Chapter 10
Nanoparticles: Interaction with Microorganisms

Heiko Schwegmann and Fritz H. Frimmel

10.1 Introduction

Nanotechnology is concerned with materials and systems whose structures and components exhibit novel physical, chemical and biological properties and processes due to their nanoscale size (1–100 nm) (U.S. National Nanotechnology Initiative). The promising new technology supplies the basis for many new products and processes offering the ability to reduce pollution and minimise the use of resources. These immense improvements will impact society, especially in the health and manufacturing technologies. It is expected to become a US $1 trillion market by 2015 (Nel et al., 2006). In this context, nanotechnology is already discussed as the new key technology of the 21st century (Woyke, 2007).

Reason enough also to care about the backstage of the scene, i.e. used nanoparticle (NP) treatment and impact on the environment, to avoid or at least minimise adverse effects and to guarantee a sustainable NP application.

10.2 Biological Impact of Nano-products

Different products with advertised nanoparticles are already in the market (Table 10.1). They find use in a variety of different products such as electronics, cosmetics, pharmaceuticals and fields of biomedical, energy, environmental, catalytic and material applications (Nowack and Bucheli, 2007).

Single-walled carbon nanotubes (SWCNTs) have a very broad commercial application potential due to their superior mechanical, electrical and magnetic properties (Dreher, 2004). By 2011 the annual worldwide production of SWCNT is estimated to exceed 1000 t (Lekas, 2005). They are used in water purification, as catalysts and in the automobile industry. The increased production can lead to an enhanced

F.H. Frimmel (✉)
Engler Bunte Institut, Chair of Water Chemistry, Karlsruhe Institute of Technology (KIT),
Engler-Bunte-Ring 1, D-76131 Karlsruhe, Germany
e-mail: fritz.frimmel@kit.edu

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Table 10.1 Nanoparticles in industrial products and possible exposure pathways into the water cycle

Nanoparticle	Products	Potential release into the water cycle
Ag	Coatings of textiles	Abrasion during washing
	Cosmetics, bandages	Application and removal through washing
	Paints, sprays, cleaning agents	Runoff
TiO_2	UV-protector in sunscreens	Application and removal through washing
	Paints	Runoff
CeO_2	Additive in fuels	Diesel exhausts
SiO_2	Additive in polymers, dental fillings	
Al_2O_3	Energy production	Disposal
C_{60}	Lubrication grease	Abrasion
CNT	Plastics, sporting equipment	Disposal
	Electronics	By-product emission into wastewater
Fe^0	Soil remediation	

risk of releasing the NP in the environment by discharges or spillages (Klaine et al., 2008).

The thermodynamically stable form of most metals are their oxides. Metal oxides have been produced as bulk material for many years by the industry. Nowadays nanoparticulate versions of these materials find their way into products and are widely used in a number of chemical and biological applications and in food industry. Several of the economically most important nanoparticulate metal oxides are TiO_2, ZnO, Fe_2O_3, Fe_3O_4, CeO_2 and SiO_2 (Nam and Lead, 2008).

TiO_2 and ZnO show powerful photocatalytic properties and due to their ultraviolet-blocking ability they are part of sunscreens, paints and coatings. The production of these metal oxides is estimated to reach 1000 t/year in 2010 (Pitkethly, 2004).

Iron oxide NPs have been widely used in biological applications and manufacturing pigments (Cornell and Schwertmann, 1997), but due to the superparamagnetic properties there are also arising markets in biomedical applications, e.g. as magnetic contrast agents (Thierry et al., 2007).

SiO_2 is mainly used as an additive for polymers and the bulk material is regarded to be safe. It plays an important role in the ecotoxicological assessment due to the broad usage. Most companies which use NP, work in their production steps with SiO_2-NP (Schmied and Riediker, 2007).

Zero valent metal nanoparticles are of major concern because of their ability to function in a particle-specific way. They are typically made by reduction of solutions of metal salts. The most prominent agents of these NP are Ag, Fe and Au.

Ag-nanometal has been used in many consumer applications, mostly because of its well-demonstrated use as an antimicrobial agent. Although they have been widely found in a variety of products, a concrete assessment of Ag-NP regarding environmental implications is still missing (Carlson et al., 2008). They are perhaps the most worrying NP because of their bactericidal capability and most likely access into the environment through the consumer products (Eckelman and Graedel, 2007). The most publications referring to the toxicity of NP report the effects of silver to microorganisms.

