

Review

UV Disinfection Systems for Wastewater Treatment: Emphasis on Reactivation of Microorganisms

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Abstract: UV disinfection is cost-effective and easy to maintain for decentralized areas. However, to ensure its effectiveness, some parameters need to be considered. In this study, a general search of Web of Science articles was performed to determine the possible influence of these parameters on the reactivation of microorganisms in UV systems; in addition, different search strings were used focusing exclusively on wastewater treatment, UV systems and Advanced Oxidation Processes (AOPs). It was found that in order to maintain low transmittance, it is essential to remove suspended solids and reduce water hardness. It is recommended to control the zeta potential in the range of 0–5 mV to avoid the aggregation of particles and bacteria. Determining the appropriate UV dose is essential to mitigate the reactivation of microorganisms. A minimum dose of 40 mJ/cm² can contribute to effective disinfection and reduce the likelihood of reactivation. In addition, maintaining a residual chlorine level of at least 0.5 mg/L provides an additional barrier to reactivation. It is also important to optimize the design flow rate of the UV system as recommended for each individual unit. These measures, together with the combination of UV disinfection and chemical or AOPs, can effectively reduce the reactivation.

Keywords: disinfection; microorganisms; solids; reactivation; ultraviolet; wastewater



Citation: González, Y.; Gómez, G.; Moeller-Chávez, G.E.; Vidal, G. UV Disinfection Systems for Wastewater Treatment: Emphasis on Reactivation of Microorganisms. *Sustainability* **2023**, *15*, 11262. <https://doi.org/10.3390/su151411262>

Academic Editor: Silvia Fiore

Received: 9 June 2023

Revised: 23 June 2023

Accepted: 29 June 2023

Published: 19 July 2023



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1. Introduction

Rapid urbanization and industrialization have generated increasingly severe water stress, along with massive wastewater discharge [1]. In 2010, the United Nations General Assembly recognized the right to safe and clean drinking water and sanitation as an essential right for the full enjoyment of life and all human rights, thereby endorsing the right of everyone to have sufficient, continuous, safe, physically accessible, affordable, and clean water for personal and domestic use, along with adequate sanitation [2]. Against this backdrop, the objective of wastewater treatment plants (WWTPs) is to adequately sanitize effluents, decreasing contaminant concentrations to increase water resource quality [3].

In WWTPs, disinfection systems play a fundamental role, removing harmful microorganisms, anthropogenic contaminants, and bromide/iodide, compounds found naturally in most water bodies. They are also powerful oxidants, helping oxidize the organic matter present [4,5]. Ozone, chlorination, and ultraviolet radiation (UV) are the three most used disinfection systems worldwide. The use of each system depends on the type of microorganism to be disinfected, the suspended solid content, temperature, pH, turbidity, and contact time, that is, the time for which the disinfectant and the wastewater interact [6].

Ozone disinfection has been found to have a high biocidal power in a broad microbial spectrum, in addition to offering taste, discoloration, and odor control and the reduction of

trace organics in treated water, increasing the biodegradability of the organic contaminants present [7,8]. Ozone presents high oxidation power and has the advantage of being able to easily diffuse through the microorganism membrane in the cytoplasm; the inactivation activity generated by this method is caused mainly by the direct oxidation of dissolved ozone molecules. However, the generation of disinfection by-products (DBPs) such as aldehyde and brominated compounds that can cause kidney cancer has been documented [9]. Chlorine, meanwhile, acts by destroying the nucleic acids and cell membranes of organisms, although DBPs are produced as a result of its reaction with organic components in the water.

DBP generation has been established for four disinfectants: chlorine, chloramines, ozone, and chlorine dioxide (including combinations of them) and recently for UV treatment with post-chlorination. The best known DBPs are trihalomethanes (THMs) and haloacetic acids (HAAs), which are regulated in the United States and other countries. The most studied route of DBP exposure has been ingestion, but bathing, showering, and swimming are also routes, as are dermal exposure and inhalation [10]. To date, almost one thousand DBPs have been detected, and it has been reported that they could generate adverse reproductive and developmental effects, genotoxicity, and carcinogenicity [11].

It has been estimated that over 50% of the total organic halide (TOX) resulting from water chlorination and over 50% of the assimilable organic carbon (AOC) formed during ozonation have not been counted in the identified DBPs [4].

Ultraviolet radiation, meanwhile, has been used as a disinfectant due to the broad spectrum of sterilization generated. Unlike traditional disinfection (e.g., chlorination), ultraviolet disinfection does not require the addition of chemicals and avoids DBP formation and the possible generation of disinfection resistance in bacteria [12]. It has been shown to be effective at inactivating various microorganisms present in effluents, including *Cryptosporidium* and *Giardia*, the protozoa found most frequently in water meant for human consumption. However, the organisms most resistant to UV rays are viruses, specifically adenoviruses and bacterial spores [13].

Unlike chlorine, which requires a contact time of 20 to 60 min with a dose of 0.5 mg/L to guarantee that the residual is sufficient for the continuous inactivation of microorganisms, the UV system tends to have a lower contact time and disinfection can be effective at hydraulic retention times (HRTs) of 5 to 30 s with doses of 30 to 60 mWs/cm² [6]. However, UV disinfection does not have a lasting residual disinfection and it has been shown that some pathogenic microorganisms are capable of reactivating in the presence of light—known as photoreactivation—or through dark repair, limiting the effectiveness of this disinfection method [14].

To meet this need, it is essential to optimize operational parameters and to promote research on UV disinfection systems. A baseline needs to be established to provide guidance on recommended parameters to achieve effective disinfection and reduce the reactivation of exposed microorganisms. This publication reviews the available literature to provide answers and applies this knowledge specifically to wastewater treatment; however, its applicability extends to a number of areas, such as drinking water supply and water treatment in industrial and food processes. Ultimately, UV disinfection systems offer a wide range of practical applications that contribute to the protection of human health and the improvement of water and air quality in a variety of environments, but it is essential to continue to investigate the appropriate operational parameters and to assess whether they need to be complemented by other disinfection systems.

2. Methods

In the first instance, a general search of scientific publications was carried out in Web of Science (WoS), in order to understand the possible influence of parameters, such as suspended solids, zeta potential, particle size, and UV dose, on the reactivation of microorganisms in the UV system and new disinfection systems.

Then, to glimpse where the research is going in the study area, more search criteria were specified in WoS. A search for publications was performed by entering search strings (using the words “wastewater treatment” and “UV” or “ultraviolet”). The search criteria were:

- All search fields are included.
- Only scientific articles are included, excluding reviews, books, book chapters, proceedings, and others.
- Includes articles published from 2003 to 2022.

Similarly, a search was carried out to visualize the evolution of AOPs combined with UV. We proceeded in the same way as described above but added as criteria in the search strings of the most well-known AOPs, which include UV/ozone, UV/hydrogen peroxide, UV/PS, UV/chlorine, UV/titanium dioxide, and UV/ozone/hydrogen peroxide. As an example, for UV/ozone, the search fields were “wastewater treatment” and “UV” or “ultraviolet” and “ozone” or “O₃” and scientific articles between the years 2003 and 2022 were selected.

