UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Higher Polytechnic School of Alcoi

Enhancing Fenton process efficiency in presence of Cu(I) / Cu(II) for hydroponic water treatment. Role of high valent metals and reactive species

Master's Thesis

Master's Degree in Materials Engineering, Science Processing, and Characterisation

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ACADEMIC YEAR: 2023/2024

ABSTRACT

Aquaponics combines hydroponic and recirculating aquaculture systems, allowing plants to utilise nutrients from aquaculture waste. However, treating their wastewater remains a challenge. So far, methods such as ultraviolet radiation and ozonisation have been proposed to disinfect and reduce emerging contaminants such as antibiotics and phytotoxic substances (e.g. benzoic acid, BA). Since aquaponics contains Fe(III), Cu(II), Mn(II) and Mo(VI) ions, it is suggested to explore Fenton-type reaction and metal interactions. This study focuses on the use of hydroxylamine (HA) as a reducing agent to induce an in-situ Fenton process by forming H_2O_2 from Cu(I)/O_2. Cu(II)/HA ($[Cu(II)]_0 = 100\ \mu M$) was investigated to degrade 50 μM of BA, evaluating anions, chelating agents (EDTA), dissolved organic matter, H_2O_2 addition and the presence of iron, manganese and molybdenum. The results showed that 250 μM HA degraded 60% of BA in 1 h, increasing to more than 95% with 1 mM H_2O_2. The main reactive species were $^1O_2$ and Cu(III), not $OH^\bullet$. The presence of EDTA (100 μM) inhibited the Cu(II)/HA(/H_2O_2) processes by forming a reduction-resistant Cu(II)-EDTA complex. This study aims to clarify the interactions in aquaponic systems by inducing an in-situ Fenton process.

Keywords: Advanced oxidation processes; high-valent metal species; reactive oxygen species; water treatment.

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RESUMEN

La acuaponía combina sistemas hidropónicos y de acuicultura recirculada, permitiendo que las plantas aprovechen los nutrientes de los desechos acuícolas. Sin embargo, tratar sus aguas residuales sigue siendo un desafío. Hasta ahora, se han propuesto métodos como la radiación ultravioleta y la ozonización para desinfectar y reducir contaminantes emergentes, como antibióticos y sustancias fitotóxicas (e.g., ácido benzoico, BA). Dado que la acuaponía contiene iones Fe(III), Cu(II), Mn(II) y Mo(VI), se sugiere explorar la reacción tipo Fenton y las interacciones metálicas. Este estudio se enfoca en el uso de hidroxilamina (HA) como agente reductor para inducir un proceso Fenton in situ mediante la formación de H_2O_2 a partir de Cu(I)/O_2. Se investigó Cu(II)/HA ($[Cu(II)]_0$ = 100 µM) para degradar 50 µM de BA, evaluando aniones, agentes quelantes (EDTA), materia orgánica disuelta, adición de H_2O_2 y presencia de hierro, manganeso y molibdeno. Los resultados mostraron que 250 µM de HA degradan el 60% de BA en 1 h, aumentando a más del 95% con 1 mM de H_2O_2. Las especies reactivas principales fueron el $^1O_2$ y el Cu(III), no el OH$^{\bullet}$. La presencia de EDTA (100 µM) inhibió los procesos Cu(II)/HA(/H_2O_2) al formar un complejo Cu(II)-EDTA resistente a la reducción. Este estudio busca esclarecer las interacciones en sistemas acuapónicos al inducir un proceso Fenton in situ.

Palabras clave: Procesos oxidación avanzada; especies metálicas altamente valentes; especies reactivas de oxígeno; tratamiento de aguas.

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RESUM

L'aquaponia combina sistemes hidropònics i d'aqüicultura recirculada, permetent que les plantes aprofitin els nutrients de les deixalles aqüícoles. Tot i això, tractar les seves aigües residuals continua sent un desafiament. Fins ara, s'han proposat mètodes com la radiació ultraviolada i l'ozonització per desinfectar i reduir contaminants emergents, com ara antibiòtics i substàncies fitotòxiques (e.g., àcid benzoic, BA). Atès que l'aquaponia conté ions $Fe(III)$, $Cu(II)$, $Mn(II)$ i $Mo(VI)$, se suggereix explorar la reacció tipus Fenton i les interaccions metàl·liques. Aquest estudi s'enfoca a l'ús d'hidroxilamina (HA) com a agent reductor per induir un procés Fenton in situ mitjançant la formació de H_2O_2 a partir de $Cu(I)/O_2$. Es va investigar $Cu(II)/HA$ ($[Cu(II)]_0 = 100$ μM) per degradar 50 μM de BA, avaluant anions, agents quelants (EDTA), matèria orgànica dissolta, addició de H_2O_2 i presència de ferro, manganès i molibdè. Els resultats van mostrar que 250 μM d'HA degraden el 60% de BA en 1 h, augmentant a més del 95% amb 1 mM de H_2O_2. Les espècies reactives principals van ser el $^1O_2$ i el $Cu(III)$, no l'$OH^·$. La presència d'EDTA (100 μM) va inhibir els processos $Cu(II)/HA(/H_2O_2)$ en formar un complex $Cu(II)$-EDTA resistent a la reducció. Aquest estudi busca aclarir les interaccions en sistemes aquapònics en induir un procés Fenton in situ.

Paraules clau: Procesos oxidación avanzada; espècies metàl·liques altament valentes; espècies reactivas d'oxigen; tractament d'aigües.

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Abbreviations List

AOPs	Advanced Oxidation Processes
BA	Benzoic Acid
CECs	Contaminants of Emerging Concern
DMPO	5,5-Dimethylpyrrolidine-N-oxide
DOM	Dissolved Organic Matter
DWC	Deep-Water Culture
EDTA	Ethylenediaminetetraacetic Acid
EPR	Electron paramagnetic resonance
EU	European Union
FFA	Furfuryl alcohol
HA	Hydroxylamine
HPLC	High Performance Liquid Chromatography
IC	Inorganic Carbon
IPA	Isopropyl alcohol
NFT	Nutrient Film Technique
p-BQ	p-Benzoquinone
ROS	Reactive oxygen species
SDGs	Sustainable Development Goals
SMX	Sulfamethoxazole
TBZ	Thiabendazole
TC	Total Carbon
TEMP	2,2,6,6-Tetramethylpiperidine
TOC	Total Organic Carbon
UV	Ultraviolet radiation

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1. INTRODUCTION

1.1 Global water resources overview

Water is an essential resource for the survival of humanity. It is widely recognised that water scarcity is growing at an alarming rate worldwide. This phenomenon has emerged as one of the major contemporary challenges, generating a global concern that calls for innovative and effective solutions[1]. The importance of water is such that its availability or lack thereof directly determines the continuity of human existence.

Although our planet is known as the "Blue Planet", the amount of fresh water is only 3%, while about 97% is salt water. Among all this fresh water, approximately 68.7% is found in glaciers, around 30.1% in aquifers and only 0.9% is found on the surface and is accessible to living organisms[2]. This distribution (represented in Figure 1) highlights the vulnerability of our access to freshwater and the urgent need for effective strategies for its conservation and sustainable use.
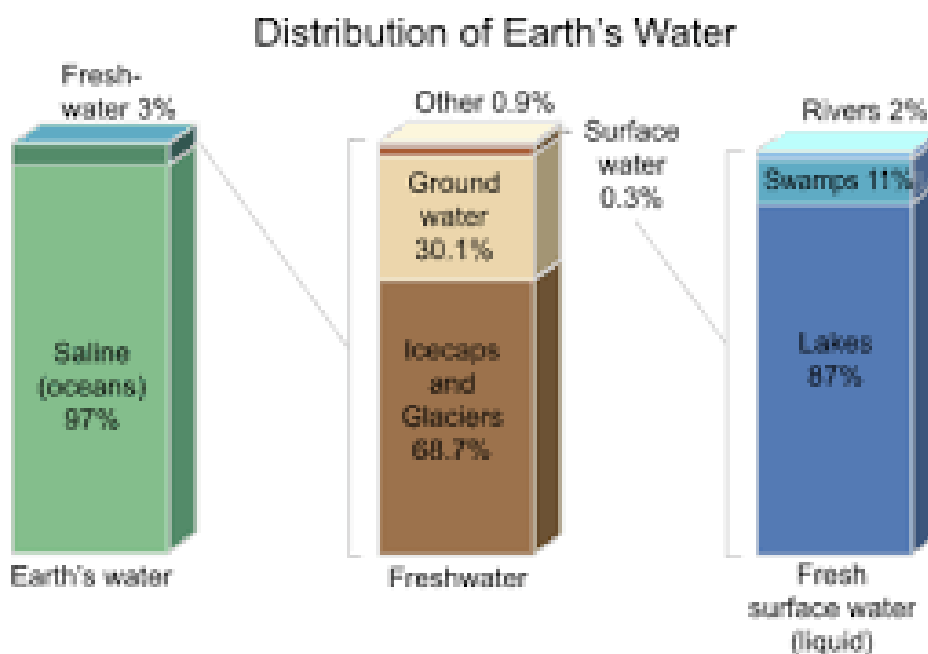
Figure 1. Distribution of water portions on the planet[3], [4]

For this reason, the constant population growth experienced by cities demands a greater number of resources to satisfy the basic needs of the population. An obvious example of this is the increasing scarcity of drinking water, affecting approximately 2.2 billion people worldwide who lack access to safe water[5]. This problem is interlinked with the geographical location of each locality, its proximity to sources such as rivers or reservoirs, and the availability of water treatment plants.

In addition, climate change has intensified water scarcity and drought conditions in several regions, further complicating the task of meeting the growing demand for water for population and agriculture. Moreover, certain areas, particularly in developing countries, face more significant challenges, with more than 80% of wastewater being

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discharged without treatment (see Figure 2). World Bank data reveals that, in 2014, between 70-75% of this wastewater was re-consumed, resulting in the spread of disease in adults and children [6]. Furthermore, growing environmental awareness and resource scarcity have raised the importance of water reuse as a crucial measure in sustainable management.
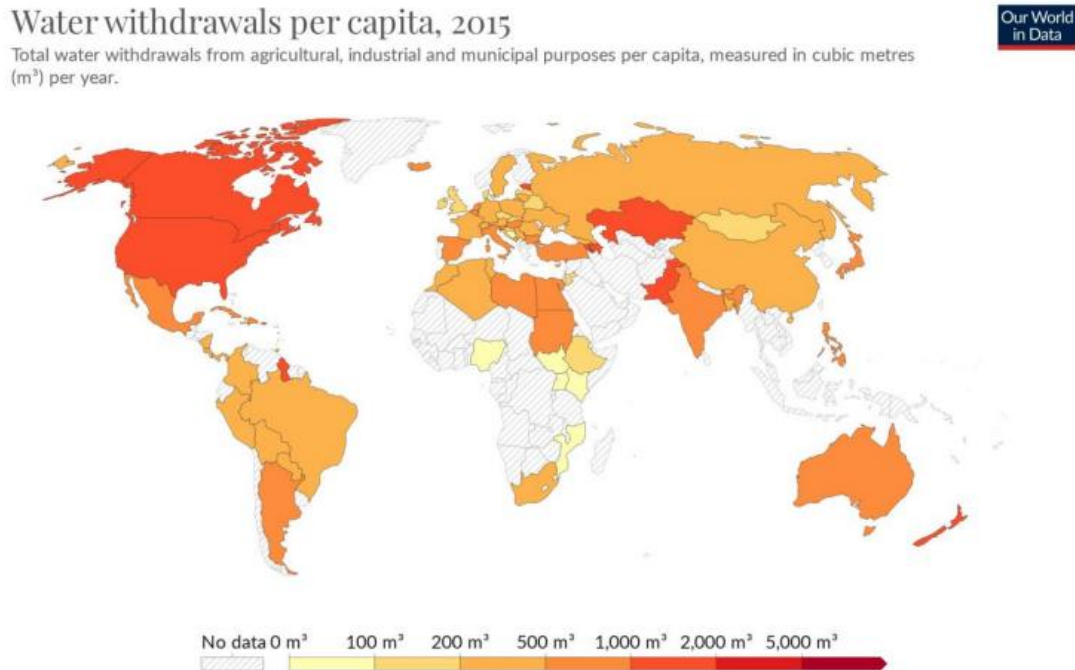
Figure 2.Water withdrawals per capita [4]

1.2 Circular economy: A sustainable approach to water management

The circular economy is an economic concept closely linked to sustainability, which aims to maintain the value of products, materials and resources (such as water, glass, paper, metals and energy) in the economy for as long as possible, while minimising the generation of waste. This approach advocates the adoption of a renewed, circular rather than linear economy, based on the principle of closing the life cycle of products, services, waste, materials, water and energy [7]. A scheme of the circular economy model is shown in Figure 3.

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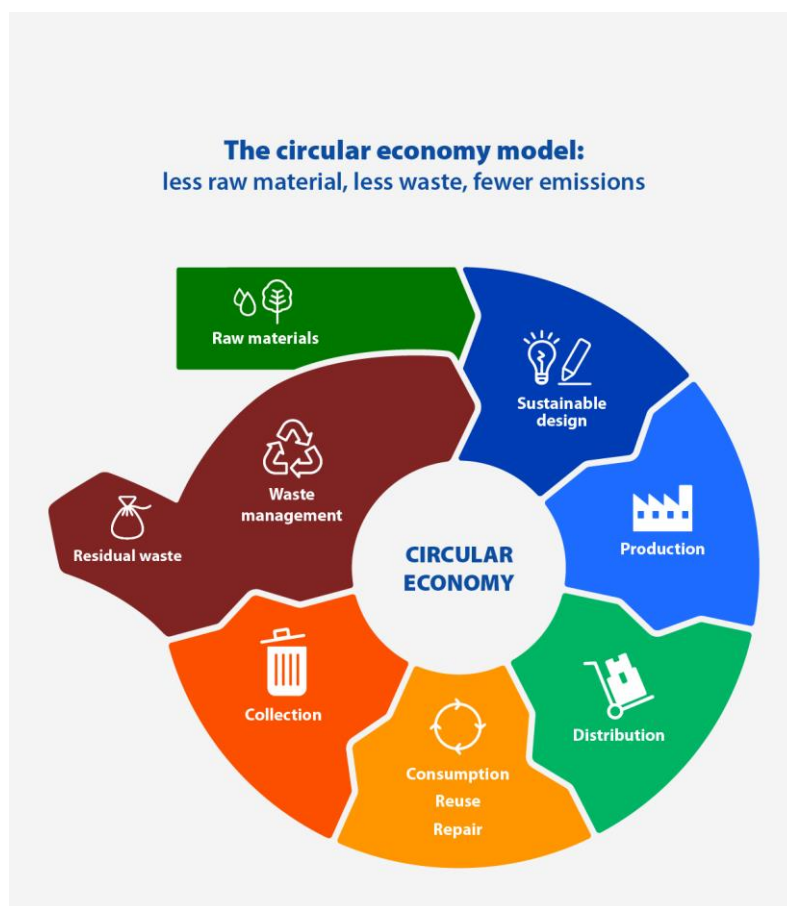
Figure 3. Circular economy model [8]

Resource efficiency in European Union (EU) is one of the central initiatives in the European continent's strategy to boost growth and jobs, endorsed by the European Parliament. This initiative aims to establish a policy framework that supports the transition to a resource-efficient and low-carbon economy, which is designed to: improve economic performance while reducing resource consumption, identifying and creating new opportunities for economic growth, boosting innovation and EU competitiveness. Therefore, the resource efficiency in EU seeks to ensure security of supply of essential resources while combating climate change, limiting the environmental impacts of resource use[9].

This initiative proposes a series of medium and long-term measures including the integration of the circular economy in the European Union. This model is based on a recycling-centred society, with the aim of reducing the production of waste by using it as a resource to produce added-value products [10]. This strategic approach not only addresses current problems, such as wastewater[10] management , but also lays the foundations for a more sustainable and resilient Europe in the future.

Several studies have demonstrated the potential of circular economy perspectives in the field of water reuse. For instance, Ortega Pozo et al., 2022, demonstrated that wastewater reclamation reduces water stress in agriculture and increases the crop yields [11]. Other works reported that the environmental and economic benefits of circular economy models applied to water reuse in industrial processes, led to significant reductions in water consumption, wastewater discharge and associated costs [2], [8].

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This context allows us to explore the potential of circular economy models in relation to water reuse and to analyse their benefits for an adequate management of this vital resource.

1.3 Aquaponics: a sustainable example of total water reuse.

Aquaponics is a bio-integrated recirculating system consisting of two key elements: an aquaculture subsystem and a hydroponic subsystem. Aquaculture is the cultivation of fish and other aquatic organisms in various aquatic environments. Hydroponics, on the other hand, is the cultivation of plants using mineral nutrient solutions in water, avoiding traditional land-based cultivation[12]. These two systems are interconnected through an aquatic medium that cycles perpetually between them, establishing a closed, self-sufficient system (see scheme in Figure 4). This method is effective and efficient for the simultaneous production of plant and fish cultures.