In recent years, zero valent Fe-NPs have increasingly been utilized in groundwater remediation and hazardous waste treatment (Sun et al., 2007). The power of Fe-NP is their ability to degrade chlorinated organic solvents and organic dyes (Zhang, 2003).

Besides the technical potential of NP and their promising properties for daily life applications there is a severe lack of reliable procedures and standardised methods (Mueller and Nowack, 2008) to follow their fate in the environment and their influence on complex technical systems like waste water treatment plants.

10.3 Nanoparticle Characteristics and Entry into the Environment

As of now, the risk posed by NP for the environment has not been well examined. However, it is beyond doubt, that an increased entry of these particles into soil, water and air at some stage between the production and the disposal has to be expected due to the increasing use of NP (Krug, 2005). Due to the lack of regulations and because the bulk material was regarded as safe, the majority of NP-producing companies have not done any risk assessment for NP so far (Helland et al., 2008). However, changes in size at the nanometre scale imply changes in the surface properties of the particles, which in turn can lead to a quite different behaviour in comparison to the one of the bulk material. Therefore, it is crucial to investigate the fate and behaviour of NP in the environment. Some of the main questions are Do the NP maintain their size and structure or do they agglomerate under environmental conditions? Do they undergo chemical or microbial transformations due to oxidation and reduction reactions? The distinct properties of NP, e.g. large surface to volume ratio and functional surfaces, which make them attractive in technical and economic applications, can also present a potential hazard after application.

Another important aspect is the gradual change of the NP and their properties in the environment. In case of Al_2O_3, it was shown that natural organic matter (NOM) as humic acids can build an outer layer around the NP, increasing the colloidal stability at neutral pH values (Ghosh et al., 2008). The adsorption of NOM onto the

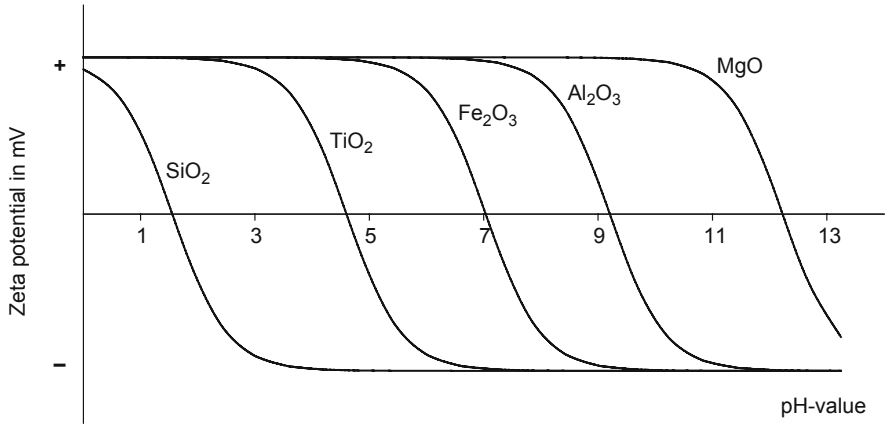

Fig. 10.1 Idealized zeta potential characteristics of various metal oxides at different pH values

surface of the NP also leads to changes in the surface charge. For example, Fe_2O_3 has a zeta potential of +19 mV in a 10 mM KCl solution at pH 8.0, but the addition of 0.5 mg/L NOM changes the potential at the same pH to –37 mV (Westerhoff et al., 2008). Absolute values of zeta potentials above 30 mV are indicators for a stable dispersion, because the electrostatic rejection inhibits the particles to agglomerate. Water bodies with a high ionic strength reduce the zeta potential of the NP resulting in larger aggregates (Müller, 1996). Figure 10.1 depicts typical zeta potential characteristics of some metal oxides.

