TiO2-Based Nanostructures for Microbial Inactivation

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Pathogenic microorganisms can spread throughout the world population, as the current COVID-19 pandemic has dramatically demonstrated. In this scenario, a protection against pathogens and other microorganisms can come from the use of photoactive materials as antimicrobial agents able to hinder, or at least limit, their spreading by means of photocatalytically assisted processes activated by light—possibly sunlight—promoting the formation of reactive oxygen species (ROS) that can kill microorganisms in different matrices such as water or different surfaces without affecting human health. Here, we focus the attention on TiO2 nanoparticle-based antimicrobial materials, intending to provide an overview of the most promising synthetic techniques, toward possible large-scale production, critically review the capability of such materials to promote pathogen (i.e., bacteria, virus, and fungi) inactivation, and, finally, take a look at selected technological applications.

Keywords: TiO2 ; photocatalysis ; nanoparticles ; pathogens ; bacteria ; virus ; fungi

1. Introduction

Over the last decades, titanium dioxide (TiO_2) has been extensively investigated for its physical-chemical properties, that, combined with its high stability, low cost, and safety for the environment and humans, have resulted in a range of environmental and energy applications.

When TiO_2 is obtained at nanoscale, many relevant properties of this semiconductor are enhanced, due to the increased surface area, which results from the high surface-to-volume ratio, the excellent surface morphology, and the band edge modulation, that, overall, turn into a control on the photocatalytic behavior and performance of the nanostructured materials [1][2][3].

Upon illumination, TiO_2 nanoparticles (NPs) convert incoming photons into excitons, or electron/hole pairs, which can migrate to the surface and participate in redox reactions and generate reactive oxygen species (ROS), such as hydroxyl radicals (OH·) and superoxide(O_2^-) [3][4].

Indeed, the photocatalytic behavior of TiO_2 NPs has been widely exploited for removal of water and air contaminants and self-cleaning surfaces [1][2][3][4]. Currently, increasing concerns regarding the COVID-19 pandemic are drawing the attention of researchers and general public more and more to photocatalytic antimicrobial and antiviral treatments with the purpose of hindering virus spreading, by using light (possibly solar light) activated systems.

TiO_2 NPs are among the most studied materials in the area of photocatalytic antimicrobial applications, having demonstrated a great potential for the disinfection/inactivation of harmful pathogens, including bacteria, viruses, and fungi [5]. However, aside from the superior advantage of TiO_2 nanostructured materials, some drawbacks have been identified, i.e., high recombination rate of the photogenerated species and limited solar light sensitivity, therefore various modifications have been developed to enhance the photocatalytic efficiency [3][4].

Upon photoactivation of TiO_2 NPs, the biocidal action is a result of the modulation of charge carriers, electrons, and holes at the surface of the material, resulting in powerful and long-lasting capabilities [1], since the process does not rely on the release of metal ions, and hence the material consumption, as it happens in the case of Ag-based antimicrobial material. Moreover, TiO_2 NPs-based systems have a substantial advantage due to their non-contact biocidal action. Finally, since any possible release into the environment of potentially toxic NPs—with unpredictable effects on human health—from the material or device for the final application must be prevented, the TiO_2-based structures are required to be suited for immobilization onto substrate and/or incorporation in matrices. In this regard, this class of materials could be considered reasonably environmentally friendly.

The antimicrobial activity of TiO_2 NPs is primarily attributed to the photocatalytic generation, under band-gap irradiation, of ROS with high oxidative potentials. However, other possible factors may be considered to explain their biocidal effect, such as free metal ions formation or synergistic effects deriving from the combination of TiO_2 NPs with other materials and compounds in nanocomposite systems [1][2][5].

