Chapter 20
Nanotechnology for Aquaculture

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Abstract As a part of sustainable culture, aquaculture is objectively a very promising activity comparing to other livestock production industries. Practically, aquaculture encounters serious challenges causing numerous drawbacks at multiple levels such as water infection, pond contamination, biofouling, chronic/acute diseases, and postharvest preservation. Researchers and overseers in the aquaculture industry have continually adopted new technologies to overcome most of these serious challenges; nanotechnology is among the prominent technologies to be applied in many aquaculture pundits. The different practical applications of nanotechnology in aquaculture disciplines are to be presented throughout this chapter. The application of nanotechnology in water and wastewater remediation, e.g., disinfection, sterilization, detoxification, and monitoring, is also discussed. The involvement of nanotechnology in aquatic organisms' performance and health in terms of vaccination, drug delivery, monitoring, antimicrobial application, reproduction control, and functional feeding is also mentioned. Additionally, the role of nanotechnology in harvested fish manufacturing, preservation, packaging, and commercialization is emphasized. The current chapter gives an overview about the current and potential nanotechnology applications in aquaculture and the suggestions to get the maximum benefit from it.

Keywords Nanotechnology · Aquaculture · Seafood · Vaccination · Disinfection · Feeding · Fish preservation

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20.1 Introduction

Nanotechnology is atomic and molecular matter controlling at the nanometer scale of 1–100 nm to study, design, create, synthesize, manipulate, and applicate of functional materials, devices as well as systems for matter exploitation of novel phenomena and properties. Worthy mentioning, the US National Nanotechnology Initiative (NNI) defined nanotechnology as "understanding and control of matter at dimensions of roughly 1–100 nm where unique phenomena enable novel applications". The numbers of nanoscience applications or authorization of patents for inventions were increased significantly in the world (FAO 2016a).

Nanotechnology has vast potential in electronic, materials science, humans, animal food, and agriculture sectors including aquaculture and its application in biological and biomedical sciences for analysis of biomolecules, development of non-viral vectors for gene therapy, cancer therapy, clinical diagnosis, and therapeutics. The majority of the investment in nanomaterial research is in nano-electronics, nanomedicine as well as nanopharmaceutics research. While in agricultural field, nanotechnologies investment is lesser than others in mentioned sectors and thus few nano-technologies and nano products are available in agriculture, aquaculture industry and animal husbandry. However, there is a huge potential for nanotechnology in this important sector which is required for revolution in socioeconomic status for huge population.

Aquaculture is a major global industry with annual production exceeding 50 million tones as well as estimated value of US$ 80 billion (FAO 2009). Global aquaculture production is growing at 6.6% as an average annual rate since 1995. In 2015, the business reached 76.6 and 29.4 million tons of aquatic animals and aquatic plants, respectively. Aquaculture sector is expected to play a significantly vital role in contributing to food security, economic development, and poverty alleviation (FAO 2016b), and will soon overtake capture fisheries (FAO 2008). Benefits of aquaculture include a production of high-quality food for growing population and jobs. But there are many problems associated with the environmental issues (Martinez-Porchas and Martinez-Cordova 2012a, b). Some of the problems that have negative impacts on aquaculture industry include a destruction of natural ecosystems (Rajitha et al. 2007; DeWalt et al. 2002); water pollution (Paez-Osuna 2001; Avnimelech and Kochba 2009); acidification/salinization of soils (Martínez-córdova et al. 2009; Rodríguez-Valencia et al. 2010); eutrophication and nitrification of ecosystems receiving effluents (Crab et al. 2009a, b; Fenaroli et al. 2014; Martinez-Porchas and Martinez-Cordova 2012a, b); chemical contamination (Justino et al. 2016; Sapkota et al. 2008a, b); biological contamination due to non-native species introduction (Krkošek et al. 2007; Molnar et al. 2008); landscape modification (Bentley 2015; Dumbauld and McCoy 2015); and negative effects on fisheries (Granada et al. 2016; Natale et al. 2013) (Fig. 20.1).

Nanotechnology shows many interdisciplinary activities in both agriculture and aquaculture sectors. Aquaculture industry and the fisheries can be revolutionized by nanotechnology with new tools for rapid disease detection, enhancement of fish

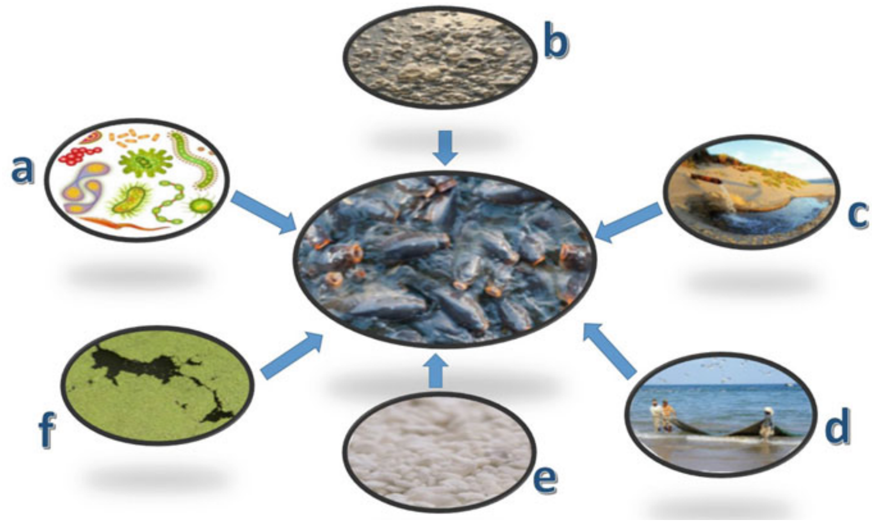

Fig. 20.1 Examples of aquaculture problems concerning environmental issues: **a** biological contamination; **b** water pollution; **c** chemical contamination; **d** fishery problems; **e** soil salinization; and **f** eutrophication. Adopted from Luis et al. (2017)

ability to absorb drugs, vaccines, and nutrients, etc (Rather et al. 2011a, b). Several nanotechnology applications for aquaculture are being developed. The highly integrated fish farming industry may be considered among the best to incorporate

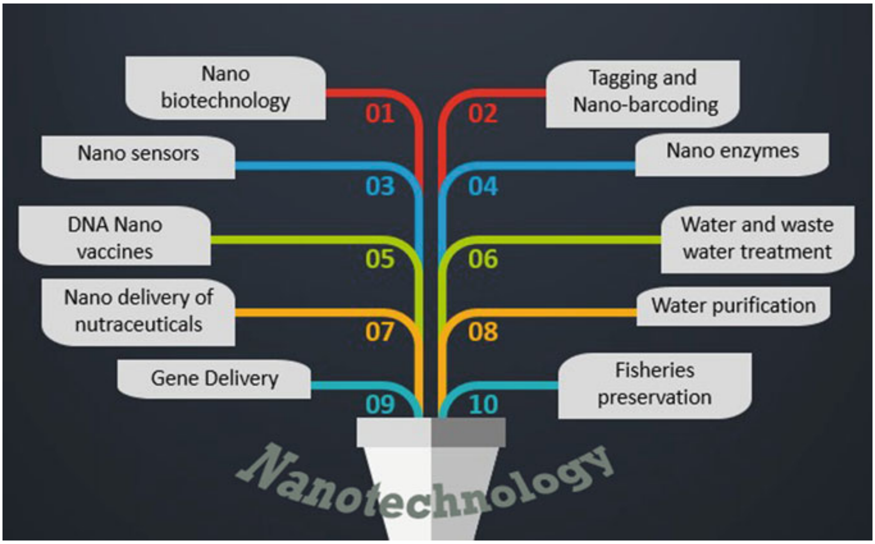

Fig. 20.2 Multidisciplinary activities of nanotechnology in agriculture and aquaculture

and commercialize nanotechnological products (Rather et al. 2011a, b). Moreover, nanotechnology applications in fish processing can also be used to detect fish bacteria in packaging, product safety by increasing the protection during processing (Fig. 20.2).

At present, although the use of nanotechnology in aquaculture needs much of development research, there are many glimpses of the future nanotechnological applications in water treatment in aquaculture, fish health management, animal breeding as well as harvest and postharvest technology.

20.2 Nanotechnology for Clean Water

Water is the life-supporting substance and a precious resource for survival of civilization. Clean water is one of the major global challenges for this century. Aquatic ecosystems and water quality face many old and new challenges. The major factors responsible for water pollution are the human activities; this pollution severely affects fish and may be lethal. So, the need for paying attention to this issue and the necessary corrective measures should be considered (Wang et al. 2015).

Most of fish farm's water is received directly from reservoirs or rivers and is discharged in rivers or reservoirs unfortunately without any previous treatments (Tavares and Santeiro 2013). Fish farms are divided into compartments, such as laboratories for larvae culture, plankton (natural feed) production ponds, fish breeding ponds, fish fattening ponds, and others. Water quality of the mentioned compartments may deteriorate directly because of fertilization and fish feeding management.

Fish farms are maintained by biological interactions as well as complex physical and chemical factors which directly depend on the water quality. Fish farms' wastewater discharged into streams, rivers, or lakes can cause important impacts on the environment (Konsowa 2007). The current water distribution, treatment, and discharge practices are no longer sustainable as these practices heavily depend on conveyance and centralized systems. Nanotechnology not only enable efficient, modular and multifunctional processes, but also provide affordable, high performance to wastewater treatment solutions with minimizing the large infrastructures dependence (Qu et al. 2012). Water treatment technologies based on nanotechnology are used for safe reuse of wastewater, improving sea and saline water desalination, decontamination and disinfection of water, i.e., nano-adsorption and biosorption for contaminant removal, nanophotocatalysis for contaminant chemical degradation, nanosensors for contaminant detection, various membrane technologies including nanofiltration, reverse osmosis, and photocatalysis (Bora and Dutta 2014; Kumar et al. 2014). A basic application of nanotechnology for water resource management has been shown in Fig. 20.3. Some of the aspects are being discussed below.

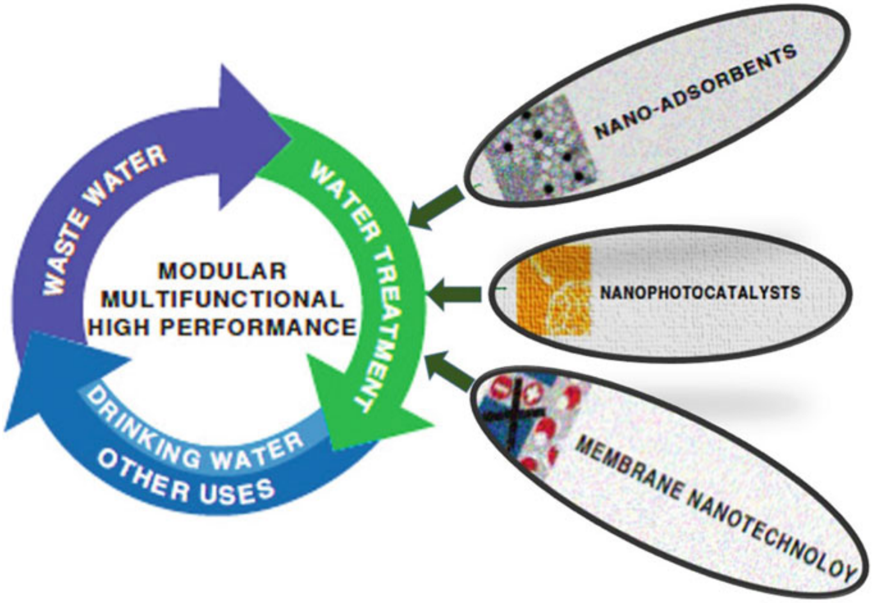

Fig. 20.3 A basic flowchart of nanotechnology for water resource management. Redrawn from Qu et al. (2013)

20.2.1 Disinfection of Water

Water supplies for aquaculture and seed production are often considered as one of the easiest means for infectious disease introduction and spread. A pathogen-free water source is vital for aquaculture success. Surface waters used in aquaculture that come from coastal waters or rivers may contain fish pathogens, so such open water supplies should be treated before use. Parameters such as environmental pollution, soil composition, and food waste can affect the physicochemical properties of pond water in aquaculture (Venkat 2011; 2014a, b), while water quality in open sea or coastal cages is affected by the natural environment (Khosravi-Katuli et al. 2017).

Fish culture facilities should depend on a disease prevention program that includes water quality, quarantine and sanitation of new animals as well as nutritional management. Sanitation practices include disinfection between fish groups, cleanliness during fish growth, and disease transmission prevention by equipment, personnel, or water. Disinfection is a common disease management tool in aquaculture and considered as a part of biosecurity plan by prevention of the target pathogenic agents' entry or exit from an aquaculture establishment or compartment, as well as the pathogenic agents spreading within aquaculture establishments. Disinfection may be used in emergency disease response for supporting the disease control zone maintenance and for eradication of the disease (stamping-out procedures) from affected aquaculture establishments. Disinfection-specific objective will determine the used strategy and how the strategy is applied.

Traditional and time-consuming disinfection process in aquaculture includes surfaces and equipment cleaning and washing for solid waste removal, which may reduce the disinfectants' effectiveness, disinfectant application and chemical residue removal or inactivation to avoid toxicity to aquatic animals as well as corrosion of equipment and environmental impacts. Processes that may be used for chemical residue removal or inactivation may include rinsing of surfaces with water, dilution until the acceptable levels, chemical agents' treatment to inactivate the disinfectant or keeping it for time needed for active compound deactivation or dissipation. Disinfectants may be risks to the users' health and aquatic animals in addition to the environment. Also, there is a need of trained labors, as chemical disinfectants should be used, stored, and disposed of in accordance with manufacturer's regulations and instructions.

Traditional disinfectant methods in aquaculture, such as pH modifiers (alkalis and acids), oxidizing agents, biguanides, quaternary ammonium compounds (QACs), aldehydes, ultraviolet (UV) irradiation, heat treatment, hydrogen peroxide and malachite green, desiccation, or even combined disinfection methods, have several limitations such as high cost, toxicity to aquatic animals, increased pathogen resistance, and negative effects on nontarget organisms (Romero et al. 2012). Still, aquatic establishments including fishponds, tanks, cage nets, pipes, vehicles, containers, buildings, boats, biofilters as well as husbandry equipment need to be disinfected. Moreover, bad water quality may lead to injury to the marine organisms and so results in poor performance (Boyd and Tucker 2012).

Nanotechnology can improve disinfection efficiency, decreasing toxic effects of traditional disinfectants and costs or through augment water supply by unconventional water sources' safe use (Qu et al. 2013). Nanotechnology use in water treatment offers several applications that could be specific to the user, which include nano-adsorbents, nanomembranes, nanometals, nanophotocatalysis, and others. In addition to one useful aspect which is the ability of various properties, integration in multifunctional materials, such as nanomaterials, can be used for simultaneous particle removal and contaminant elimination; hence, greater process efficiency is provided (Gehrke et al. 2015).

