Technologies in Wastewater Treatment Plants for the Removal of Antibiotics, Resistant Bacteria and Antibiotic Resistance Genes: a Review of the Current Literature*

Tecnologías en plantas de tratamiento de agua residual para la remoción de antibióticos, bacterias resistentes y genes de resistencia antibiótica: una revisión de literatura actual

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Abstract

This study aimed to evaluate the efficiency of technologies for removing antibiotics, antibiotic-resistant bacteria and their antibiotic resistance genes, and the countries where they have been developed. For this purpose, was conducted a systematic review to identify the tertiary treatments to remove the above-mentioned pollutants. The ScienceDirect and Scopus databases were used as sources of information, taking into account only experimental research from 2006 to 2019 and technologies with removal rates higher than 70% to the information analyses. From the analysis of 9 technologies evaluated, in a set of 47 investigations, photo-Fenton, and electrochemical treatments were found to be the most efficient in the removal of antibiotics; gamma radiation and photocatalysis with TiO2 and UV revealed better results in the removal of resistant microbial agents and their resistance genes, with efficiencies of 99.9%. As one of the largest producers and consumers of antibiotics, China appears to be the country with the most scientific research on the area. The importance of innovation in wastewater treatment processes to achieve better results in the remotion of antibiotics, antibiotic-resistant bacteria, and their resistance genes is highlighted, given the effects on the aquatic ecosystems and public health.

Keywords: Antibiotics, sewage treatment, antibiotic resistance bacteria, antibiotic resistance genes.

Resumen

El objetivo de este estudio fue evaluar la eficiencia de tecnologías de eliminación de antibióticos, bacterias resistentes a los antibióticos, sus genes de resistencia antibiótica, y los países en donde dichas tecnologías se han desarrollado. Para ello se realizó una revisión sistemática consistente en la identificación de los tratamientos terciarios implementados en la remoción de los contaminantes mencionados. Se hizo uso de las bases de ScienceDirect y Scopus como fuentes de información, teniendo en cuenta únicamente investigaciones experimentales realizadas en el periodo de 2006 a 2019; en el análisis de la información se seleccionaron aquellas tecnologías con tasas de remoción superiores al 70%. A partir del análisis de 9 tecnologías evaluadas, en un conjunto de 47 investigaciones, se obtuvo que los tratamientos foto-fenton y la electroquímica son los más eficientes en la remoción de antibióticos; por su parte, la radiación gamma y la fotocatálisis con TiO2 y UV resultaron ser superiores en la remoción de los agentes microbianos resistentes y sus genes de resistencia, con eficiencias del 99.9%. Se encontró que China, que es uno de los mayores productores y consumidores de antibióticos, es el país con más investigaciones científicas realizadas frente al tema. Se destaca la importancia de la innovación de procesos de tratamiento de aguas residuales, para alcanzar mejores resultados en la remoción de los antibióticos, bacterias resistentes a estos y sus genes de resistencia dados los problemas al ambiente acuático y la salud pública.

Palabras clave: Antibióticos, tratamiento de aguas residuales, bacterias de resistencia antibiótica, genes de resistencia antibiótica.
Introduction

A concern about the presence of emerging pollutants in wastewater, has increased in recent years, due to the potential unfavorable consequences to human health; among these, several classifications are presented, one of them being pharmaceutical products comprising of antibiotics, analgesics, antidepressants, among others [1]. Antibiotics are compounds used in humans and animals to treat infectious diseases; however, in recent decades these compounds have been used excessively, whose presence has increased in wastewater, with alterations of the aquatic environment, increased risks to human health, geno-toxic effects and the development and proliferation of bacteria which is resistant to antibiotics (ARB). The ARB are microorganisms that resist the antibiotic effects and their antibiotic resistance genes (ARG) can be transferred from one bacterium to another and contain the genetic coding that makes them resistant [2]. In that sense, the World Health Organization (WHO) has classified the bacterial resistance phenomenon as a global emergency [3]. Due to the public health problems that it causes, there is a reduction in the effectiveness of medical treatments against diseases, the increase in morbidity and mortality, that is reflected in 25000 annual deaths in Europe and 19000 in the United States, as well as the increase in costs for medical care and the time required for treatment [4].

Among the countries with the highest rate of antibiotic use are: Turkey, Tunisia and Spain, with a defined daily dose (DDD) per 1000 inhabitants of 48.1, 47.8 and 40.1 respectively [5], as well as China which also positioned itself as the largest producer of antibiotics. In 2013, this country used about 162,000 tons of them [6]. On the other hand, Latin American countries such as Brazil, Cuba, Ecuador, Uruguay, Venezuela, Dominican Republic, Mexico, Peru and Colombia, have a consumption lesser than 20 DDD per 1000 inhabitants. This value is below 50% compared to the values registered by the aforementioned countries of Europe and Africa. Colombia has an average rate of 8.1 DDD per 1000 population [4]; considering that in outpatient prescriptions and highly complex treatments, there are doses of 1.58 and 22.5 [7], [8]. However, 34.6% of antibiotic prescriptions correspond to inappropriate use, which contributes to the problem as described previously [9].

