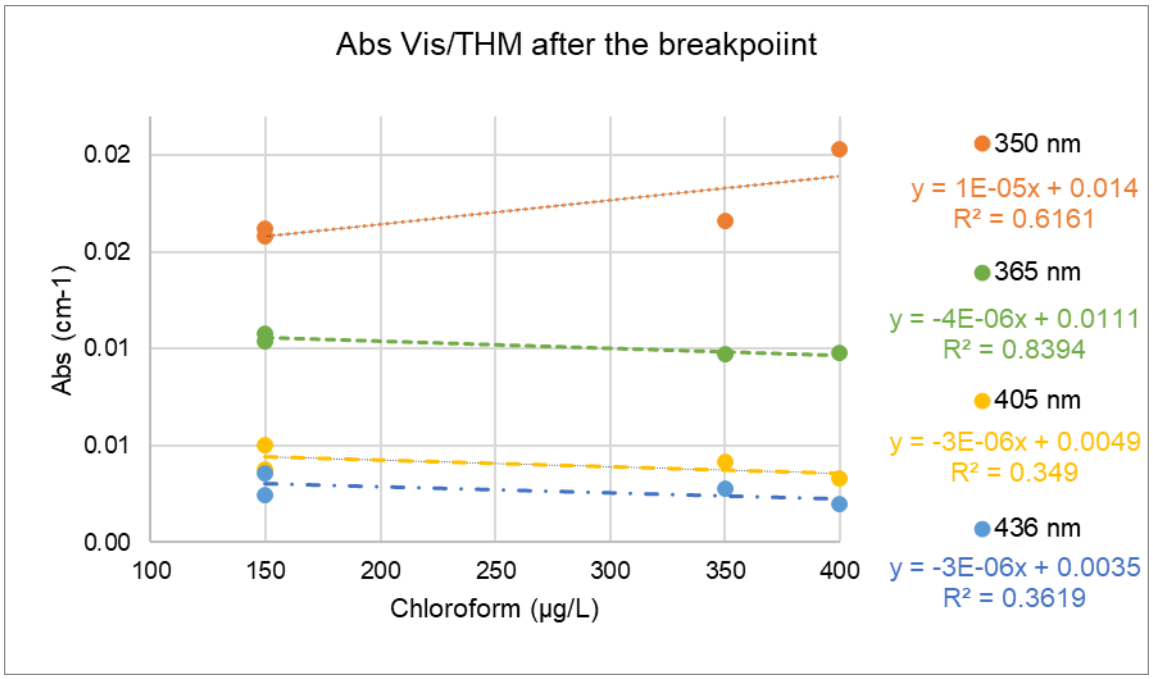

Figure 25 - Absorbance vs THM for visible wavelengths after the breakpoint

As already explained in section 2.6.1, absorbance ratios can be correlated with different characteristics of organic matter. In this section, the absorbance ratios A340/A254, A300/A400, A254/A365, A254/A436 were analysed and correlated with the Cl_2/N-NH_4 ratios and the results obtained from the THM analysis.

With regard to the A340/A254, usually used to predict DOC concentration (1), the results from the chlorinated water samples of the breakpoint assay (Figure 26 a) showed a linear positive correlation with chlorine dosing, for ratio values from 9-10 onwards. That ratio also correlated quite well with THM, for concentrations ≥ 100 µg/L (Figure 26 1b). For chlorination conditions upstream breakpoint (Cl_2/N-N-NH_4^+ ≤ 10), an exponential decrease of A340/A254 was observed for increasing doses of chlorine.

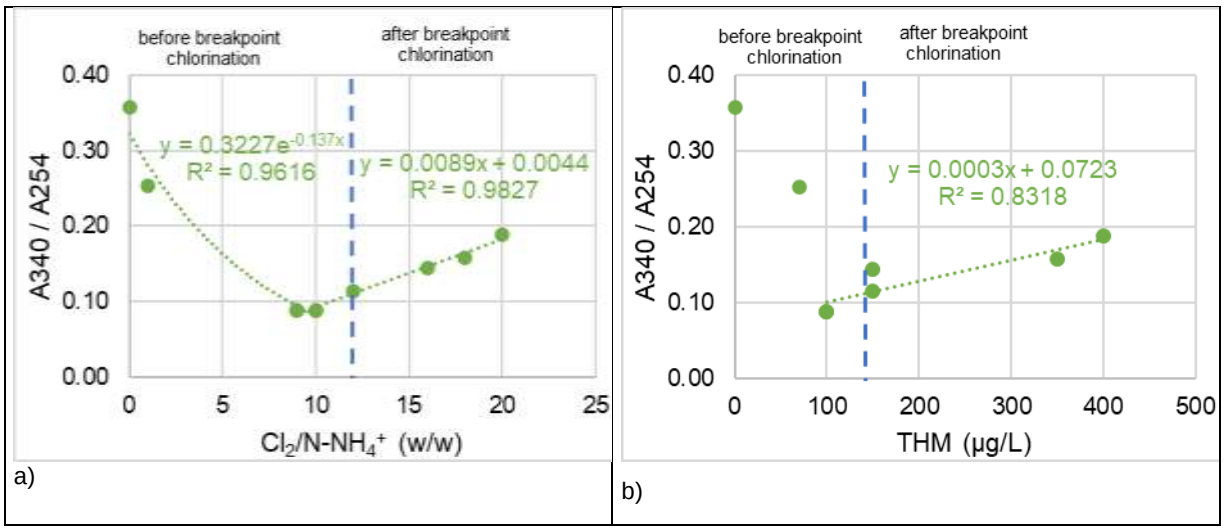

Figure 26 - Correlations of A340/A254 with Cl_2/N-NH_4 (a) and with THM concentration (b), before and after breakpoint chlorination conditions

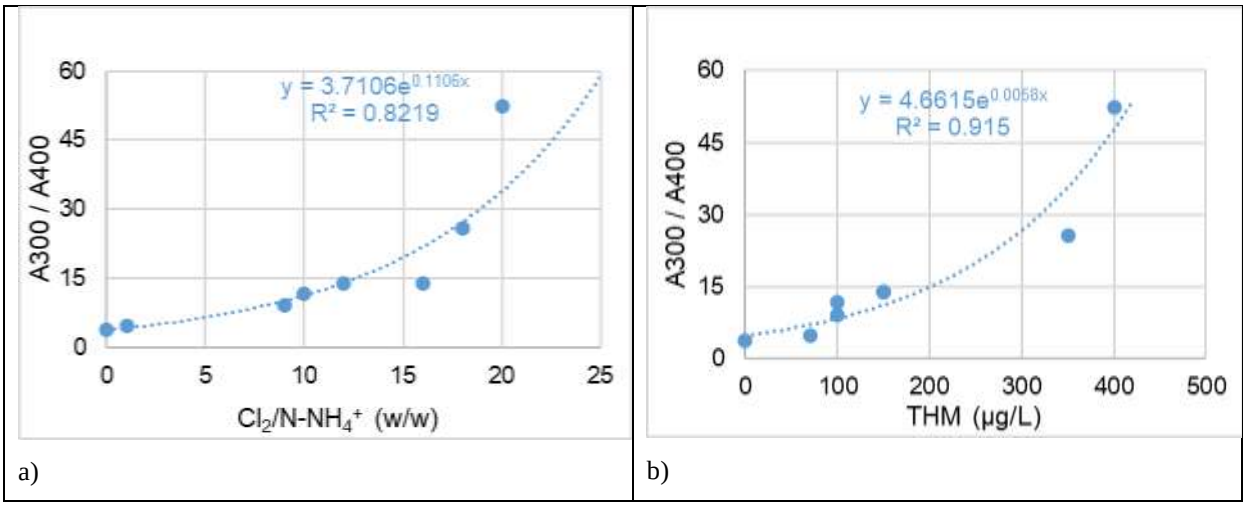

Figure 27 - Correlations of A300/A400 with Cl_2/N-NH_4 (a) and with THM concentration (b) at the chlorinated samples

The ratio A300/A400 of the chlorinated samples (Figure 27) showed an exponential correlation with the increase of chlorine doses, and a similar trend type was observed for the THM concentration in the samples. This ratio is typically considered negatively correlated with humification in natural waters (1). Although only values for Cl_2/N-NH_4 ratios up to 9-10 were in the typical range referred by Penghui et al. (1), these results suggests that for a linear increase of chlorine dosing, an exponential decrease of the humic-like compounds might have occurred and that was accomplished by an increase of the THM concentrations in the chlorinated samples.

