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Chapter 15

Advanced Oxidation Process Applied to Actinobacterium Disinfection

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Abstract

Actinobacteria, such as *Mycobacterium*, that constitute one of the main phyla within the bacteria and some genus of this phylum are reported to be a pathogen of or associated with nosocomial infections and pseudoinfections promoting health risks for immunocompromised people, particularly AIDS patients. They are also related to lower quality of surface water due to their odor production (*Actinomycineae* and *Streptomycetaceae*). These bacteria have been isolated from hospital water distribution systems, municipal drinking water, freshwater, and among other environmental samples. Their biofilm formation, amoeba-associated lifestyle, and resistance to chlorine/ozone have been recognized as important factors that contribute to persistence of these bacteria in water distribution systems. Research for new disinfection methods that are able to promote complete inactivation of these bacteria has currently increased. Among them is the use of advanced oxidation process that has demonstrated promising results; the production of ·OH radicals with high oxidizing power are capable to kill bacteria and can also destroy the products generated from lyse cell. The goal of the present work is to review the main processes based on advanced oxidation process that are able to promote actinobacterium disinfection. The fundaments of this process are also reviewed. Special emphasis was done for the photocatalysis and photoelectrocatalysis methods and the phenomena occurring at the electrode/electrolyte interface.

Keywords: Actinobacteria, photocatalysis, Mycobacterium, disinfection, TiO_2

1. Introduction

Actninobacteria or actinomycetes constitute a vast phylum within the domain Bacteria and is formed by bacteria with high content of guanine and cytosine in the DNA [1–3]. Actinobacteria

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have some similarities with the microorganisms of the domain Fungi, such as the formation of hyphal and spore dissemination. However, the absence of nuclear membrane, the presence of the typical bacterial flagellum, and sensitivity to antibiotics provided the migration of actinobacteria to the domain Bacteria [4].

There are various different lifestyles among *Actinobacteria*, and the phylum includes pathogens (e.g., *Mycobacterium spp*., *Nocardia spp*., *Tropheryma spp*., *Corynebacterium spp*., and *Propionibacterium spp*.), soil inhabitants (*Streptomyces spp*.), plant commensals (*Leifsonia spp*.), nitrogen-fixing symbionts (*Frankia*), and gastrointestinal tract inhabitants (*Bifidobacterium spp*.) [3].

They are widely distributed in terrestrial [5] and freshwater environmentals [6]. The initial hierarchical classification system of *Actinobacteria* embraced more than 222 genera belonging to 53 families and 23 suborders. A wide variety of morphologies are exhibited by actinobacteria, from coccoid (*Micrococcus*) or rod-coccoid (e.g., *Arthrobacter*) to fragmenting hyphal forms (e.g., *Nocardia spp*.) or permanent and highly differentiated branched mycelium (e.g., *Streptomyces spp*.) [7].

Mycobacteria are a genus belonging to the class of Actinomycetes and are among the most important microorganism pathogens, causing diseases such as leprosy and tuberculosis. Although it has been more than 100 years since the discovery of its etiological agents, these diseases are still associated with high levels of morbidity and mortality, respectively [7, 8]. These bacteria usually occur as straight or slightly curved rods, although they may also appear in the form of hyphae that are fragmented in coccoid elements or rods. Although considered as Gram-positive bacteria, studies confirm the presence of an external membrane in the mycobacterial envelope, resembling in this respect a Gram-negative bacteria [9]. Another outstanding feature for mycobacteria is the significant presence of mycolic acids in the cell wall, forming a waxy, water resistant layer, making for a great resistance to adverse conditions such as dryness. In addition, mycolic acids are also responsible for alcohol- and acid-resistance, another outstanding feature of this genus [10].

The two major human mycobacteriosis are leprosy and tuberculosis, caused by *Mycobacterium leprae* and *Mycobacterium tuberculosis,* respectively. However, other species of the same genus known as atypical mycobacteria is capable of causing opportunistic infections not only in humans but also in animals [11]. For example, the mycobacteria of the *M. avium* complex, due to their varied mechanisms, allow this group to efficiently infect macrophages, monocytes, and epithelial cells. These pathogens can cause various lung damage in immunocompromised patients or even in immunocompetent persons [11]. In contrast to the much opportunistic mycobacteria, these bacilli are able to join, and later invade host cells, suppress the local inflammatory response, and translocate through the wall of the respiratory tract [12]. This translocation ability through the epithelial cells has been investigated, as it represents a form of bacillus that spread to other body organs [13]. The isolation of *M avium, M. kansasi*, and *M. xenopihave*, known as nontuberculosus mycobacteria (NTM), in drinking water and water distribution systems in a hospital has been reported in the literature. Factors such as biofilm formation, amoeba-associated lifestyle, and resistance to chlorine may have contributed to the survival and colonization of these species in these environments [10].

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It is important to note that not all actinobacteria are pathogenic. It is highlighted that the genus *Streptomyces*, which as previously mentioned, is grown as filaments, similar to fungi. They are Gram-positive and have the ability to produce bioactive secondary metabolites such as antifungals, antivirals, anti-tumor, anti-hypertensives, and mainly antibiotics and immunosuppressives [14].

The presence of *Actinomycineae* and *Streptomycetaceae* in drinking water may produce odors, geosmin, and 2-methylisoborneol (MIB), which contribute to the decrease of the quality of surface water when used for drinking [15]. In this form, the presence of actinobacteria has been related to a lot of environmental issues. The abundance of the above-mentioned microorganisms in water could stem from their resistance of conventional disinfection methods such as chlorination and/or ozonation [5]. Research for new disinfection methods has been increasing and among them, the use of an advanced oxidation process has been giving promising results.