It is important to make emphasis that once antibiotics are ingested undermedical prescription, they are eliminated through urine and human feces, reaching the bodies of water, although it is possible that they reach them by simple contact with the environment, which generates their presence in wastewater [6]. In this resource, the transfer of ARG (causes of antibiotic resistance) is possible, due to the deposition of different bacteria that already developed resistant genes and others that come from different tributaries, such as urban wastewater and livestock [10]. It has been found that human feces have the presence of β-lactam antibiotics and sulfonamides, with values of 10.86 μg / kg and 8.2μg / kg, respectively [6].
Therefore, the treatment of wastewater is a determining factor in the distribution of these emerging pollutants, which in global terms includes central urban waters centralized treatment systems deploying primary treatment; and in some cases secondary treatment. According to the global wastewater treatment indicator, which was developed in 2017 by members of Yale University and the Columbia University Land Institute, the highest water treatment levels are centered in Europe and the lowest ones correspond to Latin America and the Caribbean, sub-Saharan Africa and South Asia [11].

As a result of the low treatment levels, in New Mexico, the United States and the Czech Republic, were found antibiotic concentrations such as norfloxacin, ciprofloxacin and sulfamethoxazole, were found in the effluent of a Wastewater Treatment Plants (WWTP). Only 40 to 86% of antibiotics are eliminated in the case of the Czech Republic and 20 to 77% in New Mexico [12],[13]. In addition, in Sfax, Tunisia (considered the second country consuming the most number of antibiotics [5]) 13 antibiotics were present in both, the tributary and the effluent, since the highest removal obtained in the plant was 88% for trimethoprim and less than 33% for sulfapyridine [14]. In Latin America, the presence of azithromycin, ciprofloxacin, clarithromycin, norfloxacin and sulfamethoxazole is found in tributaries and effluents of two WWTP, one in Medellin and the other one in Bogotá, Colombia [15].

Despite the available information and advanced techniques, a study that included the removal of antibiotics, ARB and ARG in parallel, was not found among the references consulted. That is why, given the need to mitigate the risk of exposure in water sources, this review is carried out based on the analysis of 9 technologies used in different countries for the removal of antibiotics, ARB and ARG, each with their respective modifications and innovation of conventional processes, to obtain better efficiencies. Among the 9 technologies 8 are advanced oxidation processes, membrane bio-reaction and absorption with activated carbon, due to the removal efficiencies, obtained through them and their constant research. In the development of this review, the removal efficiency of each technology was analyzed, according to the research carried out on them, as well as their influence on the countries where they have been implemented.

**Materials and methods**

This document outlines a systematic review of the technologies applied around the world, for the removal of antibiotics from wastewater, along with resistant bacteria and their genes. This was possible by the means of searching for research and review articles in which the broadest and most relevant issues of the problem were identified, in order to understand its scope and the importance of its management.
Due to its high application potential and removal efficiencies that change depending on the technology during the preliminary search, it was possible to identify the implementation of tertiary treatments for the removal of antibiotics ARG, and ARB, such as advanced oxidation, membrane filtration, and adsorption processes [16]. These processes increase the efficiency of conventional treatments during the removal of contaminants, to reach the limits defined by the regulation [16]. In the same way, membrane filtration and adsorption processes, due to its high application potential and removal efficiencies, differ depending on the technology [17]. Once the removal technologies were identified, their search was carried out by considering in each case the following keywords: “Degradation of antibiotics by ozone”, “Removal of antibiotic resistance genes by ozone”, and “Removal of bacteria resistant to antibiotics by ozone”. The search was carried out in the ScienceDirect and Scopus databases by taking into account only experimental researches (but literature reviews), in which the removal of each contaminant was evidenced and were in a range of 2006 and 2019. Besides, to make a comparison between the best methodologies in terms of the removal of the studied contaminants, the researches with the highest removal rates (> 70%) were selected. However, due to the implementation of conventional methodologies (without variations or additional ones), few results are presented with lower efficiency, this to show the considerable improvement of this variable, through process innovation. About the inclusion criterion mentioned, no research was rejected due to the nature of their scale; more than 75% of research belongs to tests carried out in the laboratory, and the remaining percentage corresponds to real and pilot size scales.

During the systematic review, 47 researches of 66, which were examined, were extracted; however, the review articles, as well as other documents, were considered and used to discuss the obtained results.

All information was written on a basis that contemplated the following fields: title of the article, author(s), year of publication, journal, country, type of research, its purpose, place where it was developed, methodology implemented and finally its results and discussion. A quantitative analysis of the information was carried out according to the number of publications per country, and the efficiency of each technology in the removal of contaminants.

**Advanced oxidation processes**

Advanced oxidation (AO) processes, in addition to removing other compounds, facilitate the removal of antibiotics from wastewater. These provide high efficiency due to the high oxidation potentials, which destabilize certain bonds, thus generating less harmful materials. Within these processes are ozonation, Fenton oxidation, photo-catalysis, electro-chemistry, UV radiation and gamma radiation. Below is a brief description of each case.
Ozonation

Ozonation belongs to AO technologies, within non-photochemical processes, and is used in wastewater to oxidize contaminants [18]. The ozone has two ways to react with pollutants; one by direct reaction and the other one by means of free radicals that are formed during the decomposition of ozone in water (indirect) [19]. Hydroxyl radicals are produced through free radicals, which are highly reactive and lead to the degradation of pollutants, when are attacked by ozone molecules [20].

The focus on the removal of antibiotics using this technology began in 2005. According to the review carried out, this technology has efficiencies greater than 84%; in addition, it shows the interest of countries such as Germany to eliminate antibiotic resistance genes from the waters, since these are transmitted horizontally from one bacterium to another increasing the public health problem. Table 1 presents the results of research conducted in three different countries where the target pollutants are mainly ARG and some antibiotics.