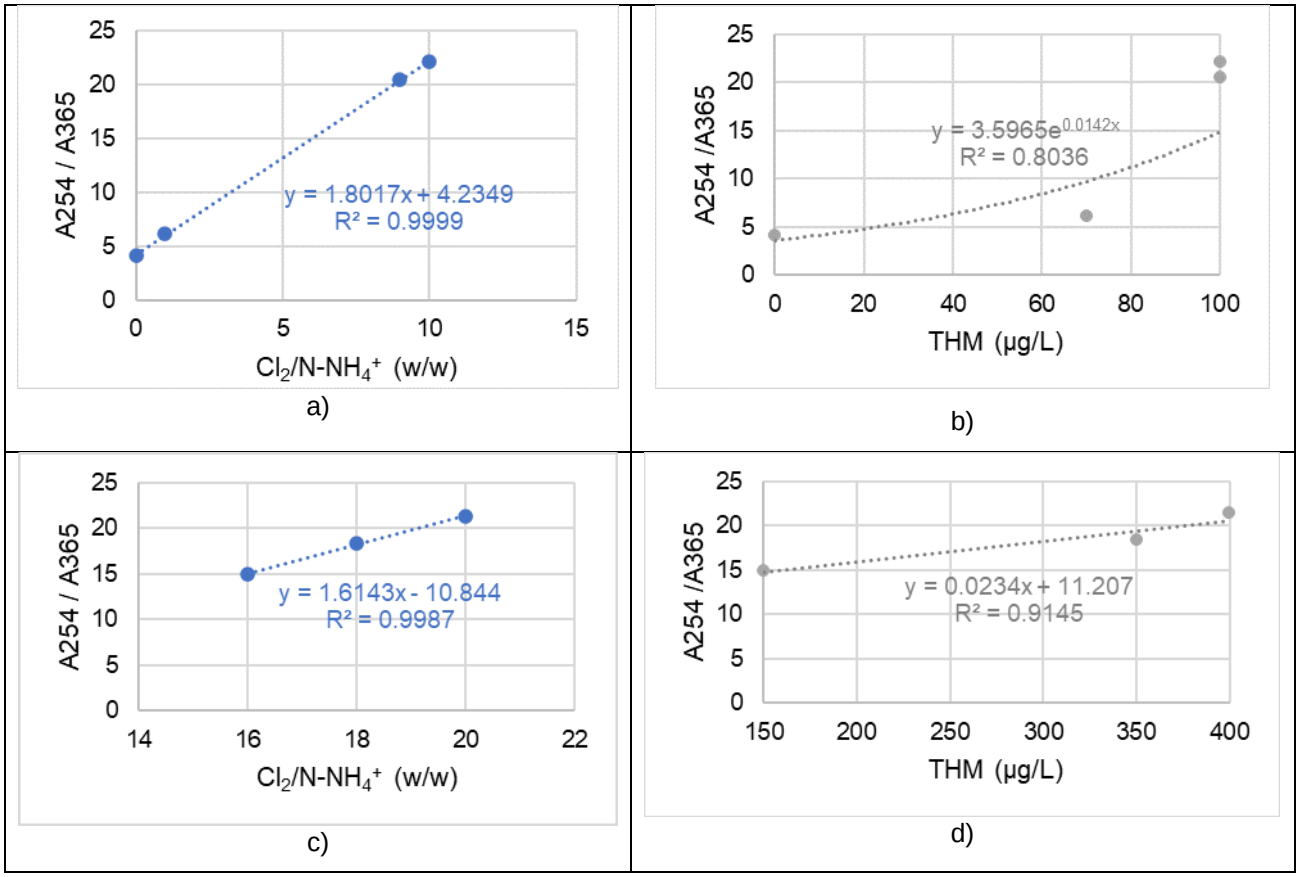

Figure 28 - Correlations of A340/A254 with Cl_2/N-NH_4 and THM concentration, before (a, b) and after (c, d) breakpoint chlorination conditions

The A254/A365 ratio, which have been shown to have a negative correlation with the aromaticity of the compounds present in water (1), showed linearly correlations with increasing chlorine doses, although different trends were observed before and after the breakpoint, as can be seen in the Figure 28 a) and c), respectively. Regarding THM, an exponential correlation was observed for samples before chlorination breakpoint (Figure 28 b), while after breakpoint a linear correlation was noticed (Figure 28 d).

The A254/A436 ratio, generally correlated with the sources and aromaticity of the compounds (1), showed values within the characteristic range only for Cl_2/N-NH_4 values of 0 and 1. Exponential correlations were obtained between A254/A436 and increasing chlorine dosing, before and after the breakpoint (Figure 29 a) and c), respectively). Regarding THM, an exponential correlation was observed for samples before chlorination breakpoint (Figure 29 b), while after breakpoint a linear correlation was noticed (Figure 29d).

This results are in accordance with the state of the art, since the increase of THM concentrations in the water samples was accomplished by the decrease/consumption of the humic like compounds and aromatic organic matter, considered the major precursors of THM.

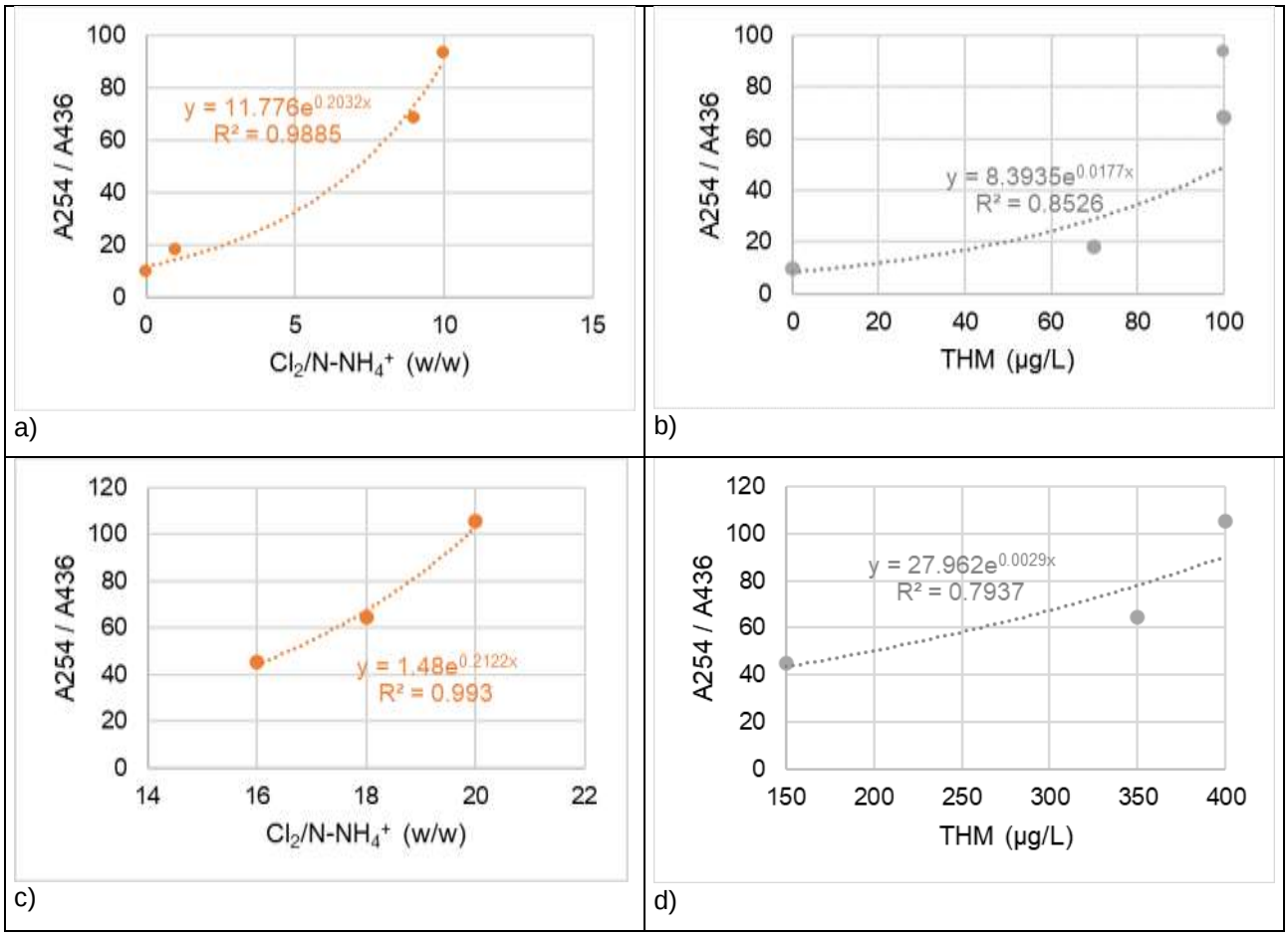

Figure 29 - Correlations of A254/A436 with Cl_2/N-NH_4 and THM concentration, before(a, b) and after(c, d) breakpoint chlorination conditions

4.5. Assessing chlorine decay from breakpoint chlorination tests

The decay of residual chlorine concentration observed in the breakpoint chlorination tests (assay 2) is herein discussed. The changes in the residual total chlorine and residual chlorine species concentration, for different chlorination conditions (Cl_2/N-NH_4 ratios of 1, 6, 10, 14 and 20), over the course of 24 hours is depicted in Figure 30 and Figure 31.

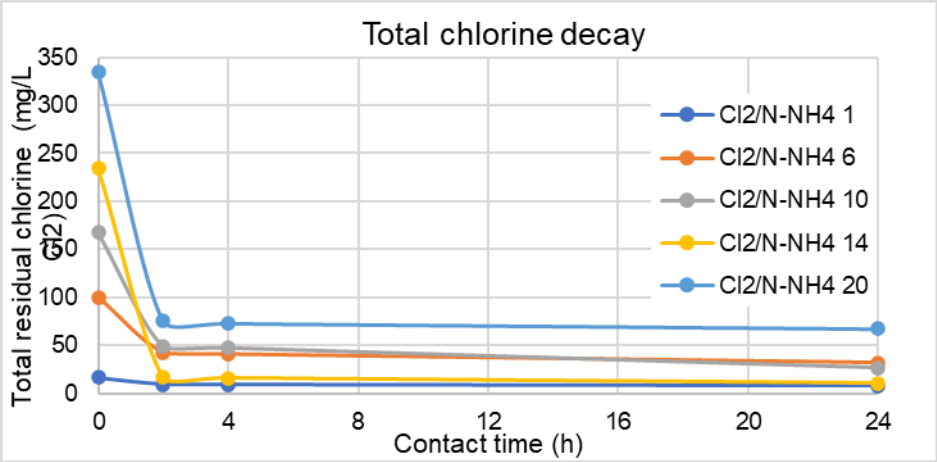

Figure 30 - Total residual chlorine decay over 24h at breakpoint chlorination test conditions

From Figure 30 it can be seen that, for all chlorination dosing considered, the decay occurred mainly in the first two hours of contact time in all samples. In the following 22 hours the total residual chlorine almost stabilized.

The chlorine decay rate was estimated, using equation (22) at the different chlorine decay phases observed. The results are presents in Table 10.

$$\frac{(C_{ti}-C_{tf})}{t_f-t_i} \quad (22)$$

where C_{ti}, C_{tf} is the residual chlorine concentration at contact time t_i and t_f, respectively

At the first two hours of contact time, (Table 10), as the concentration of added chlorine increases, the rate of chlorine decay increased from 3.67 mg Cl_2/h for the Cl_2/N-NH_4 ratio of 1 to 129.51 mg Cl_2/h for the Cl_2/N-NH_4 ratio of 20. Whereas, after the first two hours of contact time, the decay rate was much lower and no noticeable increase is noticed with increasing chlorine dosage.