Figure 4. Aquaponic system [13]

The aquaponics system, designed for the intensive rearing of fish in a recirculating environment, is at the heart of an aquaponic installation. Many modern systems incorporate water treatment processes to remove toxic waste and facilitate reuse. During this recycling process, non-toxic nutrients and organic matter tend to accumulate in large quantities [14]. Unfortunately, these valuable resources are often wasted through traditional cleaning and frequent water changes, which require significant inputs of new water [15]. However, by redirecting these aquaculture by-products to a secondary crop that both utilises them directly and supports the main fish production system, these nutrients can be used effectively. This is where the hydroponic component of aquaponic systems becomes crucial. The main aquaculture system can be adapted to channel water flow to another unit that is adjacent to the hydroponic plant production component [16]. This is highly beneficial for overall crop health and performance, as aquaculture wastewater is rich in nitrogen and other nutrients essential for plant growth. Unlike traditional hydroponic systems, which often rely on artificial fertilisers due to the low

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nutrient content of pure water, the incorporation of aquaculture wastewater provides plants with a natural source of these essential elements [17].
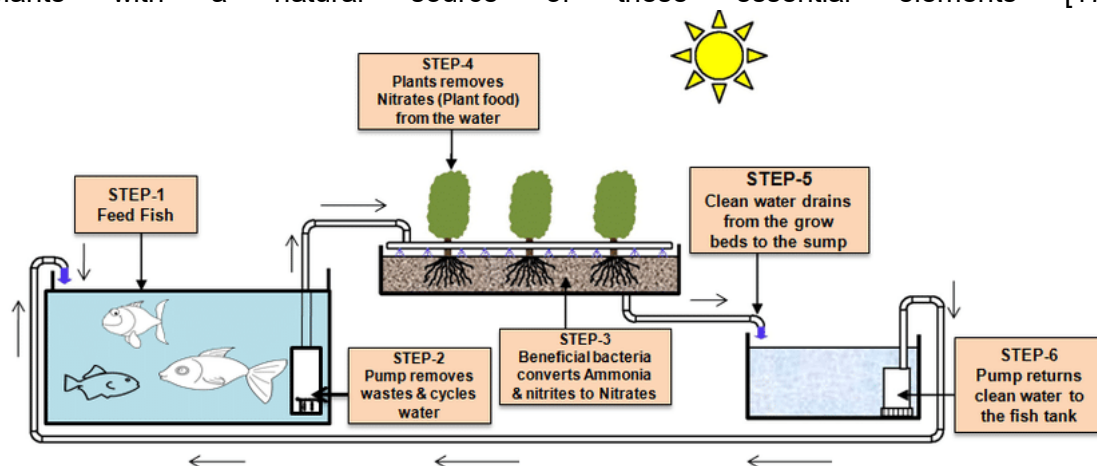
Figure 5. How an aquaponic system works. [18]

The figure 5 [18] shows an aquaponic system that integrates a smart farming method, illustrating the flow of water and nutrients in a closed cycle. The process starts in the fish tank, where the fish produce nutrient-rich waste. Water from the fish tank, loaded with these nutrients, is directed to a biofilter. Here, nitrifying bacteria convert the toxic ammonia waste into nitrates, which are essential plant nutrients.

From the biofilter, the nutrient-enriched water is channelled into hydroponic growing beds. In these beds, plant roots absorb nutrients from the water, facilitating plant growth. This efficient use of nutrients improves the health and performance of the plants.

After passing through the hydroponic growing beds, the water flows into a treatment tank. In this tank, purification processes are applied to clean the water of any remaining residue, ensuring that it is in optimal condition for the fish.

Finally, the purified water is returned to the fish tank, completing the cycle. This intelligent system of water and nutrient flow ensures continuous and efficient recirculation, benefiting both fish and plants in the process.

Aquaponics offers a sustainable solution for growing food, especially in water-scarce areas. However, as with many technologies, aquaponics is not exempt from challenges. One of the most prominent problems facing this method is the generation of wastewater, which demands effective management strategies to mitigate its impact on the environment [19] A list of the macro- and micro-elements present in these waters can be found in**Error! Reference source not found.** [20]. A summary of the hydroponic nutrient solution contains nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), boron (B), copper (Cu), manganese (Mn), molybdenum (Mo) and zinc (Zn) [21], [22].

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Table 1. List of general microelements and macroelements recommended for growing plants in hydroponics [20].

Element	Employed salts	Concentration aquaponic (mg/L)	Concentration hydroponic (mg/L)
Cu^{2+}	$CuSO_4 \cdot 5H_2O$	0.06 - 0.08	0.02 - 0.05
$Fe^{2+/3+}$	$FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$	0.09 - 0.07	5.1 - 5.2
Mn^{2+}	$MnSO_4 \cdot 4H_2O$, $MnCl_2 \cdot 4H_2O$	1.19 - 1.2	1.80 - 1.85
Mo^{6+}	Na_2MoO_4	0.003 - 0.005	0.08 - 0.09
Zn^{2+}	$ZnSO_4 \cdot 7H_2O$, $ZnCl_2$	0.16 - 0.17	0.4 - 0.5
PO_4^{3-}	$NH_2H_3PO_4$, $(NH_4)_2HPO_4$, KH_2PO_4 $Ca(H_2PO_4)_2$	6.5 - 6.6	36.5 - 37.0
$B[OH_4]^-$	H_3BO_3, $Na_2BO13 \cdot 4H_2O$, $Na_2B_4O_7 \cdot 10H_2O$	0.07 - 0.09	0.55 - 0.58
Ca^{2+}	$Ca(NO_3)_2$, $Ca(H_2PO_4)_2$, $CaCl$, $CaSO_4 \cdot 2H_2O$	125 - 130	155 - 165
Mg^{2+}	$MgSO_4 \cdot 7H2O$	20.5 - 21.5	40.5 - 42.5
K^+	KCl, K_2SO_4	50 - 51	335 - 350

1.3.1 Types of aquaponic systems

Aquaponic systems vary significantly in structure and design, each responding to specific needs and scales. When choosing a design, it is essential to consider the intended use, the size of the system and its capacity, and to match it to the desired scale and purpose. The choice of crops is equally vital, as it must be adjusted to specific growing conditions, such as temperature ranges, nutrient requirements, growth rates, root weight and density. Environmental factors, such as annual, seasonal and daily temperature fluctuations, also directly influence the productivity of the system. The three most common system designs are Media Beds, the Nutrient Film Technique, and Deep-Water Culture (DWC).

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1.3.1.1 Media Beds

Media Beds, also called Flood and Drain, aquaponic systems are user-friendly and well suited for hobbyists and home gardeners. Media Beds are also the easiest systems to build and maintain as they are simple. In a media-based system, plants are grown in a planting media like gravel, expanded clay beads, and lava rock. The media functions as a filter for organisms, parasites, and older solid waste materials. The media also provides excellent surface area for the necessary biofilter to grow, which helps reduce ammonia-waste[23]. The plant media is often held in large containers or grow beds, and the water from a separate fish tank is pumped or drained into the grow beds. The plants have immediate access to water, and the media often holds onto water longer, which ensures that plants have plenty of time to absorb necessary nutrients [23]. Thanks to the double filtration, the water is purified and then drained back into the fish tank. Figure 6
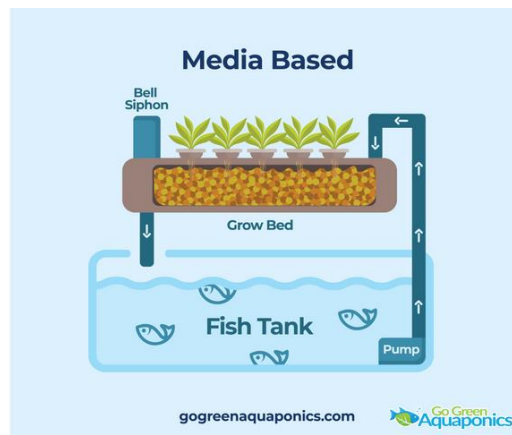
Figure 6. Media beds system [24]

1.3.1.2 Nutrient Film Technique (NFT)

The Nutrient Film Technique is a hydroponic growing technique that has been adapted to work within an aquaponics system due to the simplicity of its design and flexibility within many environments [23]. In NFT, plants are grown in long narrow channels that run horizontally with small holes that are spaced evenly apart. The plants are suspended in grow baskets, and the grow baskets placed in these holes, which allow the roots to dangle down into the channel. A pump sends a continuous thin stream of water into the bottom of each channel, where it flows over the plant roots. This provides the plant roots with adequate levels of water, nutrients, and oxygen. Once the water reaches the end of the channel, it flows back into the fish tank via a downward channel caused by a slight incline.(Figure 7)

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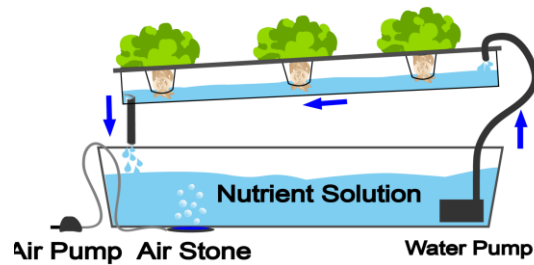
Figure 7. NFT system [25]

The NFT system requires a separate filtration system to clean the water of any solids or fish waster before it enters the channels. Otherwise, the waste can build up, and the roots can be blocked from getting oxygen[23]. The NFT system also requires an additional biofiltration component because the system does not have enough surface area to support a bacteria colony that is essential to system health. This is usually accomplished by introducing another container into the system that is situated after the solid filter, but before the NFT channels. This container can take any form, if it holds a porous medium that is heavily aerated, like the expanded clay pebbles mentioned before.(Figure 7)

1.3.1.3 Deep-Water Culture (DWC)

The Deep-Water Culture system, also known as the Raft System or Floating System, of aquaponics is one of the simplest and most efficient methods of growing produce. In DWC, plants are grown on raft boards (commonly foam boards) that float on the surface of a container where fish are housed. Plants are supported in the raft boards by net pots filled with growing media, and the plant roots hang down into the nutrient water. This allows the plants to absorb large amounts of nutrients and oxygen, aiding in rapid growth. An air pump is necessary to oxygenate the water for the fish and help the roots breathe [16].(Figure 8)

Figure 8. DWC System [26]

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Given its sustainable nature, aquaponics has the potential to revolutionise the food production industry. Conventional food production systems, such as plant cultivation and fish farming, contribute significantly to negative environmental impacts such as soil erosion, pollution and greenhouse gas emissions. In contrast, aquaponics emerges as a highly sustainable and intricate food production technology, offering a valuable solution in an increasingly urbanised world grappling with natural space limitations and food insecurity. Adapted to both urban and rural environments, aquaponic systems enable the intensive production of high-quality fresh food with minimal water use and negligible impact on climate and biodiversity[12].

1.4 Contaminants of emerging concern in water from aquaponics systems.

1.4.1 Contaminants of emerging concern definition and problematic

Contaminants of Emerging Concern (CECs) represent a growing challenge for environmental management and public health [27], [28]. These compounds, which include personal care products, pharmaceuticals, food additives, pesticides, insecticides and flame retardants, among others, are present in urban wastewater and reflect societal consumption patterns. Several scientific studies have detected the presence of these substances in lakes, rivers and oceans around the world, in concentrations ranging from nanograms per litre (ng/L) to micrograms per litre (μg/L), and in some extreme cases, milligrams per litre (mg/L)[29]. The ecological damage of the continuous release of CECs into the environment is still largely unknown, however, many of these compounds have been shown to be hazardous even at low concentrations. This issue has led to their designation as pollutants of emerging concern, underlining the need for specific attention and regulation.

In the European Union (EU), water policy legislation has started to address CECs since 2001 with the inclusion of a list of priority substances. This list, which originally contained 33 compounds, has been revised and expanded to include 45 chemicals. In addition, a watch list has been established to monitor up to 10 relevant CECs across the EU. The Joint Research Centre (JRC) has highlighted the presence of compounds such as diclofenac, ibuprofen and ciprofloxacin in urban wastewater[30]. Regarding their regulation, in drinking water, the recent revised EU directive 2020/2184, which entered into force on 12 January 2021, sets maximum tolerable limits for some CECs in drinking water, including PFASs (< 0.5 μg/L) and bisphenol A (< 2.5 μg/L). Member States have two years to transpose this directive into national legislation, underlining the growing importance of tackling CEC contamination [28]. A similar regulation has been recently entered in force for reclaimed wastewater quality [27,28].

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1.4.2 Typical CECs in Aquaponics

The most frequent CECs in hydroponic waters include pesticides, antibiotics and plant root exudates. Regarding the last ones, these are organic compounds that are released by roots into the soil or aquatic environment. These exudates are a complex mixture of chemical compounds, including carbohydrates, amino acids, organic acids, enzymes, vitamins, plant hormones (phytohormones), and other bioactive molecules [20].

Table 2. Examples of vegetable crops, bioactive compounds found to be released by their roots in hydroponics and reported nitrification. [20]

Compound	Concentration (mgL^{-1})	Sub-system found
Benzoic Acid	2	Hydroponic Systems
Phthalic Acid	3.32	Hydroponic Systems
Tebuconazole	6.15	Hydroponic Systems
Propamocarb	3.76	Hydroponic Systems
Thiabendazole	2.44	Hydroponic Systems
Sulfamethoxazole	1.15	Aquaculture Systems
Sulfapyridine	1.24	Aquaculture Systems
Trimethopim	0.95	Aquaculture Systems
Caffeine	0.97	Aquaculture Systems
Trigonelline	6.8	Aquaculture Systems
Salicylic acid	10.3	Aquaculture Systems

1.5 Present strategies for removal of emerging organic compounds in aquaponics.

Traditional wastewater treatment plants follow a series of stages to purify the influent before it is released into the environment or reused. These stages include pre-treatment, where large solids are removed by screening and sieving; primary treatment, where water is settled so that suspended solids are deposited as sludge; secondary treatment, which uses biological processes to break down dissolved organic matter using microorganisms; tertiary treatment, which may include filtration, disinfection and removal of specific nutrients; and finally, sludge treatment, where sludge is treated and dewatered for final disposal [31].

As previously mentioned, CECs present a special challenge in terms of water treatment because conventional methods (previously explained) are not sufficient for their effective removal. This is because of their low concentrations and display chemical characteristics that make them recalcitrant to standard treatment processes. Therefore, special operations and advanced technologies are required to adequately address their presence in the environment [32].

Among the most employed treatments for the removal of these pollutants are membrane technology and advanced oxidation processes (OAPs):

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1.5.1 Membrane technology

A membrane, is a physical barrier that allows the separation of two fluids, enabling the movement of different components across the physical barrier, allowing some components to pass through and restricting the passage of other components[33], [34] (Figure 9).
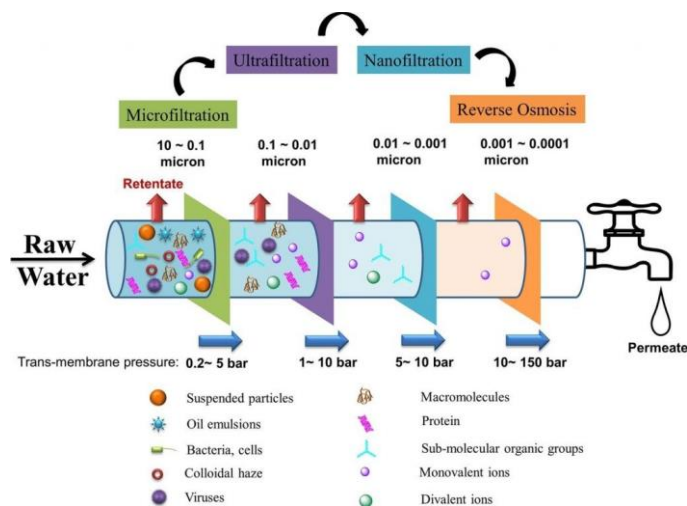
Figure 9.How a membrane works [34]*.*

These membranes can be used in ultrafiltration, nanofiltration or reverse osmosis processes to separate and concentrate contaminants in water. However, this is not a water treatment itself, specifically being a separation of pollutants instead. One must afterwards deal with the generated concentrate stream, which requires further treatment. Besides, filtration processes have high energy consumption, suffer from constant fouling, and not all the pollutants are retained.

1.5.2 Advanced Oxidation Processes (AOPs):

AOPs are a group of highly effective techniques for the removal of persistent organic pollutants in water. These techniques involve the application of potent oxidising agents, such as ozone (O_3), hydrogen peroxide (H_2O_2) and ultraviolet radiation among others to induce oxidation reactions in the contaminants present in the water [35].

The potential of advanced oxidation processes (AOPs) to remove toxic organic compounds has been extensively and preliminarily demonstrated through experimental research[36]. The application of these advanced purification techniques is becoming increasingly important as stricter legislation, tighter controls and more parameters are considered.

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Today, AOPs are effectively applied to remove contaminants in drinking water production plants, to remove biorecalcitrant micropollutants in wastewater treatment systems, and in disinfection strategies, including light-assisted disinfection[37]. These processes are based on the production of powerful reactive species (usually radicals), which can attack and mineralise almost all oxidisable substances. The advantage of AOPs is notable in engineered solutions where different processes need to be integrated to abate a large variety and concentration of target pollutants in a multi-barrier treatment strategy.