2. Occurrence of actinobacterium in water samples

The nature of the microbiology of tap water delivered to consumers via public drinking water distribution systems has been studied by Holinger et al. [16]. The authors studied tap water from 17 different cities between the headwaters of the Arkansas River and the mouth of the Mississippi River. The occurrence of Actinobacteria was detected in 24% of the samples, of which 85% were *Mycobacterium spp.* Other bacteria such as Proteobacteria (35%), Cyanobacteria (29%, including chloroplasts), Firmicutes (6%), and Bacteroidetes (3.4%) were also obtained.

The presence of mycobacteria has also been reported in municipal drinking water distribution systems, hospital water systems, and in ice machines, swimming pools, and whirlpools [17–21].

Klanicova et al. [22] have analyzed 124 samples of four drinking water supply systems in the Czech Republic, 52 dam sediments, 34 water treatment plant sludge samples, and 38 tap water household sediments. Actinobacteria of 11 different species were isolated by culture from 42 (33.9%) samples; the most prevalent were *M. gordonae* (16.7%), *M. triplex* (14.3 %), *M. lentiflavum* (9.5%), *M. avium* (7.1%), *M. montefiorenase* (7.1 %), and *M. nonchromogenicum* (7.1%). The presence of *M. paratuberculosis* in water treatment stations for potable water production is also described [23]. They have found *M. paratuberculos* in the final treated water and concluded that the public might be exposed through water supplies.

The analysis of freshwater lakes in China showed the bacterial diversity and were identified as Proteobacteria (40.9%), followed by Actinobacteria (15.9%), Cyanobacteria (11.4%), Verrucomicrobia (11.4%), Plantomycetes (6.8%), Bacteroidetes (4.5%), and Chloroflexi (4.5%) [24]. The investigation of the composition and diversity of biofilm bacterial community present in real drinking water distribution systems (DWDSs) with different purification strategies (conventional treatment and integrated treatment) show that actinobacteria is the major component of the biofilm bacterial community [25]. The abundance, identity, and activity of uncultured actinobacterium present in a drinking water reservoir (North Pine Dam, Brisbane,

Australia) has also been shown [26]. The structures and dynamics of bacterial communities from raw source water, groundwater, and drinking water before and after filtration were studied in four seasons of a year. The bacterial communities of different seasons from the four sampling sites were clustered into two major groups: water before and after filtration and source water and groundwater; representatives of the phyla Actinobacteria were found at all four sampling sites [27].

Analysis of bacterial communities associated with different drinking water treatment processes (e.g., coagulation, sedimentation, sand filtration, and chloramine disinfection) and from distantly piped water showed a large proportion of the phyla Actinobacteria and Bacteroidetes. The piped water exhibited increasing taxonomic diversity, including human pathogens such as the *Mycobacterium*, which revealed a threat to the safety of drinking water. In addition, actinobacteria was relatively consistent throughout the processes [28].

The presence of NTM was also reported in waters of a hemodialysis center [29]. Analysis of 210 samples of water from the hydric system of the unit (post-osmosis system, hemodialysis rooms, reuse system, and hemodialysis equipment) and from the municipal supply network was studied. The results showed that 24.3% of the collected samples tested positive for NTM; both the municipal supply network (2 samples, 3.2%) and the hydric system of the hemodialysis center (49 samples, 96.1%) contained NTM. Among the mycobacteria isolated, the authors highlight the presence of *Mycobacterium lentiflavum* (59.0%), *M. kansasii* (5.0%), *M. gordonae* (24.0%), *M. gastri* (8.0%), and *M. szulgai* (4.0%). The results show the need of more effective water disinfection procedures in this unit.

The incidence of NTM was also obtained in hot water systems of hospital settings with disinfection using hydrogen peroxide and silver; thermal disinfection or chlorine dioxide showed the persistence of NTM in 47% of the samples, equivalent to the remaining concentration between 10–1625 CFU L^{-1}. Among the NTM species that were isolated are *M. kansassi*, *M. xenopi*, and *M. fortuitum*. The lowest incidence was observed in the system treated by thermal disinfection and chlorine dioxide [30].

It was confirmed that drinking water supply systems (watershed–reservoir–drinking water treatment plant–household) might be a potential transmission route for actinobacteria, where mycobacteria are most reported. Some of these mycobacteria can cause various disseminated infections, tuberculosis-like illnesses, lymphadenitis, osteomyelitis in animals and in immunocompromised humans, paratuberculosis (Johne's disease), and chronic enteritis in ruminants [28].

The abundance of the above-mentioned microorganisms in water could stem from their biofilm formation, amoeba-associated lifestyle, and resistance to chlorine, which have been recognized as important factors that contribute to the survival, colonization, and persistence of actinobacteria in water distribution systems. The presence of actinobacterium in tap water, mainly mycobacteria, has been linked to nosocomial infections and pseudoinfections and provides a health risk for immunocompromised people, particularly AIDS patients. The research for new disinfection methods has been increasing and among them, the use of advanced oxidation process has been give promising results.

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3. Advanced Oxidation Processes (AOPs): Basic concepts

Chemical oxidation may be defined as a reaction in which electrons are removed from a substance, increasing their oxidation state [31]. The reactions involving oxidizing agents, such as H_2O_2 and O_3, are thermodynamically favored. However, they can be kinetically slow. In the presence of highly-oxidizing free radicals, such as hydroxyl radicals (·OH), could be found reaction rates of a million to a billion times faster compared with the oxidizing agents mentioned above [32]. Thus, the generation of hydroxyl radicals (·OH) is an essential step in the efficiency of advanced oxidation processes [33]. They are formed via the oxidation of water on the anode surface, as shown in Equation 1.

$$H_2O \rightarrow \bullet OH + H^+ + e^- \quad (1)$$

These chemical species are effective in destroying organic chemicals because they are reactive electrophiles (electron preferring), which react rapidly and are non-selective in relation to all electron-rich organic compounds [33, 34]. Furthermore, these react unselectively with a wide range of recalcitrant organics, often at diffusion-limited rates [35–38].