Table 10 – Total chlorine decay rate at the different interval contact times (Δt)

Ratio	Total chlorine decay rates (mg Cl_2/h)		
	Δt 0h - 2 h	Δt 2 h - 4 h	Δt 4h - 24 h
Cl_2/N-NH_4 1	3.67	0.22	0.07
Cl_2/N-NH_4 6	28.74	0.89	0.45
Cl_2/N-NH_4 10	59.06	0.89	1.03
Cl_2/N-NH_4 14	108.80	0.22	0.27
Cl_2/N-NH_4 20	129.51	1.12	0.29

For contact times between 2h and 4 h, the decay of total residual chlorine was similar for the Cl_2/N-NH_4 ratios of 1 and 14 (at the breakpoint) and corresponded to the lowest residual concentration observed. The decay of total residual chlorine for the ratios 6 and 10, both before the breakpoint, were quite similar and 3 times higher than those observed in Cl_2/N-NH_4 ratios of 1 and 14. The decay rate at ratio 20, far beyond the breakpoint, was higher than for the other ratios.

The Figure 31 shows the chlorine decay observed for total, combined and free chlorine and the respective decay rates, calculated using equation (22), are in Table 11.

Considering the chlorine decay rate shown in Table 11 and Figure 31, it can be seen that, for monochloramine, which is prevalent for Cl_2/N-NH_4 ratios of 1, 6 and 10, except for ratio 1, there is an appreciable decay between 2 and 4 hours. Between 4 and 24 hours the decay rate decreases appreciably, except in the case of ratio 10, where it remains around 0.73. On the other hand, free chlorine, which was detected at appreciable concentrations for Cl_2/N-NH_4 ratios of 14 and 20, showed a decay of 0.5 mg Cl_2/h and 1.25 mg Cl_2/h, respectively, between 2 and 4 hours, while the rate decreased to 0.21 and 0.28 mg Cl_2/h over the following 20 hours.

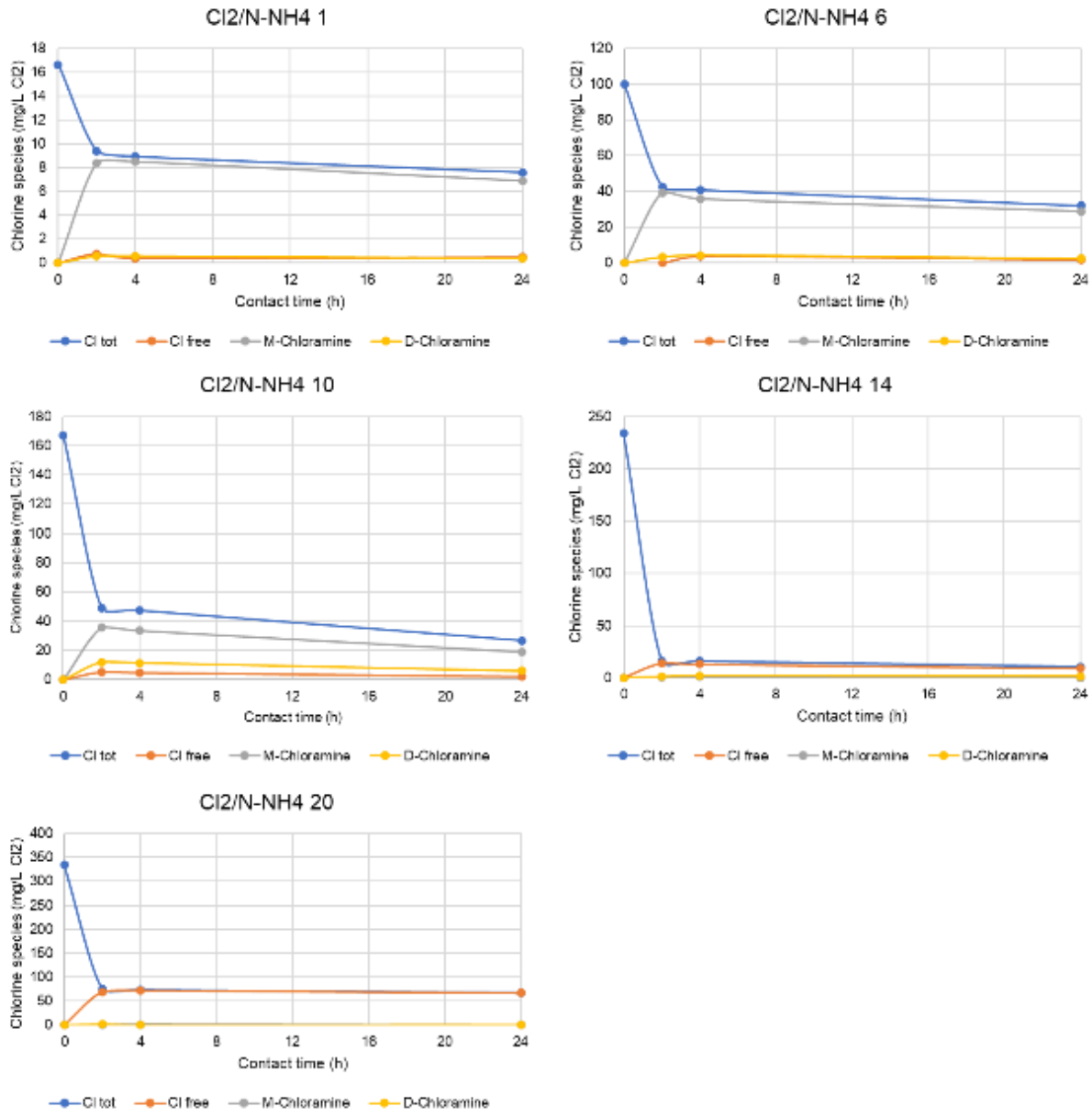

Figure 31 Decay of residual chlorine species over 24h at breakpoint chlorination test conditions

Table 11 - Chlorine species decay

Ratio	Free chlorine decay rate (mg Cl_2/h)		Monochloramine decay rate (mg Cl_2/h)		Dichloramine decay rate (mg Cl_2/h)	
	Between 2h and 4h	Between 4h and 24h	Between 2h and 4h	Between 4h and 24h	Between 2 h and 4 h	Between 4h and 24 h
Cl_2/N-NH_4 1			0.05	0.08		
Cl_2/N-NH_4 6			1.75	0.35	0.5	0
Cl_2/N-NH_4 10			1	0.73		
Cl_2/N-NH_4 14	0.5	0.21			0.5	0
Cl_2/N-NH_4 20	1.25	0.28			0.75	0

4.6. Chlorine decay and changes in water organic matter content

4.5.1. Chlorine decay tests

The residual chlorine decay in chlorination conditions near those used in real chlorination scenarios (Cl_2/N-NH_4 0.25, 0.5 and 1) was assessed and compared with the chlorine decay for chlorination conditions ahead the breakpoint (Cl_2/N-NH_4 16), for a period of 96 h. The results are depicted in Figure 32 as well as the changes in N-NH_4 concentration over the first 24 h of contact time.

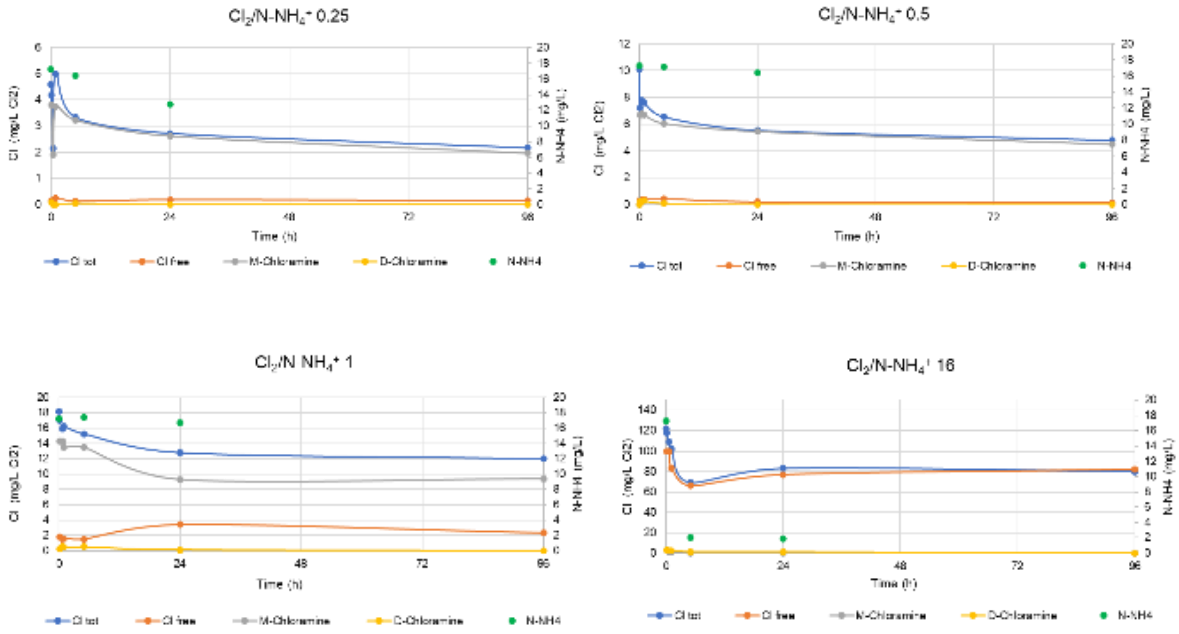

Figure 32 Decay of residual chlorine species over 96h at different chlorination conditions

The chlorine decay occurs in two distinct phases, a fast one that took place in the first 2 h, and slower phase that occurred at least until 96 h of contact time. In chlorination conditions behind the chlorination breakpoint, the residual chlorine was mainly monochloramine, so this discussion will prevail on this chlorine specie. The residual free chlorine detected in Cl_2/N-NH_4 1 testing condition is not expected and corresponds probably to organic chlorine compounds and not as free chlorine, ad explained in section 4.2.2.

