Chapter

Antimicrobial Effect of Titanium Dioxide Nanoparticles

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Abstract

The widespread use of antibiotics has led to the emergence of multidrug-resistant bacterial strains, and therefore a current concern for food safety and human health. The interest for new antimicrobial substances has been focused toward metal oxide nanoparticles. Specifically, titanium dioxide (TiO_2) has been considered as an attractive antimicrobial compound due to its photocatalytic nature and because it is a chemically stable, non-toxic, inexpensive, and Generally Recognized as Safe (GRAS) substance. Several studies have revealed this metal oxide demonstrates excellent antifungal and antibacterial properties against a broad range of both Gram-positive and Gram-negative bacteria. These properties were significantly improved by titanium dioxide nanoparticles (TiO_2 NPs) synthesis. In this chapter, latest developments on routes of synthesis of TiO_2 NPs and antimicrobial activity of these nanostructures are presented. Furthermore, TiO_2 NPs favor the inactivation of microorganisms due to their strong oxidizing power by free radical generation, such as hydroxyl and superoxide anion radicals, showing reductions growth against several microorganisms, such as *Escherichia coli* and *Staphylococcus aureus*. Understanding the main mechanisms of antimicrobial action of these nanoparticles was the second main purpose of this chapter.

Keywords: titanium dioxide, nanoparticles, green synthesis, antimicrobial activity

1. Introduction

The incidence of microbial attack in different sectors such as food, textiles, medicine, water disinfection, and food packaging leads to a constant trend in the search for new antimicrobial substances. The increased resistance of some bacteria to some antibiotics and the toxicity to the human body of some organic antimicrobial substances has increased the interest in the development of inorganic antimicrobial substances. Among these compounds, metal and metal oxide compounds have attracted significant attention due to their broad-spectrum antibacterial activities. On the other hand, nanoscale materials are well known thanks to their increased properties due to their high surface area-to-volume ratio. Antimicrobial NPs have shown excellent and different activities from their bulk properties [1, 2].

During last decades, metal oxide nanoparticles, such as zinc oxide (ZnO), manganese oxide (MgO), titanium dioxide (TiO_2), and iron oxide (Fe_2O_3), have been extensively applicable thanks to their unique physiochemical properties in biological applications. Among metal oxide antimicrobial agents, TiO_2 is a valuable semiconducting transition metal oxide material and shows special features, such as easy control, reduced cost, non-toxicity, and good resistance to chemical erosion, that allow its application in optics, solar cells, chemical sensors, electronics, antibacterial and antifungal agents [3]. In general, TiO_2 nanoparticles (TiO_2 NPs) present large surface area, excellent surface morphology, and non-toxicity in nature. Several authors have reported that TiO_2 NPs have been one of the most studied NPs thanks to their photocatalytic antimicrobial activity, exerting excellent bio-related activity against bacterial contamination [4–7].

Antimicrobial activity of nanoparticles is highly influenced by several intrinsic factors such as their morphology, size, chemistry, source, and nanostructure [8–11]. Specifically, antimicrobial activity of TiO_2 NPs is greatly dependent on photocatalytic performance of TiO_2, which depends strongly on its morphological, structural, and textural properties [12]. Several TiO_2 NPs have been developed through different methods of synthesis. Specifically, in this chapter, eco-friendly synthesis based on biological sources, such as natural plant extracts and metabolites from microorganisms, which have resulted in TiO_2 NPs with different size, shape, morphology, and crystalline structures will be presented. Titanium dioxide produces amorphous and crystalline forms and primarily can occur in three crystalline polymorphous: anatase, rutile, and brookite. Studies on synthesis have stated that the crystalline structure and morphology of TiO_2 NPs is influenced by process parameters such as hydrothermal temperatures, starting concentration of acids, etc. [13]. The crystal structures and the shape of TiO_2 NPs are both the most important properties that affect their physicochemical properties, and therefore their antimicrobial properties [14]. Regarding the crystal structures, anatase presents the highest photocatalytic and antimicrobial activity. Some works have shown that anatase structure can produce $OH^{\cdot}$ radicals in a photocatalytic reaction, and as it will be clearly explained below, bacteria wall and membranes can be deadly affected [15, 16].