3. UV Systems Implemented in WWTPs

With industrialization and the increase in effluents, they were discharged into water bodies, increasing their load, and the lack of adequate sanitation contaminated the environment. As a result, the solution was dilution, but soon it was accepted that treatment was necessary. The UN has stated that every USD spent on treatment provides an estimated societal benefit of USD 5.5 [15]. Wastewater treatment continues to be a global challenge; in the case of India as of 2017, there was a total treatment capacity of 18.6%, with only 13.5% of wastewater treated effectively. Treatment must consider the discharge standards established by each country for effluent disposal. Discharge can be based on concentration or load, with the former being more common. Concentration standards indicate an allowed contaminant mass per liter. Their limitation is that dilution can be used to comply with the discharge limit and wastewater treatment is not promoted. Meanwhile, the load-based standard allows risks to the water body to be modeled, establishing the contaminant value at which water quality at a standard appropriate for the use preference for that body is guaranteed to be maintained; thus, short- and long-term effects are considered [16].

Lack of sanitation or an insufficient level of treatment generates risks in the population such as infections and diseases, growth delays, and appearance and propagation of antimicrobial resistance [17]. Therefore, a treatment based on primary, secondary, and tertiary treatment is vital to decrease risks related to inadequate effluent disposal. For this reason, there are WWTPs that have incorporated the use of UV disinfection systems to increase the efficiency of disinfection and decrease the aforementioned risks.

Ultraviolet radiation has been used since 1910 in Marseille to disinfect water [18]. In 2000, the United States Food and Drug Administration approved UV disinfection as an effective method that can inactivate the replication of pathogenic microorganisms. The increase in the applicability of the UV system as a disinfection method in the United States and Europe occurred after the discovery of its high efficiency against *Cryptosporidium* and *Giardia*, as chlorine is not as effective against these pathogens [13]. As of 2019, there were more than 7000 municipal installations with UV disinfection in the world, and by 2020, the UV system market was expected to reach USD 2.8 billion [19]. UV systems have been installed in various countries and under different conditions. In general, they have been installed after secondary treatments based on activated sludge. The number of lamps used is usually one, and influent transmittance is a datum mentioned in studies on WWTPs that use UV radiation due to its impact on system efficiency. According to the EPA [20], the choice of UV disinfection system depends on three critical factors:

- Hydraulic properties of the reactor. There should be a uniform flow with sufficient axial movement, preventing dead zones that can decrease the contact time and divert the trajectory of the organism;
- UV radiation intensity. Lamp age, fouling, and placement inside the reactor must be considered;

- Wastewater characteristics. Suspended and colloidal solids, flow rate, and bacterial density are parameters to consider for UV system implantation. The higher these parameters are, the less radiation the organisms will absorb, affecting final disinfection.

3.1. UV System Disinfection Method

Ultraviolet radiation is divided into four wavelength bands: UV-A that acts between 315 and 400 nm, UV-B between 280 and 315 nm, and UV-C between 200 and 280 nm; the vacuum UV that acts between 100 and 200 nm cannot be used for wastewater disinfection because the wastewater absorbs it. In contrast, UV-B and UV-C have a high bactericidal power; it is estimated that 253.7 nm is the most effective wavelength for UV disinfection. At higher or lower wavelengths, absorbance decreases. Below 230 nm absorbance, it increases again [13,18].

The UV system is a physical agent based on radiation, and it has the characteristic of not generating DBPs that can affect human or biotic organism health. The method is based on the fact that at high doses (>80 mWs/cm²), the proteins in microorganisms absorb this radiation, which will lead to their death or inactivation [21]. UV light comes from mercury lamps. There are low-pressure (LP) mercury lamps that emit nearly monochromatic light at 254 nm and medium-pressure (MP) lamps that emit in a polychromatic spectrum. The disadvantages of these lamps are that they are fragile and contain toxic mercury, an element that is dangerous for the environment and requires proper disposal. They also require large amounts of energy to function, as wall plug efficiency is around 15–35%, and they have a useful life of approximately 10,000 h, which is considered low [12]. Recently, the use of UV light-emitting diodes (UV LEDs), which can adjust the wavelength and emit radiation from 210 nm to visible light, has been studied [19]. These LEDs are mercury free and have small sizes, along with a fast startup time. However, depending on the wavelength, they can present limitations such as low radiation flux and energy efficiency, generating low UV LED fluence rates. It has been recommended that the design of new reactors that use UV LEDs consider the hydrodynamics of the reactor, radiation distribution, and kinetics [12,22].

The method of action of ultraviolet radiation is shown in Figure 1; it is based on the fact that UV light is directly absorbed by the nucleic acid, attacking the DNA of the microorganisms, resulting in the formation of photoproducts, with cyclobutene-pyrimidine dimers (CPD) and 4–6 photoproducts (6–4 PP) standing out. The former are formed between adjacent pyrimidine molecules in the same DNA chain, which can affect DNA transcription or replication, generally a pair of thymines [9,23].

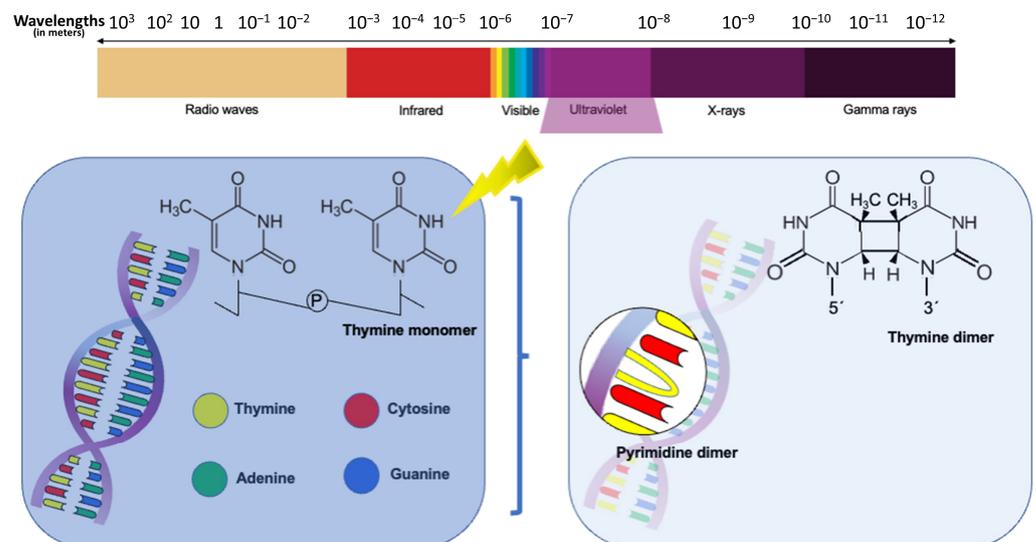


Figure 1. Representative scheme of the mechanism of cell damage by the UV system. Source: self-made based on Lee et al. [9].

Above 320 nm, the action spectrum shifts from the pattern of the DNA absorption spectrum, suggesting the contribution of other action mechanisms such as photochemicals. This damage occurs when the light is absorbed by a sensitizer, which enters an excited state and generates damaging reactions; in the presence of oxygen, reactive species such as singlet oxygen and hydrogen peroxide can form [24].