Table 1 lists the standard reduction potential of some oxidants. It is observed that the radical ·OH is a strong oxidant, only lower than fluoride, surpassing O_3 and H_2O_2.

Chemical Specie	Standard reduction Potential
F_2	+3.06
●OH	+2.77
O_3	+2.07
H_2O_2	+1.78
ClO_4-	+1.43
Cl_2	+1.36
O_2	+1.23

Table 1. Reduction potential standard for different chemical species [36–38].

For the generation of hydroxyl radicals in a reaction medium, there are different methods to classify them as homogenous and heterogeneous, and they may occur with or without light irradiation. Homogeneous systems are characterized by the absence of a solid catalyst utilized to initiate the reaction. In contrast, heterogeneous processes make use of solid catalysts to contribute to the formation of hydroxyl radicals [39]. Figure 1 summarizes the most common of AOPs systems.

Among the different AOPs systems, heterogeneous catalysis has received prominence over the years. Photocatalytic oxidation can be a good option for water disinfection, its performance is greatly restricted by fast electron-hole (e/h^+) recombination. The strategy to increase photocatalytic efficiency is photoelectrocatalysis (PEC), which consists of introducing a reverse

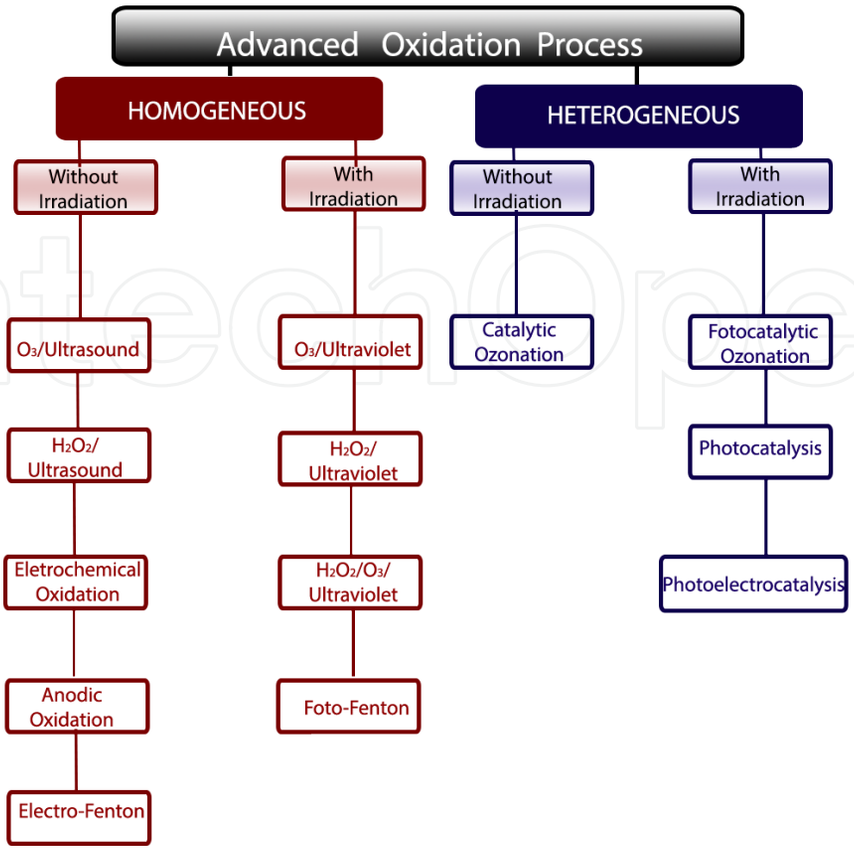

Figure 1. Schematic representation of different treatment based on AOPs

bias potential to the anode coated with the photocatalyst [40–42]. The PEC process minimizes recombination, this process exists under bias potential, creating the potential gradient on the anode that is enough to remove the electrons from the conduction band to an external counter electrode. This increases the availability of ·OH radicals and other reactive oxygen species (ROS), which can attack the bacterium cell wall and organics compounds when the bacteria gets in contact with the catalyst [42].

The principle of photocatalysis is based on the activation of a semiconductor (commonly titanium dioxide (TiO_2)) by light irradiation. In order for the semiconductor to become a conductive material, charge carriers need to be created, usually by photoexcitation. The principle of the process is the generation of a pair of electron/hole (e^-/h^+) by promotion of an electron from the valence band (VB) to the conduction band (CB) (Equation 2). A semiconductor material is characterized by the presence of a VB and a CB separated by a band gap energy (E_g) [36]. Figure 2 shows a schematic representation of a photoexcited semiconductor.

$$Semiconductor \xrightarrow{hv} Semiconductor(e^-_{CB} + h^+_{VB}) \quad (2)$$

In a semicondutor, the Fermi level (Ef) is a very important parameter and can be defined as the electrochemical potential of an electron in a metal or semiconductor, which can be

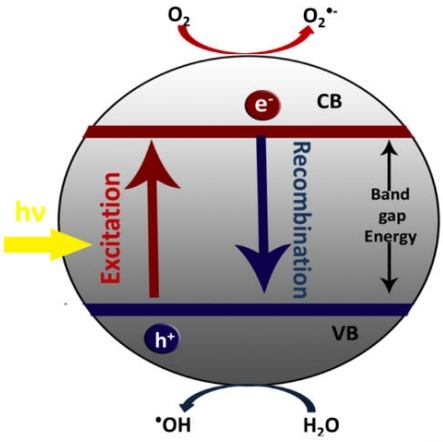

Figure 2. Schematic representation of a photoexcited semiconductor

modulated by the applied external potential. In order to promote electrical conduction in a semiconductor, the charge transport should be obtained by one of the following mechanisms: thermal generation, doping, or photoexcitation [43]. Thus, metals and semiconductors are distinguished by the Fermi level position, which is related to the concentration of charge carriers [43–44]. In the n-type semiconductor, the Fermi level is closer to the band conduction, while for the p-type semiconductor it is close to the valence band.