The decay rate of residual monochloramine was 0.133 mg Cl_2/h, 0.163 mg/h and 0.188 mg/h, in the first 5 hours, for Cl_2/N-NH_4 ratios of 0.25, 0.5 and 1, respectively. After wards those decay were 0.01 mg Cl_2/h, 0.01 mg Cl_2/h, 0.001 mg Cl_2/h, after 96 hours, that is about 14, 12 and 130 times slower, respectively. A small or even a nule decrease on NH_4 concentration was observed in the first 5 h of contact time and even at 24 h contact time the variation on NH4 concetration was only oserved for Cl_2/N-NH_4 ratios of 0.25.

A different behaviour was observed at chlorination conditions ahead the breakpoint, in wich the residual chlorine was mainly free chlorine. In this case the residual chlorine decay occurred at 4.434

mg/h in the firs 5 h, and no further decay was observed within the 96 h of the experiment. In this conditions a noticeble reduction on NH4 levels were observed.

Costa et al. [1] constructed a model of chlorine decay in treated wastewater. This model comprises a mechanistic part, which includes properties related to inorganic components such as pH, conductivity, alkalinity and ammonia concentration, and a semi-empirical part that considers chlorine decay due to reactions with organic matter.

The results obtained confirm what has already been observed by Costa et al., namely that the monochloramine concentration formed depends on the dosed chlorine concentration and that for low chlorine concentrations, all dosed chlorine is converted into monochloramine.

The monochloramine decay profiles obtained by Costa et al. are similar to those obtained in Assay 2 and Assay 3. In fact, there is a very rapid first decay, already in the first few minutes, and a slower decay for longer contact times. This suggests the existence of two different phases, one due to reactions with organic matter and one due to the decomposition of monochloramine.

In the results of Costa et al. it was found that for higher concentrations of organic matter, the decay of monochloramine was higher for the same initial chlorine dose.

4.6.1. Assessing organic matter changes in the chlorine decay tests through UV-Vis spectra and EEM

As with Assay 2, also for the Assay 3, the EEMs of the non-chlorinated sample and the chlorinated samples at the different concentrations, both after 24 and after 96 hours of contact, were performed. The results are illustrated in Figure 33, Figure 34 and Figure 35. Again, to make the contour plots easier to read, the graphs were normalized and then rescaled. It is therefore not possible to directly compare the peaks of the highlighted regions in each figure.

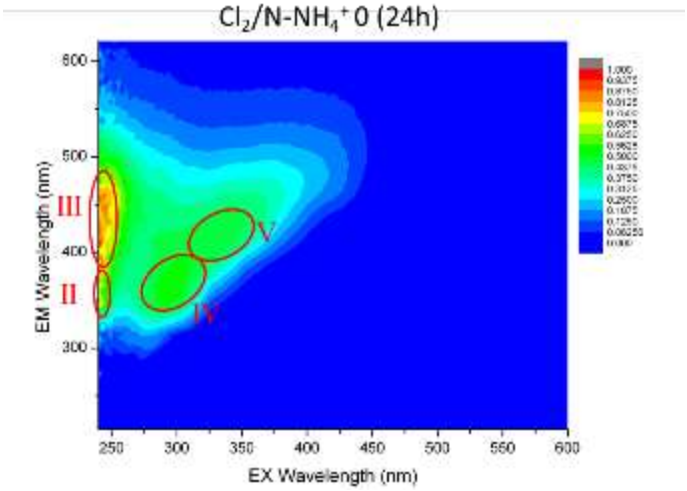

Figure 33 - Non-chlorinated water sample EEM contour plot

The presence of regions II, III, IV and V can be seen in the non-chlorinated sample and, as in Assay 2, the regions present with higher intensities are II and III, but they appear to be present to a much

greater extent than in Assay 2. It can therefore be concluded that the organic matter of the wastewater sample included compounds such as aromatic proteins, tryptophan-like, fulvic acid-like substances and hydrophobic acids, tyrosine and tryptophan-like and SMP-like species and humic acid-like organics, hydrophobic acids.

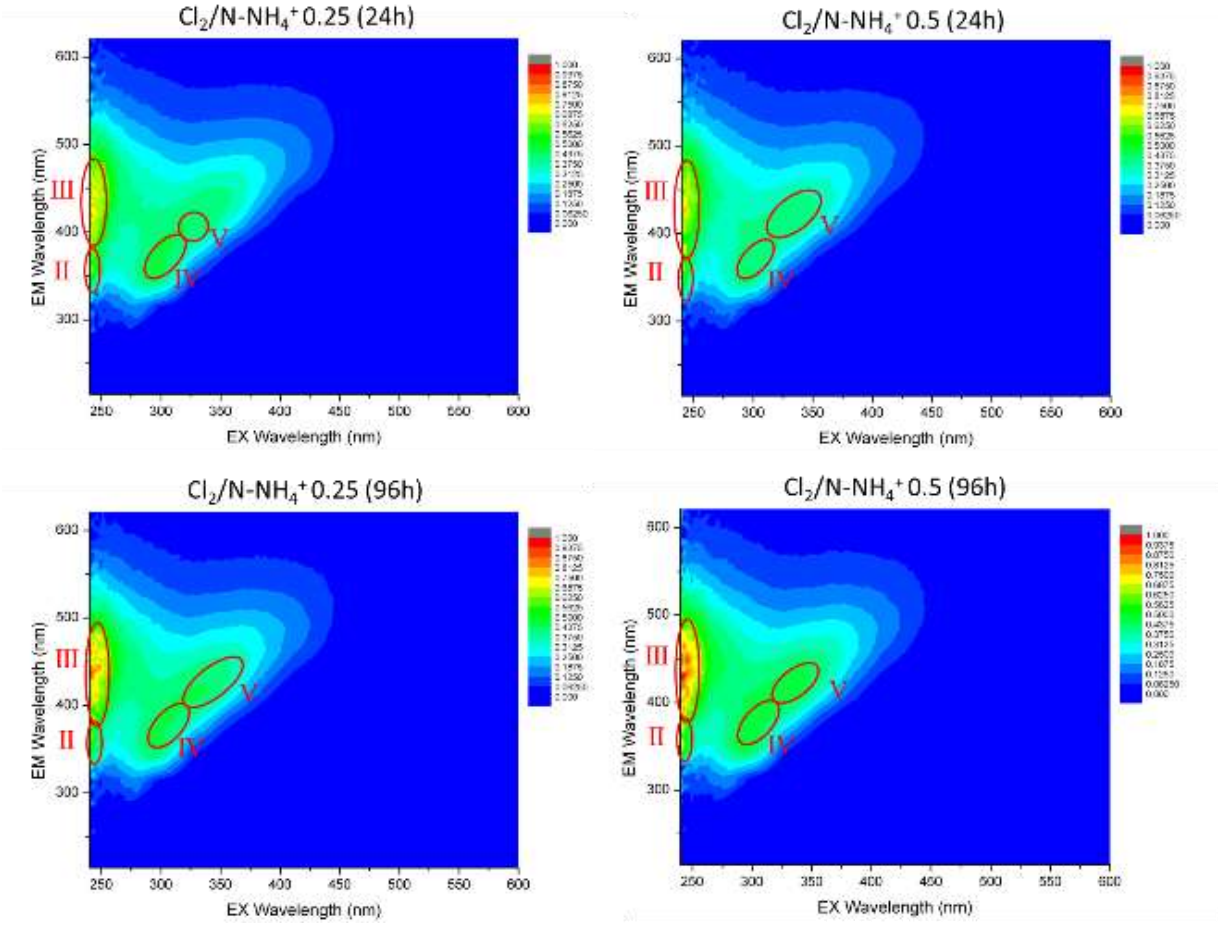

Figure 34 – EEM of Cl_2/N-NH_4 0.25 and 0.5 chlorinated water samples at 24 h and 96 h contact time

With the addition of chlorine a decrease in the extent of all four zones was observed and for the same Cl_2/N-NH_4 ratio an increase in the peaks intensity from 24 to 96 hours was always observed (Figure 34, Figure 35).

Remarkable differences can be seen for the Cl_2/N-NH_4 ratio of 1, between 24 and 96 hours, where the regions appear to be present with much greater intensities, however, as already mentioned, this result will have to be confirmed by analyzing the intensities specifically.

Also in this assay, region I, the one relating to aromatic proteins such as tyrosines, is never present, and at Cl_2/N-NH_4 ratio 16 all the fluorescent organic matter is oxidized.