2. Antimicrobial activity of titanium dioxide NPs

2.1 Latest tendencies on TiO_2 nanoparticle synthesis

The potential health impact and toxicity to the environment of NPs is currently an important matter to be addressed. Several works have confirmed that metal oxide NPs conventionally synthesized using chemical methods, such as sol–gel synthesis and chemical vapor deposition, have shown different levels of toxicity to test organisms [17–20]. In recent years, researchers have emphasized on the development of nanoparticles promoted through environmental sustainability and processes characterized by an ecological view, mild reaction conditions, and non-toxic precursors. Due to this growing sensitivity toward green chemistry and biological processes, ecological processes are currently being investigated for the synthesis of non-toxic nanoparticles.

These biological methods are considered safe, cost-effective, biocompatible, non-toxic, sustainable, and environmentally friendly processes [20]. Furthermore, it has been described that chemically synthesized NPs have exhibited less stability and added agglomeration, resulting in biologically synthesized NPs that are more dispersible, stable in size, and the processes consuming less energy [21].

These biosynthetic methods, also called "green synthesis," use various biological resources available in nature, including live plant [22], plant products, plant extracts, algae, fungi, yeasts [23], bacteria [24], and virus for the synthesis of NPs. Among these methods, the processes that use plant-based materials are considered the most suitable for large-scale green synthesis of NPs with respect to their ease and safety [25]. On the other hand, the reduction rate of metal ions in the presence of the plant extract is much faster compared to microorganisms, and provides stable particles [26]. Plants contain biomolecules that have been highly studied by researchers like phenols, nitrogen compounds, terpenoids, and other metabolites. It is well known that the hydroxyl and carboxylic groups present in these biocompounds act as stabilizers and reducing agents due to their high antioxidant activity [12]. Thus, plant extracts have been studied as one of the best green alternatives for metal oxide nanoparticles synthesis [27]. In recent years, TiO_2 nanoparticles have been obtained by using different plant extracts, but not all of them have been studied for their antimicrobial activity. **Table 1** presents a compilation of synthesized TiO2 nanoparticles from green synthesis by using plant extracts that were tested against different microorganisms.

Different factors need to be evaluated in this research field in order to obtain TiO_2 NPs with better properties and to maintain their biocompatibility. It has been shown that nanoparticles obtained from green synthesis can have a better morphology and size translated into better antimicrobial activity. Mobeen and Sundaram have obtained TiO_2 NPs from titanium tetrachloride precursor through a chemical and a green synthesis method. Sulfuric acid and ammonium hydroxide were used

Source	Titanium precursor	Size (nm)	Shape/ crystal structure	Target microorganism (method)
Azadirachta indica leaves extract [28]	TiO_2	25–87 (SEM)	Spherical/ anatase-rutile	*S. typhi*, *E. coli*, and *K. pneumoniae* (broth micro dilution method)
Psidium guajava leaves extract [29]	$TiO(OH)_2$	32.58 (FESEM)	Spherical shape and clusters/ anatase-rutile	*S. aureus* and *E. coli* (agar diffusion)
Vitex negundo Linn leaves extract [30]	$Ti\{OCH(CH_3)_2\}_4$	26–15 (TEM)	Spherical and rod shaped/ tetragonal phase anatase	*S. aureus* and *E. coli* (agar diffusion)
Morinda citrifolia leaves extract [31]	$TiCl_4$	15–19 (SEM)	Quasi-spherical shape/rutile	*S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger* (agar diffusion)
Trigonella foenum-graecum leaf extract [21]	$TiOSO_4$	20–90 (HR-SEM)	Spherical/ anatase	*E. faecalis*, *S. aureus*, *S. faecalis*, *B. subtilis.*, *Y. enterocolitica*, *P. vulgaris*, *E. coli*, *P. aeruginosa*, *K. pneumoniae,* and *C. albicans* (agar diffusion)
Orange peel extract [32]	$TiCl_4$	20–50 (SEM)	Irregular and angular structure with high porous net/ anatase	*S. aureus*, *E. coli,* and *P. aeruginosa* (agar diffusion)
Glycyrrhiza glabra root extracts [33]	TiO_2	60–140 (FESEM)	Spherical shape/ anatase	*S. aureus* and *K. pneumoniae* (agar diffusion)

Table 1.
Synthesis of TiO_2 NPs by using plant extracts.

in the chemical-based method and, in the green synthesis, those chemical reagents were replaced by an orange peel extract [32]. The nanoparticles obtained by using the natural extract presented a well-defined and smaller crystalline nature (approx. 17.30 nm) compared to the nanoparticles synthesized through the chemical method (21.61 nm). Both methods resulted in anatase crystalline structures, and, when evaluating the antimicrobial activity, the more eco-friendly NPs revealed higher bactericidal activity against Gram-positive and Gram-negative bacteria compared to the chemically synthesized nanoparticles.