10.4 Importance of Microorganisms

The effect of NP on different cell lines of mammalian cells has been investigated in various studies in recent years. An internalisation of NP was demonstrated resulting in miscellaneous effects (Bennat and Müller-Goymann, 2000; de la Fuente et al., 2006; Limbach et al., 2005; Limbach et al., 2007). However, the impact of NP on microorganisms is sparsely researched. Nevertheless, the effect of nanoparticles on bacteria is important because bacteria constitute the lowest level of life and hence the entrance to the food chain in many ecosystems. Furthermore, microorganisms are major contributors in the carbon-, nitrogen- and phosphorus cycle for recycling biomass in nutrients (Lyon et al., 2007). For example, nitrification and denitrification are important steps in the nitrogen cycle performed by microorganisms and therefore play a major role in wastewater treatment processes (ter Haseborg and Frimmel, 2007).

The major difference between mammalian cells (epithelial cells, fibroblast cells) and microorganisms (*E. coli*, *B. subtilis*) in connection with the impact of NP is

the cell wall. While, microorganisms possess a cell wall consisting of a rigid structure surrounding the cell membrane, mammalian cells lack this envelope. The rigid structure protects the cell from environmental influences and provides their shape. Bacteria are divided into two classes according to the structure of the cell wall: Gram-positive bacteria (*B. subtilis*) exhibit a cell wall consisting of a thick peptidoglycan layer (20–80 nm); Gram-negative bacteria (*E. coli*) have only a small layer of peptidoglycan (5–10 nm), but possess an outer membrane with porins.

A further remarkable difference between bacterial and mammalian cells is the ability of endocytosis by mammalian cells. Endocytosis encompasses several diverse mechanisms by which cells internalise macromolecules and particles into transport vesicles derived from the plasma membrane. It controls entry into the cell and enables cells to take up particles up to 120 nm (Conner and Schmid, 2003). Bacteria only have porins in their membrane with a diameter of up to 5 nm.

Most eukaryotes, gram-positive and gram-negative bacteria have at neutral pH value a negative surface charge and several of these bacteria do not exhibit an isoelectric point, they are negatively charged at all pH values (Kleijn and van Leeuwen, 2000). The electrostatic attraction and repulsion between the NP and microorganisms play an important role in the adhesion of the NP to the microorganisms, and hence the observed toxicity. Goodman et al. (2004) demonstrated this with Au-NP functionalised with cationic and anionic side chains. They used vesicles composed of phosphatidylcholine (SOPC) and phosphatidylserine (SOPS) (net negative charge), and a second preparation of SOPC only (no net charge) as a reference model for cell membranes. The positively charged Au-NP lysed the (SOPC/SOPS) vesicles more efficiently. Dillen et al. (2008) also showed a greater adhesion of positively charged NP on *Pseudomonas aeruginosa* and *Staphylococcus aureus* resulting in an increase of size of the microorganisms.

The bacteriostatic and bactericidal effects of various NPs on microorganisms are presented in Tables 10.2 and 10.3. Many NPs do not exhibit a bacteriostatic effect at the applied concentrations during growth measurements. In complex growth media, high contents of proteins, polysaccharides and salts can influence the stability of the NP leading to agglomeration. Additionally, the intracellular substances of the first killed bacteria can interact with the NP causing aggregation and consequently a growth delay (Sondi and Salopek-Sondi, 2004). It seems that zero valent metals have the highest impact on microorganisms during their growth phase. While the methods of growth determination vary as depicted in Table 10.2, measurements for the determination of the lethal concentration (LC) are dominated by the principle of colony forming units (cfu) (Table 10.3). However, there are also differences in the test design for cfu. In some cases the NP are dispersed in the agar in prior to the transfer into the petri dishes. Otherwise, the NP and the microorganism are mixed in different solutions for various contact times. Afterwards the suspensions are diluted and inoculated on agar. Due to the varying cell concentrations and diverse NP dispersions with diverse ionic strengths, a direct comparison of the toxicity of the individual NP is hardly possible.