Table 1 presents the research related to technology, detailing in the first column the country where it was carried out, and in the second, the antibiotic, ARG, or ARB removed of the wastewater. The third column details the type of sample studied, in the fourth, the pollutant removal efficiency, and in the fifth column, the methodology used in the research is provided.

| Country | Antibiotic /ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|-----------------------|--------|--------------------|-------------------------|------|
| Portugal | Ampicillin (AMP), Azithromycin (AZI), Clarithromycin (CLR), Erythromycin (E), Ofloxacin (OFX), Sulfamethoxazole (SMX), Tetracycline (TC), Trimethoprim (TMP) | Municipal wastewater | >84% | Ozonation operated in continuous mode at different hydraulic retention times | [21] |
| China | Antibiotic resistant genes sul1, tet G e intl1 | Municipal wastewater | 91.4% | Ozonation in the ozone contact reaction tower | [22] |
| Germany | Resistant genes tetA, tetM, tetO, tetQ, tetW, sulI and sulII | Municipal wastewater | 99% | The WEDECO ozone system, type GSO. It comprises two columns of bubbles that operate in parallel flow. | [23] |
| Germany | Escherichia coli y Enterococcus faecium carriers of ARG | Drinking water | 99% | Discontinued experiments in 250 ml glass containers filled with drinking water. The ozone stock solution was prepared by spraying | [24] |
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| Country  | Antibiotic /ARG / ARB                                           | Sample                  | Removal efficiency | Implemented methodology                                                                 | Study |
|----------|---------------------------------------------------------------|-------------------------|--------------------|------------------------------------------------------------------------------------------|-------|
| Germany  | *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis* and *Estafilococos* | Municipal wastewater    | 3.7 log            | Molecular ozone disintegrates into three phases and dissolves in water.                   | [25]  |

**Source: Own source.**

The WEDECO system is used for high continuous oxygen production through a low energy consumption compared with a conventional generator since it produces up to 9% more ozone in its efficient line and 4% in its standard line, with the same consumption [26]. Additionally, there are removal efficiencies in logarithmic units (log), where 3.7 log is equivalent to a reduction greater than 99.9%.

**Fenton**

This AO process is carried out through the use of hydrogen peroxide (H$_2$O$_2$) and a transition metal as a catalyst, generating chain reactions that allow the H$_2$O$_2$ to be consumed; thus a hydroxyl radical is produced, which is capable of removing substances quickly [27]. Additionally, one of AO process advantages is the easy use, the low cost of its usage and the fact that it does not represent a threat to the environment [28]. This process has been implemented since 1984 with more than 344 publications to current date; and 21% of this have been published in 2019.

Fenton oxidation achieves better removal results when supplemented with other methods (see Table 2). However, the best efficiencies are obtained by implementing photo-fenton in an antibiotic solution, since it achieves better results in less time, converting this into greater efficiency within a WWTP, due to the possibility of treating a high flow rate in a short time.

| Country  | Antibiotic /ARG / ARB                                           | Sample                  | Removal efficiency | Implemented methodology                                                                 | Study |
|----------|---------------------------------------------------------------|-------------------------|--------------------|------------------------------------------------------------------------------------------|-------|
| Germany  | *Escherichia coli*, *Enterococcus faecium*, *Enterococcus faecalis* and *Estafilococos* | Municipal wastewater    | 3.7 log            | Molecular ozone disintegrates into three phases and dissolves in water.                   | [25]  |

**Source: Own source.**

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Table 2. Results of fenton treatment.

| Country   | Antibiotic / ARG / ARB                                      | Sample                                      | Removal efficiency                      | Implemented methodology                              | Study |
|-----------|------------------------------------------------------------|---------------------------------------------|-----------------------------------------|-----------------------------------------------------|-------|
| Switzerland | Azithromycin (AZI), ciprofloxacin (CIP), clarithromycin (CLR), metronidazole (MTZ), norfloxacin (NOR), ofloxacin (OFX), sulfamethoxazole (SMX) and trimethoprim (TMP) | Efluent municipal wastewater plant          | 100% for all antibiotics except clarithromycin with 91% | Photo-fenton treatment for 30 min                    | [29]   |
| China     | Erythromycin (E) and resistant genes \textit{ermG}, \textit{ermC} and \textit{ermB} | Artificial wastewater                     | 88.73% erythromycin 63.5\% \textit{ermG} 77.6\% \textit{ermC} 100\% \textit{ermB} | Electro-fenton system with stirring for 48 h.        | [30]   |
| China     | Tetracycline (TC), tylosin (TLS) and sulfamethoxazoline (SMX) | Mixed solution with 10 mg / L of each antibiotic | 100%                                    | Electro-fenton microbial system catalyzed with strontium hexaferrite nanoparticles type M. The results were after 24 hours of treatment | [31]   |
| India     | Amoxicillin (AML)                                         | Synthetic Amoxicillin Solution              | 100%                                    | Photo-fenton treatment for 3.5 minutes.              | [32]   |
| Brazil    | Norfloxacin (NOR)                                         | Norfloxacin solution 15 mg / L             | 60%                                     | Fenton oxidation treatment for 60 minutes            | [33]   |
| Colombia  | Klebsiella pneumonia                                      | Hospital wastewater                         | 6 log                                   | Photo-fenton with $S_{2}O_{8}$ for 60 minutes         | [34]   |

Source: Own source.