The presence of an essentially filled energy band, a separate almost empty band, leads to semiconductor photosensitivity. The absorption of photons with energy greater than the energy of the "band gap" results in the promotion of electron from the valence band to the conduction band with concomitant generation of a hole (h^+) in the valence band. This can be visualized in the Equation (2).

The hole formed on the surface of the catalyst (n-type for instance) can usually promote oxidation of adsorbed species on the semiconductor surface and/or water oxidation leading to the formation of hydroxyl radical, and both can act on organic material degradation. Different mechanisms can be proposed:

a. The organic matter (OM) from the actinobacteria cell wall, for example, adsorbs on the surface of the photocatalyst and can be oxidized by the holes (h^+) in the valence band, forming a cation that can react rapidly with oxygen [45].

$$OM_{ads} + h^+_{VB} \rightarrow OM^{\bullet+}_{ads} \quad (3)$$

b. Depending on the chosen semiconductor and the pH of the system, the water adsorbed on the surface is oxidized to hydroxyl radical through the hole from the valence band [45]. As previously mentioned, the hydroxyl radical has a high oxidation power and can promote the oxidation of the species present in the system, subsequently.

$$Semiconductor - h^+_{VB} + H_2O_{ads} \rightarrow Semiconductor - OH_{ads} + H^+ \quad (4)$$

c. Other radical oxygen species formed by the capture of photogenareted electrons can promote the oxidation process [24, 25].

$$e^- + O_2 \rightarrow O_2^{\bullet -} \tag{5}$$

$$O_2^{\bullet -} + H^+ \rightarrow HO_2^{\bullet} \tag{6}$$

The efficiency of photocatalysis depends on the competition between the effectiveness of the electron removal from the semiconductor surface and the recombination process involving electron/h_{vb}^+ generating heat as a product [36, 46, 47]:

$$Semiconductor\left(e_{CB}^- + h_{VB}^+\right) \rightarrow Semicondutor + heat \tag{7}$$

Therefore, there is high demand for methods that are able to minimize the recombination process of electrons returning from the conduction band to the valence band. Studies reported in the literature have shown that the photocatalytic oxidation can be improved by an anodic bias potential [19]. In the under bias potential, there is a formation of a potential gradient in the interface substrate/electrolyte and the electrons are removed, leading to the decrease in recombination of the pair electron/hole [36, 42]. Figure 3 illustrates the mechanism of PEC.

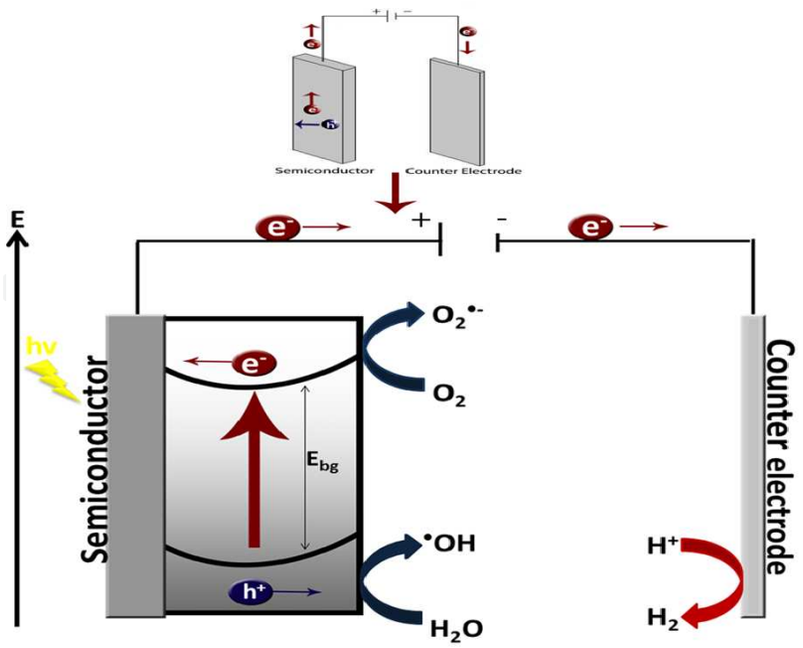

Figure 3. Mechanism of photoelectrocatalysis and charge separation in a photoelectrochemical system, where a gradient of potential is created.

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Understanding the efficient charge separation mechanism in PEC is essential. The moment a semiconductor is in contact with the electrolyte, a junction semiconductor/electrolyte interface is formed in charge of determining the electron hole separation kinetics. Due to the different potential present in the interface, there is a change in the potential Fermi of the semiconductor. Thus, the equilibrium of this interface requires a power flow between the phases, providing the creation of band-bending in the semiconductor phase. The space charge layer (SCL) is the region where there is bending and is characterized by the presence of electrons or holes in the surface [36, 48, 49].

The application of a bias potential also contributes in controlling the Fermi level [48]. Flat band potential is conceptualized as an exact potential for which the potential drops between the surface and the bulk of the electrode is zero. Thus, applying a greater potential than the flat-band potential will provide an increase in band-bending in an n-type semiconductor. In this situation, electrons are depleted and holes are enriched at the surface. The electron that has been excited in the conduction band flows through an external circuit to the counter electrode where reduction reactions may occur such as the reduction of H^+ to H_2. Figure 4 shows an energy diagram of an n-type (TiO_2) semiconductor in different situations: before semiconductor-electrolyte contact, equilibrium established after semiconductor-electrolyte contact, and applying anodic bias.