Table 10.2 Overview of EC_{50} values of various NPs on different microorganisms dependent on the particle size measurement method

Microorganism	[a]Growth media	NP	Size in nm	[b]Size determination	[e]EC_{50} ($\mu g\,mL^{-1}$)	Method	References
Chlamydomonas reinhardtii	MOPS 10 mM	Ag	44	DLS[c]	3.6	Photosynthetic yield	Navarro et al. (2008)
Escherichia coli	Luria Bertani	SiO_2	300	DLS[c]	>33,000	Optical density	Williams et al. (2006)
		SiO_2/Fe_3O_4	500		>2,200		
		Au	45		110		
Nitrifying culture	NH_4NO_3 8.3 mM	Ag	9	TEM[d]	0.5	Oxygen uptake rate	Choi and Hu (2008)
Pseudokirchneriella subcapitata	According to OECD	SiO_2	12.5	DLS[c]	>20	Cell density	Van Hoecke et al. (2008)
			27		>28.8		
Escherichia coli	BBL	Ag	14	STEM	0.45	Membrane damage by SYTO9/PI	Choi et al. (2008)
Nitrifying culture	NH_4NO_3 8.3 mM				0.3	Oxygen uptake rate	
Desmodesmus subspicatus	According to OECD	TiO_2 (irradiated in sun light simulator)	25	TEM[d]	44	Fluorescence	Hund-Rinke and Simon (2006)
			100		>50		
Escherichia coli	Luria Bertani	ZnO	250	DLS[c]	100–250	Optical density	Zhang et al. (2007)

Table 10.2 (continued)

Microorganism	[a]Growth media	NP	Size in nm	[b]Size determination	[e]EC_{50} ($\mu g\,mL^{-1}$)	Method	References
Escherichia coli	M9	Ag	9	TEM[d]	1–2	Optical density	Lok et al. (2006)
Bacillus subtilis	Nutrient broth	C_{60}/PVP (irradiated)	–	–	<13	Optical density	Kai et al. (2003)
Vibrio fischeri		C_{60}	–	–	1	Luminescence	Lyon et al. (2005)

[a] Growth media: mainly the abbreviations of the used growth media are mentioned. The exact composition of the media can be found in each article.
[b] Size determination: type of measurement to determine the size of the nanoparticles.
[c] DLS: dynamic light scattering determines the hydrodynamic diameter of the nanoparticles in the suspension.
[d] TEM: transmission electron microscopy determines the size of the primary particles in a dried state. Size distribution can be calculated by image processing.
[e] EC_{50}: refers to the concentration at which 50% of the population shows a defined effect other than lethality.

Table 10.3 Examples for the measured toxicity of various NPs on different microorganisms dependent on the particle size, the cell concentration and the type of dispersant

Microorganism	Cells (mL^{-1})	Solution	NP	Size (nm)	[a]Size determination	[b]LC_{90} ($\mu g\ mL^{-1}$)	[c]$LC_{99.9}$ ($\mu g\ mL^{-1}$)	Method	References
E. coli	10^5	Luria Bertani Agar[g]	Ag		TEM[e]	15	60	Cfu[f]	Sondi and Salopek-Sondi (2004)
E. coli	10^7	Nutrient broth	Ag (rod)	150*16	TEM[e]	8	35	Cfu[f]	Pal et al. (2007)
			Ag (spherical)	39		5	11		
Ag (triangular)	40	4	6						
E. coli	1.5×10^9	KNO_3 100 mM	CeO_2	7	DLS[d]	100–230	<500	Cfu[f]	Thill et al. (2006)
E. coli	5×10^7	Water (ultrapure)	Fe_2O_3	380	DLS[d]	>700	>700	Cfu[f]	Auffan et al. (2008)
			Fe_3O_4	50	DLS[d]	700	>700		
Fe^0	320	DLS[d]	175	>700					
E. coli	10^6	Carbonate buffer	Fe^0 (air saturated)	35	TEM[e]	70	80	Cfu[f]	Lee et al. (2008)
			Fe^0 (deaerated)			1.2	4		
S. aureus	10^3	Phosphate buffer	MgO	14	TEM[e]	10,000	10,000	Cfu[f]	Huang et al. (2005)
E. coli	6.4×10^7	NaCl 150 mM	MgO	11	TEM[e]	<1,000	<1,000	Cfu[f]	Makhluf et al. (2005)
				18		<1,000	<1,000		
				23		<1,000	>1,000		
S. aureus	9×10^7			11		<1,000	<1,000		
				18		<1,000	<1,000		
				23		<1,000	>1,000		

Table 10.3 (continued)