Photocatalysis

Photocatalytic oxidation or photocatalysis has been the subject of more than 654 research papers in recent decades, which increased exponentially in 2014 according to Scopus analysis. This process is based on the adsorption of light by a material used as a photocatalyst, which generates electron-hole pair, and these, in turn, hydroxyl radicals that lead to the degradation of pollutants [35]. Titanium dioxide ($\text{TiO}_2$) is the most researched and tested photocatalyst in studies conducted with this treatment [36]. It is emphasized that despite
being considered a promising treatment, the results of the registered research do not show high removal efficiencies for some antibiotics such as erythromycin and clarithromycin (see Table 3); the same goes for *Escherichia coli* HB101. However, it is noticed that in China the implementation of photocatalysis for the removal of ARB and ARG, showed important results, where a 99% removal efficiency for genes was achieved *ampC, mecA*, and the bacteria *Staphylococcus, Pseudomonas aeruginosa*.

Table 3. Photocatalysis treatment results.

| Country | Antibiotic /ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|-----------------------|--------|--------------------|-------------------------|-------|
| China   | Resistant bacteria *Staphylococcus* y *Pseudomonas aeruginosa* y resistant genes *mecA* and *ampC* | Solution prepared with the bacteria | 4.7 log *ampC* 5.8 log *mecA* 4.5 log *Staphylococcus* 5.8 log *Pseudomonas aeruginosa* | Treatment with TiO$_2$ / UV | [37] |
| Brazil  | Sulfamethoxazole (SMX) | Sulfamethoxazole solution | 97% | Heterogeneous photocatalysis using heterojunction between TiO$_2$ and CeO$_2$ | [38] |
| Cypress | Sulfamethoxazole (SMX), erythromycin (E) and clarithromycin (CLR) | Municipal wastewater | 87%, 84% and 86% respectively | A laboratory scale solar simulator was used and for photocatalytic treatment with TiO2-rGO | [39] |
| China   | *Escherichia coli* HB101, HB10663, HB10667 | Solution prepared | 12.2%, 59.1%, 43.4% respectively | Inactivation through photocatalysis. | [40] |
| Brazil  | Resistant bacteria *Pseudomonas aeruginosa* and *Bacillus subtilis* | Solution with bacterial strains | 100% | Inactivation with photocatalytic treatment UVA/TiO$_2$ P25 | [41] |

Source: Own elaboration.

**Ultrasound**

In the implementation of ultrasound, degradation of contaminants is performed by acoustic cavitation. In this phenomenon micro-bubbles filled with gas or steam, are formed by acoustic waves that are introduced into a liquid body. Thanks to the high pressure and temperature conditions within the microbubbles (which can reach 5000 °C and 1000 bar), the water molecules decompose generating H+ and OH- radicals, which lead to the...
degradation of pollutants [42]. Among the advantages of this method, the treatment in ambient conditions of temperature and pressure can be mentioned, and additionally to not requiring oxidizing agents; however, its energy costs for wave creation are high [43]. That is why, this treatment is rarely used for antibiotic removal. It was implemented from 2001 and its use expanded until 2018 with a decrease in publications. However, 3 of the existing publications were selected because of their relevance to the topic to be researched. Their results are found in Table 4 and show a removal above 90% with the use of the method. It is important to mention that it is not consistent to generalize these results, since the number of studies that provide information on the use of the method, are limited.

Table 4. Results of ultrasound treatment.

| Country | Antibiotic / ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|------------------------|--------|--------------------|-------------------------|-------|
| Colombia | Oxacillin (OX) | Simulated pharmaceutical wastewater | 99.9% | High frequency ultrasound was applied to water containing the antibiotic. | [44] |
| Korea | Tetracycline (TC) | Tetracycline solution | 90.7% | Pieces of the carbon nanotube sponge prepared with Ag₃PO₄ in a glass containing the solution. The ferrocene (catalyst) was dispersed by ultrasound and irradiated with visible light. The result was obtained by a space of photocatalyst band at 1.98 eV | [45] |

Source: Own source

Electrochemical

The electrochemical consists of the implementation of electrodes and electrolytes that allow the execution of the electrochemical process, where oxyradicals are generated and capable of oxidizing the pollutants, producing CO₂ and H₂O [46]. This method has been widely documented since 1978, with exponential growth in the last 5 years, leaving a total of 266 studies. From the latest research, five studies were selected (see Table 5). As per the results, the most widely used method with a removal efficiency greater than 90% is obtained by anodic oxidation in an electrochemical cell. This method allows the degradation of antibiotics directly and indirectly. However, a common denominator of the research is that the greatest degradation was obtained indirectly. This was thanks to the formation of oxidative agents during the treatment, such as electro-generated active chlorine, which decreases during the time of action, allowing the treatment of a higher flow rate in less time. Additionally, in the presence of chloride ions and basic pH the best removal efficiencies are obtained.
Within the applications of electrochemistry, there is electrolysis, a process that has been implemented in the treatment of wastewater which, through it, the decomposition of a substance is affected thanks to the chemical reactions induced by electricity. During electrolysis, a source in charge of supplying energy is used, an electrolyte to conduct it, an anode and finally a cathode [47]. In this process, the cations are directed towards the cathode and get electrons from it, and the anions are directed towards the anode where they lose electrons; electrolysis is a redox reaction [48].