Bavanilatha et al. have also detailed TiO_2 NPs green synthesis with *Glycyrrhiza glabra* root extract. Antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumonia* were investigated and *in vivo* toxicity tests using the zebrafish embryonic model (*Danio rerio*) were also carried out [33]. Results have demonstrated their biocompatibility because healthy embryos of adult fish to different variations of NP and no distinctive malformations were observed at every embryonic stage with respect to embryonic controls.

Subhapriya and Gomathipriya have biosynthesized TiO_2 NPs by using *a Trigonella foenum-graecum* leaf extract, obtaining spherical NPs and their size varied between 20 and 90 nm, and their antimicrobial activity was evaluated through the standard method of disc diffusion [21]. The NPs showed significant antimicrobial activity against *Yersinia enterocolitica* (10.6 mm), *Escherichia coli* (10.8 mm), *Staphylococcus aureus* (11.2 mm), *Enterococcus faecalis* (11.4 mm), and *Streptococcus faecalis* (11.6 mm). Results confirmed developed TiO_2 NPs as an effective antimicrobial drug that can lead to the progression of new antimicrobial drugs.

Spherical TiO_2 NPs were synthesized from plants, in particular by applying a *Morinda citrifolia* leaf extract, and through advanced hydrothermal method [31]. Developed TiO_2 NPs showed a size between 15 and 19 nm in an excellent quasispherical shape. In addition, their antimicrobial activity was tested against human pathogens, such as *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Candida albicans,* and *Aspergillus niger*. TiO_2 NPs exhibited interesting antimicrobial activity, principally against Gram-positive bacteria.

In addition to plants, other organisms can produce inorganic compounds at an intra or extracellular level. The synthesis of TiO_2 NPs through microorganisms, including bacteria, fungi, and yeasts, also meets the requirements and the exponentially growing technological demand toward eco-friendly strategies, by avoiding the use of toxic chemicals in the synthesis and protocols [34]. The metabolites generated by microorganism present bioreducing, capping, and stabilizing properties that improve the NPs synthesis performance. Jayaseelan et al. have stated glycyl-L-proline, one of the most abundant metabolite from *Aeromonas hydrophilia* bacteria, as the main compound that acted as a capping and stabilizing agent during TiO_2 NPs green synthesis [35]. Moreover, the interest in fungi in green synthesis of metal oxide nanoparticles has increased over last years. Fungi enzymes and/or metabolites also present intrinsically the potential to obtain elemental or ionic state metals from their corresponding salts [34, 36]. Different works based on the green synthesis of TiO_2 NPs from bacteria and fungus are presented in **Table 2**. Some of them have been synthesized with antimicrobial and antifungal purposes, and their target microorganisms are also declared.

Two important factors that affect NPs synthesis are the type of microorganisms and their source. Some microorganisms widely used in the food industry are *Lactobacillus*, a bacterium used in dairy products and as a probiotic supplement, and *Saccharomyces cerevisiae,* a yeast commonly used in bakery. Jha et al. have investigated the effectiveness of both microorganisms to synthesize TiO_2 NPs. A comparison between synthesis through *Lactobacillus* from yogurt and probiotic tablets resulted in different NP sizes: a particle size of 15–70 nm for yogurt, and 10–25 nm

Microorganism	Titanium precursor	Size (nm)	Shape/crystal structure	Target microorganisms (method)
Aeromonas hydrophilia [46]	$TiO(OH)_2$	28–54 (SEM) ~ 40.5 (XRD)	Spherical/uneven	*S. aureus, S. pyogenes* (agar diffusion)
Aspergillus flavus [34]	TiO_2	62–74 (TEM)	Spherical/anatase and rutile	*E. coli, P. aeruginosa, K. pneumoniae, B. subtilis* (agar diffusion and MIC)
Bacillus mycoides [37]	Titanyl hydroxide	40–60 (TEM)	Spherical/anatase	*E. coli* (toxicity)
Bacillus subtilis [38]	K_2TiF_6	11–32 (TEM)	Spherical	Aquatic biofilm
Fusarium oxysporum [36]	K_2TiF_6	6–13 (TEM)	Spherical/brookite	—
Lactobacillus sp. [51]	$TiO(OH)_2$	~ 24.6 (TEM)	Spherical/ anatase-rutile	—
Planomicrobium sp. [39]	TiO_2	100–500 (SEM)	Irregular/pure crystalline	*B. subtilis, K. planticola, Aspergillus niger* (agar diffusion)
Propionibacterium jensenii [52]	$TiO(OH)_2$, 300°C	15–80 (FESEM)	Spherical	—
Saccharomyces cerevisiae [51]	$TiO(OH)_2$	~ 12.6 (TEM)	Spherical/ anatase-rutile	—