Microorganism	Cells (mL^{-1})	Solution	NP	Size (nm)	[a]Size determination	[b]LC_{90} ($\mu g\ mL^{-1}$)	[c]$LC_{99.9}$ ($\mu g\ mL^{-1}$)	Method	References
E. coli	5×10^7	NaCl 150 mM	Single-walled CNT	2,000*0.9	TEM[e]	5			Kang et al. (2008)
B. subtilis	10^3	MD Agar[g]	SiO_2	205	DLS[d]	2,000–5,000	5,000	Cfu[f]	Adams et al. (2006)
E. coli						>5,000	>5,000		
B. subtilis			TiO_2	330		1,000–2,000	2,000		
E. coli						>5,000	>5,000		
B. subtilis			ZnO	480		10	>500		
E. coli			>1,000	>1,000					
E. coli	2×10^2	Luria Bertani Agar[g]	ZnO	11	TEM[e]	210	>210	Cfu[f]	Brayner et al. (2006)
E. coli	3×10^5	MD Medium	C_{60}	50	DLS[d]		1.5 – 3	Cfu[f]	Lyon et al. (2005)
B. subtilis							2 – 4		

[a] Size determination: type of measurement to determine the size of the nanoparticles.
[b] LC_{90}: the concentration of nanoparticles at which 90% of the microorganisms are killed.
[c] $LC_{99.9}$: the concentration of nanoparticles at which 99.9% of the microorganisms are killed.
[d] DLS: dynamic light scattering determines the hydrodynamic diameter of the nanoparticles in the suspension.
[e] TEM: transmission electron microscopy determines the size of the primary particles in a dried state. Size distribution can be calculated by image processing.
[f] cfu: colony forming units.
[g] The nanoparticles were suspended in the agar resulting in a reduced diffusion of the NP in comparison to liquid media.

10.5 Test Systems for Toxicity Measurements

Most toxicological experiments with NP and microorganisms run the same way: various concentrations of different nanoparticles are mixed in a test system of pure water, model salt solutions, real environmental samples or culture medium and are exposed to the target microorganism or culture. After a distinct time, ranging from 10 min to a few days, the chosen endpoint or biomarker is measured. Depending on the biochemical hypothesis being tested, different endpoints can be used to investigate and measure the impact of nanoparticles on microorganisms in the experimental set-up. In most cases toxicity is determined by counting grown colonies on agar plates, but for clear evidence of the impairing influence of NP the tests have to be verified by further toxicity measurements. The principles of some of these methods are shortly introduced here.

Colony forming units (cfu): The plate count of cfu is the most frequently used method for the measurement of viable cells in bacterial populations. This method takes 24–48 h to form visible colonies. The count of cfu is based on the assumptions that under suitable culture conditions each bacterium grows and proliferates to produce a single colony. Therefore, it is assumed that the inoculum is homogeneous and that no agglomerates of cells are present (Tortora et al., 1989). In the case of measurements in the presence of NP one has to insure that the adsorbed NPs on the cells do not lead to an aggregation of the microorganisms. This would lead to excessively high toxicity values and positively false results (Fig. 10.2).

Optical density (OD): The optical density (OD) is the measurement of the turbidity of a bacterial suspension in a clear liquid medium and provides a quick estimation of the number of bacteria present in solution. The increase of the turbidity

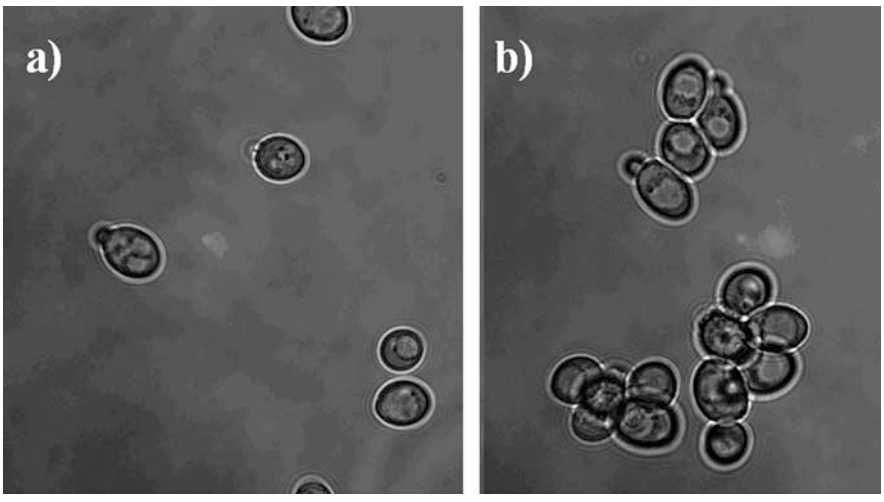

Fig. 10.2 *Saccharomyces cerevisiae* in 0.75 mM NaCl solution (**a**) without NP (**b**) with 60 mg L^{-1} Fe_2O_3