2. Technological Applications of Antimicrobial TiO_2-Based Nanostructured Materials

2.1. Environmental Applications

2.1.1. TiO_2-Based Nanostructured Materials for Water Disinfection

TiO_2 based nanocomposites are well known and extensively used materials in water treatment technologies [3][4][6]. Indeed they are able, due to their photocatalytic activity, to degrade organic pollutants such as dyes, [7] pesticides [8], pharmaceuticals [3][9], and personal care products [10], that pose a great danger for human and aquatic life. Moreover, their antimicrobial properties are effective against waterborne pathogens such as bacteria, viruses, and fungi that originate from the urban microbiome and tend to be released and accumulated in sewage and urban runoff. The mechanism is summarized in Figure 1 and will be explained in the following. Waterborne diseases derive often from bacteria such as *E. coli, Legionella pneumophila, Mycobacterium avium, S. flexneri, L. monocytogenes, V. parahaemolyticus* and, to a lesser extent, from viruses, that are typically present at lower concentration. Every year, waterborne infections cause nearly 200 million deaths worldwide, mainly localized in low income countries [11]. One of the most critical classes of bacteria in wastewater treatment plants (WWTPs) is antibiotic resistant bacteria (ARB), which derive from the extensive use and abuse of antibiotics. WWTPs offer an ideal environment for ARB proliferation and, under these conditions, the antibiotic resistance genes (ARG) that induce the resistance in the bacterial population, can spread easily and quickly. Therefore, as the use of common antibiotics becomes ineffective, it is fundamental to develop alternative strategies to treat microbial infections and to promptly take action against the spreading of ARB in water as well as in the air [12]. The present section will focus on TiO_2-based nanomaterials as an excellent alternative to conventional methods for water disinfection. In addition, nanomaterials applied to counteract the biofouling will be reviewed, as it is another great problem affecting water treatment plants as well as materials in contact with water, including distribution pipes and filtration membranes [13].

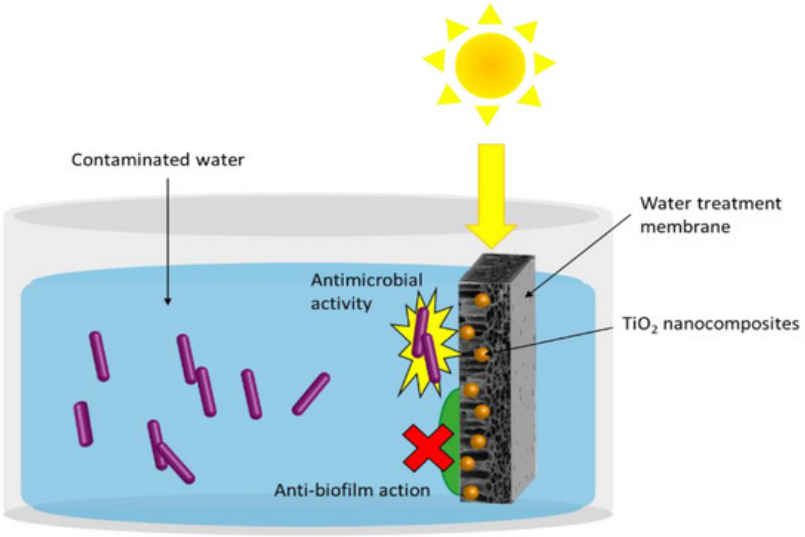

Figure 1. General scheme of TiO_2 NPs-based nanocomposite application for water disinfection, that highlights the ability to prevent biofilm formation at the surface of the substrate, such as a membrane, and the antibacterial activity against water pathogens driven by photocatalysis.

TiO_2 NPs-based nanocomposites for water disinfection are often investigated as colloidal suspensions, since, under these conditions, the whole NPs surface is available, exposing all the active catalytic sites to the aqueous environment dispersing the pathogens. However, while suspended NPs are typically found to display a higher photocatalytic activity in comparison with NPs immobilized on a substrate [14], the dispersed NPs present technological problems, as they need to be recovered to prevent them from becoming a source of pollution in the environment.

2.1.2. Immobilization of Nanocomposites on Membranes or Recoverable Supports

The immobilization of photocatalytic TiO_2 NPs and their nanocomposites onto a suitable substrate is essential to accomplish technologically feasible applications. As mentioned above, a robust and durable immobilization of NPs onto appropriate support is fundamental, not only for enabling the nanosized photocatalyst recovery, but also to prevent the accidental release of NPs in the environment, that, becoming themselves a possible contaminant, may become harmful [4]. Here, some of the most original proposed solutions for the deposition and immobilization of TiO_2 NPs-based nanomaterials specifically designed for photocatalytic microorganism inactivation are summarized.