Punctually of this process, 89 studies using this technology were recorded since 2009, from which 4 are examined and the information on the results is recorded in Table 5. These results show high removal efficiencies, 90% for chlortetracycline and 99.9% for tetracycline. However, any study presents result against the removal of ARB and ARG.

| Country  | Antibiotic /ARG / ARB | Sample                                     | Removal efficiency | Implemented methodology                                                                 | Study |
|----------|-----------------------|--------------------------------------------|--------------------|----------------------------------------------------------------------------------------|-------|
| Colombia | Oxacillin (OX), Cloxacillin (CLO), Dicloxacillin (DC) and resistant bacteria *Staphylococcus* | Antibiotic solutions prepared with deionized water | 100% of the compounds and bacterial resistance | Anodic oxidation with Ti/IrO$_2$ as a 10 cm zirconium anode and cathode. The results were obtained after 5 minutes of treatment, by indirect oxidation, thanks to reactive chlorine species generated by electrochemistry at the anode surface | [49]  |
| China    | Tetracycline (TC)     | Solution prepared with deionized water and tetracycline hydrochloride | >90%               | Anodic oxidation in electrochemical cell used as anode Ti/RuO$_2$-IrO$_2$. The results were obtained in 60 min | [50]  |
| Colombia | Norfloxacin (NOR)     | Sea water                                  | 100% in 20 minutes | Anodic oxidation in electrochemical cell with Ti/IrO$_2$ as anode in the presence of NaCl. | [51]  |
|          |                       | Municipal wastewater                       | 100% in 120 minutes|                                                                                       |       |
| México   | Amoxicillin (AML)     | Solutions prepared with distilled water    | >80%               | Formation of flocks in electrochemical cell, using Fe as an anode. The best result was obtained in 4 hours. | [52]  |
| Colombia | Cephalexin (CL), Cephadroxil (CPD), Cloxacillin (CLO), Oxacillin (OXA), | Solutions prepared with distilled water    | 100% for CIP, NOR, CLO and OXA | Anodic oxidation with Ti/IrO$_2$ as anode and Zr as cathode. The results were obtained within 20 minutes of treatment. | [53]  |
UV irradiation

Ultraviolet radiation has been used in recent years in the removal of pollutants thanks to its great effectiveness. During this process, the frequency of UV light in microorganisms, generates photochemical damage to their nucleic acids, which leads to their inactivation [58]. However, the removal of antibiotics by this method is relevant taking into account that they are photo active, which indicates that they are willing to absorb light by causing transformations in their structure [29]. To date, about 289 researches related to this method have been registered. Table 6 shows the results obtained during six researches carried out in three countries in 2015, 2016 and 2019. In these studies, there is diversity in the treated contaminants, with nine resistance genes, three resistant bacteria and ten antibiotics. Their results show greater efficiency when it was complemented with other methods such as oxidation with peroximonosulfate (PSM) and chlorination. However, it does not work in the
same and generalized way for all types of antibiotics as ARB and ARG require the previous identification of the contaminants to be treated.

### Table 6. Results of UV treatment.

| Country | Antibiotic / ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|------------------------|--------|--------------------|--------------------------|-------|
| Spain   | Resistant genes sul1, blaTEM and blaCTX-M-1 | Human and animal wastewater | 2 log | 8W germicidal lamp with a preheating of 15 min. The results were obtained after 10 min | [59] |
| China   | HLS-6 resistant bacteria and resistance genes sul1 and intl1 | Solutions prepared with the contaminants | 5.3 log HLS-6 | UVC/PMS treatment | [60] |
| China   | Antibiotic resistance genes sul1, tetG, intl1 | Municipal wastewater | 91.4% | UV irradiation with doses of 12,477 mJ/cm² | [21] |
| Spain   | Azidothymidine (AZT), clarithromycin (CLR), ciprofloxacin (CIP), ofloxacin (OFX), sulfamethoxazole (SMX), sulfadiazine (SFD), sulfapyridine (SFP), trimethoprim (TMP), metronidazole (MTZ), clindamycin (CLI) | Efluent municipal wastewater | 48% SMX, 95% SFD, 72% SFP, 92% MTZ, 42% CIP, 95% CLI, 20% AZT, <10% CLR, <10% OFX, <5% TMP | UVC/Fe(II)/PMS treatment | [61] |
|         | Resistant genes 16S, blaOXA-A, blaTEM, intl1, qnrS, sul1 | | Log 0.41 | UVC | |
| China   | Resistant genes sul1, intl1 | Drinking water | 3.5 log sul1 4.0 log intl1 | UV / Chlorination. Less than 20 minutes of treatment | [62] |
| Germany | *Escherichia coli* and *Enterococcus faecium* carriers of ARG | Drinking water | 99.9% *Escherichia coli* and *Enterococcus faecium* | 0.8 to 2.8 UV irradiation with 600 m² | [24] |

**Source: Own source.**

Most researches that implemented this technology express the removal efficiency on a logarithmic scale, where 0.41 log is equivalent to an removal greater than 85% and 5.3 log is equivalent to 99.99%; in other words, except for the case of the study carried out in Spain, all removal exceeded 90%.
Gamma irradiation

In this technology, radicals, reactive electrons, ions and neutral molecules are generated by exposing water to high-energy electromagnetic radiation; in this way, they modify the pollutant molecules and finally affect their removal [63]. This radiation has been implemented to a lesser extent compared to UV radiation, as it has a total of 59 related documents. The rates of removal of gamma radiation for certain antibiotics, and antibiotic resistance bacteria are high (see Table 7). However, not all are degraded with the same radiation dose, due to their properties and conditions. Additionally, a common factor in obtaining good results in the analyzed studies is that the solution is in an acidic condition, since it allows greater generation of H+ radicals and increases their degradation.