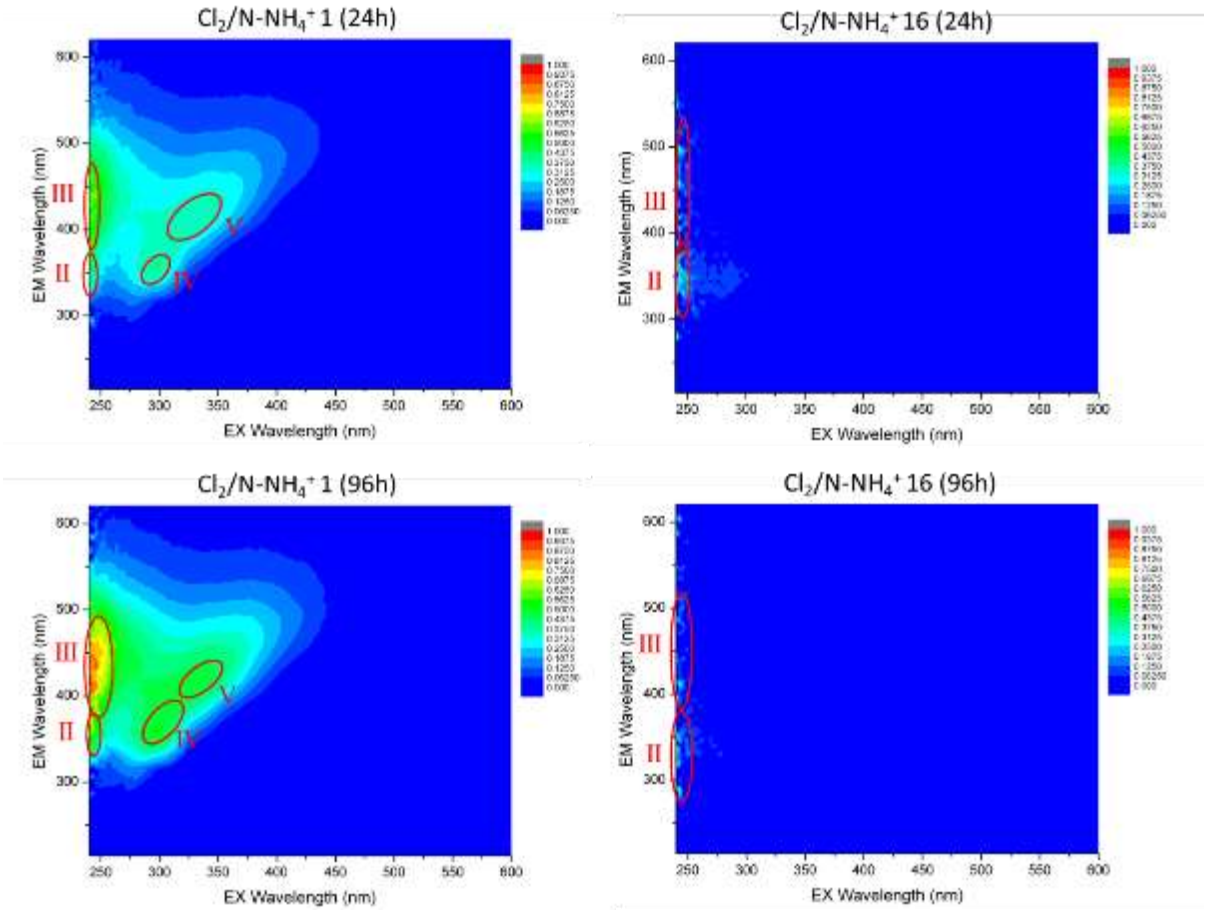

Figure 35 - EEM of Cl_2/N-NH_4 1 and 16 chlorinated water samples at 24 h and 96 h contact time

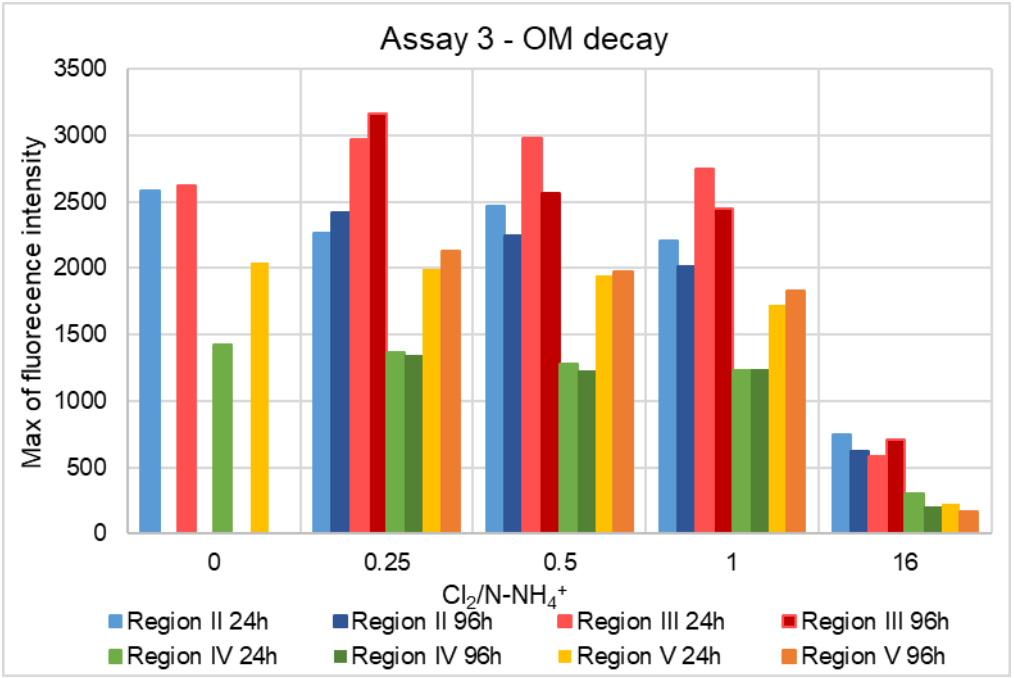

Figure 36 - Fluorescence intensity maxima in the regions detected

Figure 36 shows the fluorescence intensity maxima found in the five regions detected in each sample analyzed, for each chlorine concentration investigated, after 24 hours and 96 hours, so that a quantitative comparison of regions and peaks can be made.

Over 24 hours, in comparison to the peaks found in the non-chlorinated sample, no major differences can be seen, except for a slight increase in the peaks of region III in the samples for the 0.25 and 0.5 ratios and a drastic decrease in the peaks of all regions in the sample for the 16 ratio.

Between 24 and 96 hours, on the other hand, an increase in the peaks of regions II, III and V can be seen in sample 0.25, while in the other samples the peaks seem to decrease.

The region showing the highest peaks is the III in all samples, followed by II and V. Region IV does not show any particular variation, neither in time nor with chlorine concentration, except for high doses of added chlorine.

Figure 37 shows the UV-Vis spectra of Assay 3 water after 24 hours and 96 hours contact time, for each chlorine concentration investigated and for the non-chlorinated sample. The same spectra are also shown individually in Figure 38 for better comparison.

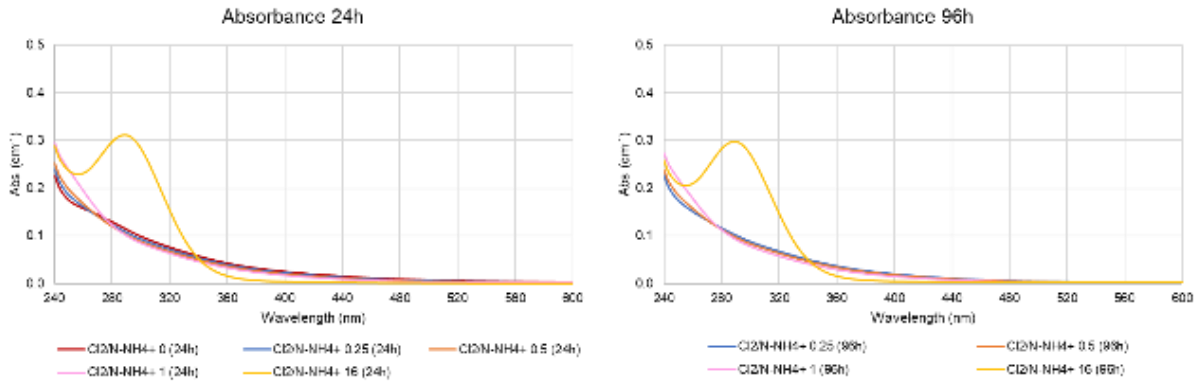

Figure 37 - UV-Vis spectra after 24 h and 96 h

Upon initial comparison of the absorbance curves, it can be seen that the trend of the spectra after 24 hours and 96 hours is very similar, with only a small decrease in absorbance values at 96 hours compared to 24 hours.

However, a gradual increase in absorbance compared to the unchlorinated sample is evident for short wavelengths, up to 270 nm, for ratios of 0.25 and 0.5 nm.

At the ratio of 1 there is instead a sharper and more evident increase, again for wavelengths from 240 to 270 nm, while for the other wavelengths the absorbance remains more or less unchanged.

At a Cl_2/N-NH_4 ratio of 16, on the other hand, the formation of a noticeable shoulder is noted at 290 nm and a slight decrease for absorbance values above 340 nm. The formation of the shoulder could suggest the formation of new compounds sensitive to these wavelengths.

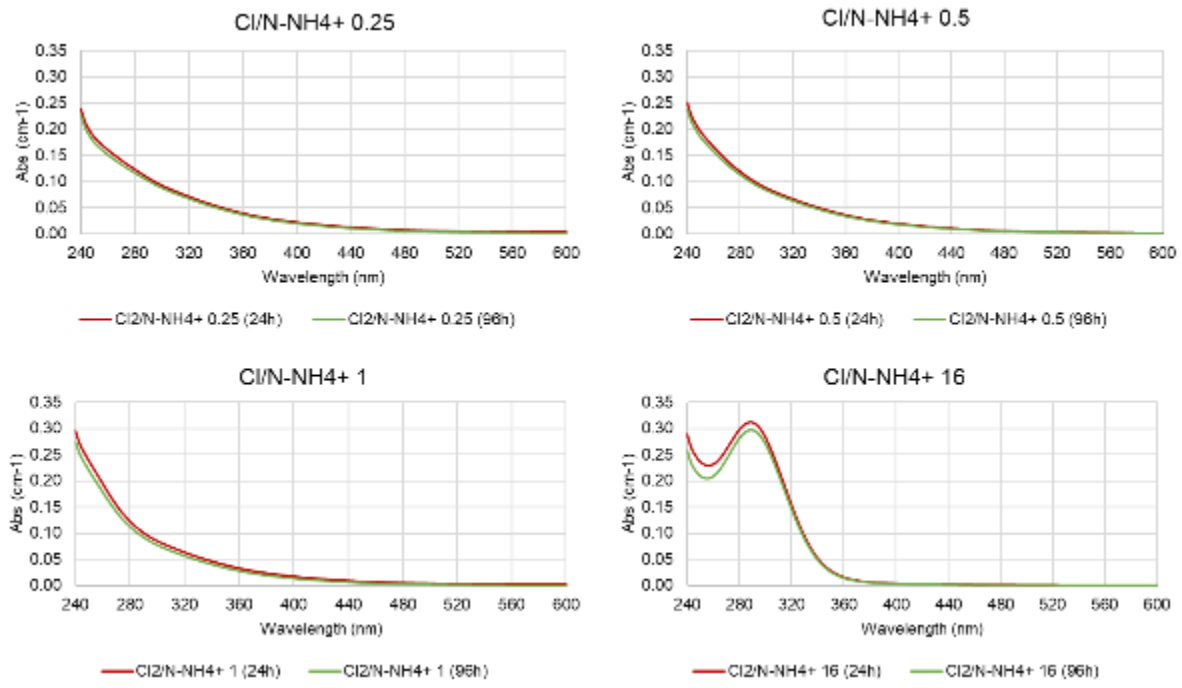

Figure 38 - UV-Vis spectra after 24 h and 96 h

In Figure 38, the changes in absorbance as the changes in concentration of added chlorine are shown to a greater extent. While for Cl_2/N-NH_4 ratios of 0.25 and 0.5 the absorbance trends are very similar, although a slight increase in absorbance values is already noticeable in the ratio of 0.5, more evident differences can be seen in the graph corresponding to ratio 1. In fact, a marked increase in absorbance from 254 nm and 360 nm and an increase in the slope of this first part of the curve can be seen. The values between 24 hours and 96 hours are almost identical for all three of these first concentrations.