As UV disinfection acts on DNA, it has also been found to cause damage to antibiotic resistance genes (ARGs). These genes have been recognized as emerging environmental contaminants, and their occurrence in WWTPs has been observed [25]. It has been found that doses of 5 mJ/cm² could eliminate resistance to antibiotics, specifically tetracycline and erythromycin, in WWTP effluents, and it has been reported that UV disinfection followed by chlorination could generate a synergistic effect, avoiding microbial regrowth [26,27].

Regarding the removal of pharmaceutical and personal care products (PPCPs), Pai and Wang [28] studied exposure to a 14 W UV lamp at 254 nm with a dose of 40 mW/cm², with which the effective removal of aspirin, ibuprofen, benzophenone, oxybenzone, caffeine, and N,N-diethyl-meta-toluamide or most estrogens was not observed. However, when using chlorine and hydrogen peroxide (H₂O₂), efficiency improved, reaching values ≥56.5% for UV/chlorine and ≥27.6% for UV/H₂O₂.

The efficiency of UV disinfection depends on UV fluence, which is defined as the product of the incident irradiance (fluence rate) and exposure time, corrected for water absorption, the Petri factor, and the reflection factor. The Bunsen–Roscoe law of dose–time reciprocity establishes that efficiency will be proportional to the applied UV fluence, but for UV disinfection, it will also depend on biological processes, affecting exposure time, wavelength, and intensity [22].

The inactivation kinetics for chemical disinfectants have commonly been described by the first-order disinfection model of Chick and Watson [29,30], shown in Equation (1), where inactivation of the N record is described by:

$$\log_{10} \left(\frac{N_t}{N} \right) = -k \cdot \text{Fluence} \quad (1)$$

where N_t is the microorganism concentration after contact time t and fluence is the product of the UV fluence (mW/cm²) and exposure t (mWs/cm² = mJ/cm²) [13].

3.2. Analysis of Factors Affecting UV Disinfection

Despite the growing interest in UV disinfection, its problems have become clear. These issues result mainly from the components presented by the influent to be disinfected, along with the equipment characteristics, as shown in Figure 2.

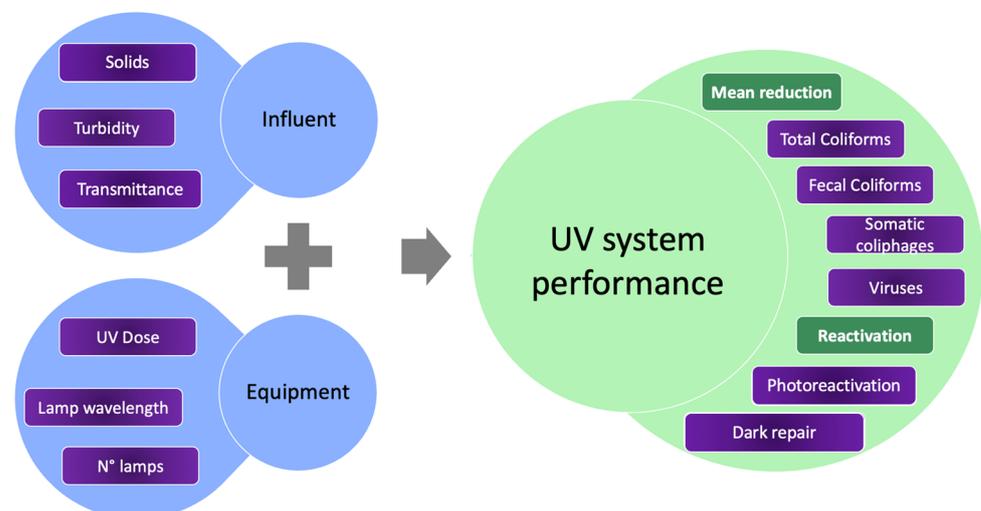


Figure 2. Conceptualization of factors affecting UV system performance. Source: own authorship.

One of the problems affecting UV disinfection is fouling on the quartz sleeves of the UV lamps, which can reduce efficiency if not cleaned properly. Fouling mechanisms are varied. It has been found that high hardness and/or high iron concentrations of heat can favor this fouling, especially Fe(III), which is dosed as FeCl_3 and is among the main inorganic components associated with fouling [31]. Meanwhile, Nessim and Gehr [32] found that UV ray intensity decreased by up to 100% when iron was present. Thus, lamp fouling mechanisms induced by UV rays were (a) $\text{Fe}(\text{OH})_3$ precipitation, (b) carbonate precipitation, and (c) the release of calcium from calico–organics complexes followed by precipitation of iron–organics. Iron and/or calcium created the most favorable conditions for fouling to occur; by contrast, when phosphorous was present, fouling in the UV section was reduced. One of the biggest problems induced by UV equipment is considered to be the dose or fluence of the system, which plays a dominant role in microorganism deactivation.

3.2.1. UV Dose

Dose plays an important role in the inactivation of microorganisms subjected to ultraviolet radiation; therefore, it is essential to provide an optimal dose of UV radiation during the disinfection process to achieve complete inhibition of cell replication. Artichowicz et al. [33] found that the most effective zone in UV reactors in terms of disinfection is the direct radiation zone, while the influence of inflow and outflow zones is insignificant. This means that UV disinfection reactors must be designed to deliver the required amount of radiation at the point at which the fluid velocity is highest.

The dose in UV disinfection plants for water, which are equipped with low-pressure mercury lamps, is defined as reduction equivalent fluence (REF), while for plants that use high-pressure mercury lamps, there are various definitions of “dose” [34].

Dose dependence has been observed with the inactivation of *Escherichia coli* O157:H7, with a high dose of $913 \text{ mJ}/\text{cm}^2$ of UV irradiation causing damage to the cell membrane, while the membrane remained intact with a relatively low dose of $0\text{--}203 \text{ mJ}/\text{cm}^2$ [35]. Meanwhile, Salcedo Dávila et al. [36] found with a pilot plant that a mean UV dose of $50 \text{ mWs}/\text{cm}^2$ was sufficient to meet required microbiological quality standards. Nguyen et al. [5] observed that inactivation varied with the UV dose applied, finding that with $47.8 \pm 1.6 \text{ mJ}/\text{cm}^2$ the coliphage MS2 reduction was $2.6 \pm 0.1 \text{ uLog}$, while with a dose of $69.4 \pm 3.8 \text{ mJ}/\text{cm}^2$ the reduction increased to $3.7 \pm 0.2 \text{ uLog}$. These findings involved the following fixed values: COD: $141.8 \pm 53.5 \text{ mg}/\text{L}$; total suspended solids (TSS): $82.7 \pm 25.0 \text{ mg}/\text{L}$; turbidity: $63.3 \pm 16.6 \text{ NTU}$; and transmittance: 25.2 ± 9.4 . Contact time for the first experiment was 82 s and for the second it was 412 s, which produced the increase in dose, with irradiance kept at $0.30 \text{ mW}/\text{cm}^2$.

With low doses of $7 \text{ mJ}/\text{cm}^2$, pharmaceutical product removal efficiencies of 26% have been found using 5 low-pressure 150 W lamps and a contact time of 33 s [37]. With a dose of $29.74 \text{ mJ}/\text{cm}^2$, there was a bacteria removal efficiency of 98.4%, with a contact time of 11.44 s [38]. Rodríguez-Chueca et al. [39] studied doses between $42\text{--}170 \text{ mJ}/\text{cm}^2$, obtaining microcontaminant removal efficiencies of 90% using 16 95 W lamps and a contact time between 4 and 18 s. Doses between 100 and $300 \text{ mJ}/\text{cm}^2$ achieved an effective total coliform removal of 99.97% using 8 20 W lamps and a contact time between 10 and 30 min [40].