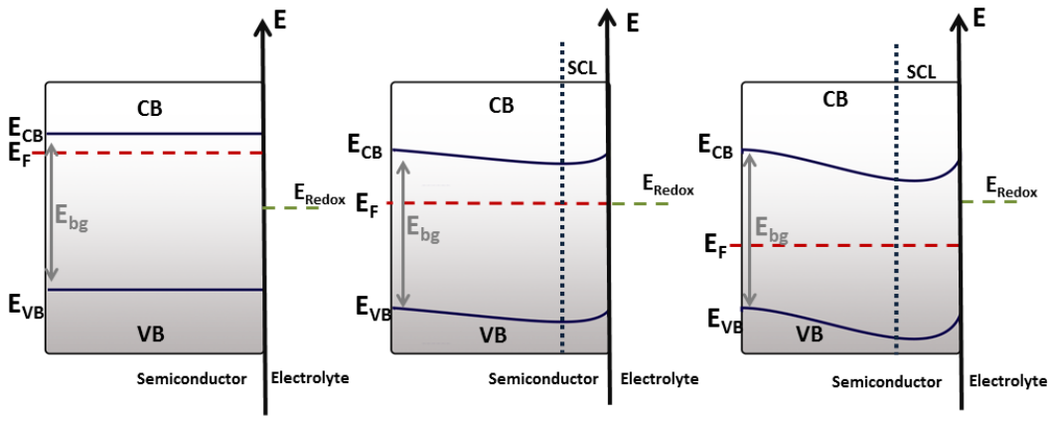

Figure 4. Energy diagram of n-type (TiO_2) semiconductor in different situations: before semiconductor-electrolyte contact, equilibrium established after semiconductor-electrolyte contact and applying anodic bias.

Thus, the high oxidizing power of hydroxyl radicals formed during photocatalytic and photoelectrocatalytic processes has gained attention in the disinfection process. Their applications in promoting actinobacterium disinfection is reported in the literature and a revision of this contribution is shown in the following sections.

4. Main advanced oxidation process applied to actinobacterium disinfection

Considering the resistance of actinobacterium to conventional disinfection treatments and the promised results of disinfection processes based on advanced oxidation process, the aim of

this work is to describe the efficiency of the advanced oxidation process applied to actinobacterium disinfection.

Among the advanced oxidation process applied to actinobacterium disinfection, the main reported are photolysis, photocatalysis, and photoelectrocatalysis specially applied to mycobacteria disinfection. Some of the works related in the literature are show below.

4.1. Photolysis

The inactivation by photolysis using mainly ultraviolet (UV) irradiation has been reported by many researches using different UV sources applied specially for *Mycobacterium* inactivation in the environmental samples. The kinetics of inactivation of *M. tuberculosis*, *M. fortuitum*, and *M. marinum* has been reported since 1971. The inactivation reached 90% for *M. tuberculosis* and *M. marinum* cells after irradiation of 7 to 22 s, respectively. However, *M. tuberculosis* and *M. marinum* are shown to be capable of photoreactivation [50]. The use of UV at 20 mJ/cm^2 inactivate more than 3 log of *M. avium*, *M. intracellulare*, and *M. lentiflavum*, whereas *M. fortuitum* required a dose of more than 50 mJ/cm^2. The study showed that mycobacteria were found 2–10 times more resistant to UV than *E. coli* [51]. The efficiency of low-pressure UV light for *Mycobacterium* complex inactivation in water is also reported. Using low-pressure UV light, the results showed greater than 4 log inactivation at fluencies of less than 20 mJ/cm^2 [52]. The 405 nm light (10 mW cm^{-2}) generated from an array of 405-nm light-emitting, inactivated 4-5 log CFU mL^{-1} *M. terrae* in liquid suspension and on exposed surfaces using a dose between 144 and 288 J cm^{-2} [53]. The use of microwave with UV lamps (peak emissions at 253.7 nm) that also generate ozone, have been tested for its ability to eradicate high populations of microbial vegetative cells and spores (of bacteria and fungi) artificially added to filter surfaces. *M. phlei* deposited on the surfaces as clumps required 30 min treatment to affect complete killing. It has been considered that longer irradiation periods are required to facilitate the complete destruction of surface microorganisms, ozone, and other oxidizing species produced by microwave UV lamps that act to enhance microbial destruction [54]. The method has been also proposed for the treatment of secondary effluents and metal working contaminated with microorganisms such as mycobacteria.

Secondary effluents spiked with *Mycobacterium terrae* filtered through nylon filters (100, 41, and 20 μm) to partition aggregate sizes were exposed to UV light doses and free chlorine; rapid inactivation with 2.5 log reduction at 14 mJ/cm^2 and 56 mg min/L for UV and free chlorine, respectively, were obtained. Mycobacteria are acid-fast and their outer waxy layer may contribute to their relative resistance to UV and chlorine disinfection. Furthermore, mycobacteria tend to aggregate (clump) and accumulate in biofilms, which may further increase their resistance to disinfectants. It has been reported that a dose of 4.2 mJ/cm^2 was required to achieve 1 log inactivation of *M. terrae*, which is higher than 1 log inactivation of any other vegetative bacteria (for example: *E. coli* 1.5 mJ/cm^2) [55]. The efficacy and rapidity of UV (192 μW/cm^2, 55 W) radiation for disinfection has been reported for the inactivation of 10^4–10^7 CFU/mL *Mycobacterium chelonae*; 74% reduction was observed after 10 min of exposure [56]. The photoinactivation of *Arthrobacter nicotinovorans* and of *Streptomyces griseus* were tested by irradiation at 222 nm and 254 nm reaching a 4-log reduction [57]. The effect of different fractions