Table 2.
Examples of TiO_2 NPs synthesis through microorganisms, both bacteria and fungus strains.

for tablets. This difference was due to the purity of the bacteria [40]. In general, TiO_2 NP synthesis through microorganisms has not provided stable sizes, being not industrially scalable compared to the synthesis of nanoparticles from plants.

2.2 Antimicrobial activity of TiO_2 NPs

Harmful bacteria, such as *Staphylococcus aureus, Burkholderia cepacia, Pseudomonas aeruginosa, Clostridium difficile, Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, Mycobacterium tuberculosis,* and *Neisseria gonorrhoeae,* are responsible for bacterial infections that can cause serious diseases in humans year after year [40]. The principal solution is the use of antibiotics, antimicrobial and antifungal agents. Nevertheless, in recent years there has been an increase in the resistance of several bacterial strains to these substances, and therefore there is currently a great interest in the search for new antimicrobial substances. The antimicrobial nanoparticles have been studied due to their high activity, specifically the metal oxide nanoparticles [41–43]. In this sense, titanium dioxide nanoparticles are one of the antimicrobial NPs whose study has gained interest during last years.

TiO_2 is a thermally stable and biocompatible chemical compound with high photocatalytic activity and has presented good results against bacterial contamination [44]. **Table 3** presents some research including the antimicrobial capacity of TiO_2 NPs.

Microorganism	NPs	Results
Methicillin-resistant *Staphylococcus aureus* [45]	Fe_3O_4-TiO_2 core/shell magnetic NPs	The survival ratio [%] of bacteria decreased from 82.40 to 7.13%.
Staphylococcus saprophyticus [45]	Fe_3O_4-TiO_2 core/shell magnetic NPs	The survival ratio [%] of bacteria decreased from 79.15 to 0.51%.
Streptococcus pyogenes [57]	Fe_3O_4-TiO_2 core/shell magnetic NPs	The survival ratio [%] of bacteria decreased from 82.87 to 4.45%.
Escherichia coli [46]	TiO_2 nanotubes ~ 20 nm	97.53% of reduction
Staphylococcus aureus [46]	TiO_2 nanotubes ~ 20 nm	99.94% of reduction
Bacillus subtilis [47]	TiO_2 NPs co-doped with silver (19–39 nm)	1% Ag-N-TiO2 had the highest antibacterial activity with antibacterial diameter reduction of 22.8 mm
Mycobacterium smegmatis [48]	Cu-doped TiO_2NPs ~20 nm	The percentage of inhibition was around 47%
Pseudomonas aeruginosa [49]	TiO_2 NPs 10–25 nm	Although it was not completely euthanized, their survival was significantly inhibited.
Shewanella oneidensis MR-1 [48]	Cu-doped TiO_2 NPs ~20 nm	The percentage of inhibition was around 11%

Table 3.
TiO_2 nanoparticles against different microorganisms and their antimicrobial activities.

The principal factors differentiating the antimicrobial activity between TiO_2 NPs were their morphology, crystal nature, and size. According to López de Dicastillo et al. [11], hollow TiO_2 nanotubes presented interesting antimicrobial reduction thanks to the enhancement of specific surface area. This fact can be explained by the nature of titanium dioxide, and one of the main mechanisms of its action is through the generation of reactive oxygen species (ROS) on its surface during the process of photocatalysis when it exposed to light at an appropriate wavelength. It is important to highlight that some research works have evidenced antimicrobial activity of TiO_2 NPs increased when they were irradiated with UV-A light due to the photocatalytic nature of this oxide. The time of irradiation varied between 20 min [45] and 3 hours [50].