In the Cl_2/N-NH_4 16 ratio, while the initial values of the curve are very similar to those at the ratio of 1, there is a sudden change and the formation of a broad shoulder from the wavelengths 250 to 300 nm, where the curve's trend becomes exponentially decreasing again, reaching much lower values after 340 nm than at lower chlorine concentrations.

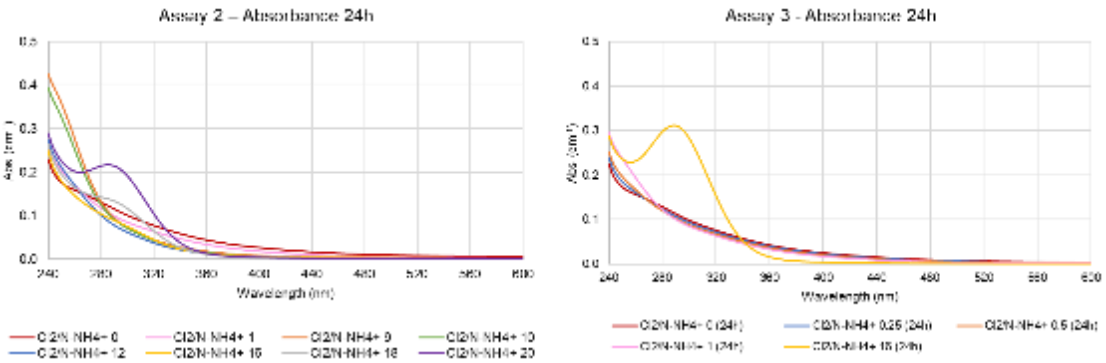

Figure 39 - UV-Vis spectra after 24 h of Assay 2 and Assay 3

Figure 39 compares the spectra of the absorbances measured in the two different assays after 24 hours of contact time.

This, in fact, shows a much higher shoulder in assay 3 than in assay 2, although the peak always occurs at a wavelength of 290 nm. The reason for this is the changes that occurred in the water during the 22 days it was kept in the refrigerator. Among the values compared, it was noted that the absorbance at 436 nm, which is related to the color of the samples, was higher in the wastewater used for Assay 3 than in Assay 2.

Finally, a relationship was sought between the total organic carbon (TOC) content and the absorbance in the UV and visible range in the water samples as the dosed chlorine increases (Figure 40, Figure 41).

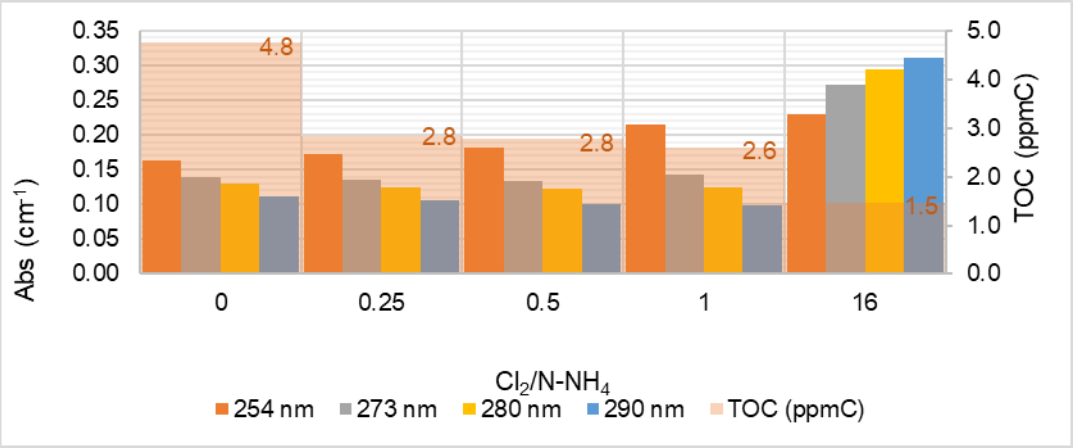

Figure 40 – Abs UV and TOC variation for different chlorine doses, after 24h contact time

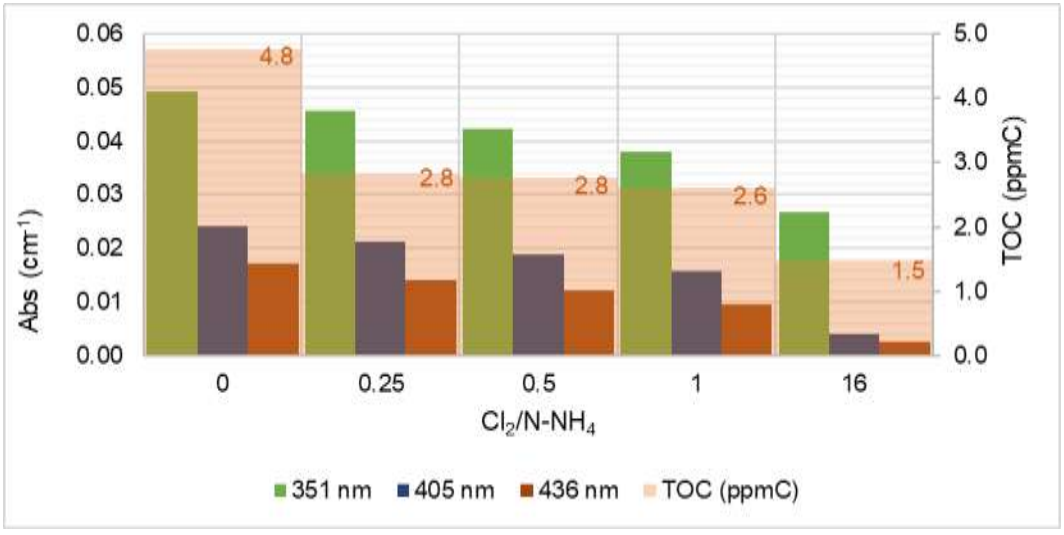

Figure 41 – Abs Vis and TOC variation for different chlorine doses, after 24h contact time

It can be seen that, as might be expected, as the dosed chlorine increases, the TOC decreases. As far as compounds absorbing in the UV range are concerned (Figure 40), there appears to be an increase only in compounds that absorbs at 254 nm. For high concentrations of chlorine, on the other hand,

compounds that absorb at other wavelengths in the UV range also appear to form. In contrast, all compounds that absorb in the visible range (Figure 41) appear to degrade.

5. Chlorination unit dimensioning

As previously mentioned, from the 22 March 2022, part of the wastewater treated at the Beirolas plant is sent for disinfection with UV and with chlorine in order to be reused to irrigate the green areas of the Parque das Nações Norte, consisting mostly of the Parque Tejo. These reach an area of almost 295,000 m^2 and an annual volume of water required for irrigation of 300,000 m^3 [61].Hence, in the Beirolas treatment plant, the disinfection treatment, for the portion of water that will be reused, consists of an ultrafiltration unit followed by UV disinfection and finally addition of chlorine to ensure residual disinfection. In the context of this thesis, which dealt with chlorine disinfection, it was thought to be of interest for academic purposes to propose an alternative approach using only a chlorination system to disinfect water after filtration. For this reason, in this chapter, the sizing of the chlorination unit required to meet the water requirement of Parque das Nações Norte was carried out.

When sizing a chlorination unit, the relevant factors that must be taken into account are: estimation of chlorine dosage, dosage control, injection and initial mixing conditions, design of contact units, maintenance of solids transport rates, effluent control and measurement of residual chlorine, chlorine storage units, containment of chemical reagent consumption and neutralization systems and dechlorination units, when present [4].

In order to determine the necessary chlorine dosage, if residual effluent concentration or number of residual coliform bacteria is known, it is a good practice to carry out analyses. In the case under study, this information was obtained from a previous study [48]. The analysis records for faecal coliform bacteria, referred to the year 2017 are shown in Table 12. As can be seen, the wastewater has high concentrations of this type of bacteria, but there is a great variation between the wastewawater entering the Beirolas treatment plant and the treated effluent, the concentration of faecal bacteria decreases by an average of 500 times, indicating a treatment efficiency of 99.8%. Nevertheless, the concentration is still very high if reuse is considered.

Table 12 - Faecal coliform bacteria year 2017 [48]

Faecal coliform bacteria Units: MPN/100 ml			
Date of collection	**Raw affluent**	**Filtered effluent**	**Efficiency**
2/Jan	$9,83x10^6$	$1,92x10^4$	99.805%
6/Feb	$8,2x10^6$	$1,15x10^4$	99.860%
1/Mar	$8,16x10^6$	$5,91x10^3$	99.928%
3/Apr	$6,31x10^6$	$6,13x10^4$	99.029%
2/May	$1,03x10^7$	$1,27x10^4$	99.877%
5/Jun	$1,79x10^7$	$3,65x10^4$	99.796%
3/Jul	$7,76x10^6$	$6,13x10^4$	99.210%
21/Aug	$1,35x10^7$	$3,87x10^4$	99.713%
11/Sept	$1,04x10^7$	$5,79x10^4$	99.443%
2/Oct	$1,15x10^7$	$8,66x10^4$	99.247%
6/Nov	$1,09x10^7$	$1,92x10^4$	99.824%
4/Dec	$1,26x10^7$	$1,72x10^4$	99.863%

The average value, i.e. $3.6x10^4$ MPN/100 ml, was taken into account for the dosage calculation. For the choice of the chlorine dosage to be used, Table 13 was considered, which shows typical chlorine dosages, based on the required effluent standards. The standard to be met was identified in the Portuguese legislation (Norma Portuguesa NP 4434 2005: reuse of reclaimed urban wastewater for irrigation), considering a class B (Public parks and gardens, sport lawns, forest with public easy access) and requiring that the limit for the parameter *E.coli* for the effluent at discharge will be inferior to 200 MPN/100 ml [62].