Table 7. Results of treatment with gamma irradiation.

| Country     | Antibiotic / ARG / ARB          | Sample                                      | Removal efficiency | Implemented methodology                                      | Study |
|-------------|--------------------------------|---------------------------------------------|--------------------|--------------------------------------------------------------|-------|
| United States | Penicillin V (PC), penicillin G (P) and amoxicillin (AML) | Solution prepared                              | 81% for penicillin V, 92% for penicillin G and 95% for amoxicillin | Linear electron accelerator and a radioactive source of cesium (Cs) | [64]   |
| China       | Cephalosporin C (CEP)           | Deionized and underground water              | 100%               | Gamma radiation at 0.4–2.0 kGy                              | [65]   |
|             | *Escherichia coli* and *Staphylococcus aureus* |                                             | 100%               | Gamma radiation at 2.5 kGy                                 |       |
| China       | Sulfamethoxazole (SMX)          | Solution with contaminant and deionized water | 100%               | Gamma radiation at 2 kGy                                    | [66]   |
| India       | Ofloxacin (OFX)                 | Ofloxacin solution                           | 75%                | Gamma radiation at 1 kGy and pH 3.0                         | [67]   |

Source: Own source.

Membrane bioreactor

Membrane bioreactors are widely used in the secondary treatment of WWTPs; these are composed of a suspended reactor, with membrane separation (micro-filtration or ultra-filtration) to perform biological treatment and solids removal [68]. In the bioreactor, the wastewater that leaves the biological reactor, passes through the membranes where compounds larger than its pore are retained thanks to the negative pressure induced by a pump; this makes possible the separation between water and biomass, where the latter
remains in the bioreactor [69]. This technology has been evaluated in the removal of antibiotics, ARB and ARG, and the results of seven researches have been extracted (see Table 8), where the efficacy in the removal of antibiotics is evidenced by means of membrane bioreactors with an added value, whether microorganisms, hollow fiber or combination of aerobic and anaerobic processes are used. Additionally, with this technology more studies are presented with a focus on ARG and ARB.

| Country | Antibiotic / ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|------------------------|--------|--------------------|-------------------------|-------|
| China   | Chloramphenicol, lincomycin, clindamycin, norfloxacin (NOR), ofloxacin (OFX), ciprofloxacin (CIP), enrofloxacin, nalidixic acid, roxithromycin, clarithromycin (CLR), erythromycin (E), trimethoprim (TMP), sulfamethoxazole (SMX), sulfadiazine (SFD) and resistance genes tetG and tetO | Sample of Permeated bioreactor | 64.79 ± 4.68% on average for all antibiotics in the summer. | Membrane bioreactor operating in a WWTP | [68] |
| Singapore | Amoxicillin (AML), azithromycin (AZI), ciprofloxacin (CIP), chloramphenicol, meropenem, minocycline, oxytetracycline (OTC), sulfametazine (SFD), vancomycin, trimethoprim (TMP), resistant genes and resistant bacteria | Sample of Permeated bioreactor | 4.8 log for resistance genes. | Membrane bioreactor operating in a WWTP | [70] |
| China   | Antibiotic resistance genes: sul1, sulII, tetC, tetX, ereA and intI | Wastewater taken from WWTP | 0.6-5.6 orders of magnitude | Tetracyclines and sulfonamides are added to the laboratory scale membrane bioreactor influencer, in order to induce ARG proliferation | [71] |
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**Table 9**

| Country | Antibiotic /ARG / ARB | Sample | Removal efficiency | Implemented methodology | Study |
|---------|-----------------------|--------|--------------------|-------------------------|-------|
| China   | Sulfametazine (SFD)   | Sample of activated sludge | 95.4±4.5% | Hollow fiber membrane bioreactor to enrich the compound’s degrading microorganisms | [72] |
| China   | Sulfonamides, tetracyclines, fluoroquinolones | Swine wastewater | > 90% for sulfonamides and tetracyclines, <70% for fluoroquinolones | Batch sequence membrane bioreactor | [73] |
| China   | Sulfonamides | Solutions prepared with 9 sulfonamides | 93.9-97.5% | Anaerobic membrane laboratory scale bioreactor/anoxic/oxide | [74] |
| Vietnam | Norfloxacin (NOR), ofloxacin (OFX), ciprofloxacin (CIP), tetracycline (TC), trimethoprim (TMP) | Hospital wastewater | 93.99%, 73.93%, 76-93%, 100% and 60-97% respectively | Sponge membrane bioreactor with hollow fiber membrane | [75] |

**Source:** Own source.

### Absorption with activated carbon

During this process, there is a solid medium (activated carbon) on which the soluble substance is arranged. This way activated carbon manages to absorb antibiotics and does not generate toxic products [76]. This is possible thanks to the calcination and physical or chemical activation of the carbon, where its porosity varies and the extraction of pollutants by adsorption, becomes effective, as it attaches to other molecules [77]. Adsorption with activated carbon for the removal of antibiotics began approximately in 2007 according to the revised documents. Table 9 shows the results obtained in five researches carried out with this method in four countries, which show the possibility of using organic waste for the preparation of activated carbon in furnaces, which gives added value and can reduce costs of raw material, still achieving removal results greater than 95%. However, its efficiency is reduced in the presence of organic matter in the environment, so it is necessary to implement it after secondary treatment within a WWTP.
In the studies, it was found that with certain doses of activated carbon, the removal of the compounds reached a constant point, which means that the amount of activated carbon is not directly proportional to the removal of the antibiotic. However, it is necessary to provide the surface required for adsorption according to the characteristics of the medium.