Recently, the nano-enabled products' application based on polymers and functionalized composites, aerogels, magnetic engineered and hydrophobic organoclay NPs for treatment and purification of water has been studied (Bhattacharyya et al. 2015; Lofrano et al. 2016). nAg, nAu, nFe, CNTs, $nTiO_2$, and lanthanum (La) were used for the removal of heavy metal pesticides and ammonia from water as well as wastewater (Ren et al. 2011; et al. 2012; Pradeep 2009; Rather et al. 2011a, b). Quantum dots (QDs) have been proposed for heavy metal detection in aquaculture (Chen et al. 2013), because of their unique optical properties (Vázquez-González and Carrillo-Carrion 2014).

Shrimp- and fish-intensive farming led to growing problems with bacterial pathogens such as *A. salmonicida, Yersinia ruckeri,* and *Flavobacterium columnare* (Pulkkinen et al. 2010). Moreover, aquaculture production has suffered severe losses due to bacterial disease (Huang et al. 2010), but the present traditional sterilization and disinfection methods for aquatic disease control such as various chemical disinfectants and antibiotics have numerous side effects and so many adverse effects (Yang 2003). It is worth mentioning that water disinfection nanotechnologies in fishpond increased water quality, fish and prawn survivals, and so the yields (Huang et al. 2015). In addition, this technology can be applied in both aquariums and commercial fishponds for reduction of water disinfection cost, and believed to provide safe and free fishponds from pollution and disease.

Effective disinfection without toxic disinfection by-product (DBP) formation is a great challenge for water industry. Conventional disinfectants, such as ozone and chlorine disinfectants, can form toxic DBPs (carcinogenic nitrosamines, halogenated disinfection by-product bromate, etc.). While UV disinfection may be an alternative for oxidative disinfection due to minimal DBPs production, but UV disinfection requires high dosage for certain microorganisms (e.g., adenoviruses). So, alternative disinfection development was an urgent need. Nanotechnology has potential role in disinfection and microbial control through many antimicrobial nanomaterials (Li et al. 2008) that have antimicrobial properties, lower tendency to DBPs formation such as nano-Ag, nano-TiO_2, nano-ZnO, CNTs, fullerenes, and nano-Ce_2O_4 (Table 20.1).

Nano-TiO_2 can be taken as an example to understand the antimicrobial mechanisms. These particles could achieve desirable sterilization on *E. coli, Aeromonas hydrophila,* and *Vibrio anguillarum*. In the presence of ultraviolet lights, the sterilizing rate of 0.1 g/L nano-TiO_2 could reach above 98% after 2 h. Moreover, this rate could be still above 96% after 2 h in the sun (Huang et al. 2010). Nano-TiO_2 photocatalytic sterilization efficiency was correlative with nano-TiO_2 concentration and reaction time. Without adequate reaction time or concentration, the sterilization would be ineffective (Huang et al. 2010). Nano-TiO_2 in natural environments has strong sterilization effects but only in sunlight presence as sunlight catalytic effect is like that of ultraviolet without a need of artificial light so this supports this technology application in aquaculture industry. Nano-TiO_2 has organic pollutant

Table 20.1 Nanomaterial antimicrobial mechanisms

Nanomaterials	Antimicrobial mechanisms
Nano-Ag	Release of silver ions, protein damage, suppression of DNA replication, membrane damage
Nano-TiO_2	Production of ROS
Nano-ZnO	Release of zinc ions, production of H_2O_2, membrane damage
Nano-MgO	Membrane damage
Nano-Ce_2O_4	Membrane damage
nC_{60}	ROS-independent oxidation
Fullerol and aminofullerene	Production of ROS
Carbon nanotubes	Membrane damage, oxidative stress
Graphene-based nanomaterials	Membrane damage, oxidative stress

degradation ability in water, disinfection, and sterilization abilities. Under ultraviolet irradiation, it is able to produce free radicals with high oxidation activity such as highly active hydroxyl –OH, peroxyl radical –OOH, superoxide ion –O, and other, which can interact with microorganisms (bacteria and viruses) and bio-macromolecules, such as proteins, lipids, enzymes, and nucleic acid, and other so destroy their cell structures through a series of chain reactions. The end result of these series of reactions is denaturation and lipolysis of bacteria and; accordingly, disinfection and sterilization with efficiency higher than traditional bactericide (Zhao et al. 2000; Yu et al. 2002; Sonawane et al. 2003).

20.2.2 Photocatalysis Using Nanoparticles for Water Purification

Photocatalytic oxidation is an advanced oxidation process for trace contaminant removal as well as microbial pathogens. This process is a useful pretreatment means for enhancing the biodegradability of non-biodegradable and hazardous contaminants. The major barrier for photocatalysis wide application is the slow kinetics due to limited light influencing and photocatalytic activity. Table 20.2 focuses on increasing photocatalytic reaction kinetics and photoactivity range by nano-TiO_2.

In water/wastewater treatment, TiO_2 is the most vastly used semiconductor photocatalyst such as low toxicity, high chemical stability, low cost, and raw material abundance. It generates an electron/hole (e^-/h^+) pair upon UV photon absorption and then later either migrates to the surface to form reactive oxygen species (ROS) or undergoes forming undesired recombination.

The photochemical reaction equation of hole/electron pair produced by photocatalyst (TiO_2) under light is as follows:

$$\text{“}TiO_2 + \text{light energy}(\text{hv}) \rightarrow \text{electron}\,(e^-) + \text{positive hole}\,(h^+)\text{”}$$

The hole/electron reacts with the object surface and water in the air, and then reactive free radicals (positive hole) are produced as follows:

$$\begin{aligned}\text{“}h^+ + \text{water molecules}\,(H_2O) &\rightarrow OH + H^+ \cdot \text{electron}\,(e^-)\,\text{and oxygen}\,(O_2)\\ &\rightarrow \text{Reactive Oxygen Species}\left(O_2^-\right)\text{”}\end{aligned}$$

The oxidation–reduction reaction occurs between the hole/electron pairs produced by photocatalyst reaction with organics on the matter surface as well as in the air, which then may be oxidized completely to other innocuous substances and water. Photocatalysis degradation is suggested to involve the (e−/h+) pair generation which is leading to radical formation such as superoxide radical anions,

Table 20.2 TiO_2 photocatalyst optimization

Optimization objects	Optimization mechanisms	Optimization approached	Waste treatment applications
Expanding photoactivity range	• Band gap narrowing	Anion doping	Low-energy cost solar/visible light-activated photocatalytic reactors
	• Impurity energy levels	Metal impurity doping	
	• Electron injection	Narrow band gap semiconductor doping	
		Dye sensitizer doping	
Enhancing kinetics of photocatalytic reaction	• Better electron–hole separation, lower electron–hole recombination	Noble metal doping	High-performance UV-activated photocatalytic reactors
	• Shorter carrier diffusion paths in the tube walls, higher reactant mass transfer rate toward tube surface	Nanotube morphology	
	• Higher reactant sorption, better electron–hole separation, lower electron–hole recombination	Reactive crystallographic facets	
	• More surface reactive sites, higher reactant adsorption, lower electron–hole recombination	Size	

hydroxyl radicals as well as hydroperoxyl radicals. These radicals act as oxidizing species in the processes of photocatalytic oxidation (Gupta and Kulkarni 2011). On the other hand, hydroxyl radicals [OH], reactive oxygen as well as other activated substances can be generated after the hole/electron pair's reaction on surface of the matter in water and air (Zhao et al. 2005). Nano-TiO_2 photoactivity can be improved by optimizing particle shape and size, reducing e^-/h^+ recombination by maximizing reactive facets, noble metal doping, and surface treatment to contaminant adsorption enhancement.

TiO_2 size plays an important role in its sorption, solid-phase transformation, and e^-/h^+ dynamics, where TiO_2 crystalline structures, the most stable for particle size larger than 35 nm, are rutile, while anatase is more efficient in ROS production and is the most stable at particle size smaller than 11 nm (Fujishima et al. 2008; Zhang and Banfield 2000).

The major cause for slow TiO_2 photocatalysis reaction kinetics is the fast e^-/h^+ recombination. Reduction of TiO_2 particle size decreases e^-/h^+ volume recombination as well as improves interfacial charge carrier transfer (Zhang et al. 1998). However, when particle size is reduced to several nanometers, surface recombination dominates, decreasing photocatalytic activity. TiO_2 nanotube organic compound decomposition was recorded to be more efficient than that of TiO_2 nanoparticles (Macak et al. 2007), where the higher photocatalytic activity was related to the shorter carrier diffusion paths in walls of the TiO_2 nanotubes in addition to reactants' faster mass transfer toward the TiO_2 nanotube surface.

In addition to TiO_2, tungsten trioxide (WO_3) and some fullerene derivatives have the potential to be used in water treatment through photocatalysis. WO_3 band gap is narrower than that of TiO_2, and this allows it to be visible light activated, <450 nm (Kominami et al. 2001). Platinum (Pt) can enhance WO_3 reactivity by facilitating O_2 multi-electron reduction and improves e^-/h^+ separation (Kim et al. 2010). Aminofullerenes generate $^1O_2$ under irradiation of the visible light (<550 nm) (Lof et al. 1995) and have been studied for pharmaceutical compound degradation and virus inactivation (Lee et al. 2010). Fullerol and C_{60} encapsulated with poly (*N*-vinylpyrrolidone) under UVA light can produce $^1O_2$ and superoxide (Brune in oxidationt et al. 2009). Aminofullerenes can be immobilized more than fullerol; moreover, aminofullerenes are more effective in disinfection purposes. This is related to their positive charge. TiO_2-produced hydroxyl radicals is higher than $^1O_2$ in oxidation potential, and it has more selective ROS that are less sensitive to nontarget background organic matter quenching. Currently, TiO_2 is much cheaper and readily available than fullerenes.

Photocatalytic water treatment process efficiency strongly depends on the photoreactor configuration and operation parameters. The commonly used two configurations are slurry reactors and immobilized TiO_2 using reactors. A different dispersion/recovery or immobilizations of catalyst techniques are being studied to maximize its efficiency. Recently, the water quality effects and operating parameters include: contaminant type and concentration, TiO_2 loading, temperature, dissolved oxygen, pH, wavelength, and intensity of light (Chong et al. 2010).

Some commercial product, e.g., Purifics Photo-Cat™ system, had treatment capacity up to 2 million gallon/day, with a 678 ft^2 minor footprint. Pilot tests indicated that this system is exceedingly efficient for eliminating organics without waste stream generation and can be operated with low power consumption (Al-Bastaki 2004; Benotti et al. 2009; Westerhoff et al. 2009).

Nano-TiO_2 expedited solar disinfection (SODIS) has been extensively examined and appears to be a possible option for safe drinking water production in developing countries' remote areas. The SODIS system can be either small scale for one person or enlarged to solar compound parabolic collectors of medium size. However, there are many technical challenges for its application on the large scale, including optimization of catalyst for improving quantum yield or visible light utilization; efficient design of photocatalytic reactor as well as catalyst recovery/immobilization techniques; and better reaction selectivity. Along with TiO_2, CeO_2 nanoparticles and carbon nanotubes have been examined in heterogeneous catalytic ozonation processes as catalysts can provide fast and relatively complete organic pollutant degradation. Both radical-mediated reaction pathway and non-radical-mediated reaction pathway have been proposed (Nawrocki and Kasprzyk-Hordern 2010). In both mechanisms, ozone and/or pollutant adsorption on the surface of catalyst plays a critical role.

Nanomaterials' high catalytic activity is related to large specific surface area in addition to an easily accessible surface. Some nanomaterials were also stated to promote ozone decomposition into hydroxyl radicals and facilitate the degradation process by radical-mediated routes (Orge et al. 2011). For industrial-scale applications, the nanomaterial enabled catalytic ozonation mechanism better understanding is a critical need. Moreover, the role of photocatalytic sterilization in aquaculture needs more research.

20.3 Use of Manufactured Nanomaterials in Aquaculture

Water pollution treating nano-adsorption material at present is broadly divided into: TiO_2 class, carbonaceous nanomaterials, iron and iron compounds, and other nanomaterials (McIntyre 2012), In the Lake Biwa, Japan, many kinds of manufactured nanomaterials (MNMs) were used to purify the lake water and the effect was significant (Cao et al. 2015). A similar experiment was carried out in some areas of Shanghai, Suzhou, and good results were achieved. MNMs are used for a month, eliminated the water body odor, and dropped NH_3–N content from 7×10^{-6} to below than 0.5×10^{-6} mg/l, without water change, no oxygen, no side effects, and no secondary pollution (Cao et al. 2015).

Insect Museum of Shanghai used MNMs in a 30 m^2 ponds of ornamental fish with stocking of 40 kg for six months without water change or oxygen addition or sewage suction but with usual feeding fodder, the water NH_3–N content fell from 0.5×10^{-6} below to 0.25×10^{-6} mg/l (Cao et al. 2015).

MNMs were also successfully used by researchers in India for industrial wastewater treatment, good results were achieved, and the investment was covered through power and all vital savings in consumption (Sreeprasad et al. 2013). MNMs were used in the seawater *Cyclina sinensis* nursery, where nanosilver powder, tourmaline nanomicron powders, and other materials were used to improve water quality, purify water, and so get better water environment, resulting in the increased survival rate and improving spat nursery production (Yang et al. 2006). Carbon nanotubes were used to adsorb industrial wastewater containing heavy metals such as zinc, copper, arsenic, chromium in addition to other heavy metals as well as nitro and amino compounds and other greater toxic substances of the dyes. The adsorption process increased with increase of both carbon nanotube dosage and the temperature (Shahryari et al. 2010).

20.3.1 Application in Aquaculture Facilities

In aquaculture field, MNMs application is most widely used in different breeding facilities. According to the physical nature of MNMs applications, they were classified into: nanocoating, nanofibers and nanofilms. In aquaculture, nanocoating has been used as functional, protective, and decorative coatings with other special features such as anti-virus, pest control, anti-fire, and others (Colvin 2003). Moreover nanocoatings' biological function includes two aspects like releasing of the component in coating for biological growth inhibition, so preventing biofouling, and slow release of nutrients that play an important role in organisms' growth (Simchi et al. 2011).

Addition of the nanoscale particle size in surface coatings improves its response to chemical catalysis and photocatalysis and the self-cleaning ability under UV. ZnO nanoscale particles added to paint make nanocoating that has bactericidal, anti-virus activities as well as has a shield UV and infrared absorbing properties (Simchi et al. 2011). Antimicrobial nanomaterial-coated sea cage mesh does not need frequent washing and cannot be fouling. The nutritious nanocoating-coated equipment in the water can breed some organisms on the surface such as unicellular algae and bait; thus increasing the production (Simchi et al. 2011).

Regarding nanofiber, there are studies that proved the strong degradation of toxic substances such as some nerve gases by Pt and TiO_2 nanoparticles; so mixing nanofibers with these groups of similar functions is a good mean for manufacturing high-performance protective suits (Bergshoef and Vancso 1999). In 1999, Professor Mac Diarmid, From the Faculty of Chemistry at Pennsylvania University, who has been awarded the Nobel Prize, proposed a composite nanofiber and reported that "a lot of metal nanoparticles can be very destructive to marine microorganisms." Nickel and copper nanoparticles have been used to make a macromolecule nanofibers acting as a coating material. Subsequently, this coating material that coats the device will produce a small current of waves which prevent marine organisms adhering on the device surfaces; thus, long-term preservation of water-exposed equipment's will not be difficult (Feng et al. 2010).