AOPs represent valuable pre-treatment options for various processes, as they effectively remove substances that can be toxic to microorganisms, which are sometimes used in subsequent biological treatments. In addition, AOPs reduce the amount of fouling that can affect membrane separation [38]. Therefore, AOPs are promising processes, especially in light of zero liquid discharge approaches, because they achieve partial or complete degradation of target substances and not simply their separation from the main effluent and their concentration in a waste stream or another phase (e.g. partitioning of contaminants onto adsorbents) [39].

The most common techniques within the field of OAPs are described in Figure 10. [40-41]The following subsections will describe them. It should be emphasised that this master thesis will focus mainly on the Fenton reaction.
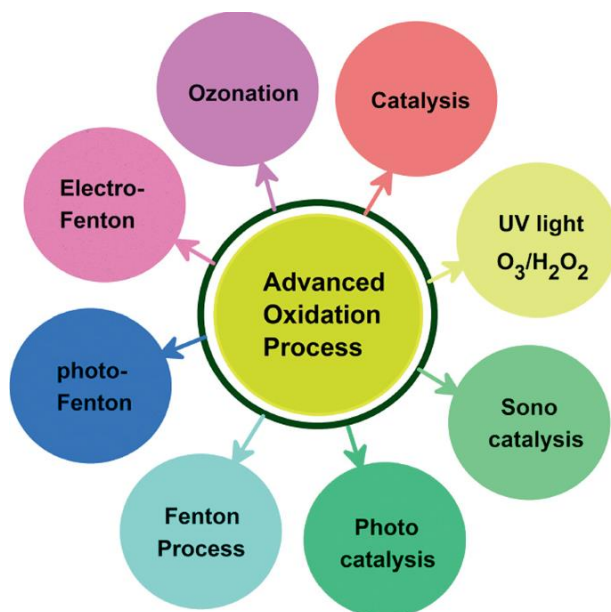
Figure 10. Most common OAPs[41]

1.5.2.1 Chlorination

Water disinfection by chlorination is a widely used procedure because chlorine possesses many of the desirable properties of an effective disinfectant (low cost, high reduction potential, and high reactivity). The main function of chlorination is to eliminate micro-organisms present in water due to the germicidal properties of chlorine. In addition to this action, chlorination is also important for its ability to destroy compounds that

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produce unpleasant odours and tastes, to remove algae and micro-organisms present in sediment, and to act as an adjuvant in the coagulation process[42].

Chlorine is a toxic gas with a pungent odour, which is denser than air and is neither combustible nor explosive. However, its residual effect is a beneficial feature, as it allows its presence in water distribution networks to be monitored, thus ensuring the potability of drinking water when its concentration is at least 0.5 mg/L [43].

Despite its advantages, chlorination has a major drawback: the generation of carcinogenic chlorinated by-products. Since the 1970s, this phenomenon has been extensively investigated, and by-products have been found to include trihalomethanes, haloacetic acids and dissolved organic halogens, some of which have been identified as potentially carcinogenic at concentrations below 0.1 mg/L. This finding underlines the need to carefully consider alternative disinfection methods and to monitor chlorine residual levels to minimise the public health risks associated with water chlorination [44].

1.5.2.2 Ultraviolet radiation (UV)

Ultraviolet (UV) radiation is a widely adopted disinfection method in water treatment due to its many advantages and desirable properties. This technique uses short wavelength electromagnetic radiation in the range of 200 to 400 nanometres (nm) to inactivate pathogenic micro-organisms present in water.

The main objective of UV disinfection is to eliminate micro-organisms, such as bacteria, viruses, algae and protozoa, by exposure to the UV spectrum[42]. This action is achieved by damaging the genetic material of the micro-organisms, which prevents their ability to reproduce and thus renders them inactive. In addition to this primary function, UV radiation can also assist in the oxidation of organic compounds present in water, although its effectiveness in this respect is limited compared to other oxidation processes.

One of the main benefits of UV radiation is that it does not require the use of additional chemicals, which makes it environmentally friendly and safe for human consumption. In addition, it does not leave chemical residues in the treated water and does not affect the taste, odour or chemical properties of the water [45]. UV radiation is also highly effective in killing a wide range of micro-organisms, even those that are resistant to other disinfection methods [46].

Nevertheless, UV radiation has limitations. As an example, its effectiveness may be reduced if the water contains high levels of turbidity, suspended solids or organic compounds that can absorb UV radiation . In addition, UV radiation does not provide a residual effect on the treated water, which means that it does not protect against microbiological contamination after disinfection. However, it is important to consider its limitations and its high cost of use.

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1.5.2.3 Ozonation (O_3)

Ozonation is a method of water disinfection and treatment that has gained popularity due to its various advantages and useful properties compared to other conventional methods.

The main objective of ozonation is to deactivate micro-organisms, including bacteria, viruses, algae and protozoa, through the oxidative action of ozone. Ozone acts as a powerful oxidant, attacking and degrading the cell membranes of micro-organisms, resulting in their inactivation and death [47]. In addition to this germicidal function, ozonation can also aid in the oxidation of organic compounds present in water, as well as in the elimination of unpleasant odours and tastes.

One of the main advantages of ozonation is its ability to disinfect water quickly and effectively, with short contact times compared to other disinfectants. In addition, ozone leaves no chemical residues in the treated water and does not adversely affect the taste, odour or chemical properties of the water. It is also effective over a wide range of pH and temperature conditions [48].

However, ozonation has some limitations. For example, ozone is a highly reactive gas and must be generated on-site using special equipment, which can increase installation and operating costs. In addition, seawater fish factories contain in its composition high levels of bromine (ca. 1 mM = 80 mg/L), and in contact with O_3 generates BrO_3^-, a harmful and carcinogenic substance [49].

1.5.2.4 Fenton process

The traditionally accepted Fenton mechanism involves the oxidation of ferrous to ferric ions to decompose H_2O_2 into hydroxyl radicals ($HO^\bullet$), represented by equations (1-9) [50]. In addition, other reactions, such as the regeneration of ferrous ions by the Fenton-like reaction (equation 2), are crucial to understand the whole process. Hydroperoxyl radicals ($HO_2^\bullet$) can also attack organic pollutants, although they are utterly less reactive than hydroxyl radicals.

The limiting step is typically the reaction between hydrogen peroxide (H_2O_2) and ferrous ion (Fe^{2+}) to produce the hydroxyl radical ($HO^\bullet$) (Equation(2)), which is the main reactive species that carries out the oxidation of organic pollutants. Equations (6)-(9) also occur during the Fenton process and are radical-radical reactions or hydrogen peroxide-radical reactions [51]. The decomposition of hydrogen peroxide into molecular oxygen and water occurs according to equation (10). The production of the desired hydroxyl radical occurs via the initiation chain reaction (Eq. (1))[52].

Hydroxyl radicals can be captured by ferrous ions (Eq. (3)), hydrogen peroxide (Eq. (7)), hydroperoxyl radicals (Eq. (9)), and can even be self-captured (Eq. (6)). The above analysis indicates that an excess of Fe(II) and/or hydrogen peroxide can act as both a radical generator and scavengers [53].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \qquad \text{k= 76 M}^{-1}\text{ s}^{-1} \text{ (1)}$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^\bullet + H^+ \qquad \text{k= 0.01 M}^{-1}\text{ s}^{-1} \text{ (2)}$$

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$$Fe^{2+} + OH^{\bullet} \rightarrow Fe^{3+} + OH^{-} \tag{3}$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{3+} + HO_2^{-} \tag{4}$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^{+} \tag{5}$$

$$OH^{\bullet} + OH^{\bullet} \rightarrow H_2O_2 \tag{6}$$

$$OH^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O \tag{7}$$

$$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \tag{8}$$

$$OH^{*} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \tag{9}$$
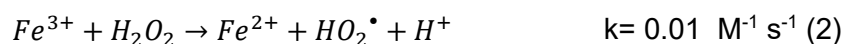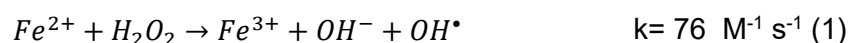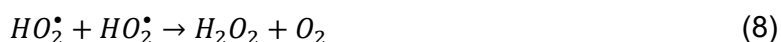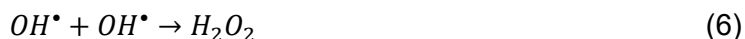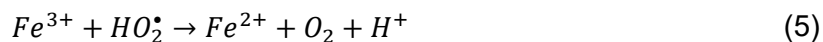
The Fenton process can be carried out at room temperature and atmospheric pressure. In addition, the required reagents are readily available, easy to store and handle, safe and do not cause environmental damage.

The Fenton reaction with iron ions shows its maximum efficiency at very low pH, approximately 2.8. This is the main problem, as at pH close to neutral (normal pH of water systems), this method loses its effectiveness due to iron precipitation at pH>4 [54]. The formation of iron sludge not only reduces the efficiency of the oxidation process, but also poses a significant environmental challenge for water management. This sludge must be properly treated and disposed of to avoid ecological problems[55].

1.5.2.5 Strategies to enhance the Fenton process

Commonly employed approaches to accelerate the reductive conversion of Fe(III) to Fe(II) in the Fe(III)/H_2O_2 system include irradiation with UV light (photo-Fenton), ultrasound (sono-Fenton), and the application of electricity (electro-Fenton)[56]. Combination of them is also a possibility (e.g., electro-photo-Fenton).

In absence of an external energy input (light, ultrasound, or electricity), to work at higher pH, the use of iron chelating and reducing substances has been studied [57]. The chelating substances form stable complexes with the iron ions, preventing their precipitation even at circumneutral pH conditions, which allows the Fenton reaction to be carried out in a wider pH range without the formation of iron sludge. Recently, it has also been shown that the addition of hydroxylamine (HA), a reducing agent, is a suitable method for the facile reduction of Fe(III). [58]

1.5.2.6 Fenton-like process with other transition metals: copper

Similar to iron, copper can also convert H_2O_2 to reactive oxidants via the Cu(II)/Cu(I) catalytic redox cycle (Eq. 14-18). Previous reports suggest that the nature of the reactive oxidants in the copper-catalysed Fenton-type system may also depend on pH; OH$^{\bullet}$ and

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cupryl ion (Cu(III)) are predominantly produced under acidic and neutral/alkaline conditions, respectively [59]. Similarly, as with iron, the reaction between Cu(I) and H_2O_2 (14 - 15) is much faster than that of Cu(II) and H_2O_2 (16), thus, the latter being the rate limiting step.

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + HO^\bullet + OH^- \qquad \text{k=4 x } 10^5 \text{ M}^{-1}\text{ s}^{-1} \text{ (14)}$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{3+} + OH^- \qquad \text{k=4 x } 10^5 \text{ M}^{-1}\text{ s}^{-1} \text{ (15)}$$

$$Cu^{2+} + H_2O_2 \rightarrow Cu^+ + HO_2^\bullet \qquad \text{k< 1 M}^{-1}\text{ s}^{-1} \text{ (16)}$$

$$Cu^+ + O_2^{\bullet-} + 2H^+ \rightarrow Cu^{2+} + H_2O_2 \qquad \text{k=2.0 x } 10^9 \text{ M}^{-1}\text{ s}^{-1}\text{(17)}$$

$$2H^+ + 2O_2^{\bullet-} \rightarrow H_2O_2 + {}^1O_2 \qquad \text{(18)}$$
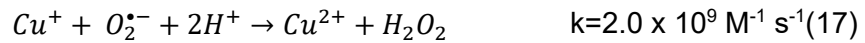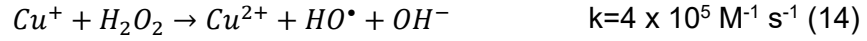
Primarily, HA reduces Cu(II) to Cu(I) according to reactions 19 and 20 [60].. Subsequently, the produced Cu(I) reduces O_2 into H_2O_2 (reactions 21 and 22). HA can be also oxidised by dissolved oxygen generating further H_2O_2 (reaction 23). However, this reaction seems to be minor due to the low reaction rate (k=9.4 x 10^{-2} M^{-1} s^{-1}) , being HA considerably stable even under aerobic conditions [61].

Finally, the reaction of Cu(I) with H_2O_2 (reactions 14 - 15) produces reactive oxidants such as OH$^\bullet$ and Cu(III), capable of oxidising CECs (reaction 25). In this series of reactions, most of the HA is released as N_2O and N_2 gases without leaving residual nitrogen products in solution (nitrites, nitrates or residual HA). HA can also act as a scavenger of reactive oxidants, its initial concentration being detrimental factor to avoid a process hindrance.

$$NH_2OH + 2Cu^{2+} \rightarrow {}^1/_2\, N_2O + {}^1/_2\, H_2O + 2Cu^+ + 2H^+ \qquad \text{(19)}$$

$$NH_2OH + 2Cu^{2+} \rightarrow {}^1/_2\, N_2 + H_2O + 2Cu^+ + 2H^+ \qquad \text{(20)}$$

$$Cu^+ + O_2 \rightarrow Cu^{2+} + O_2^{\bullet-} \qquad \text{k=3.1 x } 10^4 \text{ M}^{-1}\text{ s}^{-1} \text{ (21)}$$

$$Cu^{2+} + O_2^{\bullet-} \rightarrow O_2 + Cu^+ \qquad \text{(22)}$$

$$NH_2OH + O_2 \rightarrow N_2 + H_2O + H_2O_2 \qquad \text{k=9.4 x } 10^{-2} \text{ M}^{-1}\text{ s}^{-1} \text{ (23)}$$

$$OH^\bullet + NH_2OH \rightarrow H_2O + NHOH \qquad \text{k=5 x } 10^8 \text{ M}^{-1}\text{ s}^{-1} \text{ (24)}$$

$$CECs + OH^\bullet \text{ or } Cu^{3+} \rightarrow Products \qquad \text{(25)}$$

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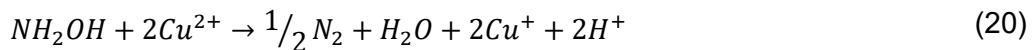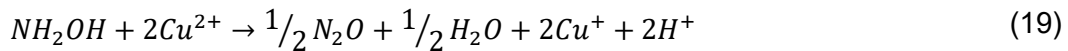
2. Objectives and Assumptions

The objective of this study is to explore the use of HA as a co-catalyst in the Fenton-like reaction with Cu(II) ions, the latter a transition metal which is already present in aquaponic systems. The purpose is to study if this AOP is effective towards the degradation of emerging pollutants present in these systems, being HA the only reagent to add. To test its performance, benzoic acid (BA) has been used as a model pollutant following its removal as a function of time.

In order to achieve this main objective, the following specific objectives have been set:

- Understand the involved mechanism in the Cu(II)/HA system.

- Explore the effect of the different operational parameters in a system containing 100 μM of Cu(II): i) initial pH, ii) initial HA concentration, iii) presence or absence of H_2O_2 1 mM at the beginning of the reaction.

- Analyse the effect of other water constituents: i) anions (Cl^-, SO_4^{2-}, HCO_3^-, HPO_4^{2-}, NO_3^- and NO_2^-), ii) cations (Fe(III), Mn(II), and Mo(VI)), iii) dissolved organic matter (by means of humic acid), iv) chelating agents (catechol and EDTA).

- Utilise the proposed system in a real aquaponics environment, as well as test a combination of emerging pollutants to evaluate the effectiveness of the process.

On the other hand, the assumptions established in this project are:

- The Cu/HA process has the ability to degrade benzoic acid, with the generation of hydroxyl radicals being the main cause of this degradation process.

- Adjusting the pH of the Cu/HA system to near neutral is expected to enhance the efficiency of benzoic acid degradation. This is due to the sensitivity of Cu ions to pH variations.

- Anions as chloride and sulphate are not expected to influence the functioning of the Cu/HA system. In contrast, nitrites are expected to completely inhibit the degradation process.

- It is considered that chelating species can form complexes with copper, which could block the reaction. However, it is suggested that in combination with iron ions, these species could improve the process.

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The Sustainable Development Goals (SDGs) are 17 interconnected global goals designed to be a "blueprint for achieving a better and more sustainable future for all". These goals were established in 2015 by the United Nations General Assembly, with the aim of achieving them by 2030 [62].Within the 17 global goals, the project focuses mainly on number 6 (clean water and sanitation) and 14 (underwater life). This is because the study aims to reduce the amount of pollutants produced in aquaponics water. In this way, it encourages the reuse of water for other purposes and contributes to keeping it clean (objective 6), as well as to the proper development of the habitat of the underwater life found in these waters (objective 14).