Table 9. Results of treatment with activated carbon.

| Country | Antibiotic /ARG / ARB | Sample                  | Removal efficiency | Implemented methodology                                                                 | Study  |
|---------|-----------------------|-------------------------|--------------------|-----------------------------------------------------------------------------------------|--------|
| Spain   | Dimetridazole (DTZ), Ronidazole (RTZ), Metronidazole (MTZ), Tinidazole (TDZ) | Municipal wastewater | >93%                | Adsorption with activated carbon combined with added microorganisms, and a nutritive medium to favor its growth | [76]    |
| Korea   | Oxytetracycline (OTC), minocycline (MNC), mecloxycline-sulfosalicylate (MCC), democloxycline (DMC) and Tetracycline (TC) | Synthetic waters      | >90%                | Granular activated carbon for 10 min                                                  | [78]    |
|         |                                                                    |                        | >90% OTC, DMC, and TC |                                                                                       |        |
|         |                                                                    |                        | <70% MNC and MCC       |                                                                                       |        |
| United States | Erythromycin (E), triclosan, Sulfamethoxazole (SMX) and Trimethoprim (TMP) | Municipal wastewater | >90%                | Granular activated carbon in scaled columns                                              | [79]    |
| Iraq    | Ciprofloxacin (CIP), Norfloxacin (NOR)                             | Solutions prepared with the compounds | 96% CIP in 90 min | Activated carbon prepared from seed pods of the Albizialebbeck tree                     | [80]    |
|         |                                                                    |                        | 98% NOR in 60 min       |                                                                                       |        |
| Iran    | Cephalexin (CL)                                                   | Compound Solution      | 100%                | Activated carbon with nut shell, prepared by chemical activation in the presence of ZnCl2. | [81]    |

Source: Own source.

Results

Among the documents reviewed, 47 researches were used and their findings were presented regarding the removal efficiency of antibiotics, ARB and ARG. The researches were grouped by continents based on the country where they were developed, resulting in three continents namely; Asia, America and Europe. Asia is the continent where the greatest number of researches were done. This result is due to China's participation is 36% of the total. The
interest of this country in testing and developing new technologies that allow the removal of antibiotics, ARB and ARG, is because as a major producer and one of the largest consumers of antibiotics, they are asked to controlling their consumption and reducing their amounts in wastewater; on the contrary, by 2050 bacterial resistance could cause the death of about 1 million people in that country [6], [82]. On the other hand, America has a 26% participation in publications on the subject, where Colombia stands out with 13% of the total research, evidencing the growing concern regarding the problem, due to its impacts on the environment and the population that increases with its low rate of wastewater treatment. Finally, the continent with the lowest participation is Europe with 19%, where Germany and Spain have the same contribution equivalent to 6% of the total studies.

The research shows an inclination towards wastewater, facing the problem that exists in it considering that they were the object of study of 51% of them. This figure was followed by the solutions previously prepared with the contaminants, which correspond to 37% of the researches. The remaining percentage belongs to drinking water, groundwater and seawater.

Among the most evaluated antibiotics for their removal are sulfamethoxazole, trimethoprim, ciprofloxacin and norfloxacin, because these are part of the most prescribed antibiotics [7], and therefore the ones with the greatest attention for removal from wastewater. However, despite being amoxicillin (belonging to the penicillin group), the most consumed antibiotic according to studies conducted in Colombia and in 25 countries in Europe [7], [83]; this research was not representative, considering that it was the object of study of 4 researches from the 47 analyzed.

Additionally, it was determined that the most studied bacterium was *Escherichia coli* with a total of five researches. The importance of this bacterium is that it is resistant to various antibiotics as confirmed by the study carried out in 2003 by Reinthaler et. al [84], where its resistance was demonstrated in 16 antibiotics out of 24 antibiotics tested. Within these, different resistance rates were found to ampicillin, piperacillin, capheotoline, trimethoprim, sulfamethoxazole and tetracycline, among other antibiotics [84]. However, given the results of this study, it is important to mention that *E. coli* is still susceptible to various antibiotics [84], [85]. Similarly, the *sul1* resistance gene is distinguished within the researches, because it is a sulfamethoxazole-resistant gene [86], which is why it is part of the *Escherichia coli* genes, increasing resistance to this antibiotic.

In the technologies studied, it is possible to identify variations in their implementation that increase or decrease their removal efficiency. This is the case of discontinued systems and the WEDECO system for ozonation, which improve the performance of the method. Similarly, the photo-fenton treatments, nano electrolysis, photocatalytic treatment with TiO₂ and UV, high frequency ultrasound, anodic oxidation with Ti / IrO₂ as an anode, UVC/PMS radiation, gamma radiation with approximately 2 kGy, bioreactor of hollow fiber membrane,
and adsorption with activated carbon made from organic elements. The results of these treatments were retrieved to make comparisons and are presented in Figure 1, where there is a graph based on the technology that shows the removal of each contaminant. This shows that the treatment with fenton was the one with the greatest removal of antibiotics, where 85% of these were completely removed by showing greater performance compared to the electro-chemistry that completely eliminated 71% of the antibiotics treated.