In aquaculture, filtering media is extremely demanded; the effectiveness of filtering is closely related to the medium fiber fineness. As the filtered material size particle should match both the filtering medium constitutional unit and the passageway, so by applying nanofibers in the medium, the nano-level granules either in the solution or in the gas can be removed (Graham et al. 2002; Tsai et al. 2002). In accordance with a relevant report (Kim 1997), microfiber filters can filtrate aerosol; therefore, these filters are suitable for purifying air. The nanofiber compound and certain selective reagents are used for manufacturing and developing molecular filters that are not only able to separate organic gas and vapor as well as O_2 and CO_2, but also can be useful in biochemical experiments for the toxic reagent removing and so on.

Nanofilm is divided into particle film and dense film. In aquaculture, nanomembrane filtration is using nanomembranes for selective permeability for a different valence ion, water bleaching, natural organic as well as synthetic organic compound removal (such as pesticides), by-products of disinfection removal (THMs and haloacetic acids) in addition to their precursors, for the water biological stability (Savage and Diallo 2005). According to reports, at early 2008 in Zhangjiagang on the anion nanofilm use to experiment, the obtained results suggested that the MNMs polyculture pool facilities of aquatic animals' use improved the quality of water, reduced the disease incidence, and accelerated the growth of animals (Albanese et al. 2012).

20.3.2 Biofouling Control

Nanotechnology may improve aquaculture production and shrimp culture through improving the disease control, feeding formulation, and biofouling control. Biofouling is unwanted bacteria (as biofilm), and invertebrates (mussels and barnacles) and algae (seaweeds and diatoms) could be controlled by coating or paint nanostructuring through metal oxide nanoparticles such as ZnO, CuO, and SiO_2 incorporation. This will be achieved by developing an efficient antifouling surface as well as improving the antifouling control performance (Rajeshkumar et al. 2008; Handy 2012).

The bacterial biofilm allows macrofouler attachment, as in maricultural cages so causing many serious problems such as weight increase, corrosion, surface alteration as well as submerged structure distortion (Champ 2003). Antifoulings are directly applied to get rid of these fouling organisms, but unfortunately on the other nontarget species there are undesired adverse effects, e.g., tributyltin or TBT (Lofrano et al. 2016). nZnO, nCuO, and nSi seem to be potential good antifouling candidates (Rather et al. 2011a, b), because of their high surface–volume ratio producing a more efficient antifouling barrier (i.e., at equal or lower concentrations). Ashraf and Edwin (2016) recorded a significant fouling reduction by using nCuO to treat nets of cage after 90 days from application.

Some commercial products (NanoCheck®) were developed for management of the fishpond using particles of 40-nm size based on lanthanum (La) compounds, which limited the growth of algae through supporting the water phosphate absorption (Mohd Ashraf et al. 2011). Moreover, NPs of La oxides were used on *Escherichia coli*, *Penicillium roqueforti*, *Staphylococcus carnosus,* and *Chlorella vulgaris* as phosphate scavenger resulted in starvation of the microorganisms (Gerber et al. 2012). Vijayan et al. (2014) examined nAg as well as nAu bacterial anti-biofilm activity. The examined nanosubstrates were synthetized from the extracts of *Turbinaria conoides.* The results indicated that nAg only was efficient in biofilm formation controlling, but nAu was not.

20.3.3 Nanodispersants

Oil spill response involves different technologies; one of them used chemical dispersants that contain surface-active agents (surfactant molecules) that agents migrate to the oil/water interface and then reduce interfacial tension between water and oil. With the wave energy abietane, tiny droplets of oil from the oil slick break away. These tiny droplets get dispersed and suspended into the water column, thereby becoming a good food source for the naturally occurring bacteria. The biodegradation process is catalyzed by the dispersants leading to spilled oil removal.

20.3.4 Nanomembranes

Manchester Institute of Technology developed nanowire membranes that have absorbent and superhydrophobic activities for oil selective absorption from an oil–water mixture. This could be achieved by using self-assembly method; this institute has constructed freestanding membranes including inorganic nanowires have the ability of oil absorption up to twenty times their weight. Moreover, MIT's SENSEable City Laboratory has recently produced robot called "Sea swarm" depending on the nanowire mesh. This autonomous oil-absorbing robot uses oil-absorbing nanowire mesh covering a conveyor belt. When the robot moves along the water surface, the conveyor belt as well as the nanomesh rotates, and selective absorption starts for water cleaning. These robots have the ability to run for weeks to clean up several oil gallons per hour using very little energy (as low as about 100 W).

Oil spills generally result in seawater contamination due to water-soluble crude oil fraction dissolution. The contaminated water is highly toxic due to high dissolved hydrocarbon concentration and able to cause ecosystem irreparable damage. The oil-contaminated water photocatalytic decomposition by using TiO_2 particle nanoscale or microscale can control the problem. Professor Feng from Tsinghua University, in collaboration with the other institutions, has produced a new functional nanomaterials (Fig. 20.4) act as an oil–water separation membrane. These nanomaterials can perform efficient separation for a series of oil–water mixture within minutes, and the efficiency of separation was over 99% (Gao et al. 2013).

20.3.5 Desalination

Seawater desalination in the near future will be a major freshwater source because of limited freshwater resources. Reverse osmosis (RO) membranes are high-cost

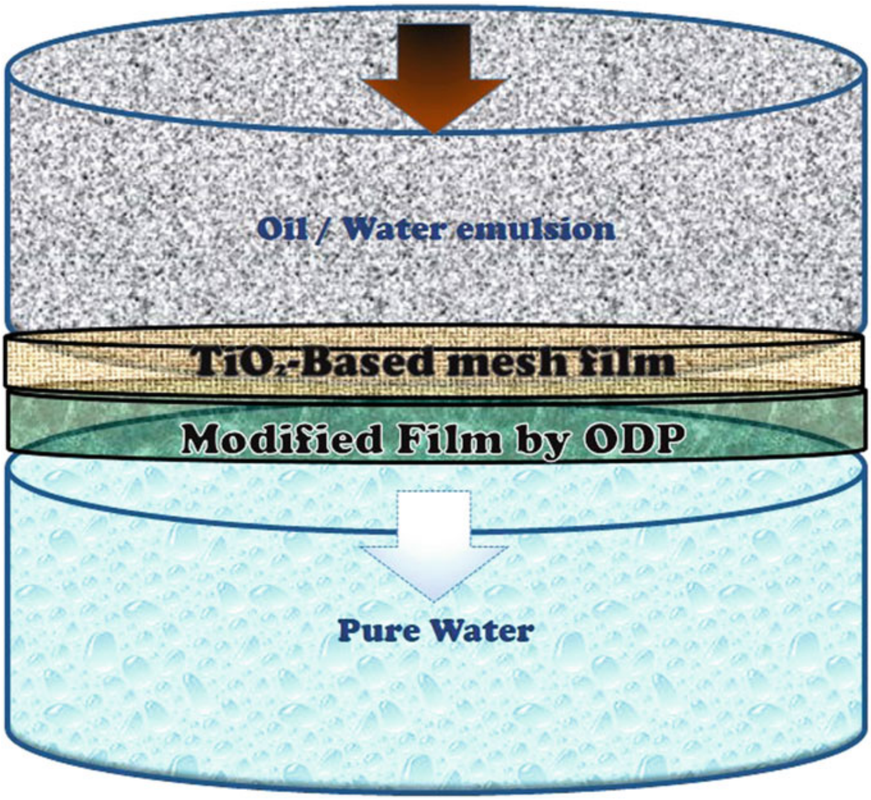

Fig. 20.4 Gravity-driven oil–water separation

conventional desalination technologies due to large amount of energy consumption. Nanotechnology has played a very vital role in creating low-energy alternatives, among which the most promising technologies are: aligned carbon nanotube membranes, protein–polymer biomimetic membranes, and thin-film nanocomposite membranes (Hoek and Ghosh 2009; Nikonenko et al. 2014). These technology desalination efficiencies are up to 1000 times better than that of RO, because it has the high permeability of water due to carbon nanotube membranes present in their structure, and integration of these membranes (some of them) in other processes such as disinfection, defouling, deodorizing, and self-cleaning. In another technology for seawater desalination, zeolite nanomembrane is used (Liu and Chen 2013). Soon, some of these desalination technologies may be present in the market place but there are critical challenges that should be considered as practical desalination effectiveness, scale-up fabrication as well as long-term stability (Ranjan et al. 2016b; Shivendu et al. 2016).

Recently, many devices have been developed with improved efficiency and performance that are self-sustained webs of polyvinylidene fluoride electrospun nanofibers (Essalhi and Khayet 2014); PVA/PVDF hollow fiber composite membrane modified with TiO_2 nanoparticles (Li et al. 2014a, b); novel integrated system coupled with nanofluid-based solar collector (Kabeel and El-Said 2013, 2014); zinc oxide micro-/nanostructures grafted on activated carbon cloth electrodes (Myint et al. 2014); tubular MFI zeolite membranes (Drobek et al. 2012); titanium oxide nanotubes/polyethersulfone blend membrane (Abdallah et al. 2014); graphene-wrapped MnO_2 nanostructures (El-Deen et al. 2014b); thin-film nanocomposite membranes (Subramani et al. 2014); graphene/SnO_2 nanocomposite (El-Deen et al. 2014a); and carbon nanotubes (Goh et al. 2013).

20.3.6 Removal of Heavy Metals

Ligand-based nanocoating can be used for effective heavy metal removal, which is related to high absorption tendency and cost-effectiveness as it can be regenerated by treating with the previously used nanocoating media bifunctional self-assembling ligand in situ. Crystal clear technology is used for water purification in this technology; multiple metal layers are bonded to one substrate (Farmen 2009). Nowadays, nanomaterials have been widely used for heavy metals removal from water/wastewater due to their high reactivity and large surface area.

Metal oxide nanoparticles, such as nanosized ferric oxides, aluminum oxides, manganese oxides, cerium oxides, magnesium oxides, and titanium oxides, have high surface area as well as specific heavy metal adsorption affinity from aqueous systems. To date, development of new technologies for metal oxide nanoparticle synthesis as well as of better possibility for practical use, i.e., composite materials or granular oxides, has become a hot topic to evaluate their heavy metal removal under different experimental conditions or to reveal the metal removal underlying mechanism based on mathematical models or analytical techniques such as XAS, NMR, and ATR-FTIR (Hua et al. 2012; Kumar and Chawla 2014).

Additionally, fulvic acid and humic acid are very common in aquatic environments in addition to have several of functional groups which help them to complex with metal ions as well as interact with nanomaterials. These interactions can alter the nanomaterials' environmental behavior and influence the heavy metal removal and transportation by nanomaterials. Thus, these interactions and their underlying mechanisms need specific investigations.

Tang et al. (2014) have recorded a detailed review on the humic acid and fulvic acid effects on heavy metal removal from aqueous solutions by different nanomaterials, mainly including iron-based nanomaterials, carbon-based nanomaterials, and photocatalytic nanomaterials. Moreover, this review discussed the interaction mechanisms and evaluated the humic acid and fulvic acid potential environmental implications to nanomaterials as well as heavy metals.

Chitosan nanoparticles as adsorbents are being used for the removal of heavy metal. Recent studies have focused on removal of heavy metal by chitosan nanoparticles with clays such as kaolinite, bentonite, and montmorillonite because of the inherent capability of clays to heavy metal removal just like chitosan and chitin. In the recent period, studies on nanochitosan–clay composite for removal of metal ion have been reported (Futalan et al. 2011; Pandey and Mishra 2011). Chitosan–magnetite nanocomposites were also stated for heavy metal removal from aqueous solution (Namdeo and Bajpai 2008; Fan et al. 2017).

20.3.7 Selective Removal of Nitrate and Phosphate

Excessive phosphorus (P) and nitrogen (N) release into runoff from activities of human is eutrophication major cause, which degrades ecosystems and freshwater. Nitrate and phosphate pollutants can be removed by biological treatment, chemical precipitation, membrane processes, ion exchange, electrolytic treatment, and adsorption process for effective removal of these pollutants from water sources (Fig. 20.5).

Ideally, the expected properties of designed zero-valent metal-containing nanoparticles (ZVMNPs) for water treatment include their low toxicity to the biota; high reactivity for the targeted contaminants removal; after injection, high reactive longevity and high mobility in porous media (Yan et al. 2013). Moreover, lowering the enhanced nitrate removal, pH is lowered by adding either buffer system or acidic solutions. Removal of phosphate by iron oxide nanoparticles and

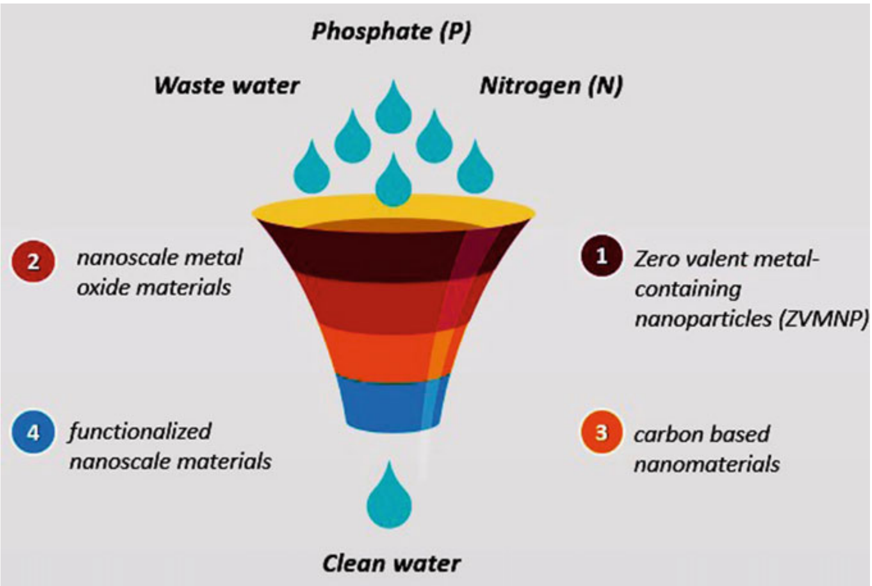

Fig. 20.5 Nanotechnological techniques for removal of nitrate and phosphate ions from wastewater

NZVI is a sorptive process where the sorbed phosphate either still in the nanoparticles or get precipitated with iron species (Prashantha Kumar et al. 2016).

Almeelbi and Bezbaruah (2012) have detected that phosphate is removed up to 100% by using of nanoscale zero-valent iron (nZVI) particles; moreover, nZVI particles had more efficient activity than larger-sized particles (micro-ZVI). Alessio (2015) have reported that nitrate removal efficiency reached up to 98, 87, and 63% in 60 min during the solution treatment from initial concentrations of nitrate nitrogen that were 50, 70, and 95 mg/L, respectively, and verified interaction was found to be a first-order kinetic type.

Among the used adsorbents for water quality standard improvement, hydrated metal oxides, e.g., Fe (III), Zr (IV), and Cu (II) nanocomposite, were extensively explored for removal of phosphate via outer-sphere complexes (Chen et al. 2015). Carbon-based materials like graphite oxide (GO), graphite, graphene, multi-walled carbon nanotube (MWCNT), carbon nanotube (CNT), and functionalized carbon-based materials have made adsorption and catalysis potential applications. Only a few studies for phosphate and nitrate ion removal from aqueous system are reported (Prashantha Kumar et al. 2016).