3.2.2. Total Suspended Solids

Another main problem affecting ultraviolet radiation is the presence of contaminants in the water to be treated, impeding the efficient transmission of radiation, especially the presence of total suspended solids (TSS), which affect characteristics such as transmittance or zeta potential and present different particle sizes, parameters that can affect UV disinfection.

- Transmittance

Like all forms of radiation, the intensity of ultraviolet radiation decreases as it penetrates a material substance, due mainly to absorption. The intensity of radiation absorbance

is known as transmittance (UVT%), which is defined as a measure of the fraction of incident light transmitted through a water column [33].

A high concentration of organic and inorganic contaminants could reduce transmittance or reduce the capacity of photons that must penetrate the water column and inactivate microorganisms. As mentioned, the wavelengths used in UV radiation fluctuate in the UV-C region (200–280 nm), and UV absorbance typically increases as wavelength decreases in this region; therefore, the UVT percentage plays an essential role [41]. Iron, meanwhile, can reduce UVT % depending on the state of oxidation and complexation of organic matter because ferric and ferrous compounds tend to absorb radiation in the UV spectrum. It has been found that the presence of humic acids and Fe(III), turbidity-causing materials (TCMs), influences the inactivation of *E. coli* and *E. faecalis* due to the decrease in UVT %. Thus, 0.2 NTU due to Fe(III) generated a 2.5 uLog reduction of *E. coli* at a dose of 10 mJ/cm², while a reduction of 3.9 uLog was achieved in the absence of TCMs. This is because materials such as chalk and Fe(III) present a more neutral surface charge, which results in a greater aggregation with bacteria compared to other TCMs. These particles represent potential vehicles for the entry of microorganisms into water treatment barriers, affecting the ecosystem and consequently human health [42]. It has also been found that contaminants of emerging concern such as personal care products in wastewater can affect the UVT % [43].

- Particle size

TSS can decrease microorganism inactivation due to UV absorption by the particles, bacterial fixation, and physical blocking of UV radiation, altering the penetration of light. These particles can be of different sizes and are classified accordingly as soluble (<0.001 µm), colloidal (0.001–1 µm), supracolloidal (1–100 µm), or settleable (>100 µm); they can also be inorganic or organic, and it has been found that the organic matter in wastewater presents a high molecular weight of up to 103 Da [44]. These particles can enter the disinfection system due to their sizes, evidencing that preliminary treatment stages can remove easily settleable inorganic particles larger than 0.01 mm, while primary sedimentation tanks are capable of removing organic and inorganic particles with sizes between 0.1 mm and 35 µm [45].

As mentioned, microorganisms can become embedded in these particles. This particle–microorganism association is regulated by the double layer theory, which states that attraction operates at two levels around the particle: the “primary energy zone” (1 nm from the particle surface), where attraction is irreversible, and the “secondary energy zone”, which occurs 5–10 nm from the particle surface, where attraction is reversible. At the first level, adhesion takes place and it is stronger, occurring when the bacterium forms a permanent bond with the surface and involving a large amount of energy; at the second level, the cell adsorbs to the particle surface, where weak electrostatic and Van der Waals forces contribute, which can be easily overcome by a change in the ionic composition of the medium or hydraulic shear forces [44,46].

Particle size plays a fundamental role; thus, bacteria usually associate with particles larger than 10 µm, and viruses associate with smaller particles of under 2 µm, with findings also indicating that aggregate particles are more resistant to UV radiation than scattered particles [47,48].

In addition, Madge and Jensen [49] found that the bacteria associated with particles in the range between 5 and 20 µm were inactivated similarly to bacteria found individually or in aggregates smaller than 5 µm; however, fecal coliforms associated with particles larger than 20 µm were removed more slowly than those associated with particles between 5 and 20 µm and small cell aggregates under 5 µm. The bacteria associated with particles larger than 20 µm accounted for 30–45% of the measured coliforms, while of the rest, 5% were associated with medium-sized particles of between 5 and 20 µm.

Filtration is considered a good pretreatment because it eliminates suspended matter; however, the production of smaller particles increases UV ray absorption, which may reduce UV system performance [50].

- Zeta potential

According to the double layer theory mentioned above, repulsion potentials are related to electrical repulsion forces, while attraction potentials are subject to London interaction forces. According to this model, there are two layers; the first presents linear decay and is fixed, while the second is diffuse with an exponential decay. There is a reference plane between the two layers, and the potential is called zeta potential (ZP) or electrokinetic potential. Thus, the measurement of zeta potential arises, a technique that provides sufficient information on the distribution of the surface charge of particles in the solid/water interface [51].

ZP provides information about particle interfacial surface and is an indirect method of estimating bacteria surface potential. It plays an important role in microorganisms' adhesion to particle surfaces and its measurement can be used to establish the feasibility of these two parts joining together [52].

Changes in electric potential have been found to be directly related to the volume of particles that pass through; that is, in diluted conditions, the surface adsorption phenomenon dominates, with ZP increasing with concentration. Meanwhile, pH is perhaps the parameter that most influences ZP. Particle surfaces usually have a negative electrostatic charge, like bacteria at neutral pH; however, bacterial adsorption to inorganic particles will increase as ZP decreases, and thus ZP will become more positive or negative in magnitude with acidic or basic pH, respectively. Another factor that affects ZP is ionic strength; as it increases, ZP decreases. Ion valence also has an influence; if ions with a greater valence are present, ZP magnitude will decrease [44,53,54]. Negative ZP values suggest that the particles have a greater repulsive energy barrier than particles with values closer to 0 mV. González et al. [55] found ZP values of -15.5 and -15.3 mV for the influent and effluent of a sample of wastewater treated by a UV system, given that due to their charge, these particles should not generate aggregates and affect UV radiation. Farrell et al. [42], meanwhile, found that in particles that present a ZP of 0–5 mV, aggregation is probable as a result of a low energy barrier, affecting final disinfection.

4. Reactivation of Pathogenic Microorganisms

Among the greatest disadvantages of the UV system is the possibility that microorganisms will survive. Exposure to UV radiation can cause damage to the nucleic acids of the cell and affect other cell components; however, microorganisms can still retain metabolic functions such as enzyme activity. Over time, microorganisms have managed to develop mechanisms to repair DNA damage. Two types of repair have been described: photoreactivation and dark repair [13]; the process involved in each mechanism is visualized in Figure 3. Zhang et al. [56] found that a viable but non-culturable state (VBNC) was induced in *Escherichia coli* and *Pseudomonas aeruginosa* strains after being subjected to UV radiation, with cell membranes remaining intact even at UV doses of 300 mJ/cm^2 , revealing the possible risks after disinfection and the need for a combined disinfection strategy.

Enumeration of waterborne pathogens has been carried out using plate count methods; however, it has been found that under external stress, bacteria can enter a VBNC state. Public health concerns have arisen because bacteria such as *Helicobacter pylori*, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Vibrio* spp., and *Yersinia enterocolitica* can enter a VBNC state. This could result in the total bacterial cell count being underestimated [56,57]. It has been found that VBNC bacteria could be induced by chlorine treatments and UV radiation; however, almost all studies focus on model or indicator strains, making it necessary to study these bacteria in isolation from the environment [58].