Photocatalytic membrane reactors (PMRs) combine the membrane technology with the advantages of photocatalysis assisted reactions. Two main configurations are reported in the literature: PMRs with the photocatalyst immobilized on the membrane or incorporated therein, and PMRs based on the photocatalyst dispersed in a colloidal suspension. In both cases the photocatalyst is confined in the reaction environment. However, the former configuration allows an effective recovery and reuse of the catalyst, thanks to its stable immobilization, while the recovery is more complicated in the latter case, although the whole NPs surface can be more effectively exploited for adsorption and catalysis [15]. Among the possible photocatalysts for PMR fabrication, TiO_2 NPs and TiO_2 NPs-based nanocomposites have been the most investigated [16]. Cheng et al. fabricated a PMR based on the combination of a polyvinylidene fluoride (PVDF) membrane and a suspension of TiO_2 P25 in tap water and studied the biocide effect for virus removal, using phage *f2* as a model. The effect of the amount of humic acid (HA) in the water dispersion on the efficiency of the system was also investigated. Moreover, HA was found to compete with phage *f2* at the adsorption sites on TiO_2 NPs surface, and the UV light absorbed by HA overall led to a reduction of biocide activity at high HA concentration [17]. A PMR based on a microfiltration hollow polyethylene (PE) fiber membrane coupled with a suspension of TiO_2 P25 was investigated for the reduction in bacterial population of secondary effluent water sample, that was assessed, down to 2 log, when a 1g/L TiO_2 NPs suspension was used [18]. Another typical and efficient approach for nanocatalyst immobilization, able to preserve their activity, relies on their immobilization onto fibers that, possessing a high surface, allow optimal interaction between the target substrate dispersed in water and the photocatalyst. Amarjargal et al. functionalized the surface of electrospun polyurethane (PU) fibers with Ag-TiO_2 NPs by simply dipping the fiber in hot colloidal photocatalyst dispersion for 4 h and 12 h. After 3 h UV irradiation (320–500nm), a 6-log cycles reduction of *E. coli* population was found for the sample obtained after 4 h immersion and a 5-log cycles reduction for that immersed for 12 h. Interestingly, only 2-log cycles reduction was detected after exposure to UV, in absence of Ag-TiO_2 and no reduction was observed upon exposure of the nanocomposite to ambient light [19]. The antibacterial activity of electrospun Ag-TiO_2 nanofibers was tested against *E. coli*. The study demonstrated that, under the investigated experimental conditions: (i) no biocidal activity was obtained upon exposure to light (solar simulator) of the bare fiber, nor in the dark for TiO_2/Ag fibers and P25-coated fibers, (ii) fibers functionalized with the synthesized TiO_2 performed better, in terms of photocatalytic antibacterial activity, than fibers coated with TiO_2 P25, and (iii) the antibacterial activity in presence of Ag NPs was found higher in the dark than under light irradiation [20]. A TiO_2/Polyamide 6 (PA-6) electrospun fiber was tested by Daels et al. for the removal of *St. aureus* and HA from a secondary effluent sample. HA reduction of 83% after 2 h irradiation with simulated solar light (300 W Osram Ultra-Vitalux lamp) with an intensity of about 5 mW/cm^2 and 99.99% bacteria reduction after 6h UV irradiation was detected. The same fibers, tested in a filtration process (flow rate 11 m^3/(m^{2}h)) in a photocatalytic experiment performed under UV irradiation achieved 37% HA reduction and 76% *St. aureus* inactivation [21]. Zheng et al. electrospun Cu-TiO_2 nanofibers and tested the biocide activity under visible light against *f2* phage and the system formed by *f2 phage* and *E. coli* as its host, and reported a photocatalytic inactivation of *E. coli* higher than that of *f2 phage*. Remarkably, the *E. coli* inactivation did not seem affected by the presence of *f2 phage*, while the inactivation of the phage *f2* in the bacteria host system was lower than that found in absence of *E. coli*. Such a result was explained by considering that *E. coli* can behave as a ROS scavenger, probably due to its membrane structure, as discussed above, thus reducing amount of ROS effective for *f2* inactivation, thus somehow preventing its inactivation [22].