20.4 Nanotechnology Devices for Aquatic Environment Management

Nanotechnology application in seawater shrimp aquaculture indicated that the nanodevice was able to reduce the water exchange rate and improve both water quality and survival rate of shrimp and so yield (Wen et al. 2003). Among many nanodevices, the best device was nanonet treatment; the results showed fish survival rate increased to 100%, decreasing in both water nitrite and nitrate; moreover, nitrite decreased to as low as 1/4 of the control group. Also, nanotechnologies increased the water pH and improved significantly the water quality (Liu et al. 2008).

In China at present, nano-863 is widely used agricultural high-tech product. This product is produced by addition of nanomaterials of high-temperature sintering and strong light-absorbing properties with a carrier of ceramic material. Nano-863 has been widely used in breeding of livestock, crop cultivation, and aquaculture (Fig. 20.6).

An experiment studied the effects of nano-863 on the water quality results indicating that nano-863 improves water quality and so is more conducive to fish growth while without water changing for 6 months, NH_3–N, NO_3–N, NO_2–N, and CD contents in the test groups were 0.58, 0.89, 0.13, and 8.95, respectively, and all of these results were lower than the control group with conventional changing of water, i.e., 1.58, 2.33, 0.28, and 19.22. While pH was 7.20 in the experimental group, that was higher than the control group (5.60).

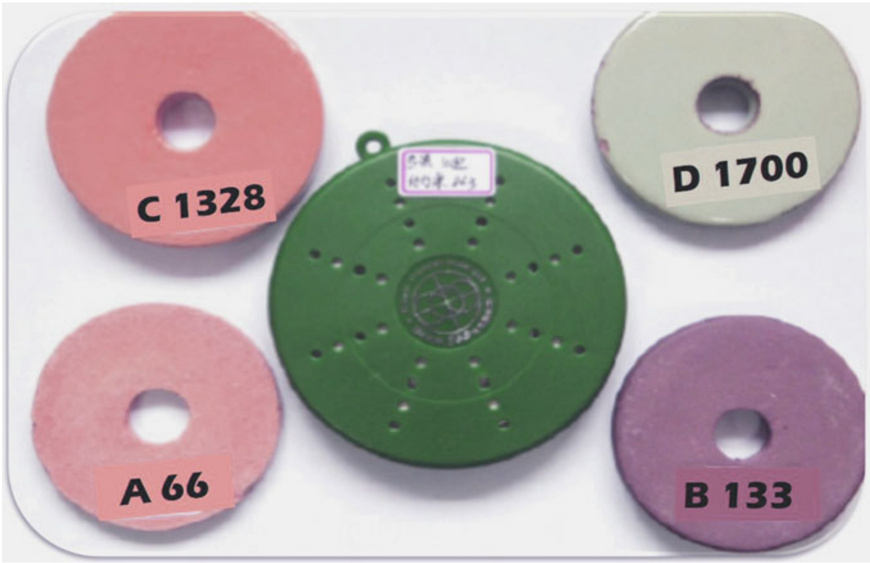

Fig. 20.6 Different types of biological assistant growth apparatus ceramic disks. Qiangdi nano-863 (the biggest green one in the center), Suzhou Zhongchi (a, b, c, and d disks around)

In shrimp farming, nano-863 was used in fishery aquatic breeding farm. A number of million tailed shrimps were tested in each of the control and experimental groups; only 360 thousand survived in the control group, while 730 thousand tailed shrimps survived in the test group; so, the test group survival rate is twice that of the control group. Nano-863 can also enhance water activity and energy, improve shrimp appetite, and so promote growth and development; it also has very strong protection efficacy such as antibacterial as well as disease and algae protection.

Nevada-based Altair Nanotechnologies produce NanoCheck which is water-cleaning product for fishponds and swimming pools. NanoCheck uses 40 nm of a lanthanum-based compound particle that absorbs phosphates from water and so inhibits algae growth (Mongillo 2007). Altair is hoping for NanoCheck use in commercial fish farms worldwide, where heavy metal removal and algae prevention are costly. Altair plans to expand the tests to confirm NanoCheck effect on fish and nanoparticle-laden runoff impacts either on human health or on the environment. Besides, nanoscale delivery of soil-wetting agents and weedicides may be very vital for aquatic weed control in large bodies of water as well as mitigation of stress due to aquatic pollution and climate change. Thai researchers have succeeded in titanium dioxide (TiO_2) nanosized particles used to coat stone or ceramic in fish bowls for water treatment. Ceramics or stones coated with TiO_2 nanoparticles in fish tanks can eliminate bacteria and moss. This technology can be applied in commercial fishponds and aquariums to reduce the water treatment cost.

20.5 Nanotechnology in Fish Health

The aquatic environment is considered as a dynamic medium able to transport pathogens over several kilometers. Furthermore, pathogens are capable of moving between farms, due to the organism's substantial movement and vehicles, which create a highly complex transmission web of disease (Munro and Gregory 2009; Murray 2013). Fish can be infected by various infectious diseases (Khoo 2000; Ramaiah 2006; Brooker et al. 2007; Jacobs et al. 2009; Gomez-Casado et al. 2011; Frans et al. 2011; Oidtmann and Stentiford 2011; Vega-Heredia et al. 2012). Environmental stress is considered a major factor affecting health of fish, such as hypoxia, ammonia poisoning, gas bubble diseases, temperature stress, nitrite poisoning, and pH stress. For infectious disease control, large quantities of veterinary drugs such as antibiotics are employed and, in some cases, resulted in the resistant pests' appearance (Gjedrem 2015; Huang et al. 2015; Lafferty et al. 2015).

There are different administration routes to treat fish, such as the medication dosing into the water (bath treatment), injection, in-feed medication, and topical administration. Bath and topical administration are used for external diseases, while injection and in-feed medication are used for internal infections (Bowker et al. 2016). Various federal as well as state regulatory agencies (such as the US Food and Drug Administration) are regulating drugs used in aquaculture. FDA-approved drugs for use with fish are presented in Tables 20.3 and 20.4 illustrating low regulatory priority (LRP) substances which present little risk to aquatic organisms, the environment, and humans. But these LRP substances can be used with the following conditions: use in the applications listed, appropriate grade when used in animals intended as food, not to exceed prescribed levels.

In addition to antibiotic use in humans, antibiotics are used in treatments of animal for growth enhancement or as prophylactics (Marshall and Levy 2011). However, in aquaculture, antibiotics are frequently used for high productivity ensurance and this has caused the antibiotic-resistant bacteria emergence; moreover, aquaculture ponds are becoming antibiotic resistance gene reservoirs that could be acquired by animal as well as human pathogens (Huang et al. 2015; Letchumanan et al. 2015a, b, c; Tomova et al. 2015; Xiong et al. 2015).

For prevention of diseases, nanotechnology applications include water treatment, pond sterilization, aquatic disease detection and control, nutrients and drug efficient delivery (including hormones and vaccines), and improvement of fish absorption ability of these substances (Bhattacharyya et al. 2015; Huang et al. 2015).

20.5.1 Drug Delivery

Today, probiotics, antibiotics, and pharmaceutical/ nutraceutical application are delivered through injection or feed either as preventive measures or when symptoms are evident. Nanoscale devices may detect as well as treat health problems and

Table 20.3 Drugs approved by the US Food and Drug Administration (FDA) for aquaculture applications

Product	Active ingredient	Manufacturer
Administration route: bath		
Finquel	Tricaine methanesulfonate	Argent Laboratories
Formacide-B	Formalin	B L Mitchell Inc.
Formalin-F™	Formalin	Natchez Animal Supply Company
Halamid® Aqua	Chloramine-T	Western Chemical Inc.
Oxymarine™	Oxytetracycline hydrochloride	Alpharma, Inc
Oxytetracycline HCL Soluble Powder-343	Oxytetracycline hydrochloride	Phoenix Scientific Inc.
Paracide F	Formalin	Argent Laboratories
Parasite-S®	Formalin	Western Chemical Inc.
Pennox 343	Oxytetracycline hydrochloride	Pennfield Oil Co.
Perox-Aid® 35%	Hydrogen peroxide	Western Chemical Inc.
TERRAMYCIN-343 (oxytetracycline HCl) soluble powder	Oxytetracycline hydrochloride	Pfizer Inc.
Tetroxy Aquatic	Oxytetracycline hydrochloride	Cross Vetpharm Group Ltd.
Tricaine-S	Tricaine methanesulfonate	Western Chemical Inc.
Administration route: injection		
Chorulon®	Chorionic gonadotropin	Intervet Inc.
Administration route: in-feed		
AquaFlor®	Florfenicol	Schering-Plough Animal Health Corporation
Romet® TC	Sulfadimethoxine/ormetoprim	Aquatic Health Resources
Romet 30®	Sulfadimethoxine/ormetoprim	Aquatic Health Resources
Terramycin® 200 for fish	Oxytetracycline dehydrate	Phibro Animal Health

infections. Nanoscale smart delivery system poses multifunctional characteristics such as pre-programming, time controlling, delivery monitoring of hormones, probiotics, vaccines and chemicals (Donbrow 1991). In medicine, many nanoparticle forms have been used (Table 20.5) such as the nanosized spherical particles which are nanospheres (De Jong and Borm 2008). Nanospheres have high surface area and small size which permit the dispersion of a specific drug on the surface of

Table 20.4 Low regulatory priority (LRP) substances used in aquaculture

Substances	Applications
Acetic acid	Parasiticide
Calcium chloride	Egg hardening and maintenance of osmotic balance in fish
Calcium oxide	Protozoacide
Carbon dioxide	Anesthetic
Garlic	Infestation control of helminths and sea lice
Ice	Metabolism reducer
Magnesium sulfate	Infestation control of trematodes and crustaceans
Onion	Infestation control of crustaceans and sea lice
Papain	Aid in removal of the gelatinous matrix from eggs
Potassium chloride	Osmoregulator and antistress agent
Povidone-iodine	Egg disinfectant
Sodium bicarbonate	Aid in fish anesthesia
Sodium chloride (salt)	Parasiticide, antistress agent, and osmoregulator
Sodium sulfide	Improved hatchability
Thiamine hydrochloride	Prevention of thiamine deficiency
Urea and tannic acid	Reduced adhesiveness

Adapted from Bowker et al. (2016)

Table 20.5 Different forms of nanoparticles used in biomedical applications

Type of nanoparticles	Structure	Application in medicine
Nanospheres	Spherical shaped	Drug delivery, tissue regeneration
Nanocapsules	Shell and core combination	Controlled drug delivery
Carbon nanotubes	Cylindrical tubes	Drug delivery, anticancer
Liposomes	Lipid bilayer globules	Drug delivery for hydrophobic and hydrophilic drugs
Dendrimers	Highly branched ends and central core	Delivery system, tissue engineering, antimicrobials
Polymeric nanoparticles	Polymers such as chitosan and PLGA	Delivery system, tissue regeneration

nanosphere and so facilitate drug delivery. Tissue regeneration is another field where nanospheres can be used (Ramalingam et al. 2013). Nanocapsule is another nanoparticle form, which consists of an inner core and an outer nanoscale shell. The core may be water or oil that contains a specific drug, while the outer shell protects that specific drug from hydrolysis and degradation (Torchilin 2006). Liposomes as an example are lipid bilayer nanosized spheres. Liposomes are ideal for both hydrophilic and lipophilic medications' drug delivery due to their structure that is like the cell membrane of eukaryotes (Lasic 1998; Cavalieri et al. 2014). Carbon nanotubes (single- or multi-walled) are nanoparticle forms commonly used in various medical applications including anti-tumor drugs as carbon nanotubes are characterized by high cell membrane penetration ability and very large surface area. Carbon nanotubes are ideal for drug delivery by acting as micro-needles (Reilly 2007). However, carbon nanotubes have high risk of inducing thrombi in the blood vessels (Gaffney et al. 2015). Dendrimer form is the fourth particle form. Dendrimers are nanoscale three-dimensional structures composed of tree branch-like units originated from a central core. These have low molecular weight with a highly branched multifunctional surface that facilitates dendrimers' use as a delivery system for vaccines, genes, and drugs as well as tissue regeneration scaffold and as antimicrobial agents (Aulenta et al. 2003; Gillies and Frechet 2005; Wu et al. 2015).

In fish medicine, the applied nanoparticle types have been limited to nanospheres or polymeric nanoparticles; other nanoparticle application forms have not been investigated yet.

20.5.1.1 Chitosan Nanoparticles for Drug Delivery

Chitosan nanoparticles are excellent drug delivery vehicles that have characteristic proprieties such as non-toxic, biocompatible, and biodegradable polymer as well as easily excreted from the kidney (De Jong and Borm 2008). Moreover, chitosan nanoparticles can be adapted for sustainable and slow drug release due to mucoadhesive property means (Dutkiewicz and Kucharska 1992). For example, in a study on rainbow trout (*Oncorhynchus mykiss*) vitamin C conjugation with chitosan nanoparticles resulted in vitamin release for 48 h after oral administration, and with led to immune system stimulation due to the potent synergism between vitamin C and chitosan (Alishahi et al. 2011). In another investigation, carried on *Cyprinus carpio*, chitosan nanoparticles were examined as a hormone delivery system. Luteinizing hormone-releasing hormone (LHRH) was bound to both chitosan nanoparticles alone in one group, and in another group LHRH conjugated with chitosan–gold nanoparticles. Both groups' results showed blood hormone levels increase with hormones sustaining release groups, in comparison with the group that injected with hormone alone. Moreover, the rates of egg fertilization were elevated after one injection dose of hormone–chitosan gold nanoparticles and hormone–chitosan nanoparticle conjugate with 83 and 87%, respectively, comparing to LHRH alone multiple injections that gave 74% fertilization rate (Rather et al. 2013).

20.5.1.2 PLGA Nanoparticles for Drug Delivery

PLGA is a copolymer composed of polylactic acid in addition to poly(glycolic) acid. PLGA is biocompatible, non-toxic, biodegradable, and FDA-approved. Hence, many researchers investigated PLGA feasibility as a drug carrier (Lü et al. 2009; Makadia and Siegel 2011). In a study on embryos of zebra fish, the

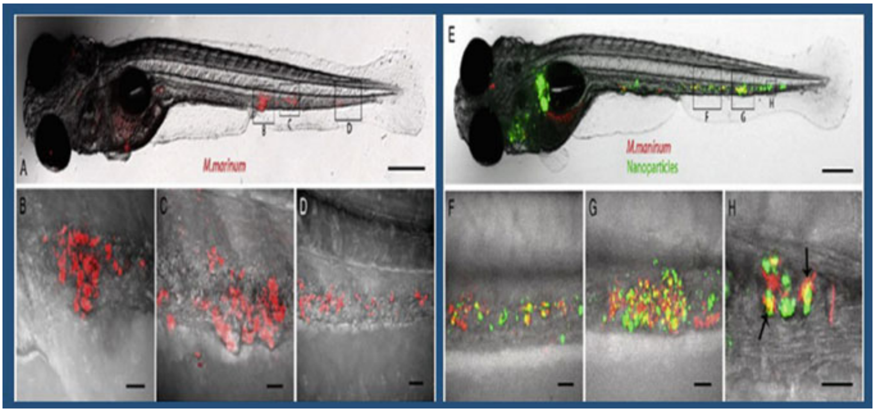

Fig. 20.7 **a–d** Lateral view showing formation of *Mycobacterium marinum* granulomas in zebra fish embryos. After injection of nanoparticles in the posterior caudal vein, panels, **e–h** show rifampicin-loaded PLGA nanoparticles targeting the granulomas. Adapted from Fenaroli et al. (2014)

anti-mycobacterial agent (rifampicin) was loaded on PLGA nanoparticles and then applied because zebra fish embryos transparency had a vital role in measuring the impact of treatment on *Mycobacterium marinum*-infection using noninvasive imaging (Fig. 20.7). The rifampicin–PLGA nanoparticles achieved increasing of therapeutic effect as well as higher embryo survival against M. marinum compared to rifampicin alone (Fenaroli et al. 2014).