With regard to the chlorine dose chosen, since the water reused in the Beirolas treatment plant undergoes activated sludge treatment and filtration before going to the disinfection unit, and given the limits imposed by regulations, the required chlorine dosage should have been in the range of 5-15 mg/L. For greater protection, the higher value was chosen in the dimensioning.

Table 13 - Typical chlorine dosage values, based on combined chlorine where not otherwise specified, required to achieve different effluent coliform concentration standards, for different types of effluent and contact time of 30 minutes [4]

		Chlorine dosage mg/l			
		Standard in effluent, MPN/100 ml			
Type of waste	**Initial coliform count MPN/100 ml**	**1000**	**200**	**23**	**<=2.2**
Untreated effluent	10^7-10^9	15-40			
Primary effluent	10^7-10^9	10-30	20-40		
Effluent from filtration	10^5-10^6	3-10	5-20	10-40	
Effluent from activated sludge	10^5-10^6	2-10	5-15	10-30	
Effluent from filtered activated sludge	10^4-10^6	4-8	5-15	6-20	8-30
Nitrified effluent	10^4-10^6	4-12	6-16	8-18	8-20
Nitrified and filtered effluent	10^4-10^6	4-10	6-12	8-14	8-16
Effluent from microfiltration	10^1-10^3	1-3	2-4	2-6	4-10
Effluent from reverse osmosis	0	0	0	0	0-2
Effluent from septic tanks	10^7-10^9	20-40	40-60		
Effluent from intermittent sand filtration	10^2-10^4	1-5	2-8	5-10	8-18

Regarding the addition of chlorine, usually in liquid sodium hypochlorite chlorination, part of the wastewater stream entering the chlorination unit is separated from the mainstream and it is chlorinated with a relatively high concentration of chlorine. Once the addition of chlorine has taken place, this current is led back to the main flow. For better mixing of the two streams, which influences the removal of bacteria, there may also be diffusers or mixers, so that the chlorine is added and mixed as quickly as possible, and the system is optimized. Once the chlorinated water has passed through the contact chamber, the residual chlorine concentration in the effluent is measured in order to adjust the feed pump. The schematic flow chart of sodium hypochlorite chlorination is shown in Figure 42.

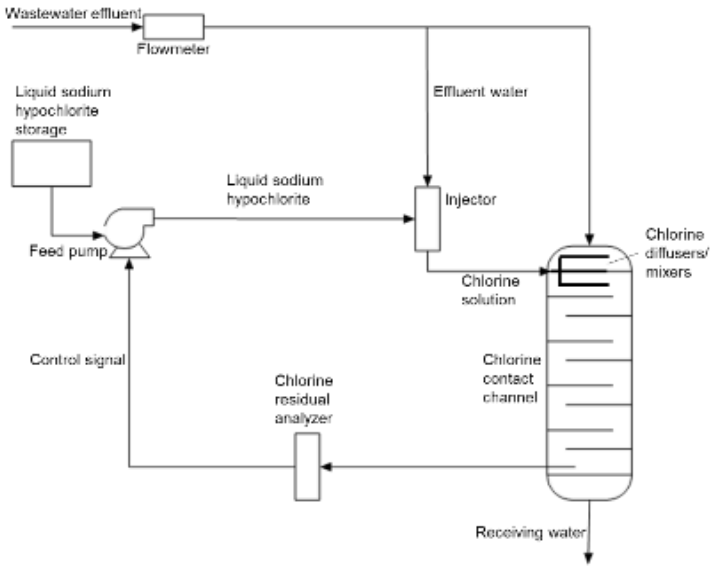

Figure 42 - Chlorination unit diagram

Typically, the dosing pumps used have a capacity of up to 50 l/h, but are adjustable for lower values, depending on the incoming flow rate and possibly the residual chlorine measurement [63].

To control the dosage, a manual control method can be adopted, manually (via an operator who varies the feed rate according to the process conditions) or automatically, via a meter with a signal transmitter and recording system.

Influencing the removal of bacteria, in addition to the good mixing of the chlorine solution with the effluent, are also the contact time and the residual chlorine concentration.

Regarding the contact time to be ensured to achieve effective disinfection, usually the standard suggests a time of about 20 minutes [4]. In the case study, given the small wastewater flow rate to be treated, a contact time of 15 minutes was considered sufficient.

To ensure that a portion of the flow rate remains in the chlorination unit for the time required for chlorination, plug-flow type reactors with a long length, or coil, or a series of interconnected basins or compartments are usually used. The objective in reactor design is to avoid the formation of dead zones, which could reduce the residence time of the effluent. These are avoided by using length to depth ratios of 20:1, or 40:1, as assumed in the dimensioning, and by using septa and deflectors [4].

Finally, it is important that the minimum flow rate in the chlorination unit is high enough to avoid sedimentation of any suspended solids still present and to keep the bottom of the tank clean. To this end, a horizontal velocity of at least 2-4.5 m/min should be achieved, although it is generally not easy to maintain such high velocities while also meeting dispersion requirements [4].

For the required irrigation flow rates, the water consumption and required irrigation flow rates estimated for the Parque Tejo gardens in a previous study [48] were used. The annual values for each month of the year 2017 are shown in Table 14.

Table 14 - Estimated flow rates needed for park irrigation per month – year 2017 [48]

Months	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Indicative value for irrigation (l/m2/day)	0.95	1.34	2.01	2.91	3.52	4.59	4.92	4.36	3.3	2.07	1.18	1
Estimated flow rate (m3/month)	6727	9489	14233	20606	24925	32502	34839	30873	23367	14658	8356	7081

In order to choose a flow rate value for sizing the disinfection unit, it would have been necessary to know the variations in water demands over a greater range, or a safety factor would have been required. This was not taken into consideration, since depending on the water demand, it would be sufficient to vary the operating hours of the chlorination unit to have a higher treated flow rate. The average flow rate required to irrigate the park is 18971 m3/month, however, conservatively, the unit has been sized on the basis of the maximum flow rate that would be required in the year 2017, i.e. that corresponds to the maximum demand for water, 34839 m^3/month. This usually occurs in the month of July. It was considered an operating time for the unit in this month of 8 hours per day. In the other months, the hourly flow rate treated by the reactor will not change, but only the plant's operating time each day will change. It is therefore considered that the reactor will operate at a constant flow rate, since only a part of the effluent of the treatment plant is treated, so the system can be considered as if an equalization tank was present. For this reason, the chlorination unit would always work with the same flow rate.

Table 15 shows the values of the maximum monthly, daily, hourly and per second flow rates.

Table 15 - Maximum flowrate

Flow rate	Values	Units of measurement
Max flow rate	34839	m^3/month
Maximum daily flow rate	1161.30	m^3/day
Maximum hourly flow rate, considering an operating time of 8h/day	145.16	m^3/h
Maximum flow rate per second	0.04	m^3/s

Table 16 shows the values of the parameters used in dimensioning.

Table 16 - Parameters for the dimensioning of the chlorination unit [4]

Input	Symbols	Values	Units of Measurement		
Flow rate	Q	0.040	m^3/s		
Hydraulic residence time	Q_H	15	min	must be fixed >10 min at maximum flow rate	
Horizontal velocity	V	between 2 and 4.5 [4]	m/min	at $Q_{m'}$	
Length/height ratio	l/H	40	40/1		Fixed
Length/distance septa ratio	l/b	40	40/1		Fixed
Chlorine dosage	$D_{Cl,m}$	15	mgCl/L	at Q_m	Fixed
Chlorine concentration of sodium hypochlorite solution	$Conc_{Cl}$	0.14	kgCl/kgNaOCl	12-15% in chlorine	Fixed
Density of sodium hypochlorite solution	D	1.2	kgNaOCl/L		Fixed

For the dimensioning of this unit, first of all the reactor volume was calculated, considering a hydraulic residence time of 15 minutes. Having set the l/B and l/H ratios at 40:1, the individual reactor dimensions were derived.

The horizontal velocity was then calculated, obtaining a value in the optimal range of 2-4.5 m/min. The number of septa was set at 7, and the volume of each compartment was then calculated. From the chlorine dosage, its consumption is calculated and the hypochlorite is obtained at the average flow rate. Finally, the actual amount of hypochlorite consumed is found, i.e. the amount to be fed into the disinfection tank.

Table 17 shows the results obtained.

As a plug flow system is being considered and chlorine is only added to a small fraction of the flow rate, a dispersion requirement must be met to ensure that there is a good mixing within the plug flow channel, between the chlorinated water fraction and the rest of the flow rate, thus ensuring contact between chlorine and effluent. Therefore, the dispersion coefficient was also checked. To calculate this, the peak coefficient was obtained with the formula $\frac{Q_{max}}{Q_{average}}$, obtaining a coefficient equal to 1.8. Having assumed a number of septa equal to 7, the number of parallel channels is 8.

Table 17 - Output of the dimensioning of the chlorination unit

Output	Symbols	Values	Units of Measurement	Formulas
Volume of disinfection reactor	V	36.29	m^3	$V = \frac{Q_{max}}{\Theta_H}$
Total length of fluid path	l	38.72	m	$l = (V * 40 * 40)^{1/3}$
Distance between two contiguous septa	b	0.97	m	$b = l/40$
Reactor height	H	0.97	m	$H = l/40$
Horizontal velocity check	v	2.58	m/min	$v = \frac{Q_m}{H * b}$
Number of septa	N	7		between 6 and 8
Reactor width	B	4.8	m	$B = \frac{l}{(N+1)}$
Reactor length	L	7.7	m	$L = (N+1) * b$
Chlorine mass flow rate at average flow rate	$C_{Cl,m}$	0.60	kgCl/d	$C_{Cl,m} = D_{Cl,m} * Q_m$
Hypochlorite mass flow rate at average flow rate	$C_{NaOCl,m}$	4.32	kgNaOCl/d	$C_{NaOCl,m} = \frac{C_{Cl,m}}{Conc_{Cl}}$
Volume flow rate of hypochlorite at average flow rate	$V_{NaOCl,m}$	3.60	LNaOCl/d	$V_{NaOCl,m} = \frac{C_{NaOCl,m}}{d}$

First, the velocity at the peak flow rate was checked, and the Reynolds number was calculated in order to determine the dispersion coefficient. The dispersion factor was then calculated, which depends on the geometry of the chlorination unit and must be such as to guarantee a dispersion coefficient of 0.015 [4].