3. Understanding the antimicrobial mechanism of TiO_2 NPs toward bacteria

Titanium dioxide nanoparticles (TiO_2 NPs) are one of the most studied materials in the area of antimicrobial applications due to its particular abilities, such as bactericidal photocatalytic activity, safety, and self-cleaning properties. The mechanism referred to the antimicrobial action of TiO_2 is commonly associated to reactive oxygen species (ROS) with high oxidative potentials produced under band-gap irradiation photo-induces charge in the presence of O_2 [51]. ROS affect bacterial cells by different mechanisms leading to their death. Antimicrobial substances with broad spectrum activity against microorganisms (Gram-negative and Gram-positive bacteria and fungi) are of particular importance to overcome the MDR (multidrug resistance) generated by traditional antibiotic site-specific.

The main photocatalytic characteristic of TiO_2 is a wide band gap of 3.2 eV, which can trigger the generation of high-energy electron–hole pair under UV-A light with wavelength of 385 nm or lower [52]. As mentioned above for bulk powder, TiO_2 NPs have the same mechanism based on the ROS generation with the advantage of being at nanoscale. This nanoscale nature implies an important increase of surface area-to-volume ratio that provides maximum contact with environment water and oxygen [53] and a minimal size, which can easily penetrate the cell wall and cell membrane, enabling the increase of the intracellular oxidative damage.

Bacteria have enzymatic antioxidant defense systems like catalases and superoxide dismutase, in addition to natural antioxidants like ascorbic acid, carotene, and tocopherol, which inhibit lipid peroxidation or O-singlet and the effects of ROS radicals such as $OH_2^{\bullet-}$ and $OH^{\bullet}$. When those systems are exceeded, a set of redox reactions can lead to the death cell by the alteration of different essential structures (cell wall, cell membrane, DNA, etc.) and metabolism routes [54]. In the following sections, several ways that cellular structures were affected in the presence of TiO_2 NPs will be described. In order to understand the genome responses of bacteria to TiO_2-photocatalysis, some biological approaches related to expression of genes encoding to defense and repair mechanism of microorganism will explained below. Different mechanisms and processes of antimicrobial activity of TiO_2 NPs are represented as a global scheme in **Figure 1**.

3.1 Cell wall

ROS are responsible for the damage by oxidation of many organic structures of microorganisms. One of them is the cell wall, which is the first defense barrier against any injury from the environment, thus being the first affected by oxidative damage. Depending on the type of microorganism, the cell wall will have different composition; that is, in fungi and yeast, cell walls are mainly composed of chitin and polysaccharides [55], Gram-positive bacteria contain many layers of peptidoglycan and teichoic acid, and Gram-negative bacteria present a thin layer of peptidoglycan surrounded by a secondary lipid membrane reinforced with transmembrane lipopolysaccharides and lipoproteins [56]. Thus, the effect of TiO_2 NPs will be slightly different depending type of microorganism.

It has been studied that the composition of the cell wall in *Pichia pastoris* (yeast) changed in the presence of TiO_2, increasing the chitin content in response to the

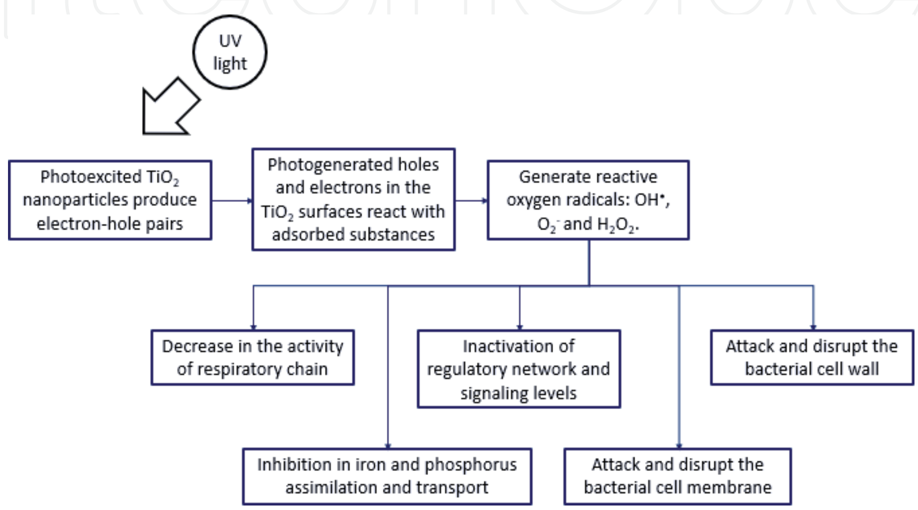

Figure 1.
Scheme of main antimicrobial activity-based processes.