20.5.2 Nanosensors

In aquaculture industry, various diseases caused huge losses annually; therefore, diseases' efficient detection and control are very important for both maximizing productivity and final product satisfactory quality ensurance (Ninawe et al. 2016). Nano-biosensor use offers an innovative means to solve problems. These devices may be depending on different nanomaterials, for example carbon nanotubes could possibly facilitate the detection of low pathogens concentrations including viruses, bacteria, parasites and pollutants (Chen et al. 2016).

Rapid pathogen detection in aquatic organisms may be very effective way to disease control, but the available detection methods are costly, time consuming, and could have detection and separation difficulties for some pathogen (Guo et al. 2016). A study designed an immunomagnetic NP-based microfluidic system for *Staphylococcus aureus* detection producing a microfluidic chip with indium tin oxide. The obtained results evidenced that the detection system sensitivity and specificity were the same results of the colony counting method, with a whole shorter time of detection without colony cultivation.

Sensitive and rapid disease diagnosis adopted for nanoparticles are called nanodiagnostics (Jain 2003). Gold nanoparticles are widely used in diagnostics and considered one of the most used particles, which are suitable for use in different methods (Baptista et al. 2008; Saleh et al. 2015). The first gold nanoparticle report for fish pathogen detection was with *Aeromonas salmonicida* antibody-gold nanoparticles conjugated for furunculosis-specific immunodiagnosis in fish tissues (Saleh et al. 2011). Kuan et al. (2013) prepared an electrochemical DNA biosensor for *Aphanomyces invadans* detection in fish with a DNA reporter probe and based on conjugation of Au-NPs and so fungi which are at a lower level than PCR could be detected.

Jaroenram et al. (2012) combined colorimetric assay of Au-NPs with loop-mediated isothermal amplification (LAMP) for yellow head virus visual detection in shrimp. This was high sensitive, rapid, and specific method. A similar combination of LAMP with DNA-functionalized Au-NPs was developed for white spot syndrome virus (WSSV) detection in shrimp (Seetang-Nun et al. 2013). This method was sensitive, specific, and suitable for field applications. In another study, Toubanaki et al. (2015) designed a method for detection of nervous necrosis virus (NNV) by using gold nanoparticle-based biosensor for viral nucleic acid detection after RT-PCR amplification, but this was quite cost-effective, as this method did not require antibody conjugation.

Yang et al. (2012) recorded an immunomagnetic reduction assay in grouper fish for nervous necrosis virus (NNV) using magnetic nanoparticles covered with rabbit anti-NNV antibody. Application of an external magnetic field, for immunodiagnosis was based on magnetic nanoparticle motility; if the viral antigen bound to antibody coated-nanoparticles and form clusters, that will decrease their motility. The virus titer could be detected using a magnetic immunoassay analyzer. Spring viremia of carp virus (SVCV) detection by a colorimetric assay was developed using unmodified Au-NPs. At first addition of the probe, that was complementary for SVCV and then followed by gold nanoparticles. If target RNA of the virus was present, it hybridized to the probe, so the probe is prevented from stabilizing the gold nanoparticles. The gold nanoparticles could aggregate resulting in the solution change from red to blue. If there was not viral nucleic acid, the probe could be freely adsorbed onto the gold nanoparticle surface; this could prevent nanoparticles aggregation and keep the red color of solution (Figs. 20.8 and 20.9) (Saleh et al. 2011). This method was highly specific and rapid, and there was not a need for prior viral nucleic acid amplification. The same principle was used for developing a specific, rapid as well as sensitive assay for the DNA virus detection, cyprinid herpesvirus 3 (CyHV-3) (Saleh and El-Matbouli 2015).

20.5.3 Nanovaccines

In many infectious fish diseases, main gateway is ecosystem. The natural biological cycle includes one or more hosts' stable association to provide a medium for the

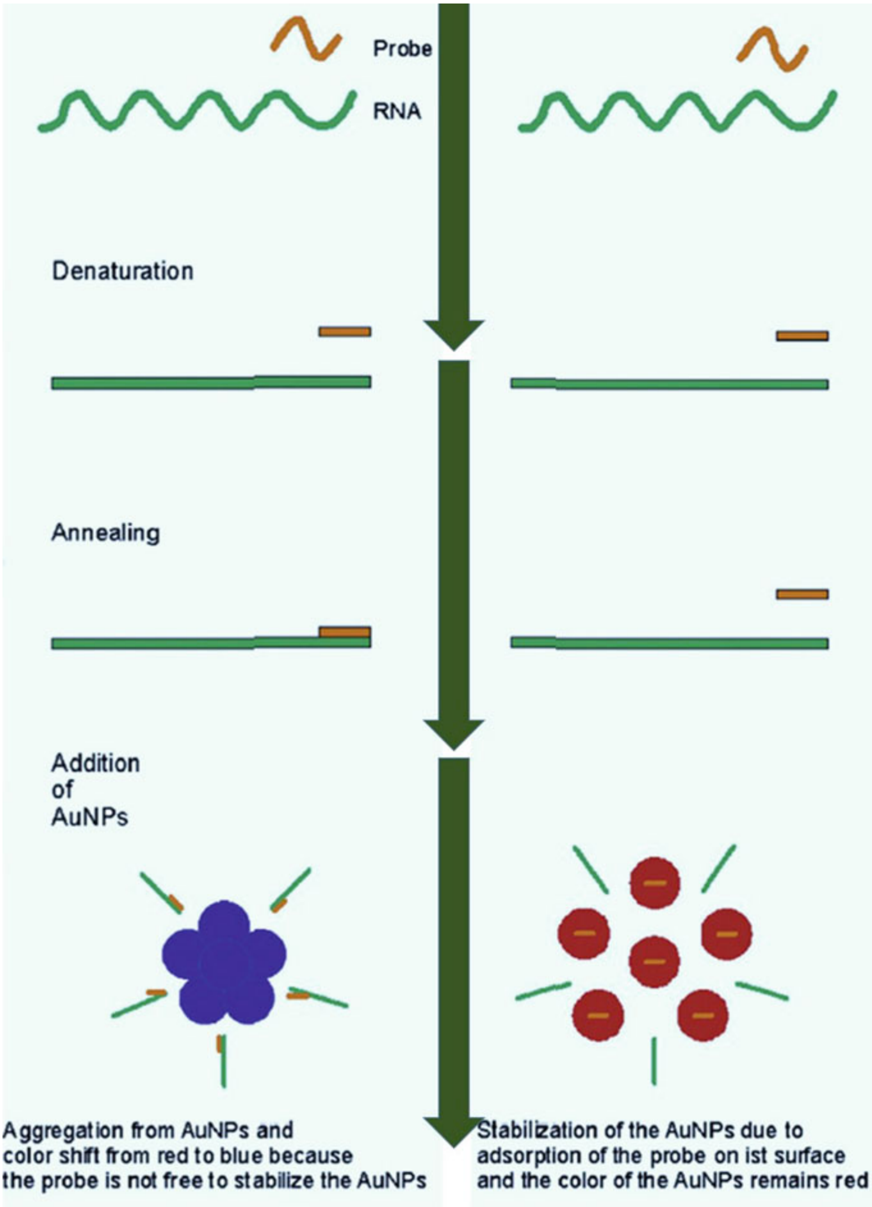

Fig. 20.8 Principle of direct detection of SVCV RNA using unmodified gold nanoparticles. Redrawn after Saleh et al. (2012)

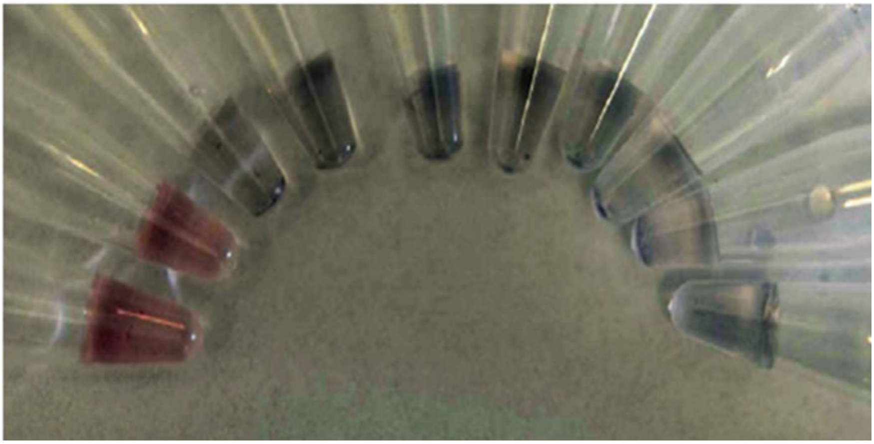

Fig. 20.9 Results of a gold nanoparticle-based assay for detection of SVCV: In a positive test, the color of the solution changes from red to blue. Adapted from Saleh et al. (2012)

pathogen progressive transmission and efficient replication for causing the infection. As infection proceeds, minor variations encounter in the pathogen natural environment, such as host genetics individual variations as well as the immunological response to infection (Alexander and Mewhinney 2008).

The nanotechnological formulation development for aquaculture application has been a major focus of research. An important characteristic of these systems is multiple application suitability, such as the administration of antibiotics, vaccines, nutraceuticals, and other pharmaceuticals (Rather et al. 2011b). Advantages typically include: enhancing the active agents' bioavailability through low absorption, sustained release of the active agent, decrease in application frequency, and high molecular level of dispersion, because of nanoscale size, that can be used in the disease treatment (Can et al. 2011; Bhattacharyya et al. 2015).

Conventionally, the active substance application is achieved either by injections or in the fish food, which can be associated with side effects because of excessive use and substantial losses during the process (Sheridan et al. 2013). The biodegradable nanoformulation production with essential oils can play a vital role in solving the disease control efficiency problems, as well as the contamination problems. The contamination can be decreased by reducing the used traditional chemical in disease control because nanoparticles can act as controlled released systems and thus sustain the biological active compounds amount to treat diseases. Many studies have recorded the synergism between using of essential oils encapsulated in biodegradable nanoparticles (Chifiriuc et al. 2017; Pavela et al. 2017; Sotelo-Boyas et al. 2017a, b).

Several of these studies can play a role in solving aquaculture real problems, where they evaluate the antifungal and bactericidal efficacy and so could be used in fish disease treatment. Recently, Rai et al. (2017) discussed the prospects and emerging trends of the synergism between antimicrobial potentials of essential oils and nanoparticles. Researchers described that the essential oil encapsulation in nanoparticles is a promising strategy where these systems can facilitate these compounds' application as antimicrobial agents. In addition, researchers cited that the active compounds sustained, and controlled release can improve the efficacy and bioavailability against multi-resistant pathogens. Moreover, nanodrugs as well as vaccines might be costless and more effective to prevent/ treat diseases than current technologies (Chen and Yada 2011).

Naturally, vaccination has an important role in fish farming. Nanoparticles use for fish vaccination became a vital criterion, particularly for the farmed fish. Nanoparticles have multifaceted advantages in the administration of drug such as vaccine delivery and improving the farmed fish protection against pathogens that caused diseases. However, the benefits accompanied with nanoparticle distribution may be associated with health and environment risks (Walker 2004). Naturally, polymeric nanoparticles have many advantages in vaccine delivery, such as sustained vaccine delivery, drug solubility for intravascular delivery, and vaccine antigen solubility improving against enzymatic degradation. In aquaculture, the nanoparticles used for vaccine delivery are an interesting possibility but still the precautionary principle needs to be the ideal of the best scientific practice for fish vaccination (Nielsen et al. 2011).

In general, salmon is vulnerable to diseases. Pathogens of fish transmit well in water as well as high stocking densities so the shorter the distances between farms the higher transmission occurs. An excessive antibiotic use may prevent bacterial diseases but again was not environmentally sustainable. The motivation trail for nanoparticle utilization in salmon vaccines relates to that oil-adjuvant vaccines have not been effective against some of intracellular pathogens (importantly viruses) as well as it is difficult to use a strong alternative adjuvant system. Moreover, oil-adjuvant vaccines have unwanted side effects in salmon such as autoimmunity, internal organ adhesion in the abdomen and, less frequently, the deformations of skeleton. Producers of vaccine are continuously trying application of oil adjuvant but with lesser side effects.

Recently, fish vaccine can be developed with poly(lactic-co-glycolic) acid (PLGA) which is a synthetic organic polymer. These particles maintain antigens from premature degradation, i.e., before producing an effective immune response. The PLGA particle degrades in vivo and so releases the antigens over time. The degradation rate of the particles can be prepared by changing copolymer composition as well as particle size, properties that the vaccine efficiency can be sensitive. Au-NPs also play a vital role in fish vaccine release.

Through chitosan nano, vaccine can be delivered. Chitosan nano is able to wrap around vaccines which act as a carrier and are used in nano-encapsulation in the fish physiology for treatment delivery. The bacterium *Listonella anguillarum* nano-encapsulated vaccines can be introduced in Asian Carp (Myhr and Myskja 2011; Rajeshkumar et al. 2009).

Nanocapsules containing nanoparticles can be used in mass vaccination of fish. These will be resistant to degradation and digestion. Nanocapsules contain short

DNA strand that are absorbed into the cells of fish when administrated to water containing fishes. For breaking the capsules, the ultrasound mechanism is used and then the DNA is released, thus improving an immune response to fish by the vaccination. Similarly, these vaccines' oral administration and the active agent site-specific release for vaccination will reduce disease management effort, the cost, drug, vaccine application delivery, etc., at the same feeding cost leading to sustainable aquaculture.

Polyanhydride NPs were used for vaccine antigen encapsulation, and release determines shrimp immunization with feed or via immersion (Ross et al. 2014). Moreover, for drug administration silica-based NPs can be suitable (i.e., pharmaceuticals or other therapeutics) due to the porous structure as well as high dose incorporation ability (Strømme et al. 2009).

Briefly, nanoparticles are applied as oral drug carriers for many reasons, like drug bioavailability improvement with poor absorption characteristics (Florence et al. 1995), residence time prolongation and drug digestive stabilization in the intestine, efficient absorption due to high dispersion at the molecular level, vaccine antigen delivery to gut-associated lymphoid tissue (Jani et al. 1990), and drug release control (Eldridge et al. 1990).