The calculations used are grouped in Table 18.

For calculating Reynolds number (NR), the Coefficient of dispersion and the Dispersion factor the following formulas were used:

$$N_R = \frac{4uR}{v} \quad (23)$$

Where:

- R is the hydraulic radius (wetted area/perimeter)
- u is the velocity at peak flow
- v is the velocity in free channel

$$D = 1.01 v {N_R}^{0.875} \quad (24)$$

Where:

- v is the kinematic viscosity

$$d = \frac{D}{uL} = \frac{D\Theta_H}{L^2} \quad (25)$$

Where:

- Θ_H is the hydraulic residence time
- L is the reactor lenght

Table 18 - Checking of the dispersion coefficient

Dimensions of the transverse section		
Peak coefficient	1.8	
Width	4.8	m
Depth	7.7	m
Number of parallel channels	8	
Velocity verification: u	8.35E-05	m/s
Verification of dispersion coefficient		
Kinematic viscosity: v	1.00E-06	m^2/s
N_R	6.09E+02	
Coefficient of dispersion		
D	2.77E-04	
Calculation of the dispersion factor		
d	0.0042	<0.015

Therefore, the required chlorination chamber should have the dimensions shown in Table 19and Figure 43 and an effective volume of 35.85 m3.

Table 19 - Final dimensions of the chlorination chamber

Dimensions	Symbols	Values	Units of Measurement
Reactor width	B	4.8	m
Reactor length	L	7.7	m
Reactor height	H	0.97	m
Reactor volume	V	35.85	m^3

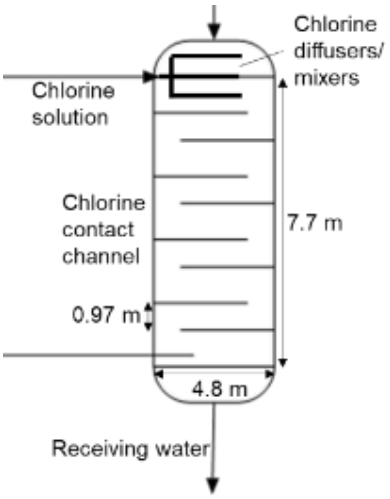

Figure 43 - Final dimensions of the chlorination chamber

Finally, a check was made on the operating conditions of the unit (Table 20), assuming a situation like that of 2017 and one with a 20% heavier water demand. For each year, three months were taken into account, corresponding in 2017 to the driest month, the average annual water demand and the wettest month, and the same demands plus an additional 20% for the case corresponding to a more onerous year. For each month, the daily operating hours of the chlorination chamber were calculated. Considering that the reactor would work with a fixed flow rate of 145.16m3/h, first the daily flow rate that should be treated for each month was calculated and then the daily hours in which the chlorination unit should operate.

Table 20 – Operating condizions' check

Wet month		Average month		Dry month	
6727	m3/month	18971.33	m3/month	34839	m3/month
224.23	m3/day	632.38	m3/day	1161.30	m3/day
2	h/day	4	h/day	8	h/day
20% wetter year					
Wet month		**Average month**		**Dry month**	
8072.4	m3/month	22765.6	m3/month	41806.8	m3/month
269.08	m3/day	758.8533	m3/day	1393.56	m3/day
2	h/day	5	h/day	10	h/day

6. Conclusions

This work evaluated the effects of chlorination, one of the most common disinfection techniques, on an effluent of a wastewater treatment plant. In particular, the influence of nitrogen on the shape of the breakpoint curve, the changes in the content and in the nature of organic matter and the formation of by-products were investigated using UV-Vis absorbance and fluorescence spectroscopy methods. An attempt was made to ascertain whether trihalomethane formation could be detected with these two techniques, so that they could be proposed as a preliminary method of analysis for detecting by-product precursors and by-products in chlorinated samples, given the cost and extensive time required for laboratory analysis.

Given the large difference in the nitrogen content of the two samples used in the chlorination breakpoint assays, which differed approximately 10-fold, it was possible to see how nitrogen influences both the residual combined chlorine maxima, which appear to be reached at lower Cl_2/N-NH_4 ratios as the nitrogen present increases, and the breakpoint, which, for low contact times appears to occur for higher Cl_2/N-NH_4 ratios, whereas it appears to be reached at lower Cl_2/N-NH_4 ratios for longer contact times.

However, the breakpoint curve also seems to vary with changing contact time and not only due to chlorine decay: in Assay 1, it appears that for lower nitrogen contents and longer contact times, the breakpoint occurs at higher Cl_2/N-NH_4 ratios; whereas in Assay 2, these differences are not noticeable

Another difference that is noticeable in the two Assays is the different chlorine demand before and after the breakpoint; a difference that is probably also due to the different concentration and nature of the organic matter contained in the samples. The difference between before and after the breakpoint is much greater in Assay 2 than in Assay 1, going from 105 to 244 mg/L Cl_2, whereas in Assay 1, it went from 6 to 22 mg/L Cl_2.

Comparing the results obtained in the two assays with those of other authors [38], who reconstructed the breakpoint curves on samples with similar nitrogen content, but different organic composition (DOC higher than our samples), a much higher chlorine demand was noted in the samples analysed in our work. Comparing the SUVAs, these indicated a predominance of hydrophobic compounds in our samples, which could justify the higher chlorine demand and would seem to suggest that the chlorine demand is not only dependent on the concentration of organic matter, but also on its nature.

The UV-vis absorbance spectra of the wastewater sample 2 after the contact of 24 h with different chlorine doses showed that as the chlorine dose increased, effective changes in the spectra trend were discernible. Indeed, at Cl_2/N-NH_4 ratios of 9-10, corresponding to the maximium formation of chloramines, an increase in absorbance at 240-280 nm was noted, probably attributable to the increase in organic chloramines. As the chlorine dose increases, up to the breakpoint, a decrease in the absorbance spectra is observed instead, to be attributed to the oxidation of the compounds present by the chlorine. After the breakpoint, for high doses of chlorine, however, a rather high peak is clearly seen at 290 nm, which can be attributed to the formation of disinfection by-products.

Absorbance spectra therefore seem useful for detecting possible by-products, but on their own they are not sufficient to identify and quantify them.

From the EEMs, the presence of compounds such as aromatic proteins, tryptophan-like substances, fulvic acid-like substances and hydrophobic acids, tyrosine and tryptophan-like and SMP-like species and humic acid-like organics, hydrophobic acids were assessed in the non-chlorinated and chlorinated samples after 24 h of contact time. With this technique, it was possible to characterise the raw sample by identifying and quantifying the above-mentioned compounds present in it, which are major precursors of disinfection by-products. Fluorescence spectroscopy, however, did not prove to be a good method for detecting these chlorination by-products. On the contrary, this method allowed to detect a progressive degradation and oxidation of the compounds present in the water,as the added chlorine dose increased. This does not mean that no by-products were formed, on the contrary, only that no fluorescent by-products are noted, as this technique is only capable of detecting fluorophores. It can therefore be used to characterize wastewater samples as such, to detect precursors and to follow their degradation, as these are fluorescent, but not to detect by-products.

From the THM analyses carried out in the laboratory, it was noted, that chloroform was the predominant THM species within all chlorinated samples and that its concentration increased with increasing chlorine dose, peaking after the breakpoint. The results highlight the need for further studies on this subject, as although lower chlorine concentrations are used in real cases than those used in this case study, chloroform was also detected for chlorine concentrations below the breakpoint, i.e. when chlorine were in the form of chloramine and not free chlorine. Although THM are expected to be mainly formed in the presence of free chlorine, these results showed that they can also be formed when the oxidant are chloramines. Also demonstrating the need for further investigation is the increase observed in the concentration of THMs, for the same Cl_2/N-NH_4 ratios, over time, between 2 and 24 hours, indicating the need to monitor the formation of these by-products over time.

As already mentioned, an attempt was made to find correlations between the formation of THM in water samples and the respective UV-Vis absorbance or absorbance ratios at specific wavelengths, which correlated with certain OM characteristics. With regard to the relationships with individual absorbance wavelengths, no good correlation was noted with the absorbance at 254 nm and with none of the all wavelengths taken into consideration in the visible range. A discrete linear correlation was only found with the absorbance at 291 nm, which is linked to the presence of organic matter.

Good correlations were found with the absorbance ratios A340/A254, A300/A400, A254/A365, A254/A436, which confirmed what was expected, i.e. that the increase in THMs is accompanied in chlorinated samples by a consumption of humic-like compounds and aromatic organic matter, as these are among the major precursors of THMs. These parameters could therefore effectively be used to detect the formation of THMs as the chlorine dosage in a water sample increases.

Chlorine decay tests showed that the decay of residual chlorine occurred in two distinct phases, one with a higher decay rate in the first two hours and a slower rate up to 24 or 96 hours, where the chlorine concentration seemed to have stabilized, for all chlorine species. This suggests the existence

of two different phases, one due to reactions with organic matter and one due to the decomposition of monochloramine.

With regard to the chlorination unit that has been dimensioned, given the importance that nitrogen has proven to have, it will be very important to know the nitrogen fluctuations within the wastewater treated by the plant, so that the chlorine dosage and consequently the most appropriate Cl_2/N-NH_4 ratio can be chosen appropriately.

Chlorine disinfection is certainly widely used, but when used as either primary or secondary disinfection, a risk/benefit analysis would need to be carried out, considering the by-products it forms. However, more studies should be carried out on the by-products formed by low chlorine concentrations in order to assess whether it could be a safe way to reuse treated wastewater.

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