ROS effects [57]. The cell wall of *Escherichia coli* (Gram-negative) composed of lipo-polysaccharide, phosphatidyl-ethanolamine, and peptidoglycan has been reported to be sensitive to the peroxidation caused by TiO_2 [58]. The damage can be quantified by assessing the production of malondialdehyde (MDA), which is a biomarker of lipid peroxidation, or through ATR-FTIR of the supernatant of cell culture, which evidenced the way that porins and proteins on the outer membrane were affected, probably as a result of greater exposure to the surface of TiO_2 [59]. In fungi, the release of $OH^{\bullet}$ captured hydrogen atoms from sugar subunits of polysaccharides, which composed the cell wall, leading to the cleavage of polysaccharide chain and the exposition of cell membrane [60].

In terms of genetic issues, there is evidence that the bacteria change the level expression of certain genes encoding for proteins involved in lipopolysaccharide and peptidoglycan metabolism, pilus biosynthesis, and protein insertion related to the cell wall which values were lower-expressed after exposition to TiO_2 NPs [61].

3.2 Cell membrane

The second usual cellular target of most of antibiotics is the cell membrane mainly composed by phospholipids, which grant the cell a non-rigid cover, permeability, and protection. Most of the studies with TiO_2 NPs have been focused to the loss of membrane integrity caused by oxidation of phospholipids due to ROS such hydroxyl radicals and hydrogen peroxide [62, 63], which led to an increase in the membrane fluidity, leakage of cellular content, and eventually cell lysis.

Gram-positive bacteria present only one membrane protected by many layers of peptidoglycan, whereas Gram-negative bacteria are composed by two membranes, inner and outer, and a thin layer of peptidoglycan between them. The outer membrane is exposed, thus, more liable to mechanical breakage due to the lack of peptidoglycan protective cover, like in Gram-positive bacteria [64]. Some studies have demonstrated a better antimicrobial performance of TiO_2 NPs against Gram-positive bacteria [65] while others reported that Gram-negative bacteria were more resistant [66, 67]. It can be concluded that the bacterial inactivation effectiveness depends mainly on the resistant capacity of cell wall structures and the damage level of ROS generation [68].

In contrast with the lower expression of genes related to the cell wall seen before, the level expression of genes encoding for enzymes involved in metabolism of lipid essential for the cell membrane structure, are over-expressed [61]. It would be concluded that cells compensate the initial cell wall damage by reinforcing the second defense barrier, the cell membrane, in a way to provide support against the oxidation produced by ROS.

In fungi, the biocidal effect is not quite different. In the presence of TiO_2 NPs and UV light, hydroxyl radicals, hydrogen peroxide, and superoxide anions initially promote oxidation of the membrane, leading to an unbalance in the cell permeability, even decomposition of cell walls [69]. This oxidation can inhibit cell respiration by affecting intracellular membranes in mitochondria. Studies have demonstrated biocidal effects on *Penicillium expansum* [70], but there is still research on other strains.

Beyond the relatively well-studied initial lipoperoxidation attack of TiO_2 NPs on the outer/inner cell membrane of the microorganism, specific mechanisms are still aimed of being solved.

3.3 Inhibition of respiratory chain

As the oxidative damage generates lipoperoxidation of cell membranes due to their lipid nature, the respiratory chain, which takes place in the double-membrane mitochondria, is also affected. This organelle is a natural source of ROS in aerobic metabolism because superoxide anions are produced in the electron transfer respiratory chain process. Mitochondria can control this fact by converting them into H_2O_2 by superoxide dismutase (SOD), and finally into water by glutathione peroxidase and catalase [71]. The presence of TiO_2 NPs increases the production of ROS at levels that this enzymatic defense mechanism cannot attenuate the damage, even a dysregulation in electron transfer through the mitochondrial respiratory chain implies an increase in ROS generation [72].

The genetic approaches have indicated that changes in level expression in genes related to the energy production in mitochondria prioritize the most efficient pathway to uptake oxygen, which is through ubiquinol coenzyme [61]. This coenzyme presented a higher capacity to exchange electrons, while the coenzyme-independent oxygen uptake pathways were expressed at lower level.