Figure 11. Sustainable Development Goals [62]

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3. Methodology

3.1 Reagents

Several reagents have been used in this study, which are described below. On first, the emerging pollutant used is benzoic acid (BA, $C_7H_6O_2$), which was added to the solutions to be treated in the reactor in very small quantities, in the order of 50 µM. It belongs to the Aldrich Chemical Company and has a purity of 98%.
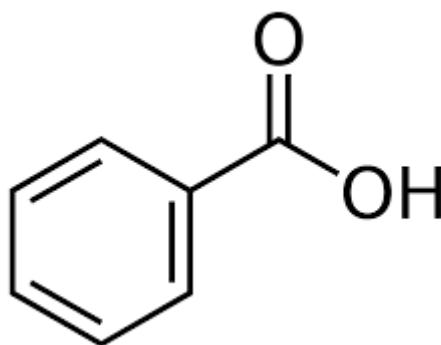
Figure 12. Structural formula of benzoic acid.

The goal, as mentioned above, is to degrade the named CEC. To succeed in the oxidation of this compound, hydroxylamine hydrochloride is incorporated into the system. In this project, hydroxylamine hydrochloride from Aldrich Chemical Company is used, with a purity of 99%.
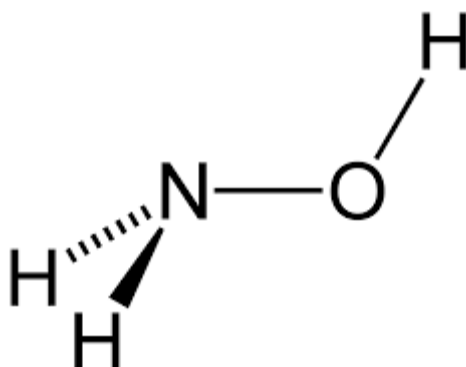
Figure 13. Structural formula of Hydroxylamine.

Due to the use of hydroxylamine in combination with the copper present in the hydroponic water, 100 µM anhydrous copper sulphate from Sigma Aldrich Chemical, with

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a purity of 98 %, is used. This compound is also used to simulate copper in aquaponic water conditions.

Once the three main reagents of our project have been mentioned, the following tables detail the other reagents used to carry out all the stages of the study. This information is essential to understand the totality of the experimental processes carried out.

Table 3. Products involved in the project.

| Products attached to the project	
Product	**Manufacturer**
H_2O_2 33% m/V	PanReac AppliChem
EDTA	Sigma Aldrich
CH_3OH, UHPLC grade	PanReac AppliChem
NaOH	PanReac AppliChem
$HClO_4$ 70%	PanReac AppliChem
$Fe(ClO_4)_3 \cdot H_2O$	Sigma Aldrich
$MnSO_4 \cdot H_2O$	Sigma Aldrich
Na_2MoO_4	Sigma Aldrich
NaCl	Sigma Aldrich
Na_2SO_4	Sigma Aldrich
$NaHCO_3$	Sigma Aldrich
$NaNO_3$	PanReac AppliChem
$NaNO_2$	Montplet & Esteban SL
Na_2HPO_4	Scharlau
NaH_2PO_4	Scharlau
Catechol	Sigma Aldrich
Humic Acids	Sigma Aldrich
5,5-Dimethyl-1-pyrroline N-oxide	Sigma Aldrich
2,2,6,6-tetramethylpiperidine	Sigma Aldrich
Isopropyl alcohol	Sigma Aldrich
Furfuryl alcohol	Sigma Aldrich
Benzoquinone	Sigma Aldrich
Ascorbic Acid	Sigma Aldrich
Neocuprine	Sigma Aldrich
Orthophenanthroline	Sigma Aldrich
Peroxidase	Sigma Aldrich
Phenol	Sigma Aldrich
4-Aminopyridine	Sigma Aldrich
H_3PO_4 85%	VWR Chemicals
Acetone, >97%	VWR Chemicals
Acetonitrile, UHPLC grade	PanReac AppliChem
Thiabendazole	Sigma Aldrich
Sulfamethoxazole	Sigma Aldrich

All the products used for the successful completion of this project having been mentioned, the preparation of the main reagents will be briefly explained. Stock solutions of benzoic acid (10 mM), hydroxylamine (1 M), copper(II) (10 mM) and H_2O_2 (1 M) were prepared. It is important to note that the hydroxylamine stock solution is prepared daily due to its degradation over time, which prevents its prolonged storage.

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3.2 Benzoic acid degradation experiments

Open batch reactors served as the experimental environment (Figure 15). Each was filled with a 100 mL solution containing a fixed concentration of 50 µM benzoic acid (BA), model CEC used for the system, and 100 µM copper (Cu(II)) ions. When needed, HA was added, ranging from 0.05 to 5 mM initial concentration, whereas H_2O_2 was employed at a fixed concentration of 1 mM. pH was adjusted employing NaOH 0.1 M or $HClO_4$ 0.1 M solutions, depending on the specific requirements of each experiment. Degradation experiments were carried out over a period of 4 hours, taking samples at different time intervals (0, 30, 60, 120, and 240 min).

Figure 14. Experimental process

In addition to the main reagents mentioned above, the concentrations of other components were taking into consideration in the water. This included the presence of: i) selected anions (Cl^-, SO_4^{2-}, HCO_3^-, HPO_4^{2-}, NO_3^- and NO_2^-) at a concentration of 1 mM, respectively, ii) transition metals (Fe(III), Mn(II), and Mo(VI)) 100 µM, respectively, iii) chelating agents, EDTA or catechol, at 100 µM, respectively, iv) humic acids at a concentration of 10 - 100 mg L^{-1}. These additional components were carefully selected to simulate all possible conditions of an aquaponics water.

3.3 Analytical methods

This subsection will detail and describe all the analysis procedures that were carried out, which are fundamental for the interpretation of the results obtained.

3.3.1 High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography (HPLC) is used to study the evolution of BA and HA evolution during the procedure, as well as the appearance of other compounds (TBZ, SMX). This equipment works based on specific interactions between the molecules (analytes) of each sample and two phases: the stationary phase (the column) and the mobile phase passing through the column.

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In reverse phase chromatography, the C18 stationary phase consists of silica particles whose surface is modified with silanol groups of 18 carbons length, whereas the mobile phase is a mixture of two liquids: a non-polar organic phase (e.g., methanol or acetonitrile) and a polar aqueous phase (e.g., H_3PO_4 solutions). Reverse phase columns, such as C18, are so called because they are used with a higher proportion of aqueous than organic phase, and the organic phase is later on increased to elute the analytes. In contrast, normal phase columns, which are hardly used any more, since they operate with a polar stationary phase (containing amino groups), chromatographic methods start with higher proportion of non-polar organic solution as mobile phase, and the aqueous phase is later on increased to elute the analytes.

In this work, benzoic acid (BA) was eluted with a mobile phase consisting of a solution of 14 mM phosphoric acid (H_3PO_4) and methanol (60:40). Detection was performed at a wavelength (λ) of 235 nm. For catechol, the mobile phase consists of a solution of 14 mM phosphoric acid (H_3PO_4) and methanol (70:30). Detection is performed at a wavelength (λ) of 280 nm. For sulfamethoxazole (SMX), the mobile phase consists of a solution of 14 mM phosphoric acid (H_3PO_4) and methanol (75:25). Detection is performed at a wavelength (λ) of 267 nm. For thiabendazole (TBZ), the mobile phase consists of a 10 mM phosphate solution ($H_2PO_4^- + HPO_4^{2-}$) in acetonitrile (50:50). Detection is performed at a wavelength (λ) of 285 nm. Hydroxylamine (HA) is derivatized with acetone. During this process, hydroxylamine reacts with acetone to form the corresponding oxime, known as hydroxylamine oxime. The mobile phase for HA consists of a solution of 10 mM phosphate ($H_2PO_4^- + HPO_4^{2-}$) in acetonitrile (97:3). Detection is performed at a wavelength (λ) of 210 nm. In all measurements, the isocratic mode with a flow rate of 1 mL/min and a column temperature of 35°C is used.

Figure 15. HPLC LaboLine YL9150 Autosampler

Figure 18 shows all the calibrating straight lines carried out.

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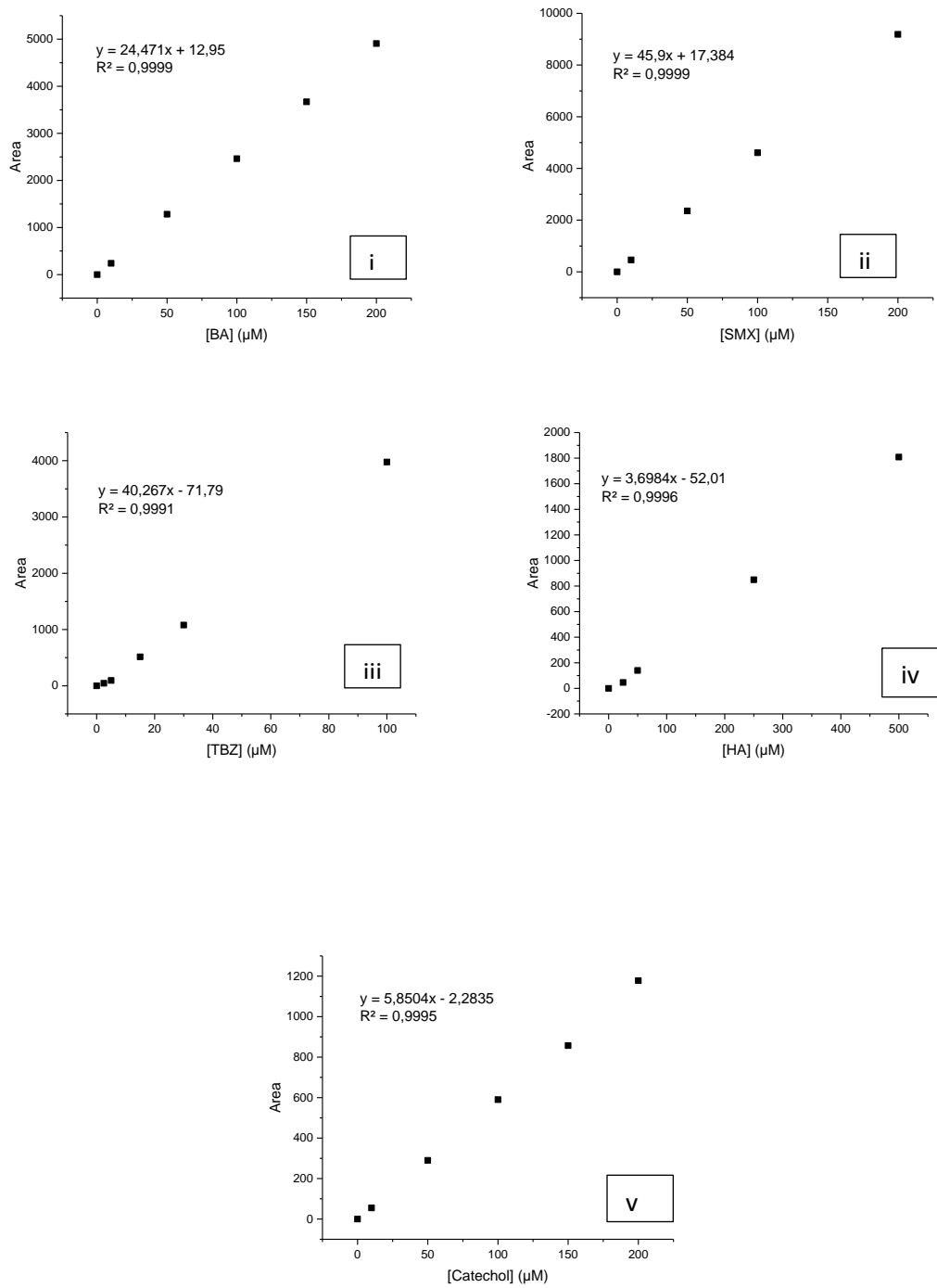
Figure 16. Calibration lines. i)BA; ii)SMX iii)TBZ; iv)HA; v)Catechol.

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3.3.2 Spectrophotometry

Absorbance is a measure of how radiation is reduced as it passes through an analyte and can be expressed as a logarithm representing the ratio between the intensity of radiation leaving and entering the substance, respectively (transmittance). According to the Lambert-Beer law:

$$A = -logT = -\log\left(\frac{I}{I_0}\right) = \varepsilon bc$$

Figure 17. Cary Scan 100 Spectrophotometer

3.3.3.1 Spectrophotometric copper determination

The quantification of copper in water samples is carried out by its reaction with ascorbic acid and Neocuprine 1.3M in an ammonia/ammonium buffered medium (pH=9). Subsequently, a colour change to yellowish is observed, measuring the resulting absorbance at a wavelength of 450 nm is detected, The colour is caused by the formation of a complex Cu(I)-Neocuprine. The addition of ascorbic acid (reducing agent) gives the value of total copper, while the sample without ascorbic acid is used to determine the Cu(I) concentration [63]. Cu(II) concentration is known by making the difference between Cu(total) and Cu(I).

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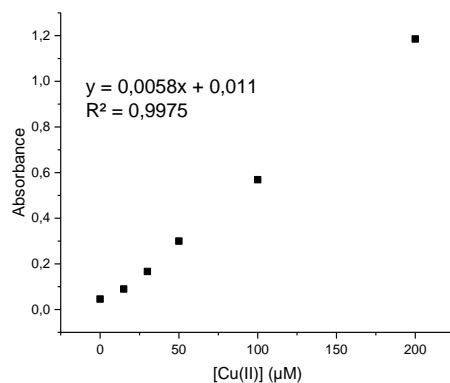
Figure 18. Calibration line Neocuprine method

3.3.3.2 Spectrophotometric iron determination

Quantification of iron in water samples is carried out by the reaction between ascorbic acid, 0.1% m/V orthophenanthroline and iron in an acetic/acetate buffered medium (pH=4). Subsequently, a colour changes to red at a wavelength of 510 nm is detected, the colour is caused by the formation of the complex Fe(II)-orthophenanthroline. Similarly as with total copper determination, the addition of ascorbic acid indicates the total iron concentration, while the sample without ascorbic acid reveals the Fe(II) concentration [64].
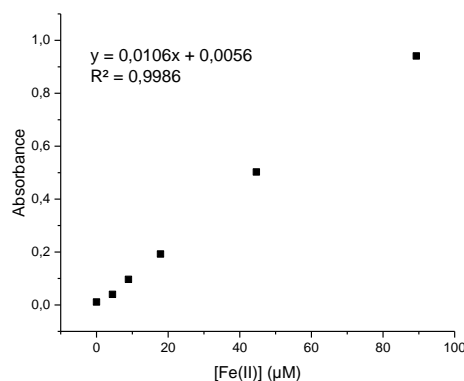
Figure 19. Calibration line Orthophenanthroline method

3.3.3.3 Spectrophotometric hydrogen peroxide determination

H_2O_2 concentration was measured by colorimetric method, adapted from [65], employing a solution containing 0.234 % m/V of phenol, 0.10 % m/V of 4-aminoantipyrine, 0.0010% m/V of peroxidase, dissolved in buffer phosphate pH 7.0, and measuring the resulting

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absorbance at 505 nm. This technique allows precise determination of the concentration of hydrogen peroxide present in the sample analysed.
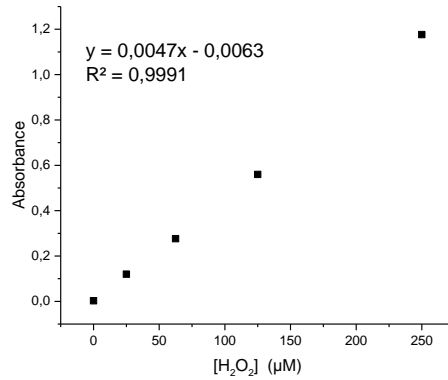
Figure 20.Calibration line peroxidase method.

3.3.3 Electron paramagnetic resonance (EPR)

Electron paramagnetic resonance (EPR) is a spectroscopy technique used to study systems with unpaired electrons or electron spins. In an external magnetic field, the unpaired electrons generate a characteristic resonance signal that can be detected and measured. EPR is used to investigate the electronic structure of molecules, the kinetics of chemical reactions, the interaction between chemical species and the dynamics of biological systems, among other fields. It is particularly useful in the characterisation of free radicals, transition metals and metal complexes.
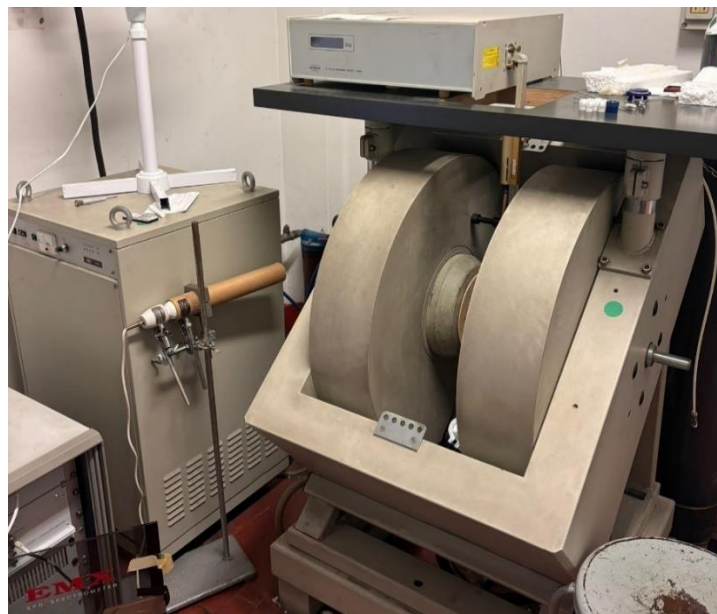
Figure 21. EMX EPR spectrometer

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In this study, electron paramagnetic resonance experiments (EPR) were performed in order to identify and characterise the radicals involved in the process in question. To carry out these experiments, two commonly used radical scavengers were employed: 5,5-dimethylpyrrolidine-N-oxide (DMPO) at a concentration of 18 mM and 2,2,6,6-tetramethylpiperidine (TEMP) at a concentration of 77 mM. These radical scavengers are used to capture and stabilise transient radicals formed during chemical reactions, allowing their subsequent detection by the EPR. TEMP was used for the detection of singlet oxygen radical and DMPO for the determination of hydroxyl radicals ($OH^{\bullet}$). In addition, it is important to note that several studies indicate that the use of DMPO and methanol allows differentiation of the main reactive species. If the hydroxyl radical ($OH^{\bullet}$) is the main reactive species, $-CH_2OH$ is formed, and the signals corresponding to DMPO-CH_2OH are observed. On the other hand, if Cu(III) is the main reactive species, CH_3O^- will be formed, and the adduct observed in the EPR will be DMPO-OCH_3.