over time at growth measurements correlates with the number of living microorganisms; therefore, OD values obtained from spectrophotometric readings are directly related to the concentration of cells in the media under the Lambert–Beer law. Consequently a NP-dependent growth inhibition can be calculated (Weir et al., 2008).

Membrane damage via fluorescence: The assessment of the membrane integrity of microorganisms is often determined by using two different fluorescence dyes. One dye, mainly green fluorophores (DAPI, SybrGreen, SYTO®), is able to penetrate into all cells and binds to the nucleic acid. When used alone, it labels all microorganisms and the cell number can be determined. The second dye, in most cases the red fluorophore propidium iodide, can penetrate only into cells with a damaged membrane and has an excitation maximum close to the emission maximum of the green fluorescent dye. Therefore damaged cells reduce the green fluorescence intensity and enhance the red fluorescence intensity. The percentage of dead cells can be estimated based on the ratio of the two intensities (Singh, 2006).

Oxygen uptake rate: In aerobic cultures the oxygen uptake rate correlates with cell concentration. The oxygen uptake can be measured in sealed flasks. The produced CO_2 is absorbed by soda lime, and hence from the drop of pressure the consumed quantity of oxygen can be calculated.

Luminescence: Bioluminescent bacteria can be used to assess toxicity of inhibitory substances. Bioluminescence is produced by the enzyme luciferase, which catalyses the oxidation of the reduced luciferin by oxygen, thereby being elevated to its excited state. In non-toxic matrices the enzyme is regenerated under light emission (Fent, 1998). Toxic matter reduces the bioluminescence underlying various mechanisms like interactions with cell surface receptors, disruption of cell membrane functions, chemical reactions with cellular components or inhibition of enzyme systems (Arain, 2006). Tests with the bioluminescent bacteria *V. fischeri* require a high salt concentration which can interfere with the stability of NP.

Measurement of reactive oxygen species (ROS): 2′,7′-Dichlorodihydrofluorescein diacetate (DCDFA) is the most popular probe for measuring ROS. DCDFA can enter the cell and accumulates in the cytosol. There it will be deacetylated by esterases to 2, 7-dichlorohydrofluorescein (DCFH). This nonfluorescent product is converted by ROS to 2, 7-dichlorofluorescein DCF, which can be visualized quantitatively by fluorescence measurements (Halliwell and Whiteman, 2004).

Bacteria with specific marker genes: The use of specified marker genes can help as an additional tool to understand the mechanisms that are the reason for toxic effects. A well-working method is based on the application of recombinant bioluminescence bacteria with different promoters fused with *lux* genes. In such a set-up the defence mechanism of the cell by expression of the specific enzyme can be measured by luminescence because of the fused *lux* genes (Hwang et al., 2008). Recombinant bacteria lacking in genes for the expression of defence enzymes (e.g. the *sod*A gene expresses the enzyme superoxide dismutase which catalyses superoxide radicals in oxygen and hydrogen peroxide) can be used as marker for the damaging effects of ROS (Ruiz-Laguna et al., 2000).

10.5.1 Mode of Antibacterial Action

Nanosilver (Ag): Ag is one of the metals most commonly used in nanoparticulate form because of its strong antimicrobial activity. The mechanism by which Ag nanoparticles kill microorganisms is a controversial issue. Some researchers claim that the dissoluted Ag^+ ions from the nanoparticle are the more active species, whereas in some studies the nanoparticle itself was assumed to lead to a higher toxicity.