of natural UV radiation doses on two Antarctic marine bacteria (*Arthrobacter* UVvi and FCB-related UVps strains) were evaluated by six treatments: DARK, PAR (with UVR shielded off), UVA360, UVA320, UVB305, and UVB280 [58]. In all UVR treatments, strains showed significant losses of viability under high and moderate irradiance and no differences were observed between UV treatments. Under high UVB dose (15.0 kJ m^{-2}) received in only two hours, the effect of UVB treatments was significantly higher than that observed under UVA. However, for both strains, UVA wavelengths are sufficient to suppress bacterial viability significantly in marine situations, principally UVA (λ< 360 nm). When exposed to UVB wavelengths, both strains lose viability exponentially with the dose. The efficiency of the photolysis in the inactivation of the actinobacterium has been based mainly on the damage caused to the nucleic acids (DNA/RNA) of the cell. However, the photolytic treatment has not showed promising results, thus, a lot of alternatives have been proposed to improve the efficiency of disinfection, especially considering the possibility of photoreactivation of actinobacterium next to a dark period. Among the alternatives, photocatalysis has shown promising results.

4.2. Photocatalysis

Photocatalytic treatments are based on an irradiation source and a photocatalyst as a semiconductor. Among them, the TiO_2 is one of the most powerful semicondutor materials used for photocatalysis due its high activity, strong oxidizing powers, and long-term stability. TiO_2 can generate strong oxidizing power when illuminated with UV light at wavelengths lower than 385 nm. The photon energy generates an electron hole pair on the TiO_2 surface. The hole in the valence band can then react with water or hydroxide ions adsorbed on the surface to produce hydroxyl radical (·OH), and the electron in the conduction band can reduce O_2 to produce superoxide ions (O_2^-). Both holes and ·OH are extremely reactive upon contacting organic compounds and microorganisms [59]. However, others semiconductors have been proposed for actinobacterium disinfection and some of the main works are described in this section.

The photocatalysis with immobilized TiO_2 and UV irradiation has been proposed as seawater disinfection technology. The method was evaluated using marine bacteria assigned as actinobacteria *Corynebacterium stationis*. The photocatalytic treatment promoted faster disinfection kinetics for both bacteria species, between 30% and 33% less UV dose to achieve the same level of disinfection [60]. Shintani et al. [61] have shown TiO_2 membrane as a photocatalyst irradiated by UV lamp to sterilize airborne microorganisms in health care. A statistical difference was observed between UV and the photocatalyst sterilization ($p<0.01$) when humidity was increased to 60–70%.This indicates that maintaining high humidity levels gives more effective sterilization results due to the higher production of OH radicals. Among representative airborne microorganisms found in the dialysis room are *Micrococcus spp.*, *Bacillus spp.*, and *Corynebacterium spp*.

The anti-bactericide activity of TiO_2 nanofilms deposited onto polyvinyl chloride and glass substrates was evaluated by monitoring *Micrococcus lutes* (odor-causing bacteria) disinfection. *M. lutes* bacteria were completely killed in 20 min over TiO_2 films under the UV solar simulator (150 W). On the other hand, about 30% of bacteria were destroyed in 20 min under UV black

light (100 W) [62]. The antibacterial activity against *Micrococcus luteus* has also been reported using apatite-coated TiO_2. Its antibacterial performance was observed under black light, visible light, and dark conditions. The number of viable bacteria decreased with irradiation time and became most prominent at 24 hours. The black-light irradiation demonstrated higher antibacterial activity than either visible-light irradiation or dark conditions. Around 50% reduction within 24 h using apatite-coated TiO_2 under black light has been observed [59].

Hetero-nanostructured film of titania nanosheets and lysozyme have been proposed as a semiconductor-like antibacterial agent for *Micrococcus lysodeikticus* with high inhibition rate. In the presence of UV light, a significant high inhibition rate was observed when the multilayer film is present in turbid bacterial suspension [63]. The photocatalytic kill of *Micrococcus* has also been reported using visible light-induced sulfur-doped TiO_2 [64].

The impregnation of TiO_2 with different metals has been proposed to improve the photocatalytic actives, such as Ag, Cu, and Pt. Su et al. [65] proposed the use of TiO_2/Ag nano-antibacterial materials prepared at low temperature using polyethylene glycol (PEG-600) as reducing and stabilizing agents. The antibacterial activity study was carried by growth inhibition rates against *E. coli* and *Streptomyces*. The growth inhibition rates against *Streptomyces* was 97.9% and the concentration of TiO_2/Ag was 10 ppm, but changes of inhibition rates are not very obvious with the decrease of concentration of TiO_2/Ag. Under photocatalytic conditions, it is expected that the energy of the photons absorbed by Ti/TiO_2-Ag will generate charges. The separation of these charges after reduction of O_2 can produce hydroxyl radicals (·OH) in the valence band, due to water oxidation and superoxide ions (O_2^-) in the conduction band. The decoration with Ag nanoparticles can trap the photogenerated electrons, increasing the lifetime of the holes that are able to produce hydroxyl radicals. Consequently, the frequency of charge recombination is lower than the usual value observed for similar processes conducted without Ag, improving the efficiency of the disinfection [66].