2.1.3. Anti-Biofouling Membranes for Water Treatment

The biofouling process is defined as the accumulation of microorganisms on a surface, and is realized in four steps: (i) organic matters are adsorbed on the surface, (ii) microorganisms adsorb and adhere to the surface, and (iii) start to grow and reproduce on the surface, and finally (iv) extracellular polymers are secreted and mutual adhesion among the clonal cells takes place [23]. These processes ultimately result in the formation of a biofilm, namely an assembly of microbial cells, irreversibly bound to a surface and embedded in a matrix of polysaccharide material [24]. When biofouling takes place at the surface of a membrane, its flux is limited, thus interfering with rejection and reducing the lifetime of the membrane itself, with the consequent drawbacks. In order to prevent biofilm formation, two main strategies can be employed. The first relies on the ability to enhance the hydrophilicity of the membrane, which reduces the affinity of the organic matter, and enhances its affinity with water, that can, thus, form a thin layer on the membrane surface, finally hampering the microorganisms' adhesion. The second route is based on the incorporation of antibacterial agents into the membrane, such as TiO_2-based nanomaterials [25]. TiO_2-based nanocomposites are greatly suggested for this application

since they display antibacterial properties and, concomitantly, convey, as a function of their chemical nature, hydrophilicity to the host membrane [26]. The combination of the intrinsic antibacterial properties with the photocatalytic properties of TiO_2 NPs-based nanocomposites offers multiple advantages for hindering the biofouling. As an example, Kim et al. deposited TiO_2 NPs on a polyamide thin film composite (TFC) membrane and tested their anti-biofouling properties, along with the antibacterial activity against *E. coli*. The water flux was measured for three days, for pristine and TiO_2 NPs-modified membrane, and with and without UV-light irradiation. After pipetting *E. coli* dispersion onto the membranes and incubating at 37 °C, a fast and significant reduction of the flux, a clear indication of the occurrence of a higher fouling, was observed for the membranes not exposed to UV, while a slow and low reduction of the flux was found for TiO_2-modified membranes, especially upon UV irradiation, finally demonstrating how the anti-biofouling activity is tightly connected to antibacterial behavior of UV-active TiO_2 photocatalysts [27].

2.2. TiO_2 NPs-Based Nanocomposite against Biofouling on Building Materials

In the construction field, nanostructured TiO_2 is commonly employed to degrade organic pollutants in the air or to protect the building materials from soot, due to its photocatalytic and hydrophobic properties. The biocide activity of TiO_2 can also be exploited to reduce the formation of biofilms formed on the surface of buildings [28]. Indeed, biofouling causes, not only aesthetic and structural degradation of construction materials and surfaces, but also bacteria and fungi proliferation that may pose a great health concern [29]. The application of photocatalytic TiO_2 NPs as a biocidal agent has considerable relevance, especially for locations strongly sensitive to biological safety, such as medical facilities and food industries, where usually ceramic tiles are used to coat walls and floor. The photocatalytic activity of TiO_2 nanomaterials was found to successfully reduce the bacterial proliferation and the fungi population (and, consequently, mycotoxins production) indoor when applied to surfaces, tiles, furniture etc. [30]. Dyshlyuk et al. tested suspensions of TiO_2, ZnO, and SiO_2 against microorganisms known for damaging construction materials, namely a bacterium (*B. subtilis*) and several common fungi (*A. niger, Aspergillus terreus, Aureobasidium pullulans* var. *pullulans, Cladosporium cladosporioides, Penicillium ochrochloron, Trichoderma viride*, and *Paecilomyces variotii*). They found TiO_2 and SiO_2 less active under sunlight than ZnO [31]. Sikora et al. fabricated core-shell nanocomposites of mesoporous silica ($mSiO_2$, core) and TiO_2 (shell) to be applied on cement mortars. The merging of the two nanomaterials in one nanocomposite was found to play the two roles of introducing in the building material a filler, $mSiO_2$, able to improve its mechanical properties, and conveying, with TiO_2, anti-biofouling and self-cleaning activity upon UV exposure. The $mSiO_2/TiO_2$ composite antibacterial activity was tested against *E. coli* and, after 2 h, a 67% and a 42% reduction were measured after exposure to UV light and at dark, respectively [32].