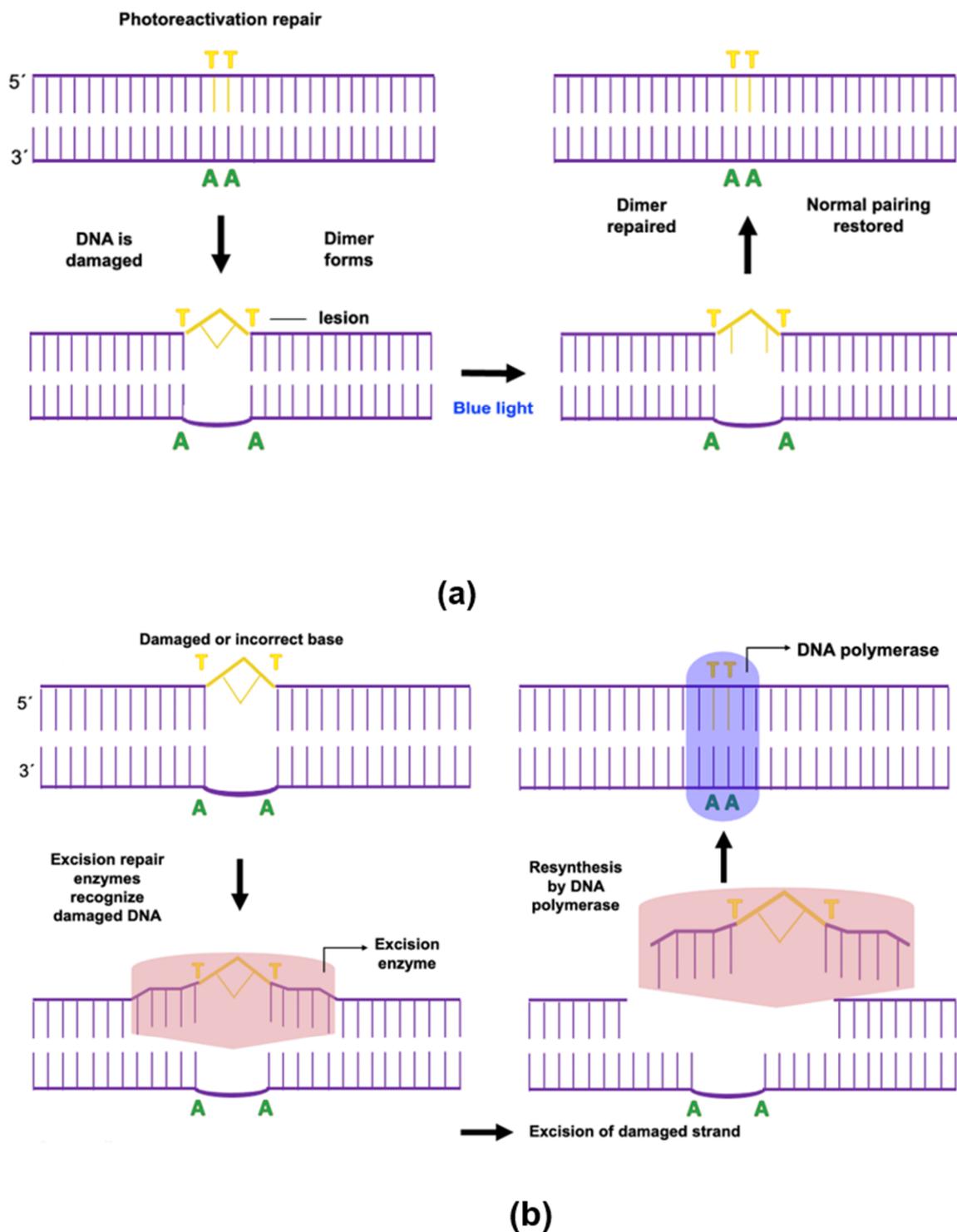


Figure 3. Representative diagram of the cell repair mechanism by (a) photoreactivation and (b) dark repair. Source: self-made based on Hijnen et al. [13].

4.1. Photoreactivation

UV radiation has been shown to be less effective against fecal coliforms such as *E. coli* due to a process called photoreactivation. In this process, *E. coli* strains can repair themselves and reverse the damaging effects of UV radiation when low doses are used [6].

Photoreactivation occurs under conditions of prolonged exposure to visible light and is specifically directed at pyrimidine dimers [13].

As previously mentioned, UV radiation generates dimer formation, but photoreactive bacteria have developed defense mechanisms for their cells. Lindenauer and Darby [59] mention two steps in which photoreactivation occurs:

1. Formation of PRE-dimer complex. The organisms present photoreactivating enzymes (PREs). Their quantity can vary by organism. In the presence or absence of light, a PRE binds with a pyrimidine dimer, forming a complex. This is a reversible step, but formation kinetics are heavily favored. Factors such as temperature, pH, and ionic strength affect the speed of complex formation;
2. Release of repaired DNA and PRE. Photoreactivation results in the monomerization of the dimer and subsequent release of the PRE. The reaction takes place in under a millisecond and the repair is perfect. The restoration of the dimer depends on the reaction kinetics and light energy intensity.

The wavelength necessary for photoreactivation varies by organism and is generally between 310 and 490 nm. The degree of photoreactivation obtained will depend on how many PRE-pyrimidine dimer complexes have been formed, which in turn will depend on the number of PREs available in each cell. A long period of exposure to photoreactivating light will result in a repaired dimer and a PRE that would be available to form new complexes with the dimers still to be corrected. These photoreactivating enzymes are known as “photolyases”, which are monomeric proteins of 53 to 66 kDa that contain flavin-adenine dinucleotide (FAD) as a cofactor.

A significant inverse relationship between mean UV dose and coliform photoreactivation in wastewater has been found at low and high UV doses [59].

Hallmich and Gehr [60] found that at low doses of 10 and 20 mJ/cm², delaying exposure to photoreactivating light for 3 h suppressed reactivation, which began upon exposure to at least 440 lux (0.065 mW/cm²) of visible light. In addition, photoreactivation decreased by almost 50% in winter samples when the samples were exposed to visible light at the same time as or before UV irradiation. The summer samples were more sensitive to inactivation and less capable of reactivating.

Table 1 brings together various works in which photoreactivation analyses have been performed, showing that the most frequently used wavelength is 360 nm and that the lamps do not exceed 15 W. Locas et al. [61], meanwhile, found that photoreactivation of *E. coli* increased significantly after exposure to 5600 lx compared to 1600 lx of visible light and was higher in warm water (25 °C) than in cold water (4 °C). This photoreactivation was also higher after disinfection using low-pressure UV lamps compared to medium-pressure lamps.

Table 1. UV system operating parameters and photoreactivation analysis.