20.5.4 Antibacterial Activity of Metal-Based Nanoparticles

The most investigated nano-antibacterial agent is silver nanoparticle. They have multiple acting mechanisms against bacteria, and so they can evade the resistance of bacteria (Knetsch and Koole 2011), in contrast to antibiotics which have one acting mechanism only (Hindi et al. 2009; Antony et al. 2013). The silver ion (Ag+) release is one mechanism [287]. Ag+ binds to cell membrane proteins of bacteria causing membrane disruption, so leading to the bacterial cell's death (Lara et al. 2010). Intracellularly, Ag+ binds to nucleic acids and cytochrome, damaging them as well as inhibiting cell division (Huang et al. 2011).

Prakash et al. (2013) illustrated that silver nanoparticles possess high antibacterial efficacy against bacterial isolates that have multi-drug resistant. Silver nanoparticles' bactericidal effect has also been demonstrated against methicillin resistant *Staphylococcus aureus* (Ayala-Núñez et al. 2009). Silver nanoparticles synthesized using a reducing agent which was citrus limon juice demonstrated antibacterial as well as anti-cyanobacterial activity against *Edwardsiella tarda*, *S. aureus*, Anabaena and Oscillatoria species, respectively (Swain et al. 2014).

Umashankari et al. (2012) used mangrove *Rhizophora mucronata* leaf bud extract for AgNPs biological synthesis and then demonstrated antimicrobial effects against *Proteus* species, *Pseudomonas fluorescens,* and *Flavobacterium* species. These "green" synthesized silver nanoparticles' efficacy was the same as that of commercial antibiotics. In a study on juvenile shrimp *Fenneropenaeus indicus* infected by *Vibrio harveyi*, silver nanoparticle long-term treatment decreased mortalities by 71% at AgNPs high doses (Vaseeharan et al. 2010).

There is great interest to investigate gold nanoparticle antimicrobial effects, and this is related to that gold nanoparticles have low toxic effect to eukaryotic cells (Li et al. 2014a, b). Gold nanoparticles can interact with biological proteins as well as nonproteins, such as lipopolysaccharides (LPS), and have biological functions (Sumbayev et al. 2013). Au-NPs supporting zeolite have bactericidal effects against *Salmonella typhi* and *E. coli* (Lima et al. 2013). Functionalized Au-NPs inhibited resistant bacterial isolate growth (Li et al. 2014a, b). "Green" synthesized gold nanoparticles possess antibacterial activity against bacteria isolated from fish (Velmurugan et al. 2014).

Gold nanoparticles have three pathways to achieve their antibacterial effects. First way is through oxidative phosphorylation process interference with changing bacterial cell membrane, which leads to decrease in F-Type ATP synthase activity and a net decrease in both ATP synthesis and metabolism. The second path is via interference with tRNA binding to the two ribosome subunits. The third pathway is through enhancing chemotaxis (Cui et al. 2012).

Zinc oxide nanoparticles have antibacterial as well as antifungal effects (Gunalan et al. 2012; Swain et al. 2014). The antibacterial activity is related to damage the particles to the cell membrane of the bacteria and so makes the contents of the cytoplasm leak from the cell (Liu et al. 2009). In the fish medicine field, ZnO-NPs can inhibit Aeromonas hydrophila, *Vibrio* species, *Edwardsiella tarda, S. aureus, Flavobacterium branchiophilum, Pseudomonas aeruginosa, Citrobacter* spp., and *Bacillus cereus* growth (Swain et al. 2014). Ramamoorthy et al. (2013) studied the ZnO nanoparticles' antibacterial effects against *Vibrio harveyi,* and the results indicated a higher bactericidal effect of ZnO-NPs in comparison with bulk ZnO.

TiO_2-NPs had a bactericidal effect, when doped with magnetic Fe_3O_4-NPs against *Edwardsiella tarda, Streptococcus iniae,* and *Photobacterium damselae*, after light activation. Moreover, these particles can be applied for water disinfection, as pathogens of fish bind with the nanoparticles, and then be easily extracted from the water using a magnet (Cheng et al. 2009, 2011). However, Jovanovic et al. (2011, 2015) concluded that TiO_2-NPs could affect immune system of fish though inhibition of fish neutrophils antibacterial activity, and accordingly making fish liable to the infection and hence fish mortality increased especially in outbreaks of the diseases.

20.5.5 Antifungal Activity of Metal-Based Nanoparticles

Silver nanoparticles as an antifungal agent exhibited high inhibitory effects similar to the Amphotericin B (commercial antifungal) against Candida species (Sanjenbam et al. 2014; Mallmann et al. 2015). Silver nanoparticles' antifungal activity against dermatophytes was recorded (Kim et al. 2008). Gold nanoparticles' fungicidal activity was reported against Candida species. Au-NPs efficacy was related to their size, as the smaller the size of gold nanoparticles the higher their antifungal activity (Wani and Ahmad 2013; Ahmad et al. 2013).

In another interesting study, *Aeromonas hydrophila* was used for ZnO nanoparticle biological synthesis, and these nanoparticles had antibacterial activity against *A. hydrophila* (the same bacterium), *P. aeruginosa*, *Enterococcus faecalis*, *E. coli*, *Candida albicans,* and *Aspergillus flavus* as shown in Fig. 20.10.

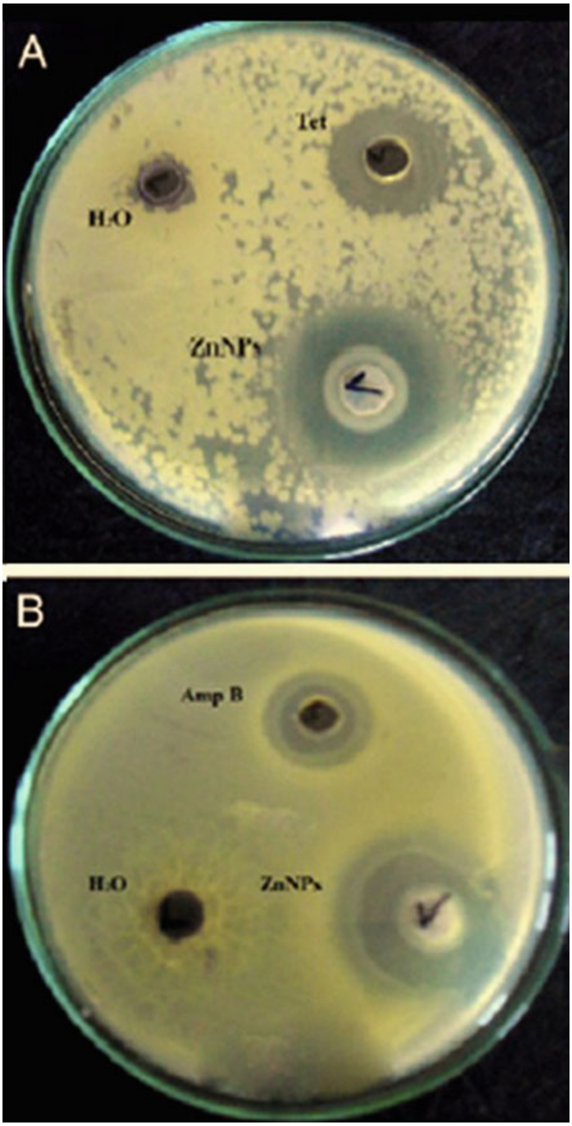

Fig. 20.10 Mueller-Hinton agar plates showing the antifungal effects of ZnO-NPs, and zones of inhibition against both **a** *Pseudomonas aeruginosa* and **b** *Aspergillus flavus*. Adapted from Jayaseelan et al. (2012)

20.5.6 Antiviral Activity of Metal-Based Nanoparticles

Silver nanoparticles also possess antiviral properties such as HIV-1 virus proteins binding activity in vitro (Elechiguerra et al. 2005), active against influenza A virus (both silver nanoparticle–chitosan composite and silver nanoparticles) (Mehrbod et al. 2009; Mori et al. 2013). In fish medicine, little work was published specifically on silver nanoparticles' antiviral and antifungal effects.

20.5.7 Gene Delivery

The new carrier system development for gene delivery acts as an enabling technology for many genetic disorders' treatment. However, a safe and efficient delivery vehicle formulation is considered a critical barrier for gene therapy success. Non-viral systems for delivery were proposed as promising alternatives to viral vectors, due to their stability, safety, and facility to be made in large amounts (Tomlinson and Rolland 1996). Some attitudes employ nanocomposites complexed with DNA containing peptide, protein, lipid, or polymeric carriers. Promising outcomes were achieved from the nanosphere complex formation between chitosan and DNA (Roy et al. 1997).

Ramya et al. (2014) showed the efficient protection of a DNA construct containing nodavirus gene which was extra small virus antisense (XSVAS) encapsulated with chitosan NPs increasing the survivability of *M. rosenbergii*. Nanomedicine in fish is in its infancy, and several gaps about the adverse effects to both target and nontarget species need to be addressed.

20.5.8 Fish Reproduction Control

In commercial aquatic animal artificial reproduction, incomplete vitellogenesis in females is considered one of the most vital and common problems, which is leading to final oocyte maturation as well as ovulation failure. So, there is a necessary need to develop methods for adequately controlling the reproductive process and so overcome this problem. Chitosan NPs can be used in a controlled way to carry and release endogenous hormone (Pulavendran et al. 2011). Rather et al. (2013) studied salmon hormone–chitosan–nAu as a trial to overcome the problem of the reproductive hormones' short life in blood, thus avoiding the multiple injection uses to enhance reproductive efficacy. The achieved results were that in treated organisms the reproductive hormones were present for a longer period in blood and both of the eggs' relative number and fertilization rate were significantly increased. Moreover, chitosan nanoconjugated salmon luteinizing hormone-releasing hormone

(CsLHRH) increased the level of expression to SOX9 transcripts in gonads as well as the levels of steroid hormonal in blood of *Clarias batrachus* female and male, moreover being helpful for proper development of gonad (Bhat et al. 2016).

20.6 Nanotechnology in Fish Feeding

Fish may suffer from nutritional deficiencies due to the improper balance, lack, or excess of food components. In aquaculture, the feed production is considered one of the most important applications of nanotechnology where NPs' use has proved to be effective for micronutrient delivery, growth promotion, and encapsulation of feed.

20.6.1 Micronutrient Delivery

In aquaculture research, an emerging area is nutraceutical use for fish health management, stress mitigation, and value addition in fish as well as shellfish. Despite nutraceutical low requirement, higher application cost is involved. Thus, wastage should be minimized for economic viability and efficient utilization of nutraceutical. Nanodelivery system development for these molecule kinds may address their application problems in aquaculture practices at the commercial level. There is a great opportunity for nanoparticle use to deliver nutraceuticals in feed of fish and nutrigenomics studies. Moreover, many feed nanoformulations help to maintain better consistency as well as feed taste.

Chitosan [poly(1,4-β-D-glucopyranosamine)] is a polysaccharide having antimicrobial potential with low toxicity and low immunogenicity that is being widely used for human and animal feed production (Rather et al. 2013; Luo and Wang 2013; Ferosekhan et al. 2014). Chitosan NPs novel applications for the unstable and/or hydrosoluble micronutrient delivery are in early development stages. Alishahi et al. (2014) showed that chitosan NPs use significantly increased vitamin C delivery as well as shelf life in rainbow trout after feeding for 20 days. Jiménez-Fernández et al. (2014) conducted a similar study for chitosan NPs application for ascorbic acid delivery (AA) in (i) the rotifer *Brachionus plicatilis* in vivo and (ii) zebra fish liver cell line (ZFL). NPs were able to penetrate intestinal epithelium of fish and significantly increase AA on both models. Rotifers that fed with AA-NPs had up to twofold increase of their AA levels comparing to the control groups.

20.6.2 Growth Promotion

Zinc (Zn) is an essential micronutrient involved in many metabolic pathways, and Zn is essential for the protein synthesis regulation, consumption of energy as well as lipid and vitamin A metabolism (Muralisankar et al. 2014). Faiz et al. (2015) investigated nZnO as a dietary Zn source evidencing improved immune response and growth in grass carp (*Ctenopharyngodon idella*). Muralisankar et al. (2014) showed that weight, antioxidant enzyme activity, and protein content increased significantly in freshwater prawn (*Macrobrachium rosenbergii*) after feeding for 90 days with nZnO improved feed.

Bhattacharyya et al. (2015) reviewed the use of nanomaterials (NMs) for growth induction in aquatic species by increasing the nutrients' proportion passing through the gut tissue and so into the organism rather than that passing across the digestive system and then excreted partially or totally unused. Ramsden et al. (2009) used $nTiO_2$ to growth performance improvement in rainbow trout (*Oncorhynchus mykiss*).

Selenium (Se) is an essential trace element for life and recently has been considered for animal nutrition in many case studies (Polettini et al. 2015; Sabbioni et al. 2015). Se is glutathione peroxidase enzyme (GSH-Px) component (Rotruck et al. 1973), which maintains the cell membrane through reduction of glutathione. Se can be supplemented through diet (Wang et al. 2013; Ilham and Munilkumar 2016); moreover, Se NPs are gaining a great attention due to its antioxidant defense properties and bioavailability (Sonkusre et al. 2014). Prussian carp (*Carassius auratus gibelio*) fed nSe supplemented diets; results showed FCR reduction with increase in final weight, muscle protein content as well as liver and blood plasma GSH-Px activity (Zhou et al. 2009). Additionally, Wang et al. (2013) stated that nSe caused increase in LDH, GSH-Px, Na^+/K^+-ATPase, cellular protein contents, and superoxide dismutase (SOD) in crucian carp (*C. auratus gibelio*); moreover, these effects were related to both dose and NPs size. Deng and Cheng (2003) reported that nSe significantly improved Nile tilapia (*Oreochromis niloticus*) growth at moderate and high Se NPs doses (0.5 mg/kg), (2.5 mg/kg), respectively, via spiked feed presenting 86.3 ± 4.7 g weight gain rate.

20.6.3 Nano-encapsulation in Fish Feed

During the direct feed administration to water, feed nutrients can be released to water from feed pellets. These nutrients can easily degrade during contact with water. Chitosan NPs can be applied as an encapsulating agent (Chatterjee and Judeh 2016; Ji et al. 2015).

Essential oils (EOs) are volatile compounds and complex mixtures (mainly monoterpenes, benzenoids, and sesquiterpenoids, others) produced by different plant species like bushy matgrass (da Cunha et al. 2010; Becker et al. 2011), mentha (Danner et al. 2011; Roohi and Imanpoor 2015; Pedrazzani and Neto 2016), cloves (Perdikaris et al. 2010; Javahery et al. 2012), and others (Benovit et al. 2012; Parodi et al. 2013; Silva et al. 2015). Essential oils' (EOs) pharmacological properties include antioxidant, anti-inflammatory, and anticarcinogenic effects. Moreover, they have biocidal activity against many organisms such as bacteria, viruses, fungi, insects, and nematodes (Raut and Karuppayil 2014; Baser and Buchbauer 2015; Calo et al. 2015). In the aquaculture industry, EOs have great potential for use due to their attractive characteristics such as low cost, ready availability, low risk of side effects, low toxicity as well as high biodegradability comparing to antibiotic use and the risk of pathogen drug-resistant strain emergence challenge (Wei and Wee 2013; Anusha et al. 2014; Acar et al. 2015; Malheiros et al. 2016). EOs can also assist in the fish diets because they can improve the functioning and development of the digestive tract, especially in the early stages of fish development, preventing pathogen adherence to the intestinal mucosa, accelerating the glucose absorption, and stimulating the digestive enzyme secretion (Freccia et al.,2014; Hernandez et al. 2016; Zeppenfeld et al. 2016).