Furthermore, together with the EPR technique, selective scavenging experiments were carried out in order to try to validate the results obtained with the EPR technique. The scavengers used were furfuryl alcohol to trap singlet oxygen, benzoquinone to trap the superoxide radical, and isopropyl alcohol to neutralise the hydroxyl radical.

3.3.4 Total Organic Carbon (TOC) and inorganic carbon (IC)

TOC analysis is a technique used to determine the total amount of organic carbon present in a sample, either liquid or solid. In TOC analysis, the sample is heated to 680°C in the presence of a platinum catalyst bonded to alumina spheres. The total carbon (TOC + IC) is converted into CO_2, which is transported by high purity air, cooled and dried with a dehumidifier. The CO_2 produced during burning is then quantified by a non-dispersive infrared (NDIR) sensor, resulting in a peak whose magnitude is related to the amount of carbon in the sample.

Figure 22. TOC-V CSH SHIMAZDU

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TOC analysis is an important tool to evaluate water quality, in this project, this equipment has been used for the characterisation of the real aquaponics water.´

Inorganic carbon (IC) refers to the carbon present in inorganic compounds in a water sample, such as dissolved carbon dioxide, carbonates and bicarbonates. To measure IC, the sample is acidified to convert carbonates and bicarbonates into CO_2. To carry out the CIT measurement, the sample is placed in a reaction vessel with 25% phosphoric acid and air is passed through it. Carbon dioxide is produced from the decomposition of carbonates and bicarbonates, which is transported by the high-quality air and analysed in the NDIR in the same way as for TC. This process provides a measure of the CI in the sample.

Total organic carbon (TOC) is calculated by subtracting the CI from the total carbon (TC) measured in the sample:

$$TOC = TC - IC$$

3.3.5 Other measuring/analytical equipment

3.3.5.1 pHmeter

The pH meter operates by measuring the voltage between two electrodes, one of which is an electrical conductor for non-metallic components. It then displays the value of this voltage transformed into pH levels. This process is carried out thanks to its structure, which consists of two rods with electrodes: one made of calomel (Ag/AgCl) and the other of glass. The glass used to construct the sensor electrode is polarisable, which makes it sensitive to the concentration of hydrogen ions in the sample. In this way, one electrode acts as a reference while the other changes the charge of the ions present in the sensor to positive.

This potential difference between the electrodes makes the pH measurement possible. It is important to note that pH represents the hydrogen potential of a substance; therefore, the concentration of hydrogen ions in a sensor and the resulting potential difference allow for accurate determinations of pH levels.

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Figure 23. pHmeter metrohm 691

In the context of this study, pH measurement is crucial firstly to adjust the solution to the acidity levels required for the experiments. Secondly, it is used to monitor the evolution of the pH during the experimental process and, finally, to ensure that the solution is brought back to appropriate pH levels according to the applicable legislation for the following procedures.

3.3.5.3 Conductivity

Conductivity is an instrument used to measure the electrical conductivity of a solution, which provides information about the concentration of ions present in that solution. Conductivity is a property that indicates the ability of a solution to conduct electricity and is measured in siemens per metre (S/m). The conductivity meter uses a probe with two electrodes that are immersed in the solution, a small alternating voltage is applied to the electrodes, generating an electric current proportional to the amount of ions present. This current is measured and converted into a conductivity reading. Before use, the conductivity meter must be calibrated with standard solutions of known conductivity to ensure accuracy. It is an essential tool in various fields, such as water quality control, agriculture, the food industry and scientific research, due to its ability to provide fast and accurate information on the concentration of ions in solutions.

Figure 24. Conductivity CRISON Basic 30.

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3.3.5.2 Ultra-pure water converter

Finally, it is worth mentioning that all these experiments have been carried out using MQ water. This choice is based on the need to ensure the purity of the reaction medium, thus avoiding the introduction of any type of contaminant that could interfere with the results obtained. By using MQ water, we ensure that we have a high-quality solvent, free of impurities that could distort the results of our research. This meticulous approach guarantees the reliability and accuracy of the data obtained at each stage of the experimental process.

Figure 25. Milllipore synthesis quantum

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4. Results and Discussion

4.1 Determination of the operational parameters.

In the following section, results related to the operating parameters that influence the maximum efficiency of the system will be discussed and analysed. Specifically, the optimal concentration of Hydroxylamine (HA) to be added to the system and the ideal pH of the process will be examined.

4.1.1 Optimal hydroxylamine concentration

A sequence of experiments was undertaken to determine the optimal concentration of HA for the process. HA additions were made from 0 µM to 1000 µM. It was observed that the highest degradation enhancement peak occurred in the experiment with a concentration of 250 µM of HA, achieving a 60% reduction in BA concentration within 60 minutes.
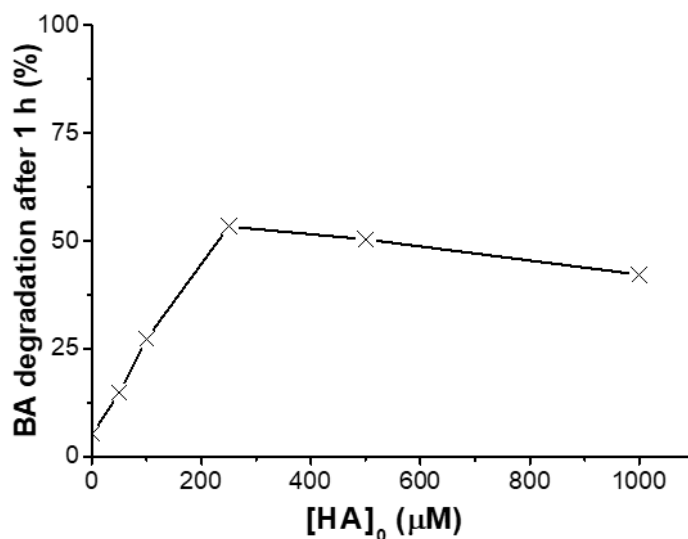

Figure 26. Optimal [HA]. [BA]=50 µM; [Cu(II)]=100 µM; pH_0=7

These results relate directly to the following Table 4, as the concentration of hydroxylamine increases, the pH becomes more acidic, moving away from the optimal pH of the mechanism, as we will see in the next subsection.

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Table 4. Final pH

[HA] (µM)	Final pH
0	7.6
50	6.84
100	6.89
250	6.5
500	3.8
1000	3.5

4.1.2 Optimal pH

According to the literature, it has been observed that Fenton-like process with copper has a higher operating efficiency at near neutral pH [67], in contrast to iron, whose effectiveness decreases as pH increases, as it tends to precipitate above pH 3 [68]. Therefore, a series of experiments were carried out to determine the best efficiency of the system at a concentration of 250 µM HA.
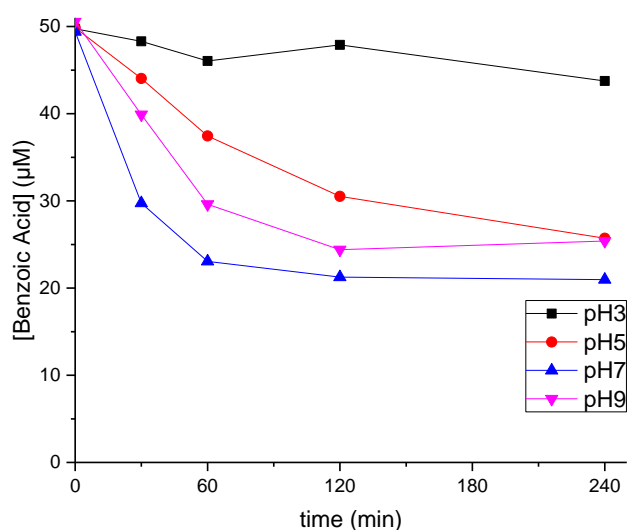
Figure 27. Optimal pH. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM

The results of the experiments revealed a clear trend: as the pH of the system moves away from neutral values towards more acid conditions, a significant decrease in the efficiency of the degradation process is observed. This suggests that the system undergoes a noticeable inhibition in acid environments, which could be related to changes in the catalytic activity of the oxidising agents involved. On the contrary, it was observed that at pH close to neutral-alkaline, specifically around 7, the best results in terms of pollutant degradation were obtained, indicating that the system is more effective in slightly acidic to neutral conditions. This is due to the following reasons:

- The Cu(II)/H_2O_2 system has a higher rate constant (k) at pH 7 compared to pH 3. This means that the process is more efficient under more neutral conditions.

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- HA needs to be in its NH_2OH form to function as a reducing agent, as the protonated NH_3OH^+ form does not reduce Cu(II) ($pK_b = 8$). Therefore, a higher concentration of HA can be problematic, as it also acts as an acid.

- The reagent used was $NH_2OH \cdot HCl$ (or $NH_3OH^+ Cl^-$), which is, evidently, an acid. As the HA concentration is increased, the pH becomes more acidic. This pH change with increasing HA concentration is crucial, as it moves away from the optimum pH for the Cu(II) reduction mechanism and affects the efficiency of the process.

Based on these observations, it was determined that the optimal combination for maximum efficiency of the system is to maintain a pH around 7 and a concentration of 250 µM HA. These conditions provide a favourable environment for copper activity against H_2O_2 as well as $[NH_2OH] > [NH_3OH^+]$, resulting in increased efficiency in the degradation of emerging contaminants. This is explained by the formation of Cu(II)-HA complex intermediate [69], [70]

$$Cu^{2+} + NH_2OH \rightarrow Cu(NH_2OH)^{2+} \qquad (26)$$

$$Cu(NH_2OH)^{2+} \rightarrow Cu^+ + H_2NO^\bullet + H^+ \qquad (27)$$

$$Cu^{2+} + H_2NO^\bullet \rightarrow Cu^+ + HNO + H^+ \qquad (28)$$
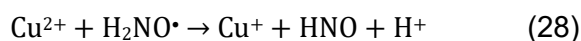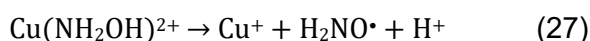
Furthermore, in the Cu/HA system after 60 minutes, the reaction stops. Therefore, it was decided to carry out a series of experiments in order to reactivate the reaction and determine the factor that could be limiting the efficiency of the process. These experiments consisted of maintaining the pH constant at 7 throughout the experiment by constant addition of NaOH (phosphate buffer pH 7 was avoided as could cause complexation with the copper ions), adding 100 µM of Cu(II) at 60 minutes, introducing 250 µM of HA at the same time and dosing HA at a rate of 62.5 µM per hour.
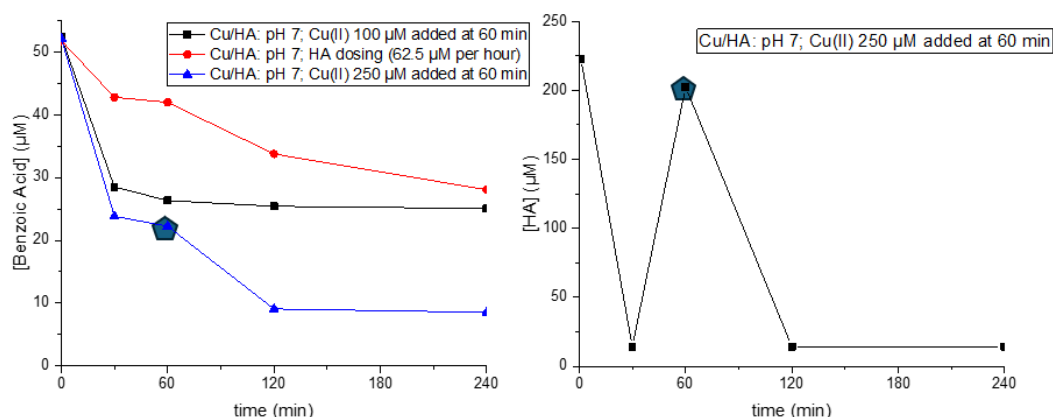
Figure 28. Experiments to determine the limiting factor. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; pH_0=7.

According to the results, only with the addition of 250 µM of hydroxylamine at 60 min leads to a final degradation of 95%, not observed with the other two cases. This is

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because hydroxylamine is completely consumed in the first 60 minutes, and the addition of another 250 µM reactivates the reaction between copper and HA, which reactivates BA degradation.

Briefly, at pH 7, HA degradation is complete and fast (<30 minutes; see consumptions on the right). This suggests that the limiting factor is the availability of HA when the pH is kept constant at 7 (and no chelating substances are present).

4.1.3 Blanks

The system exhibits a remarkable degradation efficiency when Cu/HA is incorporated, in contrast to the addition of only hydrogen peroxide, hydroxylamine or a combination of both, as shown in the figure below. It is clear that without the presence of copper in the system, together with the addition of hydroxylamine, the benzoic acid degradation effect could not be achieved.

Figure 29. Blanks. . [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [H_2O_2]=1000 µM; pH_0=7.

4.1.4 Effect of H_2O_2 addition

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Figure 30. First results of the system. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [H₂O₂]=1000 µM; pH₀=7.

The results reveal that the reaction between copper and hydrogen peroxide is not effective in degrading benzoic acid under these conditions (40% in 240 min). On the other hand, the Cu/HA syste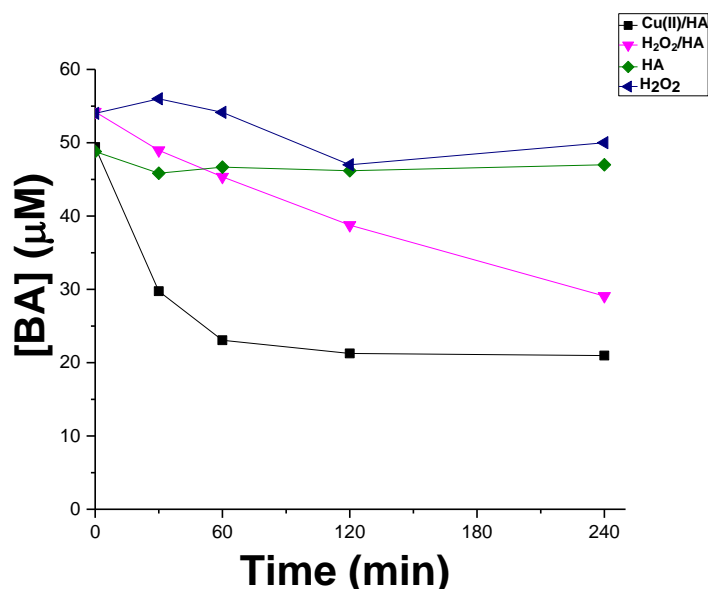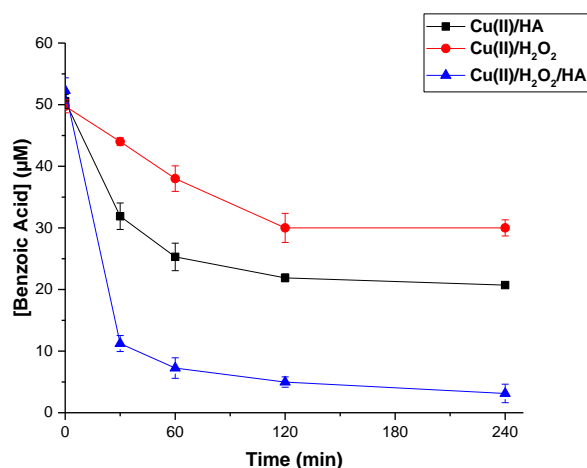m showed an efficiency of 60% after 60 minutes of reaction. However, when 1000 µM of hydrogen peroxide were added, an efficiency of 95% was achieved in the same time interval. This is because HA leads to the presence of Cu(I), and when there is also an excess of H_2O_2, BA degradation is immediate. Without HA, there is no Cu(I), and H_2O_2 alone has no considerable effect. While Cu(I) can be present in the absence of H_2O_2 but in the presence of HA, H_2O_2 is simply formed, consumed, and BA degradation is slower than when both are present simultaneously.