Most of the studies were focused on *E. coli* as an indicator microorganism. Lok et al. (2006) deduced that during growth of *E. coli* the effective antimicrobial concentration was 1,000-fold higher for Ag^+ than for Ag nanoparticles coated with BSA. The nano-Ag treated cells showed an accumulation of envelope protein precursors identified by proteomic analyses. Therefore nano-Ag may target the cell membrane, leading to a dissipation of the proton motive force. However, in growth studies with a nitrifying culture exposed to nano-Ag, there was no evidence for changes in the membrane integrity at 1 mg/L Ag measured with the fluorescence dyes SYTO 9™ and PI (Choi et al., 2008), and it was demonstrated that the inhibition of a nitrifying culture correlated well with the fraction of nano-Ag less than 5 nm from different Ag stock solutions (Choi and Hu, 2008). In addition, Morones et al. (2005) found that mainly nano-Ag in the range of 1–10 nm dispersed into different growing cultures of gram-negative bacteria, attach to the cell surface and penetrate inside the cell. Pal et al. (2007) also concluded that a reduction in the size of the nano-Ag will increase the contact area with the microorganisms. Furthermore, Smetana et al., (2008) observed that highly dispersed nanosilver coated with mercapto-1,2-propanediol could enter the cell without any toxic effects.

It is interesting to note that Ag^+ generated less intracellular ROS compared to nano-Ag. Choi and Hu, (2008) concluded that nanoparticles smaller than 5 nm can enter more easily into the cells through porins than Ag^+ because of the hydrophobic properties of the NP without damaging the cell membrane. In contrast, Sondi and Salopek-Sondi (2004) detected significant changes and damages in the cell membrane of nanosilver-treated bacteria. Obviously the formation of pits on the surfaces caused cell death by the incapability of regulating transports through the plasma membrane. Whereas Choi and Hu (2008) claim that cell death occurred through intracellular ROS generation due to nanosilver incorporation. Hwang et al. (2008) deduced from their experiments with nano-Ag and *E. coli* that Ag^+, produced by the particles outside the cell, is the main source of toxicity, because these silver ions can move into the cell and lead to a production of ROS. They further suggest that the cell dies due to the denaturation of DNA and sulphur containing proteins. The particles themselves stay outside and damage to a less extent the cell membrane, which leads to a disruption of the ion efflux system. Therefore the cell cannot extrude the harmful Ag^+ ions from the cytoplasm.

Smetana et al. (2008) reasoned that the reduced silver ions inside the cell agglomerate and reform silver metal nanoparticles. It was also concluded that very small,

irregular surfaces are necessary for the high biocidal activity as Pal et al. (2007) investigated a shape-dependent interaction.

Biofilm formation on stainless steel could be reduced by depositing silver NP embedded in an organosilicon matrix. The modified surface showed anti-adhesive properties against fungal biofilms in spite of the identical hydrophobic surface properties (Guillemot et al., 2008). However, the disinfection of biofilms in tap water was ineffective (Silvestry-Rodriguez et al., 2008), and the ineffectiveness was reasoned by complexing the silver cations with the anionic extracellular polymeric substances of the biofilm.

Carbon nano tubes (CNT) and fullerenes (C_{60}): In case of C_{60} and CNT the function of the generated ROS has been discussed as the main source of damage. Kai et al. (2003) speculated that the toxic effect is based on the light-induced generation of reactive oxygen species ($O_2^{-\bullet}$ and $^{\bullet}OH$) affecting the cell membrane. However, Lyon et al. (2006) demonstrated that in the absence of light and under anaerobic conditions toxic effects also occur. Photocatalyzed ROS production is probably not the sole antibacterial mechanism associated with C_{60}. In the case of CNT it was demonstrated by Kang et al. (2008) that damage was caused by direct cell contact and physicochemical/mechanical interaction with the outer cell membrane of *E. coli.* The higher toxicity of single-walled CNT compared to multiwalled CNT can be attributed to the smaller diameter.

Iron (Fe^0) and iron oxides (Fe_2O_3, Fe_3O_4): Iron and its oxides can act as a source of ROS through oxidation. Therefore, the highest cytotoxicity towards *E. coli* was observed by Fe^0 and further decreased for Fe_3O_4 to Fe_2O_3. Mutants of *E. coli,* where the enzyme production of superoxide dismutase, an important antioxidant enzyme, is disabled, showed increased cytotoxicity. These results support the hypothesis that the oxidation of the reduced iron (Fe^0, Fe^{II}) resulting in generation of ROS is one of the main reasons for toxic effects. But no internalised NP could be observed by TEM analysis (Auffan et al., 2008). In contrast, Lee et al. (2008) demonstrated an internalisation of Fe-NP into *E. coli* by TEM analysis suggesting that Fe^{II} ions released from metallic iron can pass into the cell and form via oxidation Fe^{III} oxide particles inside. Furthermore, it appears that nano-Fe^0 penetrates the cells through the vulnerable membranes after the chemical disruption, causing more serious physical damages.