UV System Operating Parameters		Operating Parameters for Photoreactivation							References
Wavelength (nm)	Lamp Type	Inactivating Dose (mJ/cm ²)	Irradiance (mW/cm ²)	N° Lamps	Power (W)	Model Lamps	Lamp Wavelength (nm)	Temperature (°C)	
254	UV-C	50–200	0.10	1	3.7	Philips TLD	360	5,10,15,20,25,30	[62]
222–282	UV-C/UV-LED/LP UV	1–200	0.10–0.25	1–2	3.7–15	Philips TLD	360–365	4–37	[62–65]
254–310	UV-C/UV-LED	50–200	0.10–0.384	1	3.7	Philips TLD	360	5,10,15,20,25,30	[62,66]
222–282	UV-C/UV-LED/LP UV	5–200	0.10–0.25	1–2	3.7–15	Philips TLD	360–365	4–30	[62–64,67]
254	UV-C	50–200	0.10	1	3.7	Philips TLD	360	5,10,15,20,25,30	[62]
222–310	UV-C/UV-LED/LP UV	1–200	0.10–0.25	1–2	3.7–15	Philips TLD	360–365	4–37	[62–65]
254	UV-C	50–200	0.10	1	3.7	Philips TLD	360	5–30	[62]
222–282	UV-C/LP UV	5–200	0.10–0.25	1–2	3.7–15	Philips TLD	360–365	5–37	[62–64,67]
267–310	UV-LED	-	-	-	-	-	-	-	[66]
222–282	UV-LED/LP UV	-	0.25	2	8–15	Philips, Holland	365	4–37	[63,64]
222–310	UV-LED/LP UV	-	0.25	2	8–15	Philips, Holland	365	4–37	[63,64,67]
254–280	UV-LED/LP UV	-	0.25	2	8–15	Philips, Holland	365	25	[64]
267–310	UV-LED	-	-	-	-	-	-	-	[66]
254–280	UV-LED/LP UV	1–5	0.25	2	8–15	Philips, Holland	365	25	[64,65,67]

Wan et al. [68] subjected fungal spores to 0.25 mW/cm^2 , 365 nm light, finding that the addition of advanced disinfection processes (ADPs) decreased photoreactivation, and at a wavelength of 265 nm, better inhibition of *Aspergillus niger* photoreactivation was achieved, probably because ADPs further damage cell components or the photolyase involved. It was also demonstrated that UV radiation-based ADPs almost controlled *E. coli* photoreactivation within 24 h; thus, they can be considered promising means to guarantee water biosecurity.

Research shows that UV radiation has limitations in disinfection, but it has been found that these limitations can be mitigated by using higher doses of UV radiation. It is recommended to use doses higher than 40 mJ/cm^2 and to delay exposure to photoreactivating light, as photoreactivation tends to increase over time. It has also been observed that parameters such as wavelength and the type of lamp used can influence photoreactivation. In this respect, it is suggested to use medium-pressure lamps that emit a “polychromatic” spectrum. These strategies help to improve the effectiveness of UV disinfection.

4.2. Dark Repair

Dark repair does not require light and has been found in most bacteria. Spores do not have an active metabolism, but repair occurs after germination. Viruses, meanwhile, do not have a metabolism; therefore, they cannot repair the damage to genetic material on their own. However, various viruses have been found to use host cell repair enzymes. This has been studied in the case of adenovirus and its high resistance; it is a double-stranded DNA virus that can use host cell repair enzymes, which RNA viruses do not [13].

Dark repair or excision repair is based on the use of enzymes that replace damaged DNA with undamaged nucleotides. According to Goosen and Moolenaar [69], the proteins UvrA, UvrB, and UvrC participate in the repair process, which takes place in the following steps:

1. Association of UvrA and UvrB in solution, which searches for possible lesions in the DNA. UvrA first searches for anomalies and, upon finding them, provides the DNA to UvrB, which will attempt to bind the DNA;
2. When a lesion is present, it results in a tight complex of UvrB and DNA, from which the UvrA protein dissociates;
3. UvrC joins this complex by making an incision at the fourth or fifth phosphodiester bond $3'$ to the damage, and then makes an incision at the eighth phosphodiester bond $5'$ to the damage;
4. Following the incisions, UvrD, also called helicase II, eliminates the damaged oligo and then polymerase I and ligase to restore the DNA strand.

Dark repair has been found to occur to a much lesser degree than photoreactivation, as shown in Table 2. Li et al. [70] found that for LP UV radiation, the maximum repair percentage was 0.2%. A repair percentage of 0.11% was obtained with a 280 nm LED, and 0.14% was obtained for repair after exposure to 265/280 nm irradiation. Meanwhile, no repair of *E. coli* in distilled water mixed with humic acids after a UV radiation dose of 9 mJ/cm^2 via pulsed UV (PUV) disinfection was found [71].

It has been shown that microorganism reactivation is often related to the UV-C dose applied. In addition, medium-pressure UV lamps generate a broad “polychromatic” spectrum of UV wavelengths that cause irreparable damage to DNA and other microorganism molecules such as enzymes. By contrast, low-pressure lamps emit a single wavelength peak that only affects DNA; therefore, the former could result in a lower possibility of reactivation [62,72].

Table 2. Results obtained from photoreactivation and dark repair analysis.

Reactivation Time (h)	Indicator	Photoreactivation						Dark Repair					References
		Percentage of Reactivation (%)	Survival Ratio (%)	First-Order Kinetic Parameters				Percentage of Reactivation (%)	Survival Ratio (%)	S _m (%)	First-Order Kinetic Parameters		
				K ₁ (L/min)	K ₂ (%min)	S _m (%)	R ²				K ₂ (%min)	R ²	
0.5	Total Coliforms/Fecal Coliforms <i>Aspergillus niger</i> /Total Coliforms/Fecal	-	0.01–0.12	-	0.039–1.301	0.012–0.945	0.932–0.997	-	0.004–0.062	0.004–0.073	0.341–4.737	0.821–0.987	[62]
1	Coliforms/ <i>Escherichia coli</i> O157:H7/ <i>Escherichia coli</i> HB 102	4.41–8.44	0.01–2.27	0.0041–0.0091	0.031–1.301	0.012–69.89	0.91–0.997	0.94–14.13	0.004–6.17	0.004–0.073	0.341–4.737	0.821–0.987	[62–65]
1.5	Total Coliforms/Fecal Coliforms/ <i>Escherichia coli</i> O157:H7/ <i>Aspergillus niger</i> /Total Coliforms/Fecal	1.00–1.18	0.01–0.48	-	0.031–1.301	0.012–0.945	0.932–0.997	0.12–0.41	0.004–0.061	0.004–0.073	0.341–4.737	0.821–0.987	[62,66]
2	Coliforms/ <i>Escherichia coli</i> O157:H7	5.87–12.28	0.01–2.20	0.0041–0.0091	0.031–1.301	0.012–69.89	0.91–0.997	1.53–26.59	0.004–10.84	0.004–0.073	0.341–4.737	0.821–0.987	[62–64,67]
2.5	Total Coliforms/Fecal Coliforms/ <i>Aspergillus niger</i> /Total Coliforms/Fecal	-	0.01–0.64	-	0.031–1.301	0.012–0.945	0.932–0.997	-	0.004–0.058	0.004–0.073	0.341–4.737	0.821–0.987	[62]
3	Coliforms/ <i>Escherichia coli</i> O157:H7/ <i>Escherichia coli</i> HB 102/ <i>Escherichia coli</i>	2.18–32.87	0.01–2.05	0.0041–0.0091	0.031–1.301	0.012–69.89	0.91–0.997	0.43–38.32	0.003–17.20	0.004–0.073	0.341–4.737	0.821–0.987	[62–66]
3.5	Total Coliforms/Fecal Coliforms/ <i>Aspergillus niger</i> /Total Coliforms/Fecal	-	0.01–0.94	-	0.031–1.301	0.012–0.945	0.932–0.997	-	0.003–0.049	0.004–0.073	0.341–4.737	0.821–0.987	[62]
4	Coliforms/ <i>Escherichia coli</i> O157:H7/ <i>Escherichia coli</i>	12.05–12.28	0.01–2.80	0.0041–0.0091	0.031–1.301	0.012–69.89	0.91–0.997	2.56–51.50	0.002–33.08	0.004–0.073	0.341–4.737	0.821–0.987	[62–64,67]
4.5	<i>Escherichia coli</i>	6.96–18.11	-	-	-	-	-	0.66–4.95	-	-	-	-	[66]
5	<i>Aspergillus niger</i> / <i>Escherichia coli</i> O157:H7	-	0.83–2.12	0.0041–0.0091	-	28.12–69.89	0.91–0.98	3.24–61.56	18.88–37.00	-	-	-	[63,64]
6	<i>Aspergillus niger</i> / <i>Escherichia coli</i> / <i>Escherichia coli</i> O157:H7	12.58–30.36	0.83–2.20	0.0041–0.0091	-	28.12–69.89	0.91–0.98	2.05–66.59	23.36–47.48	-	-	-	[63,64,66]
7	<i>Aspergillus niger</i>	-	0.91–2.12	0.0041–0.0091	-	28.12–69.89	0.91–0.98	-	26.36–55.14	-	-	-	[64]
7.5	<i>Escherichia coli</i>	15.93–31.19	-	-	-	-	-	2.40–7.66	-	-	-	-	[66]
8	<i>Aspergillus niger</i> /Fecal coliforms/ <i>Escherichia coli</i> HB 102/ <i>Escherichia coli</i>	9.70–43.45	0.91–2.42	0.0041–0.0091	-	28.12–69.89	0.91–0.98	-	28.79–65.98	-	-	-	[64,65,67]
9	<i>Escherichia coli</i>	15.93–31.19	-	-	-	-	-	2.40–7.85	-	-	-	-	[66]