The efficiency of undoped TiO_2 and platinized sulfated TiO_2 (Pt/TiO_2) to photocatalytic oxidation was investigated with microorganisms loaded over photocatalyst films from aerosols to load *Mycobacterium smegmatis*, *Bacillus thuringiensis*, vaccinia virus, and influenza A (H3N2) virus. About 90% inactivation is reached in 30 min UVA irradiation on TiO_2 and from 90% to 99.8% on Pt/TiO_2. It has been proposed that Pt/TiO_2 showed increased rate of mineralization, as well as increased inactivation likely due to a better charge carrier separation in the doped semiconductor photocatalyst. The results demonstrate that photocatalytic filters with deposited TiO_2 or Pt/TiO_2 are able to inactivate aerosol microorganisms and completely decompose them into inorganic products and Pt/TiO_2 provides higher disinfection and mineralization rates [67]. Pt/TiO_2 nanoarchitecture activated by a 365 nm UV light has also been proposed for the photo-driven killing of *Micrococcus lylaecells* [68]. Significant cell death is observed only for the photoactivated Pt/TiO_2 film, with which 70% of the M. *Lylaecells* were killed in 60 min. The authors proposed that oxidative species can easily diffuse out of the porous matrix to attack the cells. However, considering that the photocatalyst can not directly attack the cells that were protected by an outer peptidoglycan layer [69], the oxidative species are believed to be responsible for killing *M. lylae* cells. The detailed bactericidal mechanism of these photocatalytically-induced oxidative species is not unambiguously clear. However,

based on previous studies, the authors proposed that the plasma membrane can be first attacked by the oxidative species penetrating the outer layer of the bacteria. These reactive species can then oxidize coenzyme A and the plasma membranes. The oxidation of coenzyme A would inhibit cell respiration and directly cause cell death [70]. Meanwhile, the oxidation of the plasma membrane can break the main permeability barrier of the bacteria. This would result in the slow leakage of the intracellular materials, including RNA, protein, and minerals, leading to the subsequent death of *M. lylae*.

The use of Cu-doped TiO_2 nanoparticles (NPs) for the inactivation of *Mycobacterium smegmatis* was investigated under three light conditions (complete dark, fluorescent light, and UV light) [71]. The survival rate of *M. smegmatis* (in a minimal salt medium for 2 h) exposed to the NPs varied depending on the light irradiation conditions as well as the dopant concentrations. When TiO_2 NPs were doped with copper (>3wt% dopant), their inactivation potential was promoted and the UV-resistant cells were reduced by over 99%. Limbach et al. [72] have proposed that Cu NPs may enter the cytoplasm of the cell through a Trojan-horse mechanism and that the inactivation capability of NPs is enhanced due to an increased uptake of ions into the cell structure, disturbing its function. However, Cu-doped TiO_2 NPs tend to agglomerate in the aqueous solution and their effective sizes were not much smaller than the bacteria itself [73]. Also, mycobacteria have a very thick and waxy cell wall. Therefore, the Trojan-horse mechanism discussed earlier may not play an important role here [72]. Instead, the leached Cu^{2+} ion, which electrostatically interacts with the negatively charged cell membrane, forms a concentrated ionic zone of copper, enhancing the microbial effects.

Another catalyst that has been reported is Ag/ZnO composite NPs. This photocatalyst was applied for textile treatment. Long-term stable sols of ZnO and Ag/ZnO NPs were prepared and applied as liquid coating agent for textile treatment in combination with an inorganic-organic hybrid polymer binder sols prepared from the precursors 3-glycidyloxypropyltrimethoxysilane (GPTMS) and tetraethoxysilane (TEOS) [74]. The antimicrobial activity of the NPs applied on textile fabrics was tested against the Gram-negative bacterium *Escherichia coli* and Gram-positive *Micrococcus luteus*. It was observed that bacterial susceptibility improved considerably with the increase in the silver contents.

The mycobactericidal properties of an iron-based novel heterogeneous-modified polyacrylonitrile (PAN) catalyst in combination with hydrogen peroxide were examined against *Mycobacterium chelonae*. The 0.5% w/v hydrogen peroxide and 2-g catalyst system resulted in average log reductions of >5.80 for *M. chelonae* at 30 min exposure at room temperature. This was a significant increase in activity ($P<0.01$) compared to 0.5% w/v hydrogen peroxide alone [75].

There are many photocatalytic actinobacterium disinfection processes based on different semiconductors, mainly TiO_2 (pure and impregnate). Although photocatalytic oxidation can be a good option for water disinfection, its performance is greatly restricted by fast electron–hole (e^-/h^+) recombination. A strategy to increase photocatalytic efficiency is PEC, which consists of introducing a reverse bias potential to the anode coated with the photocatalyst. The PEC process minimizes recombination because the system exists under bias potential, creating a potential gradient on the anode that is enough to remove the electrons from the conduction band to an external counter electrode. This increases the availability of ·OH radicals and other

reactive oxygen species (ROS) that are able to attack the bacterium cell wall where the bacteria gets in contact with the catalyst. There are few researches about the actinobacteria photoelectrocatalytic inactivation and they are only described for *Mycobacteria* inactivation. These works are shown in the section below.

4.3. Photoelectrocatalysis (PEC)

The photoelectrocatalytic inactivation using TiO_2 nanotubular array electrodes of 10^3 CFU (Colony-Forming Units) mL^{-1} M*ycobaterium fortuitum*, *M. chelonae*, and *M. abscessus* was achieved after 3 min treatment. The metabolites released during cellular lysis were also degraded with 240 min of photoelectrocatalytic treatment, the monitoring was carried out by mass spectroscopy measurements. Mineralization was greater than 70% under optimum conditions. The photoelectrocatalytic method gave better results than the photolytic and photocatalytic techniques [76].

TiO_2 nanotubular array electrodes coated with 16% (w/w) Ag NPs (Ti/TiO_2-Ag) have shown excellent performance in the disinfection of water containing *Mycobacterium smegmatis* [77]. Photoelectrocatalytic disinfection of *M. smegmatis* 5.1×10^3 CFU mL^{-1} in 0.05 M Na_2SO_4 (pH 6), applied potential of +1.5 V versus Ag/AgCl and UV irradiation promoted 100% inactivation after 3 min of treatment. In addition, analytical methods such as TOC removal, sugar release, chromatography, and mass spectroscopy measurements confirmed that there was complete inactivation of mycobacteria and degradation of the by-products generated during cellular lyses by the proposed method.