3.4 DNA

Damage at molecular level in DNA affects all regulatory microorganism metabolism, replication, transcription, and cell division. DNA is particularly sensitive to oxidative damage because oxygen radicals, specially $OH^{\cdot}$ produced by Fenton reaction [73], may attack the sugar-phosphate or the nucleobases and cause saccharide fragmentation aimed to the strand break [74].

DNA strand modifications are more lethal than base modifications (punctual mutation). Mitochondrial DNA is more vulnerable to oxidative damage than nuclear DNA because it is closer to a major cellular ROS source [75].

Besides the enzymatic detoxification system (SOD, glutathione and catalase), DNA injuries are covered by a set of structures related to post-translational modification, protein turnover, chaperones (related to folding), DNA replication and repair, which are significantly over-expressed in the presence of TiO_2 NPs [61].

3.5 Assimilation and transport of iron and inorganic phosphate (Pi)

Iron is an essential ion for cell growth and survival, but it can turn potentially toxic if some malfunction in homeostatic regulation occurs (i.e., Fenton reaction that produces ROS). Bacteria are able to regulate iron concentration in order to maintain it in a physiological range [76]. This regulation involves directly siderophores to active transport of iron in cell [77], whose coding genes related to siderophore synthesis and iron transport protein are significantly lower-expressed in the presence of TiO_2 NPs, decreasing the ability to assimilate and transport it, leading to cell death [61]. The loss of homeostasis regulation was confirmed by ICP-MS analysis, which revealed that the presence of TiO_2 NPs significantly reduced the cellular iron level in *Pseudomonas brassicacearum*, directly proportional to the cell viability [78].

Regarding the functions related to Pi group (PO_4^{3-}) uptake, major differences were found in the expression of set of genes contained in Pho regulon, which were significantly lower when compared to the control [61]. The Pho regulon is a regulatory network in bacteria, yeast, plants, and animals, related to assimilation of inorganic phosphate, merely available in nature, and essential to nutritional cross-talk, secondary metabolite production, and pathogenesis [79].

This suggested that the microorganisms were highly deficient in phosphorus uptake and metabolism in the presence of TiO_2 NPs. It should be also noted that the Pho regulon has been reported to regulate biofilm synthesis capacity and pathogenicity [80].

3.6 Cell-to-cell communication

TiO_2 NPs can directly oxidize components of cell signaling pathways and even change the gene expression by interfering with transcription factors [81]. There is evidence to confirm the interference of TiO_2 NPs in biosynthesis pathways of signaling molecules that bind lipopolysaccharide, stabilize and protect the cell wall against oxidative damage [82]. Moreover, a significant decrease in the synthesis of quorum-sensing signal molecule related to functions like pathogenesis and biofilm development was observed. This was corroborated through Scanning Electron Microscopy (SEM) images of bacteria (*P. aeruginosa*) growth in the presence of TiO_2 NPs without UV irradiation. Cells appeared mainly non-aggregated and dispersed in the substratum, compared with controls without NPs where cells were mainly aggregated by lateral contact. This suggested that TiO_2 NPs not only affected microorganisms by oxidative damage, but also bacteria aggregation and biofilm formation, which directly influenced in pathogenicity [83].

In plants and algae, ROS can act as signaling intermediates in the process of transcription factor controlling stress response by H_2O_2, which is activated by a GSH peroxidase, and not by peroxides directly. But there is still lack of research in this area [84].

4. Conclusions

The control of morphology and crystal structure of TiO_2 NPs is the most important factor to enhance their antimicrobial activity. The appropriate design based on desirable surface properties given by shaped nanoparticles can improve effectiveness that is also dependent on the type of bacteria. The route of synthesis of TiO_2 NPs is also a key factor. Recent works have revealed more eco-friendly synthesis methods, principally based on plant-based compounds and microorganisms, such as bacteria and fungus. Antimicrobial activity of different TiO_2 NPs against Gram-positive and Gram-negative bacteria including antibiotic-resistant strains has been confirmed in different works.

Specific studies on antimicrobial mechanisms have evidenced that microorganism exposed to photocatalytic TiO_2 NPs exhibited cell inactivation at regulatory network and signaling levels, an important decrease in the activity of respiratory chain, and inhibition in the ability to assimilate and transport iron and phosphorous. These processes with the extensive cell wall and membrane alterations were the main factors that explain the biocidal activity of TiO_2 NPs.

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Conflict of interest

The authors declare no conflict of interest.

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