2.3. Photocatalytic TiO_2 NPs-Based Nanocomposites for Biomaterials Disinfection

Antibacterial NPs find a wide range of applications in the field of biomaterials. Materials designed for wound dressing or for tissue engineering-related applications need to comply with several requirements as summarized in Figure 2, as they need to be biocompatible, non-allergenic, easily removable, and degradable after implantation.

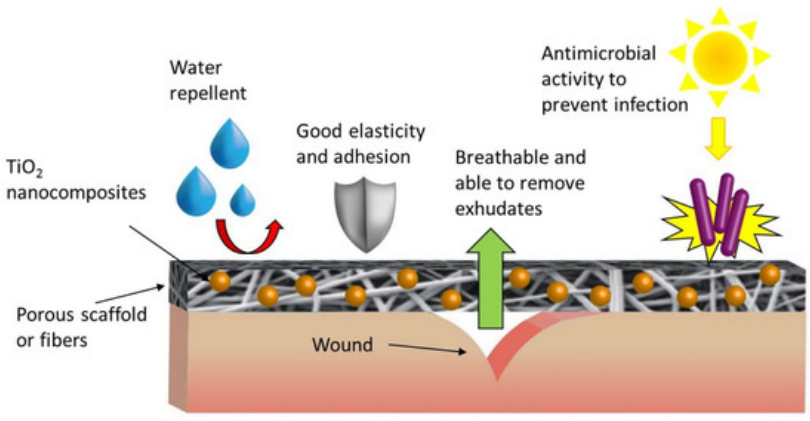

Figure 2. General scheme of TiO_2 NPs-based nanocomposite application for biomaterial-related applications highlighting the ability to repel water, to adhere to the skin following its movements, to remove exudates, to be permeable to air, and to prevent infections due to the antimicrobial activity driven by photocatalysis.

Antibacterial behavior is strongly desirable in this kind of material to prevent infections during wound recovery [33][34]. A great deal of work has been carried out in the development of biocompatible TiO_2-NPs-based nanocomposites, with intrinsic antimicrobial activity, specifically designed for tissue engineering-related applications to be used in healthcare facilities [35][36][37][38][39][40].

2.4. TiO_2 NPs-Based Nanocomposites Designed for Disinfection of Food Packaging and Processing Materials

Food packaging materials containing TiO_2 NPs-based nanocomposites have been thoroughly investigated materials for food contact-related applications and a steadily increasing number of technologies has been in development in the last years. This is in order to accomplish multiple purposes including improved mechanical, thermal, optical and antimicrobial properties, control of gas and moisture permeability, UV shielding, nutraceuticals release, and installing sensors for pathogens and harmful substances (Figure 3) [41][42][43]. Indeed, surfaces of food-processing plant components need to comply with the same requirements, as they are often in contact with food and the presence of microorganisms therein can easily lead to food contamination, causing transmission of diseases [17]. Indeed, the presence of antimicrobial substances in food packaging materials may inhibit growth of harmful microorganisms and pathogens on food, improving its safety and shelf life. NPs and nanocomposites can be integrated in food packaging materials as growth inhibitors, antimicrobial agents, or carriers [44], and are usually applied to the packaging of meat, fish, poultry, bread, cheese, fruits, and vegetables [45]. TiO_2 NPs, aside from antibacterial properties, also possess unique characteristics that may prove particularly useful for such applications. For example, TiO_2 shields UV light, a property that is generally exploited in the formulation of sunscreen lotions, and therefore may be an effective additive in packaging to preserve food from irradiation, and thus limit its deterioration [46][47]. Moreover, when TiO_2 NPs are contained in a packaging material, upon UV exposure they can photocatalytically degrade the ethylene molecules produced during the ripening process, which are responsible for food degradation [48].