The irradiation of Ti/TiO_2–Ag with visible irradiation as photoelectrode promoted 99.6% inactivation of *M. smegmatis* after 30 min. In addition, the analysis of silver in the solution along with the reaction shows a negligible lixiviation and a high stability of the surface silver deposit [78]. Ti/TiO_2-Ag photoanode has also been proposed to inactivate *Mycobacterium kansasii* and *Mycobacterium avium* for a mycobacteria population of 5×10^8 CFU mL^{-1}; it achieved 99.9% and 99.8% inactivation after 240 min. Using a mycobacteria population of 7.5×10^4 CFU mL^{-1}, total mycobacteria inactivation within 3 min of treatment was obtained. The presence of Ag nanoparticles in the electrode provided 1.5 larger degradation rate constant as compared with the Ti/TiO_2 anode [79]. An explanation for this is that in the photoelectrocatalytic process, there are reductions in the recombination of charges photogenerated since under bias potential there are formations of a gradient potential on the photoanode and flow of electrons from the conduction band to the external counter electrode connected to the photoreactor. This arrangement increases the availability of ·OH radicals and other ROS that are able to attack the bacterium cell wall where the bacteria gets in contact with the catalyst [80, 81]. This results to larger damage to all cell wall layers, allowing small components such as ions to leak. Damage at this stage may be irreversible and cell death ensues. Because peptidoglycan is a highly cross-linked molecule, damage may not be evident at this stage; indeed, visualization would require leakage of components with higher molecular weight such as proteins [82].

So, among the advanced oxidative process described until this moment, the photoelectrocatalytic treatment gave the best results, since it promotes the inactivation and also degradation of cell lyse components.

4.4. Others alternative advanced oxidation processes

Among the other advanced oxidation processes, we can highlight the use of ozone, Engineered Water Nanostructures, peroxide, and direct electrolysis.

The effect of ozone on cariogenic microorganisms has been reported for actinobacteria *Actinomyces viscosus* and other bacteria such as *Streptococcus salivarius* and *Streptococcus mutans*. The microorganisms were exposed to power levels 2, 3, or 4 of an ozone device for 20, 40, 60, or 120 s. CFU numbers of bacteria were counted after the ozone application; as a result, the number of bacterial cells decreased. In addition, the bacterial cells were evaluated with a fluorescence microscope and showed that some bacterial cells were killed instantaneously in the ozone [83].

Pyrgiotakis et al. [84] have been the first to report the use of Engineered Water Nanostructures (EWNS) for mycobacteria inactivation. The method is based on the transformation of atmospheric water vapor into EWNS. Electron paramagnetic resonance (EPR) showed that EWNS contain a large number of ROS, primarily ·OH and superoxides. *M. parafortuitum* was inactivated on surfaces eight times faster than the control. It was demonstrated that the EWNS effectively deliver the ROS encapsulated during the electrospray process to the bacteria, oxidizing their cell membrane and resulting to inactivation. Overall, this is a method with the potential to become an effective intervention technology in the battle against airborne infections.

The disinfection of *Streptococcus mutans*, which generates acid that causes mineral loss of the teeth and the so-called dental caries, has been also evaluated by hydroxyl radicals generated by photolysis of H_2O_2 [85]. Laser irradiation of suspensions in 1M H_2O_2 resulted in a >99.99% reduction of the viable counts within 3 min of treatment. Treatment of a biofilm composed by *S. mutansin* resulted in a >99.999% reduction of viable counts within 3 min.

Finally, disinfection using electrolyzed strongly acidic water (ESW) against *Mycobacteria* and the recovery of the disinfection potential of inactivated ESW by re-electrolysis was reported. The ESW was carried out using positive and negative platinum-coated titanium electrodes and 0.1% NaCl electrolyzed at a constant voltage of 24 V. ESW containing 10, 20, and 30 ppm free chlorine was applied in a solution with *M. bovis* cells (10^5–10^8 CFU/mL) for 0–7 min. To clarify the recovery of the disinfection potential of inactivated ESW by re-electrolysis, it was mixed with ESW containing 10 ppm free chlorine with *M. bovis* cells (10^7 CFU/mL) for 1 min. The number of viable cells decreased to $1/10^3$, but the cells were still detected. After re-electrolysis for 7 min, viable cells were not detected. ESW (10 ppm) showed its complete bactericidal effect against 10^5 and 10^6/mL of bacterial suspension within 1 and 3 min, respectively. Against NTM, one strain each of *M. avium*, *M. intracellulare*, and *M. kansasii* ESW (10 ppm) decreased the number of viable cells by less than $1/10^5$ within 1 min of contact [86].

Then, considering the abundance of the actinobacteria in water mainly due their resistance to conventional water treatment, such as chlorine, the advanced oxidation process is a promising alternative to the water treatment, especially to water used in therapeutic applications.

5. Final consideration

The advanced oxidation process has been reported as an excellent alternative for actinobacterium disinfection. Good results have been obtained by photolysis treatment, however, it requires longer time and there is the possibility of photo-reparation next to a dark period. The use of photocatalysis gave the best results using different photoanodes such as TiO_2 (bare and impregnated) with metals such as Au, Ag, and Pt, which can improve the photocatalytic activated. The most promising technique seems to be photoelectrocatalysis as it is able to promote inactivation at a short period of illumination and higher mineralization of products generated from the cellular lyse. Nevertheless, the technique deserves further test to improve the economic aspects involved in using the combination of bias potential and UV irradiation.

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