In aquaculture, anesthetics have been used to stress reduction in fish. Best anesthetic selection depends on several factors including availability, cost, physical conditions as well as the operative safety. Many anesthetics have not been used in aquaculture, due to toxicity and undesirable side effects (Zahl et al. 2011). As an alternative pathway, some essential oils have anesthetic activities in fish with greater biodegradability and reduced toxicity, compared to synthetic chemicals.

However, despite essential oils' (EOs) useful properties, some important limitations for aquaculture applications are present. Unfortunately, as in other aromatic and lipophilic compounds, EOs have low water solubility so their application in ponds, as well other aquatic media, is difficult. Other disadvantages of EOs are their high light sensitivity, low stability, and strong organoleptic characteristics, e.g., aroma and flavor (Turek and Stintzing 2013). Therefore, new approaches are required for EOs applications and nano-encapsulation is a technique that can improve their properties (Ghayempour and Montazer 2016).

Many studies of essential oil encapsulation by using several carrier systems, such as chitosan nanoparticles (Hosseini et al. 2013; Esmaeili and Asgari 2015; Hu et al. 2015; Mohammadi et al. 2015), zinc nanoparticles (Parris et al. 2005; Wu et al. 2012; Zhang et al. 2014a; da Rosa et al. 2015); cyclodextrins (Ciobanu et al. 2012; Hill et al. 2013; Siqueira-Lima et al. 2014; Abarca et al. 2016), polymeric nanoparticles (de Oliveira et al. 2014; Christofoli et al. 2015; Liakos et al. 2016); nanotubes (Lee and Park 2015; Kim et al. 2016); and solid lipid nanoparticles (Lai et al. 2006; Feng 2012; Moghimipour et al. 2013; Cortes-Rojas et al. 2014). Addition of single-walled nanotubes of carbon, C60, or $nTiO_2$ to rainbow trout food changed fish pellet physical properties and resulted in reduction of nutrients' leaching and their subsequent waste in fishpond (Ramsden et al. 2009).

20.7 Nanotechnology and Seafood

Fishing lures used to catch fish are painted to attract fish attention by light reflection. But conventional lures reflect light in one direction only. To overcome this problem, the lure surface is colored and then nanocoated with a polyimide film that enhances fish-catching chance 2–3 times comparing to a lure without using a polyimide coating. Before fish processing, Walha et al. (2008) applied the nanofiltration as well as reverse osmosis processes to reduce drilling water salinity

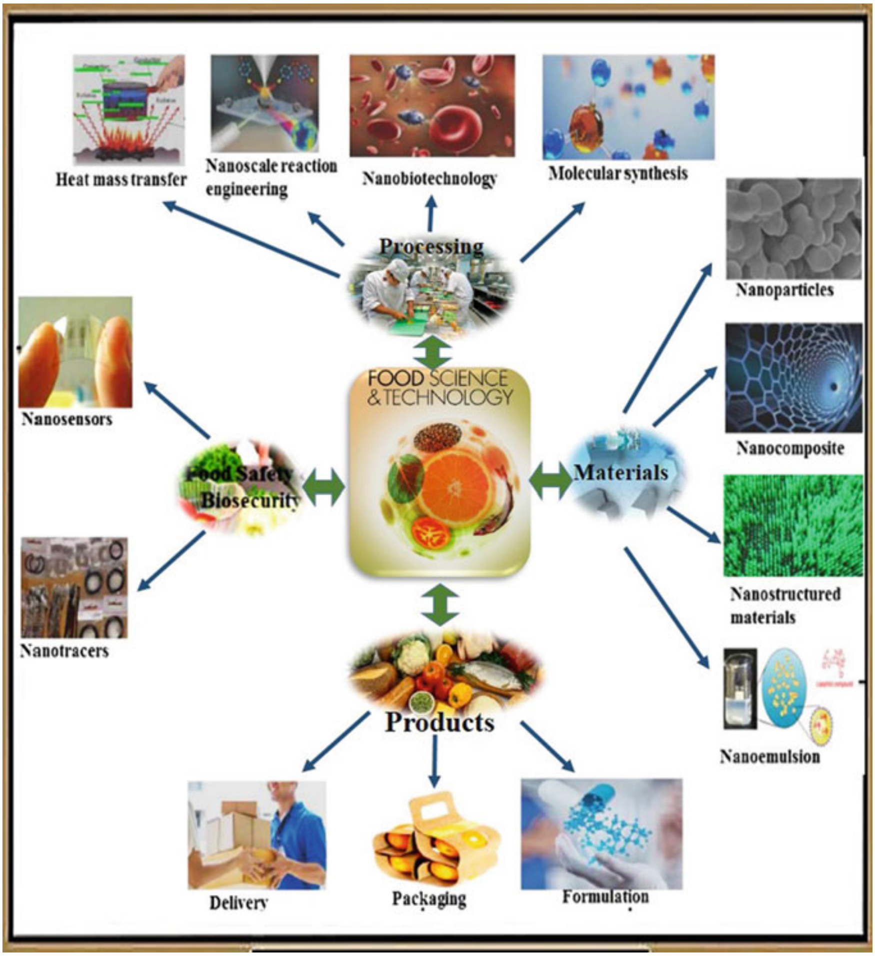

Fig. 20.11 Different steps of food management that involve several steps (processing, packaging, and preservation) and these aided by nanotechnology with the assistance of several nanomaterials. Redrawn from Thiruvengadam et al. (2018)

that is used in washing and processing of seafood. As pretreatment step, nanofiltration was used before the process of thermal and membrane seawater desalination.

Three different commercial ultrafiltration/nanofiltration (UF-NF) membranes XP117, MT03, and MT44 (cutoffs ranging between 200 and 4000 Da) are examined using a tangential flow filtration cell. The results detected that the MT03 membrane effectively removes the natural organic matter and reduces the concentration of sulfate in drilling water. Nanofiltration makes the water quality standardization possible (Fig. 20.11).

20.7.1 Harvested Fish Preservation and Packaging for Commercialization

Nanotechnology can have a vital role not only to fish production but also to the fishery product marketing, especially because of the widespread need to increase product shelf life. Hence, in the food packaging sector there have been great intensified research efforts (Omodara 2015).

Due to growing environmental concerns, the biopolymer use in the food industry has grown. However, this sector is still facing important problems related to the high costs of production and low performance, in comparison with synthetic polymers (Robertson 2016). Many scientific studies have shown that nanomaterial incorporation in biodegradable food packaging can improve the materials' thermal and mechanical properties and so can increase the performance.

Furthermore, new properties can be provided by nanomaterials, such as oxygen elimination, antimicrobial activities, antifungal activities, protection against degradation, and enzyme immobilization, hence contributing to stored products' better stability (de Azeredo 2009; Peelman et al. 2013; Rhim et al. 2013; Reig et al. 2014; Jiang et al. 2015; Kuswandi 2016). The whole nanoparticle use concept is based on their properties which are improved stability, penetrability, reactivity, surface area and strength, their mechanical, catalytic, optical, and quantum properties. The nanoparticles size allows their use in new or more efficient physical and chemical reactions comparing to larger-scale materials (Cockburn et al. 2012; Sastry et al. 2013; Ramachandraiah et al. 2015).

These useful properties will explain why nanotechnology should be implemented in food packaging. Nanopackaging can be classified to "improved" nanopackaging, which is used to improve the properties of packaging (e.g., mechanical and barrier properties); "active" nanopackaging, which allows interaction with the environment and food by absorbing or releasing substances from or into the packaged food; and "intelligent" or "smart" nanopackaging, which allows condition monitoring of the food surrounding environment or packaged food (Chaudhry and Castle 2011; Silvestre et al. 2011; Baltic et al. 2013; Prasad et al. 2017). Ramezani et al. (2015) studied chitosan and chitosan NPs effect on fillets of silver carp stored at 4 °C, and results showed that chitosan NPs had an interesting antimicrobial activity as well as inhibition ability of the total volatile nitrogen (TVB-N) content for improving a product general storage potentiality.

20.7.2 Nanobarcode and Tagging Technology

In our daily life, tags of identification have been applied in wholesale livestock and agriculture products. Due to nanoparticles' small size, nanoparticles have been used in many fields ranging from agricultural encoding to advanced biotechnology. Nanobarcodes (>million) have been applied in general encoding and multiplexed bioassays due to their ability to form a large combination number which make nanobarcodes attractive for this purpose.

The optical microscope and UV lamp are used for micrometer-sized glass barcode identification. These barcodes are formed by doping with rare earth containing different fluorescent material patterns of a specific type. The particles to be used in nanobarcodes should be machine-readable, durable, easily encodable, submicron-sized taggant particles.

For these nanobarcode particles' manufacture, the process is highly scalable and semiautomated, involving the inert metals (silver, gold, etc.) electroplating into templates defining particle diameter and then release of the resulting striped nanorods from templates. There are biological and non-biological applications for these nanobarcodes (Mathew et al. 2009). A major challenge for researchers is cost for nanobarcode technology development which can be concluded on the fact that only 18 documents are published on Scopus indexed article database (SIAD). Out of 18 articles, conference paper (six in number), notes (one in number), review article (two in number), and nine research articles were only available in last ten years by "nanobarcode" as keyword (SIAD 2014).

Similarly, with the same keyword only 32 articles are published in SciFinder® database. None of these articles were found for 2014; but only one article was found by Han et al. (2013). Similarly, three articles have been only found for 2012, but none of these articles have described nanobarcode application in agricultural field (SciFinder 2014). This shows that nanobarcode technology development for agricultural application is needed to be one of the thrust areas.

A nanobarcode is a monitoring device composed of metallic stripes containing nanoparticles as striping variations provide the encoding information method. By nanobarcoding incorporation, exporters and processing industry can monitor their aqua product source or track the delivery status until this product reaches the market. Further, coupled with nanosensors in addition to "synthetic DNA tagged with color-coded probes", nanobarcode device could monitor temperature change, detect pathogens, etc., thus improving the product quality.

Nanobarcodes have been utilized as ID tags for gene expression multiplexed analysis and intracellular histopathology. Plant resistance improvement could be achieved, against various environmental stresses as salinity, drought, diseases and others, through biotechnology advancement at the nanoscale. Soon, through nanotechnology advancement based on gene sequencing more effective utilization and identification of plant gene trait resources may introduce cost-effective and rapid capability (Branton et al. 2008). Nanobarcodes can also be used to detect pathogens from food products, and this is cost-effective (Han et al. 2013).

Nanobarcodes could also be used in many non-biological applications as -authentication or tracking in husbandry and agricultural food products. This technology will enable the development of new auto-ID technologies for item tagging that were previously not tagged with conventional barcodes (Branton et al. 2008). The entire chip can be nearly at a dust mite size—closer to microscale than nanoscale. Technology developers envision a world where any object anywhere can be automatically identified.

Radio-frequency identification (RFID) is "a chip with a radio circuit incorporating a nanoscale component with an identification code embedded in it". These tag characteristics as can be scanned from a distance hold more information as well as identify any object, anywhere, automatically as they can be embedded in the product. RFID tag may be used in juvenile fish. RFID tag can be a tracking device and a device for monitoring fish swimming pattern, metabolism, and fish feeding behavior. Moreover, a possible benefit is to permit fish processing plants to identify fish source by incorporating the "nanobarcoding" as information management system part. This technology may also be used by exporters of the whole fish for tracking the delivery status of tagged fish. RFID nanotag advantages include memory storage capacity to product identification number, product cost, price, manufactured date, characteristics, and location as well as inventory on hand. The fresh food traceability, such as fish, has become an important challenging in order to keep consumer's safety and freshness. Although this technology application on finfish aquaculture is yet to be studied, however the previously mentioned potential benefits could prompt industry participants for exploring this opportunity further (Rather et al. 2011a, b).

There are numerous bacterial diseases affecting humans such as tetanus, diphtheria, typhoid fever, cholera, syphilis, food-borne illness, tuberculosis, and leprosy caused by different bacterial species. As a remedial process, there is a need to detect bacteria, and so, dye staining method is used. For bacterial staining, organic dyes are the most commonly used biolabels, but their fluorescence degrades with time and they are expensive. So, there is a need to find economical and durable alternatives. Quantum dot fluorescent labeling with biorecognition molecules has been detected through the recent developments in luminescent nanocrystal field. Quantum dots are much better than conventional organic dyes because of their more efficient luminescence, excellent photostability, narrow emission spectra, tenability, and symmetry according to the sizes of particle and material composition.

By a single excitation light source, all colors quantum dots can be excited because of their broad absorption spectra (Warad et al. 2004). NPs biolabeled bacillus bacteria consist of ZnS and Mn^{+2} which capped with biocompatible "chitosan" that gave an orange glow when viewed by fluorescence microscope. For *E. coli* O157:H7 detection, quantum dots were utilized as a fluorescence marker that was coupled with immune magnetic separation (Su and Li 2004). For *E. coli* O157 detection, the magnetic beads were coated with anti-*E. coli* O157 antibodies for selective attachment to the target bacteria and with biotin-conjugated antibodies of anti-*E. coli* for sandwich immune complexes formation. Quantum dots were labeled by the immune complexes through biotin–streptavidin conjugation after the magnetic separation.

Another nanotechnology application possibility in seafood is using of different packaging and conservation techniques for providing food safety via delaying spoilage through enzymatic and microbial activities. Nanocomposite films are utilized mostly to foods with active/intelligent packaging (antimicrobial films) combination and edible film/coating technology. Nanocomposite films are formed from natural biopolymers, like polysaccharide, lipid, and protein. These sources are better packaging material than petrochemical-based plastics, and this is related to that they are environment-friendly, edible, and anticancerogenic (Dursun et al. 2010).