4.2 Study of the reactive species.

The study of reactive species has been carried out by EPR technique, as well as by selective scavenging experiments, as mentioned above. In both analyses, the possible radicals involved in the process have been identified, such as singlet oxygen ($^1O_2$), hydroxyl radical (HO$^•$) and superoxide radical (O$_2^{•-}$).

Experiments with selective scavengers have been fundamental to identify and characterise the different radicals involved in the process. In particular, three specific scavengers were used: isopropyl alcohol (IPA) to scavenge the HO$^•$, FFA to HO$^•$ and $^1O_2$ and p-BQ to neutralise the O$_2^{•-}$ [71]. The results of these experiments shown in Figure 26 revealed the significant contribution of $^1O_2$ as the main degrading agent in the process, followed by the participation of HO$^•$ in a minor proportion, being negligible the one of O$_2^{•-}$ (in line with the fast reaction of O$_2^{•-}$ with Cu(I), see reaction 17, to generate H_2O_2, explaining the scarce role of this radical). For instance, in the case of Cu/HA, in absence of ROS scavengers, the BA degradation was of 60% in 2 h, whereas in presence of IPA and FFA it was of 10 and 40%, respectively, in the same period. In the case of HA/H_2O_2, the three ROS contributed to the overall BA degradation.

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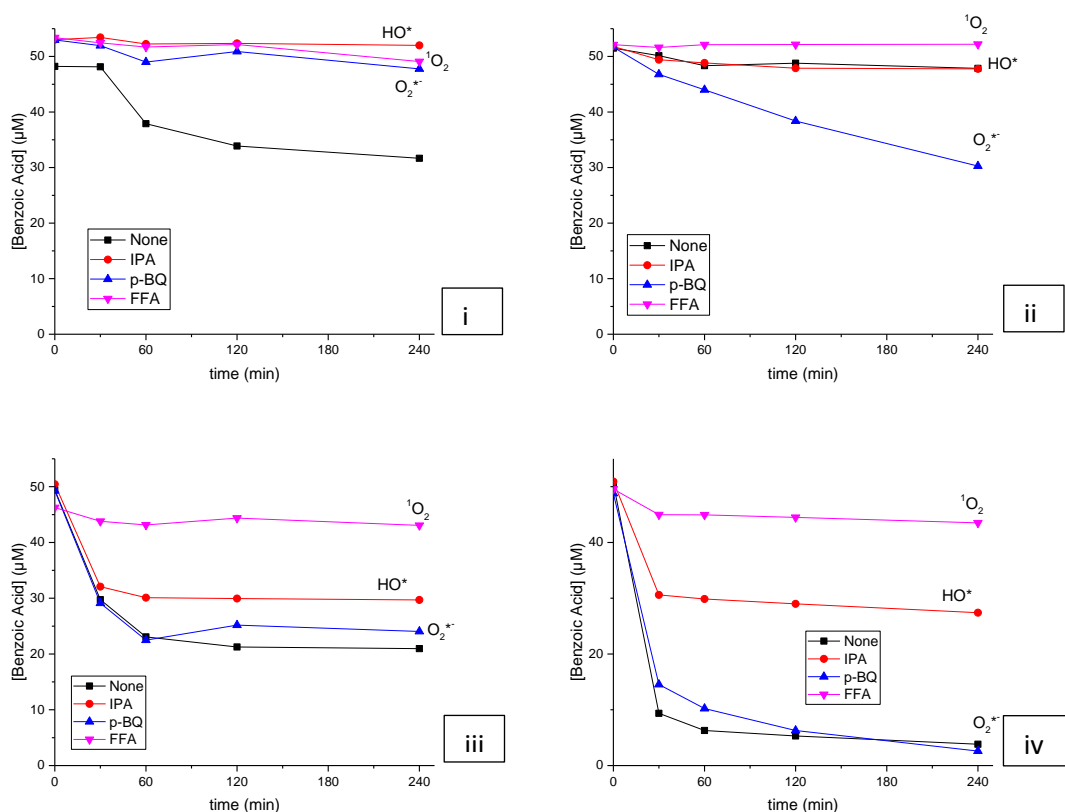
Figure 31. Selective scavenger's results i) HA/ H_2O_2; ii) Cu/ H_2O_2; iii) Cu/HA; iv) Cu/HA/ H_2O_2: [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Scavenger]=100 µM;[H_2O_2]$_0$=1000 µM; pH$_0$=7

These results contradict the findings of previous studies, which had attributed a more significant role in this process to the hydroxyl radical [72]. Furthermore, they are in line with results reported by Song et al., 2007, proposing ascorbic acid as Cu(II) reducing agent, observing also that $^1O_2$ was the main reactive specie [73], suggesting that Cu(II)/HA do not have a Haber-Wiess mechanism (the Fenton mechanism). This finding provides a new perspective on degradation mechanisms and emphasises the importance of taking other reactive species into account when assessing treatment efficacy. Besides the contribution from the reaction between $O_2^{\cdot-}$ and H_2O_2 (reaction 18), the formation of $^1O_2$ might be explained by the reaction proposed below:

$$2Cu^{2+} + H_2O_2 \rightarrow 2Cu^+ + {}^1O_2 + 2H^+ \tag{29}$$

Furthermore, we can also hypothesise that the reaction number 22 [74] should lead to $^1O_2$ instead of $^3O_2$.

To confirm the aforementioned hypothesis, experiments were repeated using the EPR technique to validate the results obtained. TEMP 77 mM was used, and the signals corresponding to TEMPO (triplet with 1:1:1 intensity) were detected (see Figure 28).

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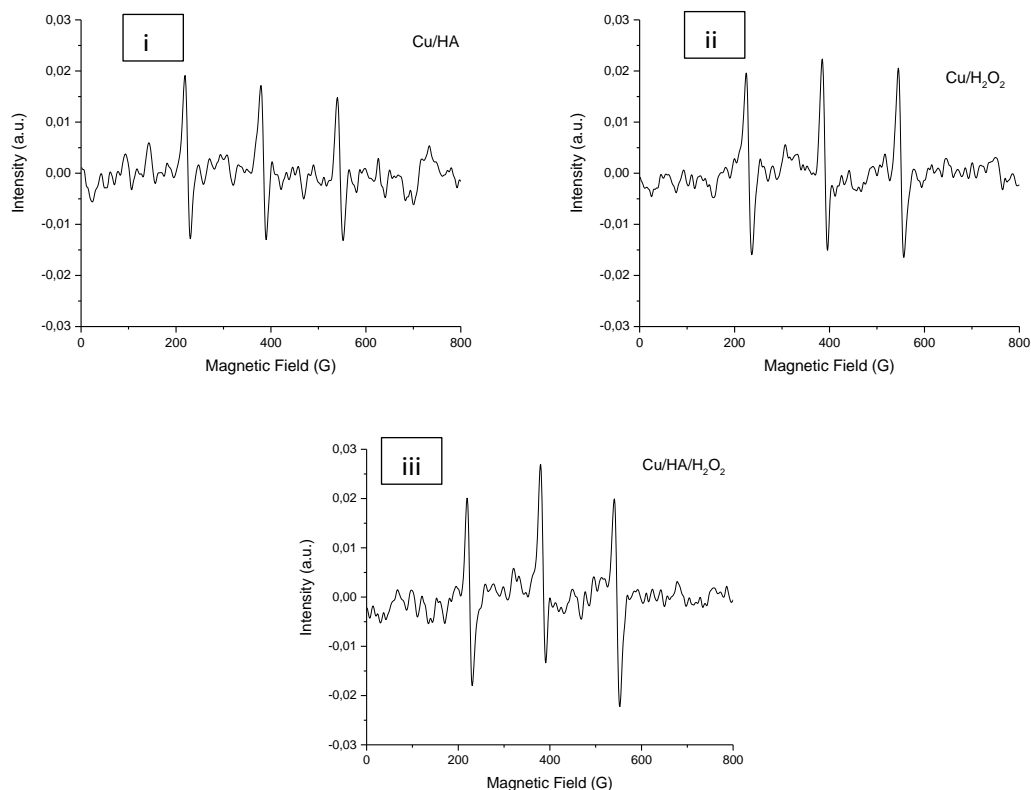
Figure 32. EPR Results. Detection of $^1O_2$ ([TEMP]=77 mM); i) Cu/HA; ii)Cu/ H_2O_2; III) Cu/HA/ H_2O_2

To confirm the formation of HO•, deduced from Cu/HA experiments in presence of IPA, analogous tests were performed in presence of DMPO 18 mM. Contrarily to our expectations, no DMPO-OH signals (quadruplet with intensity ratio 1:2:2:1) were detected in the case of Cu/HA or Cu/HA/H_2O_2, being only visible in absence of HA, this is for Cu/H_2O_2 (see Fig.29). These results indicate that the hydroxyl radical does not seem to play a relevant role as an oxidising agent in this context and/or must be quenched by HA (reaction 24, k=5 x 10^8 M^{-1} s^{-1}). Furthermore, the strange signals observed in the case of Cu/HA and Cu/HA/H_2O_2 suggests that another radical is reacting with DMPO, which could be H_2NO• (see reaction 27).

These findings confirm the role of $^1O_2$ as the main ROS for the oxidation of BA. Noteworthy, since the kinetic rate constant between $^1O_2$ and BA is very low ($\approx$ 10^6 M^{-1} s^{-1} [75], this ROS should not be the main responsible for its degradation, suggesting that Cu(III) should be the main reactive specie in our system.

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Enhancing Fenton process efficiency in presence of Cu(I)/Cu(II) for aquaponic water treatment: role of high valent metal and reactive species.
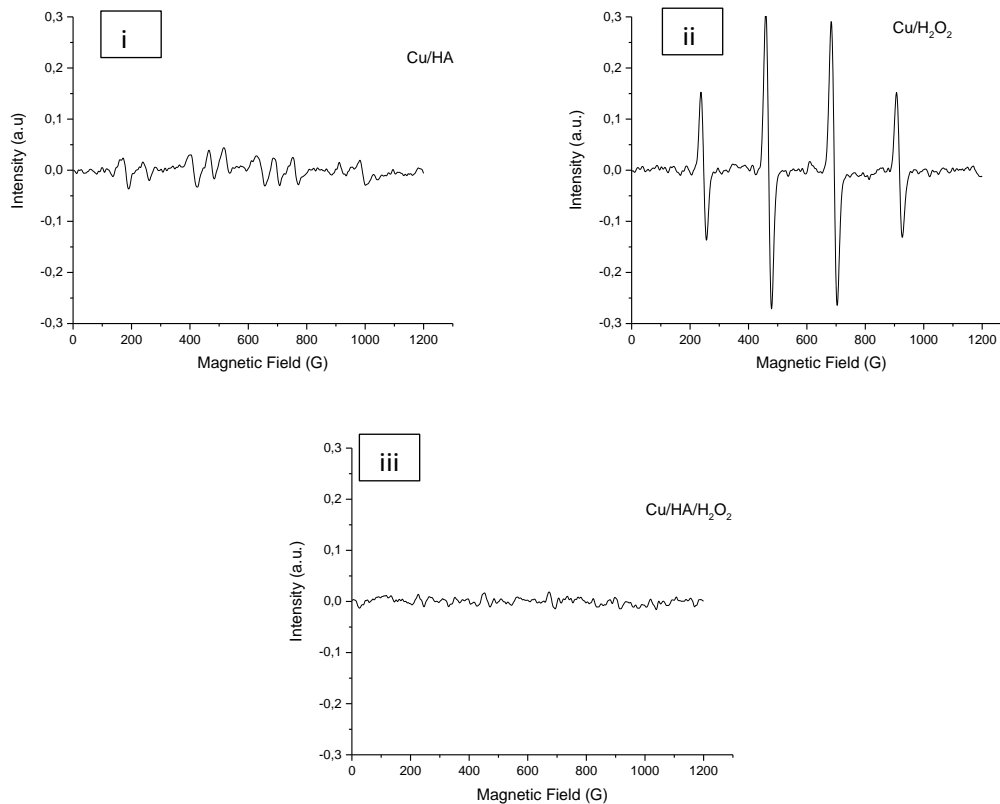
Figure 33. EPR Results. Detection of HO˙ ([DMPO]=18 mM); i) Cu/HA; ii)Cu/ H_2O_2; III) Cu/HA/ H_2O_2

Following the results obtained, the hypothesis that Cu(III) could be involved in the process might be the most probable. To demonstrate its formation, an assay using DMPO in the presence of CH_3OH 10 M was tested to address this uncertainty. If HO˙ is the main reactive specie, ˙CH_2OH is formed, observing the signals corresponding to DMPO-CH_2OH, whereas if Cu(III) is the main reactive specie, CH_3O˙ will be formed, the adduct observed in the EPR being DMPO-OCH_3. As shown in Figure 30, signals from DMPO-CH_2OH and DMPO-OCH_3 were observed, proving the formation of Cu(III), but not discarding the presence of HO• as well [66].
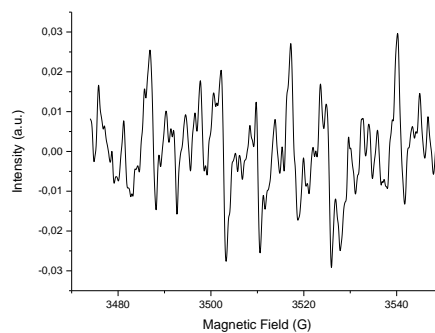
Figure 34. EPR. Methanol detection using 10M CH_3OH and 18 mM DMPO. Cu/HA/H_2O_2 experiment.

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4.3. Effect of water constituents

4.3.1. Chelating agents

The effect of the addition of chelating substances in the proposed systems, commonly present in hydroponic waters, was explored. The substance selected to simulate these conditions is ethylenediaminetetraacetic acid (EDTA), with a concentration of 100 μM.
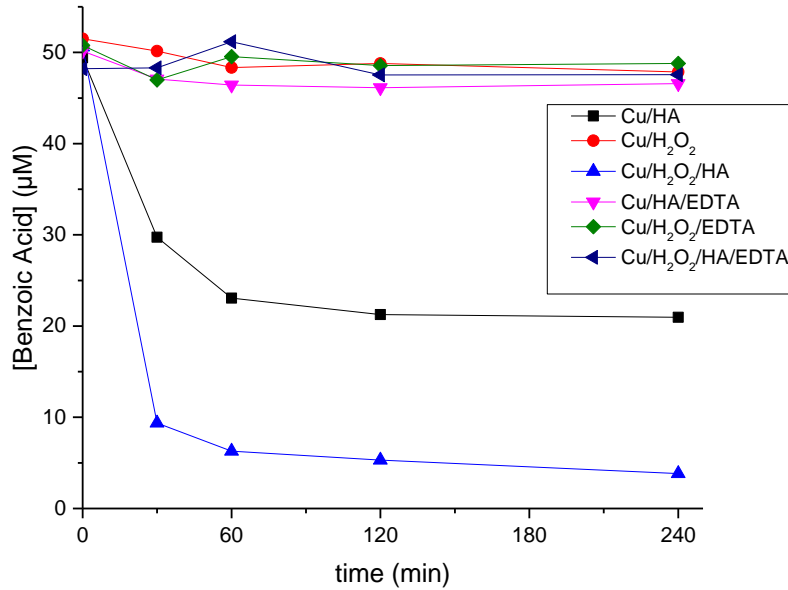
Figure 35. Experiments with chelating agents. [BA]=50 μM; [Cu(II)]=100 μM; [HA]=250 μM; [EDTA]=100 μM; pH₀=7.

The results obtained in the presence of chelating agents reveal a complete inhibition of the process in all the cases studied.
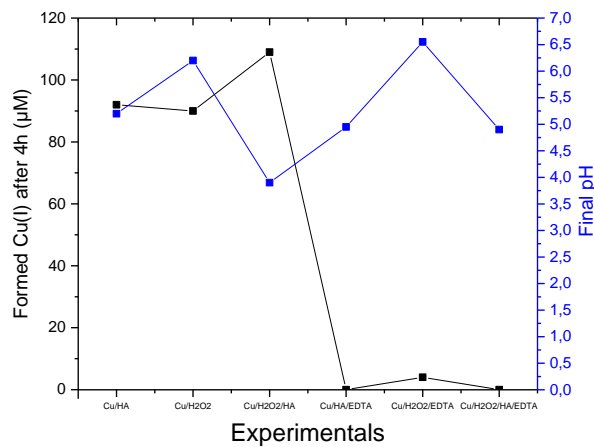
Figure 36. Experiments with chelating agents, Formed copper (I) in the process vs pH. [BA]=50 μM; [Cu(II)]=100 μM; [HA]=250 μM; [EDTA]=100 μM; pH₀=7.