Nyangaresi et al. [66] found that 275 nm UV LEDs presented better persistence against reactivation than 265, 275/310, and 265/275 nm UV LEDs, which can be attributed to the protein damage caused at 275 nm. They also mention that the mortality rate in this type of reactivation may be due to the residual effect of radiation on microorganism. This residual effect is produced by the biochemical mechanism of actuation, which needs time to be manifested. Therefore, in photoreactivation, this phenomenon may not occur because the repair of damaged DNA is more effective, even though the photo effect is the dominant reactivation mechanism.

In summary, dark reactivation is less effective than photoreactivation. However, as with photoreactivation, the type of UV lamp used and the choice of wavelength used are critical. In addition, combining the UV system with other disinfection technologies such as chlorination, ozonation, or advanced disinfection processes can help to reduce the possibility of dark reactivation and improve the overall effectiveness of the system in ensuring water quality. These recommendations apply to effluent from wastewater treatment plants as well as drinking, industrial, and food water sources.

5. Advanced Disinfection Processes: Perspectives

Because microorganisms such as bacteria can self-repair, generating subsequent reactivation, and given that they are much more complex than recalcitrant compounds that could be present in effluents, more and more work has been conducted on the incorporation of advanced oxidation processes (AOPs) into UV systems.

Figure 4a shows that there has been an increasing number of publications on UV systems in the last 20 years, growing between 52 and 59% between time periods, due mainly to growing interest in removing new contaminants present in wastewater and the easy application of UV systems. However, Figure 4b shows an important trend in the last decade related to the use of AOPs combined with UV disinfection, such as the combined use of UV/O₃/H₂O₂; in the last five years, there have been 93 publications. Another example is the shift from no research into the use of UV/PS (UV/persulfate) in the 2003–2012 period to 14 publications on this topic in the 2018–2022 period. In general, there has been growing interest in all AOPs added to UV systems because they are presented as mechanisms to prevent the reactivation of microorganisms by UV systems.

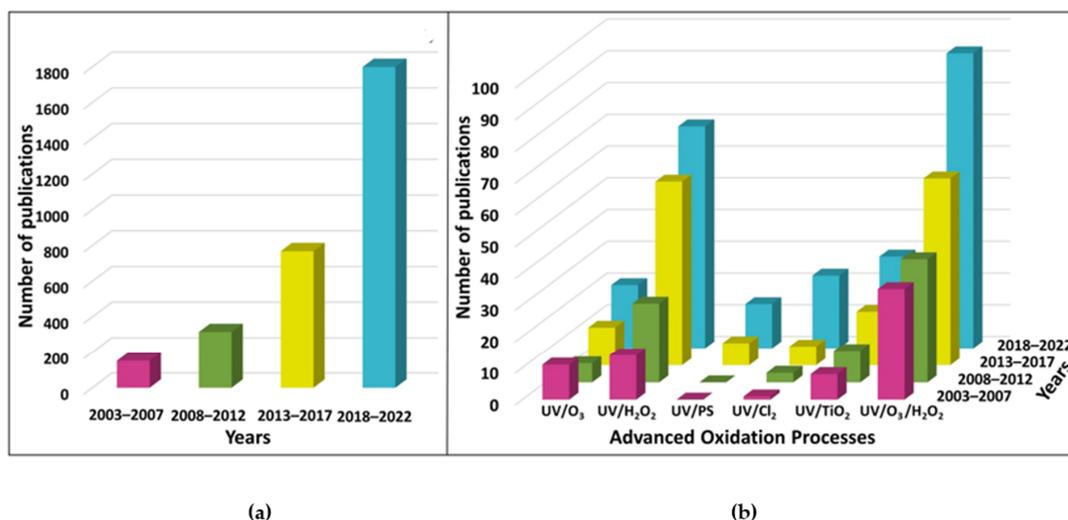


Figure 4. Evolution of publications between 2003 and 2022 related to (a) UV disinfection and wastewater treatment and (b) UV disinfection combined with advanced oxidation processes in wastewater treatment, showing the 2003–2007, 2008–2012, 2013–2017, and 2018–2022 periods, with UV: ultraviolet, O₃: ozone, H₂O₂: hydrogen peroxide, PS: persulfate, Cl₂: chlorine, TiO₂: titanium dioxide. Source: own authorship.

Specifically, these AOPs have emerged as new, potentially environmentally friendly disinfection methods that present high efficiency compared to conventional disinfectants. The method of action is based on the in situ formation of different chemical oxidants, which allows various harmful organic contaminants present in wastewater to be mineralized into CO₂, water, and inorganic ions, along with inactivation of potentially pathogenic microorganisms [73,74].

These processes are characterized by being of the redox type and their non-selectivity on the target, with their power based on the generation of highly reactive oxygen species (ROS) such as the hydroxyl radical (HO•), which is the main oxidizing agent and is highly oxidizing (2.80 eV). At the same time, it presents reaction rates between 10⁻⁶ and 10⁻⁹ M⁻¹s⁻¹ [73,75]. Other ROS that can act are singlet oxygen, triplet oxygen, anion-radical superoxide (O₂^{-•}), hydroperoxyl radical (HO₂•), and hydrogen peroxide; they act as intercellular structures, cell walls, and cytoplasmic membranes, thereby damaging microorganisms. However, the method of action of each ROS on each type of microorganism has not yet been described [76,77].