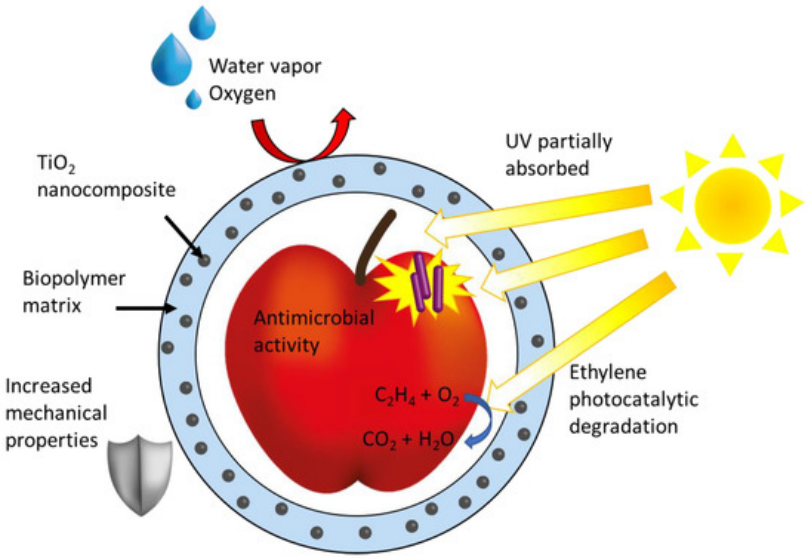

Figure 3. General scheme of TiO_2 NPs-based nanocomposite application for food packaging highlighting the advantages of TiO_2 in the coating as improved mechanical properties, water and oxygen repellence, UV shielding, and ethylene scavenger and antimicrobial activity.

In the last years, the choice of a polymer suitable and environmentally sustainable for food packaging has oriented towards biopolymers, which can be easily degraded and recycled, for a more ecological fingerprint in a field that has been, so far, heavily relying on petroleum-based polymers. Such biopolymers include poly hydroxybutyrates (PHB), polylactic acid (PLA), poly caprolactone (PCL), polyvinyl alcohol (PVA), poly butylene succinate, lipids (wax and free fatty acids), proteins (casein, whey, and gluten), polysaccharides (starch and cellulose derivatives, alginates, and chitosan) and their possible blends [49]. In particular, chitosan, a polysaccharide derived from chitin, is especially suited for food packaging, considering, as reported above, its biocompatibility, biodegradability, and antibacterial and antifungal behavior, along with good film forming properties [46][50].

Besides chitosan-based blends, many other polymers have been used recently to develop antimicrobial composite coatings containing TiO_2 NPs for food packaging. Xie et al., for example, embedded TiO_2 NPs into three different biodegradable polymers: cellulose acetate (CA), polycaprolactone (PCL), and PLA. A higher compatibility of TiO_2 with CA and PLA was observed, along with improved film forming properties, resulting in uniform films. Moreover, the CA/TiO_2-based films showed a high transparency, and good photocatalytic activity, as demonstrated by degradation of methylene blue upon irradiation with a UV-A light. The best antibacterial activity under the investigated experimental conditions, when compared to the other systems, was found against *E. coli* upon 2 h UV-A light irradiation with light intensity of 1.30 ± 0.15 mW/cm^2, and was found to increase at higher TiO_2 concentration, achieving a 1.69 log CFU/mL reduction. Very low reduction was, instead, observed, even at the highest TiO_2 concentration, without irradiation [51]. Xie et al. thoroughly

investigated the behavior of the best performing CA/TiO_2 coatings when the bacteria were inoculated under the coating itself and—upon UV-A exposure—a higher TiO_2 concentration was found to act as a screen, decreasing the intensity of the light reaching the inoculum and resulting in reduced antibacterial activity [52].

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