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This phenomenon can be attributed to the higher formation stability constant of Cu(II)-EDTA, avoiding the formation of Cu(II)-HA, which inhibits the conversion of Cu(II) to Cu(I). Consequently, this inhibition prevents the generation of hydrogen peroxide necessary for the benzoic acid degradation reaction. As can be seen in the figure above, the formation of copper(I) in the presence of chelating agents is almost zero, compared to the system without the addition of EDTA. This issue was also confirmed by the absence of HA consumption in presence of EDTA (See Figure 37).
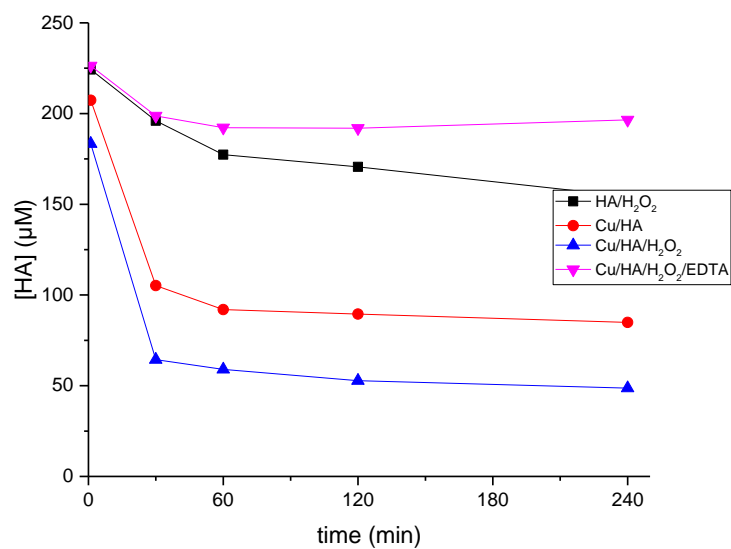
Figure 37. HA consumption

4.3.2 Effect of anions

In order to investigate the influence of anions in the system, a preliminary study was made on the salts commonly present in aquaponics systems. The most frequent anions identified were chlorides, sulphates, bicarbonates, nitrites, nitrates and phosphates [17]. A concentration of 1000 µM was set for each of these anions in order to evaluate their effect on the process.

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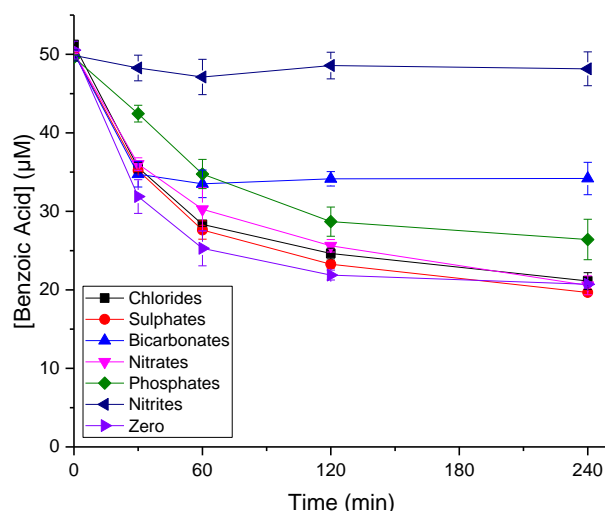
Figure 38. Effect of anions. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Anions]=1000 µM; pH$_0$=7.

The results indicate that chlorides, sulphates and nitrates do not significantly affect the efficiency of the Cu/HA process. However, nitrites, bicarbonates and phosphate did show an inhibition of the process. The inhibition produced by each case is related to three different phenomena: i) for NO_2^-, it reacts with HA by comproportionation reaction forming N_2O, as well as it also reacts with ROS, ii) HCO_3^- acts as a buffer (pKa = 6.4) and, as previously explained in section 4.1.2, at pH 7 the consumption of HA is higher than at acidic conditions, explaining its kinetics [76]; iii) phosphates, although it also acts as buffer at pH ca. 7, it form complexes with copper(II) ions, reducing the HA consumption (as markedly observed with EDTA), which slows down the degradation of benzoic acid[60]. Nitrites are infrequent, or have negligible concentrations in aquaponic water, not supposing a serious interference to the Cu/HA process. Regarding HCO_3^-, which is frequently found in high concentrations in natural or wastewater effluents, a further addition of HA to the system could solve this, without interfering with the process (see Figure 35, where second addition of HA was performed at constant pH 7).

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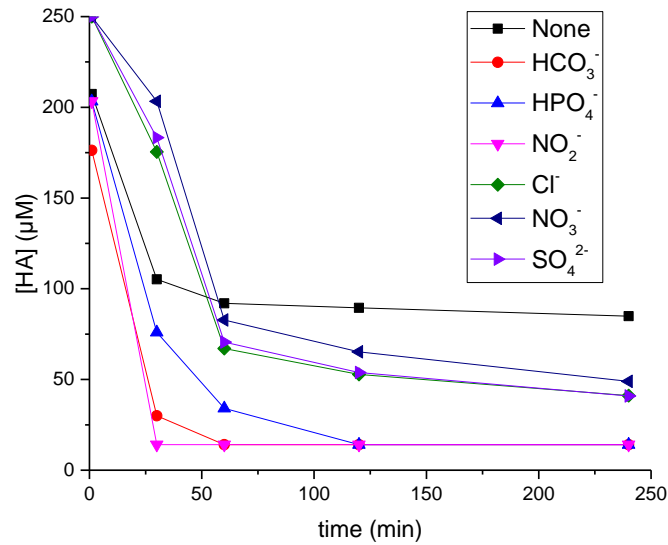
Figure 39. HA consumption in addition to bicarbonates. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Anions]=1000 µM; pH$_0$=7.

4.3.3 Effect of dissolved organic matter

In it the system response to the presence of DOM (Dissolved Organic Matter) in the aquaponics water was investigated. To recreate these conditions, catechol and humic acid were used as a DOM proxy at a concentration of 50 µM and 10 - 100 mg/L.
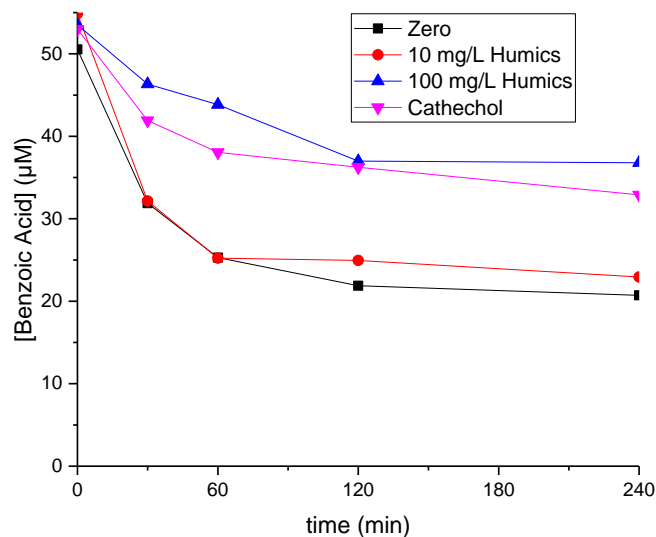
Figure 40. Effect of DOM. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Catechol]=50 µM; pH$_0$=7.

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The presence of humic acids at 10 mg/L seems to have a minimal effect on the process. However, at high concentrations, BA degradation was hindered, which was expected at a certain point as ROS are consumed either by BA and humic acid (as well as plausible chelation of Cu(II) might also occur, which was previously demonstrated that reduces the Fenton-like efficiency). For this reason, catechol 50 μM (which is able of chelating and reducing Cu(II), [60] was added. A similar effect as 100 mg/L of humic acid was observed. It was observed that catechol was removed faster than BA, indicating a preference of ROS for catechol than BA. Experiments were also performed with catechol in the absence of HA, which showed that the first is also able of reducing Cu(II) into Cu(I). Clearly this indicates the presence of the Fenton reaction in the process, even in the absence of HA.
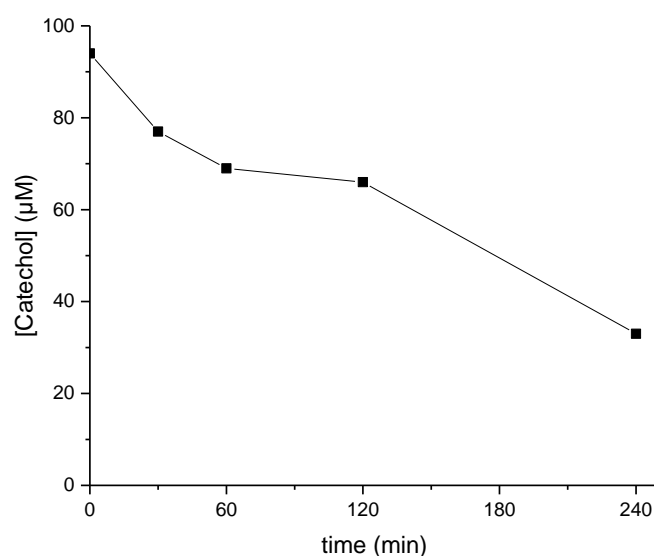
Figure 41. Degradation of catechol in Cu/HA system. [BA]=50 μM; [Cu(II)]=100 μM; [HA]=250 μM; [Catechol]=50 μM; pH_0=7.

4.3.4 Effect of transition metals

An in-depth analysis of the effect of transition metals, such as iron (Fe(III)), manganese (Mn(II)) and molybdenum (Mo(VI)), which are commonly found in waters used in aquaponics systems, on the dynamics of the copper (Cu(II)) and HA system will be examined in this section. The aim is to examine how the presence of these metals can influence the efficiency of the degradation reaction catalysed by copper and hydroxylamine. 100 μM of each transition metal will be added (same amount of copper used).

Furthermore, the role of chelating substances in this context is research by assessing their ability to form complexes with the metal ions and their possible interference in the interaction between copper and hydroxylamine. 100 μM of EDTA were added.

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4.3.4.1 Effect of Iron (III)

The classic Fenton-like reaction (Fe(III)/H_2O_2) at pH 7, only produced a 5 % in 240 min (figure 38), expected as this reaction is only effective at pH = 3 as explained in sections 4.2.1. However, by adding hydrogen peroxide and hydroxylamine, 100% efficiency is achieved after 60 minutes because of OH$^{\cdot}$. On the other hand, when iron is combined with copper ions, a significant improvement in efficacy is observed, especially in the presence of hydrogen peroxide, where 99% degradation is achieved after 30 minutes.

Figure 42. Effect of Fe(III) in the system with HA. . [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Fe(III)]=100 µM; [H_2O_2]=1000 µM; pH_0=7.

In the presence of EDTA, it is widely known that the complex Fe(III)-EDTA is Fenton active [77], contrarily to the analogous complex with Cu(II) which loses catalytic power against H_2O_2 activation, as well as proving more difficult to reduce by NH_2OH. Therefore, the presence of Fe(III) compensate the inhibition produced by EDTA in the Cu(II)/HA/H_2O_2 system (60% in 4 hours Fe(III)/Cu(II)/EDTA/HA/H_2O_2, see Figure 39). Therefore, although, Cu(II)/HA is a useful method of producing Fenton-like in-situ, it is strongly inhibited by complexing substances, Fe(III), which at pH 7 and in the absence of EDTA has an insignificant catalytic effect, compensates for the inhibition observed in Cu(II)/EDTA/HA.

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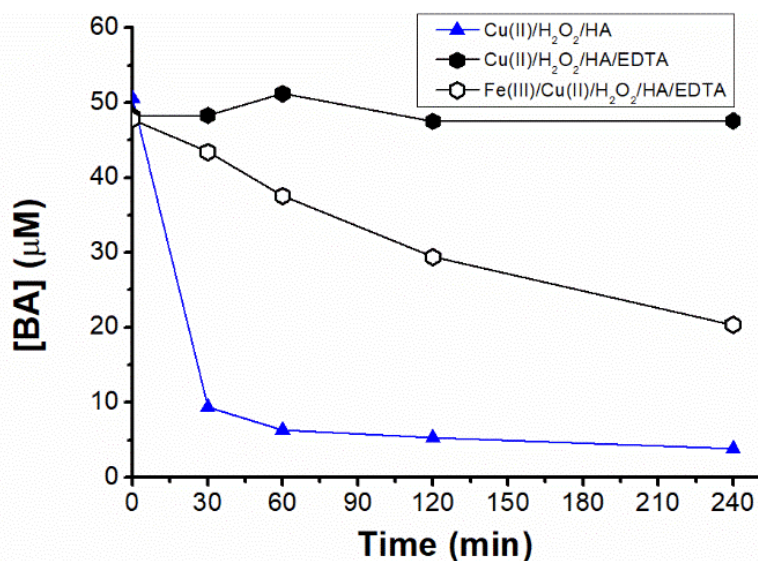
Figure 43. Effect of EDTA in presence of Cu(II) and Fe(III). [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Fe(III)]=100 µM; [H$_2$O$_2$]=1000 µM; [EDTA]=100 µM; pH$_0$=7.

4.3.4.2 Effect of Manganese (II)

Regarding the impact of manganese in the system, their results indicate that it does not enhance or hinder the catalytic reactions of Cu(II) (see Figure 40). Specifically, a lack of efficacy is observed in the Mn/HA and Mn/H$_2$O$_2$ treatments, suggesting a limited degradation capacity in the absence of copper (II) ions.

In line with the above mentioned statements, the presence of EDTA results in a complete inhibition of the degradation process (see Figure 39). This phenomenon indicates that the contribution of manganese to the process is practically zero under these experimental conditions. Furthermore, the interaction of manganese with chelating substances results also in an inhibition of the degradation process. These findings indicate that the presence of EDTA and chelating substances significantly interferes with the ability of the system to degrade the target pollutant.

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Enhancing Fenton process efficiency in presence of Cu(I)/Cu(II) for aquaponic water treatment: role of high valent metal and reactive species.
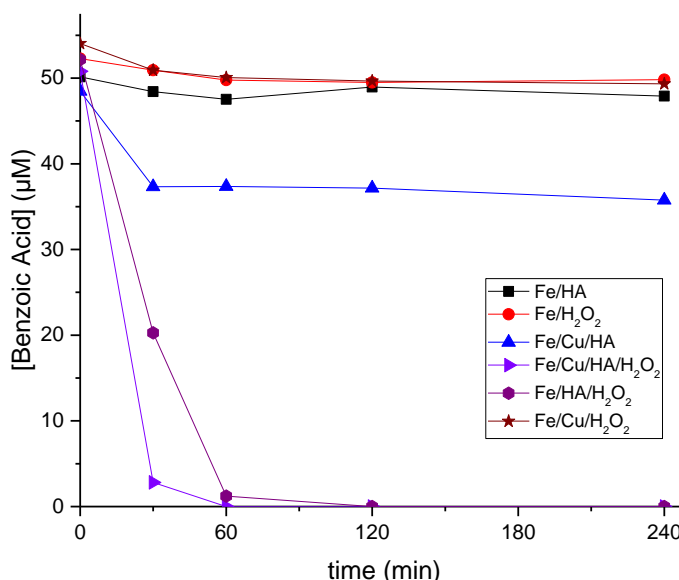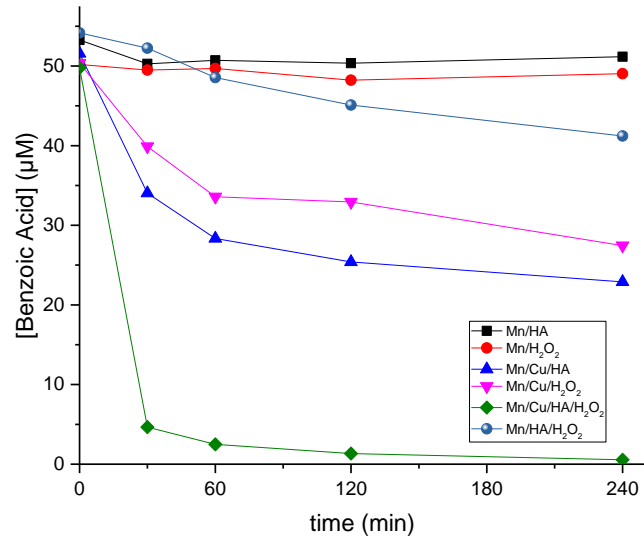

Figure 44.Effect of Mn(II) in the system with HA. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Mn(II)]=100 µM; [H₂O₂]=1000 µM; pH₀=7.
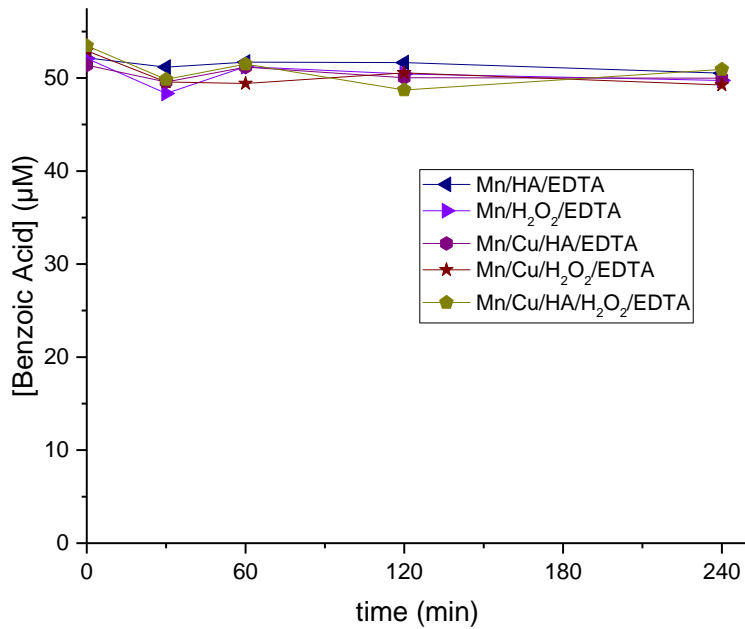
Figure 45. Effect of EDTA in presence of Cu(II) and Mn(II). [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Fe(III)]=100 µM; [H₂O₂]=1000 µM; [EDTA]=100 µM; pH₀=7.