Magnesium oxide (MgO): MgO-NPs are slowly converted to hydroxides in water. Upon dissociation and the loss of hydroxide anions the NPs become positively charged (Stoimenov and Klabunde, 2005). In combination with microorganisms, this causes a coagulation into large aggregates (Stoimenov et al., 2002). Oxygen, dissolved in the solution, can generate superoxide anions O_2^- which are stable and can exist in high concentrations at the surface of the NP covered with a layer of $Mg(OH)_2$ resulting in basic conditions (Huang et al., 2005). Therefore, the microorganisms suffer considerable cell wall damage upon contact (Stoimenov and Klabunde, 2005). Hence, it was demonstrated that MgO loaded with Cl_2 is very effective in disinfecting *B. cereus* spores (Koper et al., 2002).

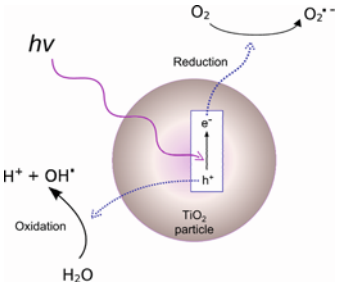

Fig. 10.3 Generation of ROS in water at irradiated surface of TiO_2

Titanium dioxide (TiO_2) and zinc oxide (ZnO): Both NPs are photosensitive and can produce ROS in the presence of light. Irradiated TiO_2 in water generates electrons in the conduction band and positive holes through irradiation with UV light. The electron excitation can produce ROS in water (Fig. 10.3). The oxidation process with the generated radicals by light irradiation is called photocatalysis.

In irradiation experiments it was demonstrated that the photocatalytic disinfection of *E. coli* is correlating with the amount of OH radicals formed (Cho et al., 2004). Additionally the toxic effect is dependent on the growth rate of the microorganism. It was shown that *E. coli* with higher specific growth rates were more susceptible than slow growing *E. coli* cells (Berney et al., 2006). Referring to Rincon and Pulgarin (2004), HPO_4^{2-} and HCO_3^- ions can retard the photocatalytic effect towards *E. coli* whereas the presence of Fe^{3+} ions in water does promote the toxic effect (Rincon and Pulgarin, 2007). In the case of ZnO, Jones et al. (2008) found a higher toxicity under normal ambient lab light and for smaller ZnO-NP (8 nm) than in darkness and with 50 nm particles. According to Brayner et al. (2006) an internalisation of ZnO-NP with a primary particle size of 11 nm into *E. coli* cells was observed by TEM analysis. In contrast, 200 nm ZnO aggregates determined by DLS measurements were not internalised into *E. coli* and led to damages at the membrane only (Zhang et al., 2007). In comparison ZnO-NP were more toxic against *B. subtilis* than TiO_2-NP. In comparison the toxicity towards *E. coli* was lower for TiO_2 and ZnO-NP. In all cases the antibacterial activity was higher under solar irradiation than in the dark (Adams et al., 2006).

10.6 Conclusion

The influence of various NPs on different microorganisms in the growth phase is evident and toxic effects in model solutions could be observed. Through the growing markets for NPs their appearance in the aquatic environment is inevitable. Lab results showed negative effects to environmentally relevant microorganisms. However, the application of the results from the lab onto the real ecosystem needs precaution. So far it is obvious that many factors will affect the toxicity of the NPs such as ionic strength of the water body, presence of NOM, surface charge and size

distribution of the particles. The comparison of the results of various publications suffers from the different experiment designs applied. The problematic situation asks for the development of sound standardized protocols and methods. With respect to the controversial findings in the mode of bactericidal action or in the internalisation of the particles into living cells more systematic research is needed to clarify the dominating particle transfer mechanisms and the NP-specific potential of ecotoxicity. So far as threat for the biologically working waste water treatment and the aquatic environment cannot be excluded. On the other hand, the bactericidal potential and applicability of NPs in water treatment has not been elucidated.

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