AOP performance can be affected by the presence of compounds such as natural organic matter (NOM), alkalinity, pH, temperature, and dissolved and suspended solids. It has also been found that chloride, bicarbonate, and carbonate ions can interfere, competing with the contaminants for hydroxyl radicals, thus decreasing the efficacy of the process [78].

There are different types of advanced oxidation processes such as that based on the photo-Fenton process, sulfate-based oxidation, photochemical oxidation, and electrochemical oxidation, among others. In the case of AOPs incorporated into UV systems, UV/ozone (UV/O₃), UV/hydrogen peroxide (UV/H₂O₂), UV/PS, UV/chlorine (UV/Cl₂), UV/titanium dioxide (UV/TiO₂), and UV/O₃/H₂O₂ combinations can be found [79].

In the UV/O₃ combination, ozone has been used as a disinfection process due to its high oxidation potential, allowing it to oxidize contaminants through direct selective reactions or decompose them through chain reactions produced by free hydroxyl radicals and oxygen radicals. The UV system/ozone combination promotes free radical activity, improving reactions, while ozone destroys organic contaminants in water and UV radiation inactivates the microorganisms present [80,81].

Various studies have been performed to assess the efficiencies of different AOPs. For example, in the case of bisphenol A degradation, it was found that the combination of photolysis and ozonation generated a degree of decomposition greater than 90% in urban wastewater effluents, higher than that obtained by the two processes separately [82]. In the case of organic substance removal, it was found that this AOP was able to demineralize them, achieving a 90% reduction in dissolved organic carbon (DOC), versus 36% achieved by ozonation alone, also removing all four fractions of dissolved organic matter (DOM) [83].

Another disinfection method that has been used is the UV/H₂O₂ combination. It has been found that this combination, through H₂O₂ photolysis, can produce hydroxyl radicals. Thus, it has become an efficient method to remove microorganisms and organic contaminants in water. However, it cannot be applied widely due to its high operating costs [73]. Another parameter to consider in this method is quantum yield and UV absorption; the latter is an indicator of the strength with which a molecule can absorb UV rays, subsequently leading to its degradation [75].

Regarding elimination efficiency, Bairagi et al. [84] obtained a 99% inactivation of microorganisms after 240 min of treatment, a value higher than that obtained using only UV radiation at 253.7 nm, which achieved 61% with an initial concentration of 1.7 × 10⁷ UFC/mL. It has also presented good COD removal results, with Yonar et al. [85] achieving a reduction of over 95% in less than 60 min of reaction time and finding that the optimal pH and H₂O₂ dose for this process were 3 and 50 mg/L, respectively, and that the electricity required for the process was 10 kWh/kg COD.

In short, traditional water disinfection approaches such as chlorination and ozonation have been widely used but are limited by the formation of toxic by-products and the ability of some microorganisms to self-repair against UV systems. In contrast, AOPs

offer a promising alternative. These processes generate chemical oxidants in situ, such as hydroxyl radicals, which break down a wide range of organic contaminants and deactivate microorganisms. AOP_S are highly efficient, do not produce toxic by-products, and show an increased ability to remove resistant contaminants. Although more research is needed, AOP_S represent an innovative and safe approach to water disinfection.

While it is challenging to integrate AOP systems in decentralized environments, the focus should be on strengthening UV systems to expand their use. These systems have found applications beyond wastewater treatment, including in hospital environments. However, the dose required in such environments to achieve a specific reduction has not yet been fully determined. According to data collected by Malayeri et al. [86] in 2016, to achieve a bacterial reduction of 3 uLog (99.9% disinfection), doses vary from 1 mJ/cm² to 170 mJ/cm².

It is important to note that a benefit is always observed as the dose increases. For example, when the dose was increased from 1.6 to 19.7 mJ/cm², photoreactivation decreased from 5.31% to 0%. Another factor to consider is relative humidity and temperature. It is recommended to keep the humidity as low as possible to avoid a decrease in UV radiation. In addition, lower temperatures lead to a reduction in cell viability, resulting in slower photoreactivation [87].

It has been observed that environmental bacteria and bacterial spores are more resistant to UV than laboratory-grown microorganisms. Therefore, although no significant increase in UV dose is required for drinking water treatment, doses may need to be adjusted for other types of environments [13].

A useful strategy could be the coupling of UV-A/UV-C lamps to achieve higher microbial reductions, or even the combination of different wavelengths, such as 280/365 nm [88]. It is important to note that the UV doses used can vary widely in different applications and contexts.

6. Conclusions

The UV system has been shown to be effective in inactivating microorganisms, but there are concerns about the possibility of reactivation, which compromises the safety of the treated water. This research focuses on addressing this issue and proposes optimal operating values to minimize reactivation.

The results highlight the importance of considering various factors to reduce the possibility of reactivation. The appropriate choice of UV lamp and wavelength is critical. The use of medium-pressure UV lamps with a polychromatic spectrum and a disinfection wavelength of 253.7 nm is recommended. In addition, it is essential to set optimal parameters, such as exposure time and radiation dose, to ensure effective disinfection without leaving room for reactivation. It is suggested that doses should be higher than 40 mJ/cm². These parameters should be adapted to the specific characteristics of the water to be treated, such as its microbiological composition, organic matter, and suspended solids content, as well as its end use. The use of more than one UV lamp is recommended and the hydraulic design should be reviewed to increase the dosage in the reactors.

Further research is needed to understand the interaction between inactivated microorganisms, disinfection by-products, and other components of the treated water that may trigger reactivation. In addition, the combination of disinfection systems, such as chlorine addition (maintaining a chlorine residual close to 0.5 mg/L) or AOP_S, may play an important role in reducing microbial reactivation.

In conclusion, although UV radiation is widely used and effective for water disinfection, the possibility of microbial reactivation poses a significant challenge. Further research and refinement of UV system operating parameters, as well as the exploration of new strategies and combinations of technologies, are needed to ensure effective disinfection and minimize the risk of reactivation, thereby ensuring water quality and protecting public health.

Author Contributions: Conceptualization, Y.G. and G.V.; methodology, Y.G., G.G. and G.V.; software, G.G.; validation, G.V., G.G. and Y.G.; formal analysis, Y.G. and G.G.; investigation, Y.G.; resources, G.V.; data curation, Y.G., G.G. and G.V.; writing—original draft preparation, Y.G. and G.G.; writing—review and editing, G.E.M.-C. and G.G.; visualization, G.G.; supervision, G.V.; project administration, G.V.; funding acquisition, G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by the following grant: ANID/FONDAP/15130015. Also, Y.G. thanks ANID/Scholarship Program/DOCTORADO BECAS CHILE/2022-21222126 for supporting her Ph.D. studies at the University of Concepción.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data available in this review was obtained from the database “Web of Science” using the keywords described in the methods section.

Acknowledgments: ANID/FONDAP/15130015. Also, Y. González thanks the National Agency for Research and Development (ANID), Chile for her Scholarship Program National Agency for Research and Development (ANID)/Scholarship Program/Doctorado Nacional/2022-21222126, for supporting her Ph.D. studies at the University of Concepción.

Conflicts of Interest: The authors declare no conflict of interest.

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