Previous studies confirm the good performance of fenton based reactions against other advanced oxidation processes because it does not produce toxic effluents and achieves high removal efficiencies even more in the presence of light, as is the case with photo-fenton [87],[88]. The latter has also been tested in the presence of oxalic acid and ultrasound, obtaining removal efficiencies of 100%, for metronidazole and clindamycin; whilst for antibiotics such as trimethoprim, ciprofloxacin, norfloxacin, among others, its removal efficiency was less than 70% [89]. The fenton process has been evaluated for the removal of other contaminants, in which it also stands out against the others, since it allows their mineralization and does not generate total organic carbon, once the reaction ends [90]. However, they also expose a series of difficulties when being implemented, due to the rigorous pH range that it handles, iron oxide generation that hinders the process and the instability of the mixture of the agents used [89]. The difficulties presented during the treatment of wastewater can be overcome by choosing the appropriate catalyst, in addition to the application of heterogeneous photo-fenton where the catalyst exhibits greater stability [91].

The results of previous reviews on the efficiency of electro-chemistry highlights its success in water remediation and its current growing research as it additionally eliminates persistent organic pollutants and presents advantages such as low costs, easy implementation and use [92]. Advantages which are due to the saving of chemical products, thanks to the generation of oxidizing agents in situ and the operation of the technology in environmental conditions [93]. However, this technology is still at an intermediate level of technological preparation, which makes it difficult to apply at the industrial level. It is necessary to study components such as the anode and cathode, in order to make an adequate selection of it which allows optimal operating conditions, as well as the development of a mechanical design, suitable for the electrochemical cell [94].
Figure 1. Removal of contaminants by technology.

(a) OZONATION
(b) FENTON
(c) PHOTOCATALYSIS
(d) ULTRASOUND
(e) ELECTROCHEMICAL
(f) UV RADIATION
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In the case of resistance genes, photocatalysis is the treatment that has the highest removal efficiency compared to the genes studied (mecA and ampC), followed by UV radiation that shows the greatest removal of the sul1 and intI1 genes. Additionally, it was found that the photocatalysis performance is again superior to ozonation in the removal of resistant bacteria, together with gamma radiation that presents a complete removal of Staphylococcus.

Photocatalysis proves to be a convenient technology for the removal of bacteria and resistance genes at the same time. This is a result consistent with previous research that shows this method as promising, by achieving the degradation of a high range of pollutants, with benefits such as stability of the catalyst, the operation under lower limitations regarding the pH of the solution and the possible decrease in operating costs due to the use of sunlight [95], [96]. During photocatalysis it is possible to implement different catalysts such as Al2O3, ZnO,
Fe₂O₃ and TiO₂ [96]. However, the efficiency of TiO₂ photocatalysis for wastewater disinfection, is highlighted, as it reaches lower toxicity and cost, as well as complementing it with other methods such as UV radiation, in order to accelerate reactions [95], [97], both variables coincide with the selection of the best application of photocatalysis in this review.

As for gamma radiation, other studies also emphasize its potential, due to by means of ionizing radiation, it is possible to attack antibiotic molecules directly and also damage microbial DNA obtaining optimal results [98]. Despite this, this technology is rarely used for the treatment of wastewater because of its high investment cost and the inputs required for its operation [99], a disadvantage that can be overcome by combining gamma radiation with biological treatments in WWTP [100].

Regarding the operating costs of the most important technologies in the elimination process, although there are few studies detailing this variable, the research carried out in 2009 by Pablo [101] showed that the treatment of wastewater by fenton processes represents a lower cost, compared with other POAs such as ozonation and electrochemistry; the cost is around 8 USD (5.75 €) per m³, versus 81 and 15 USD per m³ (58.24 € and 10.785 € per m³ approximately to date). The results were obtained in the elimination of chlorophenol [101]. This research can be compared with one carried out in 2015, where estimated operating costs of photocatalysis and photo-fenton were determined at 8.69 and 5.2 € per m³ respectively, during the treatment of wastewater from the pesticide industry [102].

The costs of each technology will vary depending on the pollutant load and the target pollutants, as well as the modifications made in each implementation. A clear example of the last variable, was evidenced in a research where the costs of the conventional fenton process were compared, with photo-fenton in the elimination of adsorbable organic halogens; reaching costs of € 70.0 and € 46.5 per m³ respectively [103].

Conclusions

In recent decades, research has been done to evaluate the technologies in the removal of antibiotics, ARB and ARG from wastewater, as they cause alterations in the aquatic environment and affect public health. In these area, there is the collaboration of several countries; however, the most representative is China, since its interest is driven by being the largest producer and one of the largest consumers of antibiotics in the world.

During the development of the article, 9 technologies were identified in the removal of the target pollutants, such as the advanced oxidation process (AOP) with ozone, fenton, photocatalysis, ultrasound, electrolysis, electrochemistry, UV radiation and gamma
radiation, as well as membrane bioreactors and activated carbon adsorption. Within these technologies, the ones that are most effective for the removal of antibiotics according to the analysis of the information are the fenton and electrochemical processes, achieving the complete removal of some of them. On the other hand, for the removal of ARB and ARG, the photocatalysis and gamma radiation processes were more efficient, respectively. In each of the mentioned technologies, they presented modifications that increased their efficiency. In that sense, the importance of innovating on conventional processes is highlighted, in order to achieve better results and meet the need to eliminate these pollutants in wastewater, given the problems that cause the environment and the population.

During the choice of technology to be implemented within a WWTP, for the removal of these contaminants, it is necessary to also characterize the type of water to be treated, in order to determine its presence in detail and select the one that best suits the conditions, including the other physicochemical parameters to remove. However, a substantial variables exist in the cost of each treatment with the respective variations or combinations that increase its efficiency; therefore, it is recommended to continue the research through a financial analysis of the technologies.

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