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4.3.4.3 Effect of Molybdenum (VI)

Experiments with Mo(VI) found a complete inhibition of the degradation process, both in the presence and in the absence of EDTA (See Figure 41). This is attributed to the high oxidative capacity of MoO_4^-, which degrades HA and H_2O_2 even before it can react to with Cu(II). It is worth mentioning that the reaction MoO_4^- should not lead to ROS formation, as well as MoO_4^- alone was not able of oxidising BA. As a result, the process is interrupted before it can actually start. (See Figure 42,43).
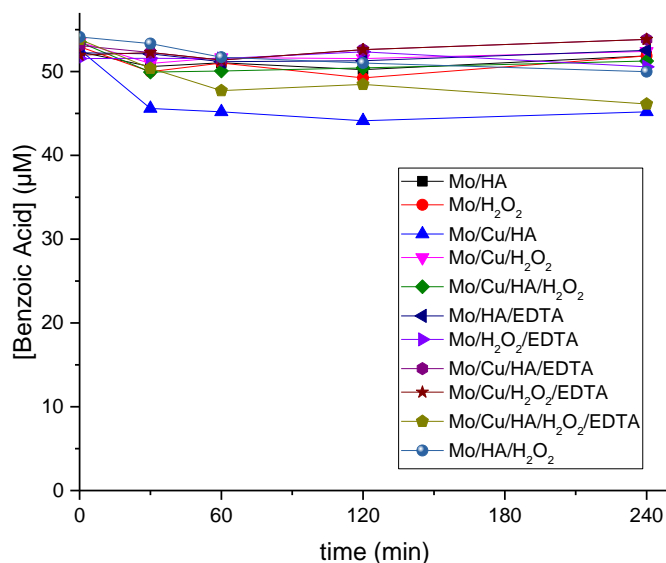
Figure 46. Effect of Mo(VI) in the system with HA. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [Mn(II)]=100 µM; [H2O2]=1000 µM; [EDTA]= 100 µM; pH0=7.

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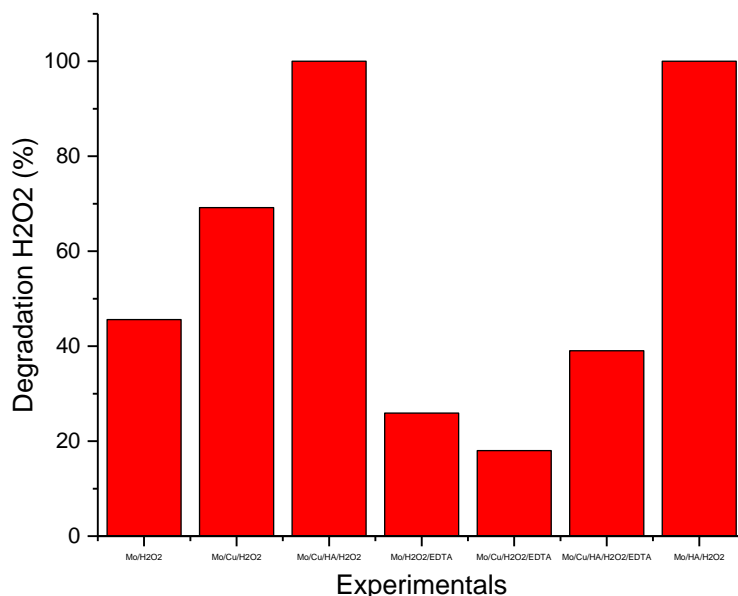
Figure 47. H_2O_2 degradation in Mo(VI) Experiments

4.4 Results obtained with real aquaponics water.

Finally, tests were performed using real water from an aquaponics system, obtained from a pilot plant located at the Department of Veterinary Medicine of the University of Turin. Before these tests, a complete characterisation of the collected water was carried out in order to understand its chemical composition and possible variables that could influence the experimental results. Afterwards, the most relevant experiments were replicated using this water in order to validate the applicability of the findings in real conditions. Moreover, additional tests were performed by adapting the concentrations of copper ions and contaminants to a real scale, and other contaminants such as thiabendazole (fungicide) and sulfamethoxazole (antibiotic) were added to evaluate the effectiveness of the treatment in a more representative context.

4.4.1 Characterisation of aquaponics water

Analysis performed to characterise our aquaponic water model shows a pH close to neutrality, which is favourable as it eliminates the need to add acid or base to adjust the system to optimal conditions. In addition, the conductivity is very low, suggesting a low salt content and a remarkably low inorganic carbon load.

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Table 5. Characterisation of aquaponics water

CHARACTERISATION	
Parameter	Value
pH	6.8
Conductivity (mS cm^{-1})	0.92
IC (mg/L)	1.782
TOC (mg/L)	12.87
TOC + BA 50µM (mg/L)	17.82

These characteristics suggest a water quality comparable to that of a controlled aquatic environment, such as a fish tank, which is highly favourable. This water quality provides a solid basis for the success of the benzoic acid degradation processes, anticipating positive results in the efficacy of the proposed treatments.

4.4.2 Experiments with model water

We applied a comprehensive replication of all copper tests using the model water as the experimental substrate, in order to subject the system to conditions that accurately simulated a representative environment of a real aquaponics installation. This arrangement allowed a detailed evaluation of the system's ability to degrade benzoic acid in a more realistic context.
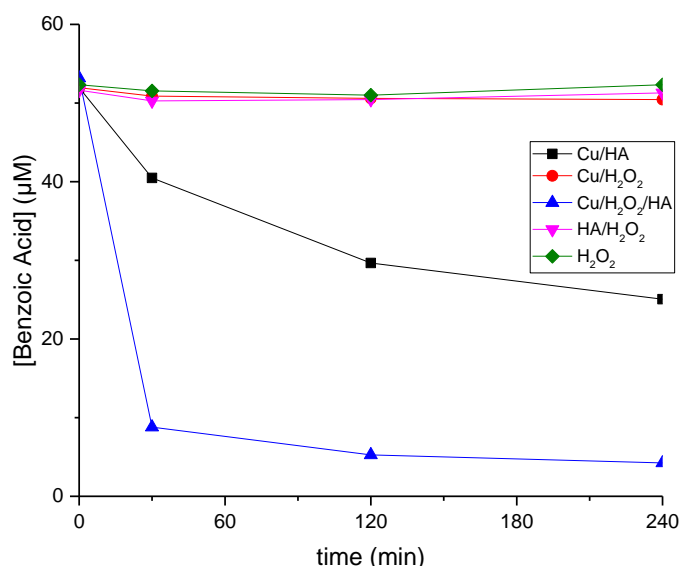
Figure 48. Experiments in real water. [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; pH$_0$=7.

The results indicate efficiencies comparable to those obtained in the simulated water. The Cu/HA/H$_2$O$_2$ system stands out as the most effective, achieving 95% degradation in four hours. On the other hand, the system under study presents an efficiency of 50% in the same period of time.

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4.4.3 Experiments with various pollutants

Finally, experiments were conducted using three emerging contaminants (CECs): the already studied BA, thiabendazole (TBZ), a fungicide widely used in aquaponics systems, and sulfamethoxazole (SMX), an antibiotic commonly used in fish farming. These experiments were carried out in conjunction with the most effective treatment system demonstrated in this study, the Cu/HA/H$_2$O$_2$ system. It is also important to note that the model water we are using has a low concentration of copper ions in the system. If they were present in significant amounts, the hydroxylamine (HA) and HA/H$_2$O$_2$ experiments would react with these ions instead of behaving as they do under current conditions.
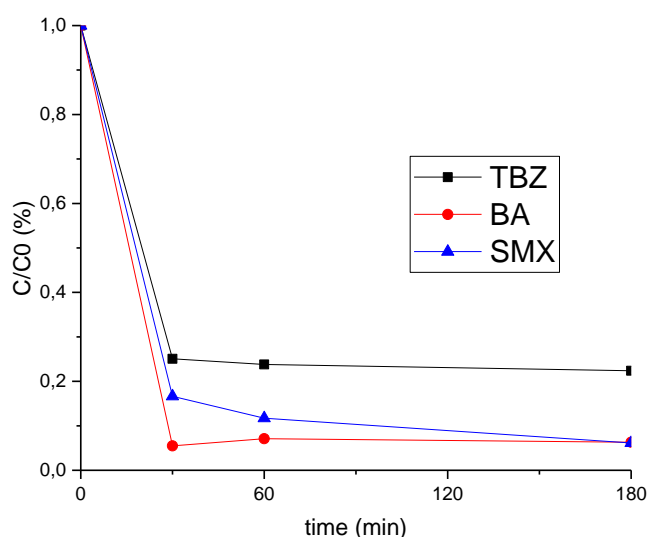
Figure 49. Experiment in real water with simulated condition. [SMX]=50 µM; [TBZ]= 50 µM; [BA]=50 µM; [Cu(II)]=100 µM; [HA]=250 µM; [H$_2$O$_2$]=1000 µM; pH$_0$=7.

Experiments carried out under simulated conditions exhibit remarkable efficiency during the first 60 minutes, degrading approximately 90% of benzoic acid, 80% of SMX and 75% of TBZ. However, after one hour, the process stops, possibly due to the depletion of HA, suggesting the need for additional incorporation of this reagent for complete degradation. Despite this, the results conclusively demonstrate the efficacy of the treatment for all the emerging pollutants evaluated.

When using conditions closer to reality ([Cu(II)] ≈ 10 µM, see Table 1), the efficiency of the system is affected. For this, the concentrations of HA and H$_2$O$_2$ were also reduced one order of magnitude in line with the lower copper content (i.e., [HA] = 25 µM and [H$_2$O$_2$] = 100 µM) as well as those of the pollutants ([CEC] = 15 µM each). With these conditions, although it exhibited favourable degradation rates for TBZ (60% degradation in three hours), the BA and SMX removals were negligible (see Figure 46). This might indicate that with low copper contents, the operational conditions (concentrations of HA

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and H_2O_2) should be optimised by performing a design of experiment – response surface methodology (DoE/RSM) in order to obtain fast degradation kinetics useful for the real application.
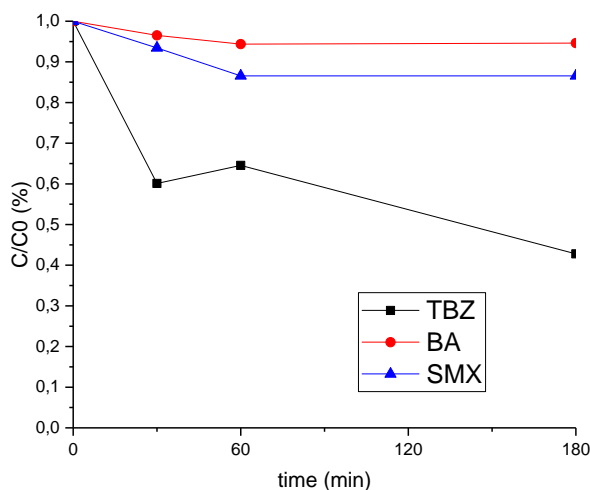
Figure 50. Experiment in real water with real condition. [SMX]=15 µM; [TBZ]= 15 µM; [BA]=15 µM; [Cu(II)]=10 µM; [HA]=100 µM; [H_2O_2]=10 µM; pH$_0$=7.

5. Conclusions and future research directions

The combination of hydroxylamine (HA) as a co-catalyst in the Fenton-type reaction, together with Cu(II) ions present in aquaponics systems, proves to be highly effective in the degradation of emerging pollutants. This suggests a promising potential for the development of a water treatment method to generate H_2O_2 in situ. This process approach would be used in an aquaponic system in a tank downstream of the two sub-systems, ensuring that all pollutants reach both sub-systems and thus improving the treatment of both sub-systems.

Regarding the operating parameters, a higher reaction efficiency is observed at pH close to 7 (an advantage compared to the Fenton process with iron), with an optimal HA percentage of 250 µM. These parameters have shown an efficiency of 60% BA degradation in 1 h, which increases to 95% with the addition of H_2O_2 because HA leads to the presence of Cu(I), and when there is also an excess of H_2O_2, BA degradation is immediate. Without HA, there is no Cu(I), and H_2O_2 alone has no considerable effect. While Cu(I) can be present in the absence of H_2O_2 but in the presence of HA, H_2O_2 is simply formed, consumed, and BA degradation is slower than when both are present simultaneously.

Furthermore, it was determined that by keeping the pH constant at 7, the consumption of HA is immediate (the HA protonated form is not reactive). Therefore, these systems

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require a fixed pH at 7 - 8 (obtained by the natural presence of HCO_3^- of real water body, being an advantage), and a constant addition of HA. In this way, the addition of H_2O_2 is not necessary, the system only requiring the addition of one single reagent.

The major interference of Cu(II)/HA AOP were not anions (not even in high concentrations) or DOM as expected, if not the presence of chelating agents (e.g. EDTA), which drastically hindered the BA degradation process. This could pose a serious disadvantage for the proposed system, considering that aquaponics present EDTA. To solve this issue, addition of Fe(III) could be a simple choice, as well as several authors proposed the irradiation with UV-visible light, breaking the Cu(II)-complex, which could reactivate the system. This will be studied in future works.

Concerning the interaction of the process with transition metals, it is observed that at neutral pH, the presence of iron ions leads to a loss of efficiency due to the precipitation of iron in that pH range, which limits the availability of ions to react. However, by adding hydrogen peroxide and combining it with copper, the process is reactivated, reaching 100% degradation in 60 minutes. On the other hand, manganese shows no significant effect and seems to have no influence on the process. In the presence of EDTA, the combination with manganese results in a complete inhibition of the process. Finally, molybdenum, being a strong oxidising agent, completely inhibits the process by consuming all the hydroxylamine before it can interact with copper.

The mechanism involved in the Cu/HA system was thoroughly studied, indicating that $O_2^{\cdot-}$ is quenched by Cu(I, II), $HO^\bullet$ is quenched by HA, and that $^1O_2$ and Cu(III) are the main reactive species responsible for the BA degradation processes. Noteworthy, since $^1O_2$ has slow kinetics with CEC, we concluded that Cu(III) is the most important reactive specie. These findings represents a significant advance in Cu-based AOPs, as they can be employed in more complex water systems due to the more selective character of Cu(III) compared to $HO\bullet$, the first one not being scavenged by typical water constituents (confirmed with the experiments performed containing anions at 1 mM concentration). Furthermore, although $^1O_2$ is not useful to degrade CEC, it is extremely useful for disinfection processes, which will be studied in future works.

Finally, an evaluation of the treatment was made using real water from an aquaponics system. Characterisation of the sample revealed a pH close to the optimum for treatment, a low salt concentration reflected in its low conductivity, and a high dissolved organic carbon (DOM) load. Tests showed that the addition of H_2O_2 at the beginning, together with the Cu/HA/H_2O_2 system, achieved 90% degradation in one hour, proving to be the most effective treatment for hydroponic water. In addition, the degradation of other compounds considered as emerging contaminants, such as thiabendazole and sulfamethoxazole, a model fungicide and antibiotic respectively, was evaluated. The results indicated a high degradation of these compounds at the concentrations used in the entire project, while a lower degradation of benzoic acid and sulfamethoxazole was observed under simulated conditions.

Concerning future lines of research for this project, several interesting possibilities emerge:

- As previously mentioned, exploring the application of sunlight to convert this process into a Photo-Fenton system, which could significantly accelerate the degradation times of the pollutants. In addition, the possibility of breaking the

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complex formed by copper and EDTA through solar irradiation could be investigated to avoid inhibition of the process.

- Perform studies to apply this treatment in disinfection of real aquaponics plant and analyse its interaction with all the emerging pollutants present in the water, which would provide a more complete understanding of its efficacy and applicability in real situations.

- Exploring the possibility of using this treatment in other wastewater systems that have a high copper content in their influent as could be the case in (add example) or employing heterogeneous systems to avoid the effluent contamination with Cu(II) (e.g. use of copper oxides). This would extend the scope and relevance of the treatment beyond aquaponics systems, offering innovative solutions for the treatment of contaminated water.

- This method could also be used to eradicate bacteria and other microorganisms that can cause problems in aquaponics systems. Since both Cu(I) and Cu(III) are biocidal, they could extend their effectiveness not only in the elimination of CECs.

6. Acknowledgments

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