There are many studies that demonstrate the formulation potential based on biopolymers for using in aquaculture and the fishing industry (Borgogna et al. 2011; Alboofetileh et al. 2016; Joukar et al. 2017). Alishahi et al. (2014) reported the advantages of using chitosan-based nanocomposites in aquaculture and seafood industry; the usage of chitosan-based nanocomposites, as well as other

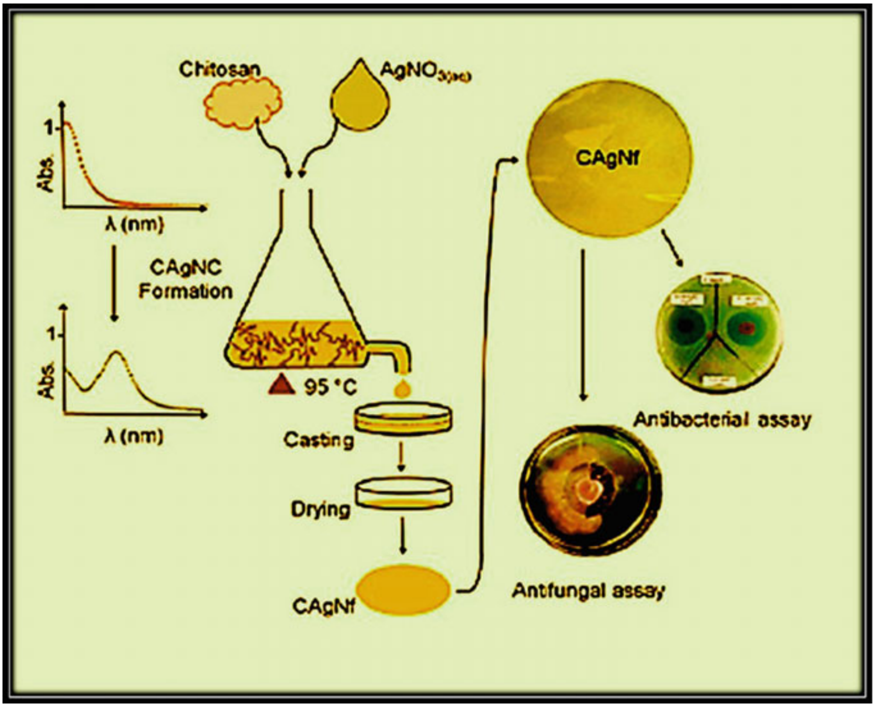

Fig. 20.12 Illustration of procedure for examining chitosan/$AgNO_3$ composites as antimicrobial film. Adopted Dananjaya et al. (2017)

biopolymers, could solve the problems faced in this sector, during a short-term period. Dananjaya et al. (2017) produced "silver nanoparticle (AgNP)-embedded chitosan films" that have antimicrobial properties. Results of antimicrobial experiment clearly detected that CAgNfs can inhibit the fish pathogenic bacteria growth such as *Vibrio tapetis*, *Vibrio* (Aliivibrio) *salmonicida*, and *E. tarda* and fungi growth such as *Fusarium oxysporum*. Moreover, CAgNfs significantly decreased the experimentally exposed levels of *V. salmonicida* in artificial seawater, and so there was a suggestion of using these CAgNfs to develop antimicrobial filters/membranes to purify water units and eliminate the pathogenic microbes (Fig. 20.12).

The emulsion is the result of mixing two or more liquids which do not readily combine together such as water and oil (Ravichandran 2010). Nanoemulsions are "liquid-in-liquid dispersions with droplet sizes on the order of 100 nm". Nanoemulsions are highly stable in comparison with conventional emulsions. Moreover, nanoemulsions cannot be easily separated by using either gravitational forces or aggregation of the droplets due to the small size of the particle and droplets coating with surfactants that prevent the droplets from coalescing because of interfacial repulsion, respectively.

In the food industry, nanoemulsions are applied to ice creams, salad dressings, etc. With the small size particle, the products are creamier, are very easily transported through the digestive system epithelium, and therefore enhance the component adsorption better than other emulsions. Nanoemulsion has several activities against microorganisms either pathogenic or nonpathogenic (MNIMBS 2010). Nanoemulsions are commonly utilized for the phytochemical's delivery (Pradhan et al. 2015). Joe et al. (2011) tested the sunflower oil-based nanoemulsion (AUSN-4) influence on the quality and shelf life of steaks stored at 20 °C of Indo-Pacific king mackerel "*Scomberomorus guttatus*".

Among metal nanomaterials, Ag is the most promising one as it has both viricidal and bactericidal activities due to reactive oxygen species production that cleaves DNA as well as can be used for a wide range of applications in addition to other properties such as low toxicity, its charge capacity, ease of use, crystallographic structure, high surface-to-volume ratios, and adaptability to various substrates (Nangmenyi et al. 2009; Chen and Yada 2011; Faunce et al. 2014). Recently, researches have been tried to vary gold and silver nanoparticles' size with simple approaches, i.e., changing the reactant concentration. The improved anticancerous and antimicrobial activities were observed (Sireesh et al. 2015, 2017; Dasgupta et al. 2016a; Jain et al. 2016; Shukla et al. 2017; Siripireddy et al. 2017; Tammina et al. 2017). Recently, trends are changing in computational and silico as well as in vitro approaches for inorganic nanoparticle toxicity evaluation at biomolecular level (Ranjan et al. 2015, 2016a; Dasgupta et al. 2016b; Jain et al. 2016).

Fresh Box® is a developed antimicrobial container for food; it is produced by unique nanotechnology using fine polymers and shows tremendous properties against numerous fungi and bacteria due to its contents from finely dispersed Ag nanoparticles. Also, Rai and Bai (2018) reported a wide variety of "nanoproducts"

Fig. 20.13 Examples of some commercialized nanosilver-based products

achieved good sellers in markets, and these products contained metallic nanoparticles (Fig. 20.13).

20.7.3 Biosensors and Electronic Nose

Microorganisms produced various characteristic volatile compounds that are either useful or harmful to human beings; for example, yeasts' use makes fermentation while when bacteria eat sugar alcohol is produced as a by-product. Bacteria are the most common food rotting causal organisms. Foul odor is a clear indication of food degradation that may be detected by nasal and visual sensation; this sometimes may be impractical as well as for further causes of poisoning. Therefore, for these odors' detection it is more sensible to utilize an instrument as rapid detection biosensors (Compagnone et al. 1995).

The nano-biosensors' future applications were recently developed by Zhang et al. (2014b, c), in fields other than agriculture and food nano-biosensor area. Several sensors have been developed due to its importance; a review on this has been prepared by Rocha-Santos (2014).

20.7.3.1 Rapid Detection Biosensors

These instruments can reduce the time required for immunoassays and lengthy microbial testing. These instruments' applications include contaminant detection in different bodies such as food products, raw food materials, and water supplies (Compagnone et al. 1995). Recently, nano-biosensors are developed for IgG and metabolites' rapid detection (Turkoglu et al. 2013; Labroo and Cui 2014).

20.7.3.2 Enzymatic Biosensors

Enzymes are very specific for certain biomolecule attachment so they can act as a sensing element. According to Patel (2002), enzymatic biosensors on the immobilization surface basis are classified into controlled pore glass beads with optical transducer element, polyurethane foam with photothermal transducer element, ion-selective membrane with either potentiometric or amperometric transducer element, screen-printed electrode with amperometric transducer element. Regarding microbial contamination, an electrogenerated chemiluminescence immunosensor was developed to detect *Bacillus thuringiensis* using Fe_3O_4-Au nanoparticles (Li et al. 2013). An optical fiber-based micro-analyzer in aquaculture was used to measure fish volatile amine levels. This has future aspect for developing such nano-biosensor instead of micro (Silva et al. 2010).

Electronic nose is an instrument based on the human nose operation and is applied for odors' different types of identification; electronic nose uses a response pattern across a gas sensor array. Electronic nose can identify the odorant, find the odor characteristic properties, and estimate the odorant concentration in the same way as that of the human nose. It is mainly composed of gas sensors which consist of nanoparticles and ZnO nanowires, for example (Hossain et al. 2005; Sugunan et al. 2005). ZnO nanorods are utilized to develop electronic nose that can detect the impurities from vapor mixture (Ko et al. 2013). Their resistance changes with certain gas passage and generates a characteristic change in electrical signal which forms the fingerprint pattern for detection of gas. This pattern is applied to determine the type, quantity, and quality of the detected odor. An improved surface area is also present that helps in better gas absorption.

20.8 Safety Concerns of Nanotechnology in Aquaculture

Nanotechnology use can revolutionize the aquaculture industry (Fig. 20.13), but further research is still a vital need for an effective and viable commercial implementation of this technology (Fig. 20.14). Some of the main problems which should be addressed are cost analysis, the scalability of these systems, and the possible environmental impacts (Fig. 20.15). Aquaculture and fishery industries

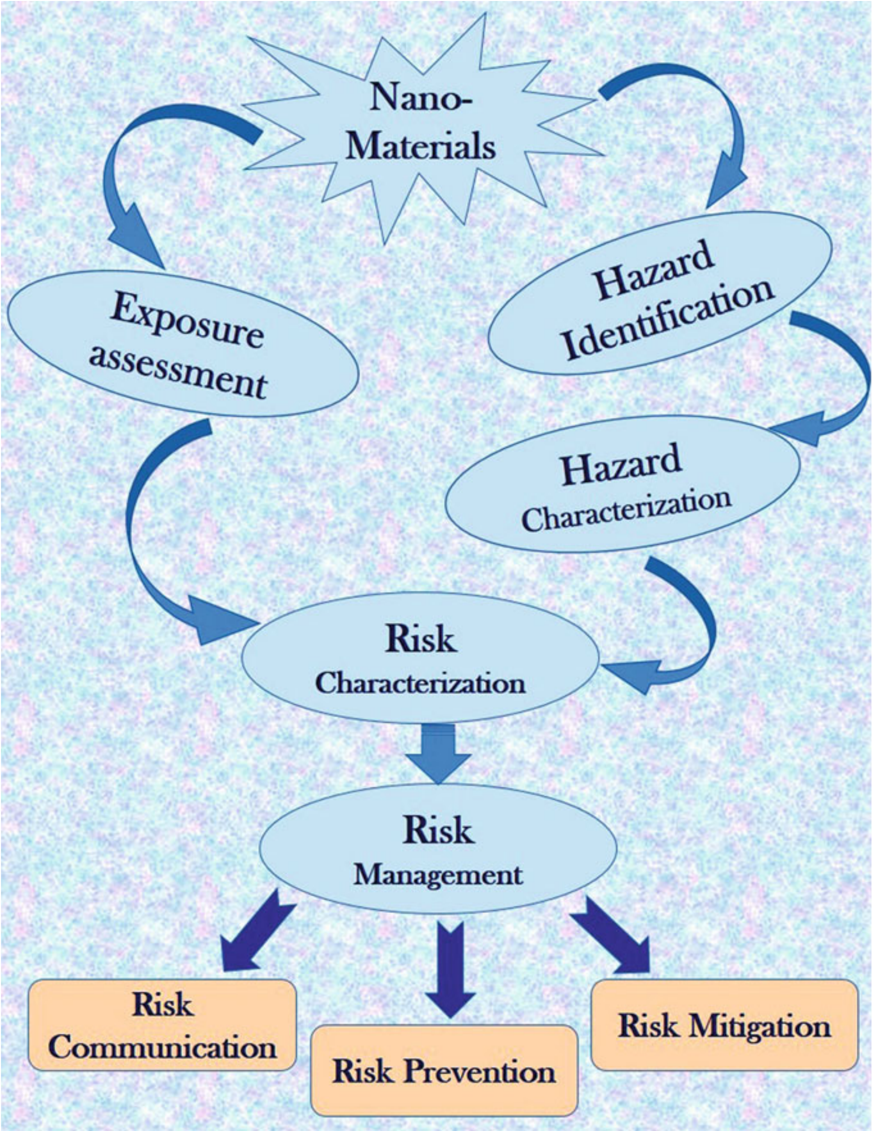

Fig. 20.14 Toxicological aspect of nanomaterials on humans, animals, environment, and whole ecosystem. Diagrammatic representation of nanotoxicological analysis. Redrawn after Dasgupta et al. (2017)

will have to absorb the new technologies to move forward. Moreover, safe use and the rational of nanotechnology will contribute to progress.

When it comes to nanotechnology application in the industrial scale, it is very important to estimate the subsequent exposure levels to these materials and to

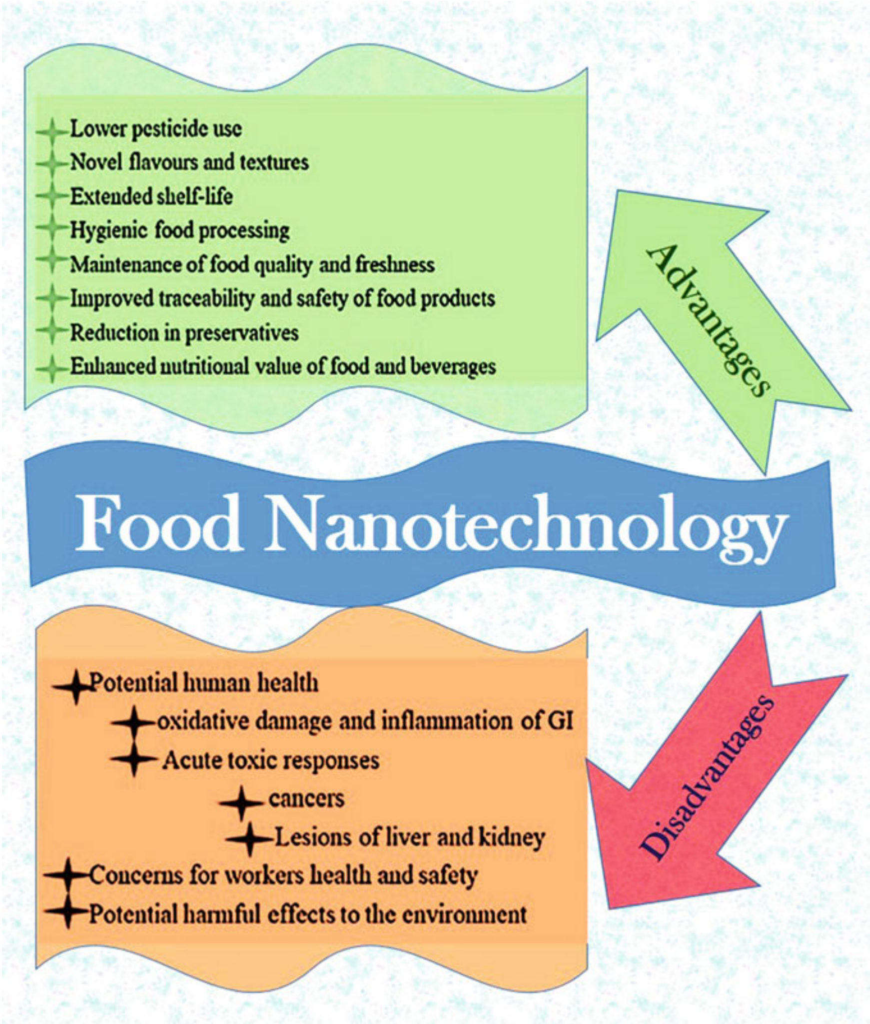

Fig. 20.15 Benefits and risks of nanotechnology applications in food and related products. Redrawn from Thiruvengadam et al. (2018)

evaluate nanoparticle release into the environment because nanoparticles can easily penetrate organ and organelles of the human, so exposure concentrations, exposure time, immune response, sites of penetration as well as nanoparticle retention and accumulation in body and so their subsequent effects should be evaluated carefully.

Even though the research regarding nanotechnology application is growing every day, in the naturally occurring nanosystems still insufficient scientific examination is available. Treated water and/or nanomodified agricultural products' compulsory testing should be performed before they are introduced into the market.

Standardized test procedures are required for studying nanoparticle impacts on living cells to evaluate the risks on human exposed to nanoparticles. Nanoparticle toxicology is poorly understood due to the lack of validated test methods as well as the inconsistency in the reported information. The inconsistency in the reported data is related to nanoparticles' improper characterization and the nanoparticle interferences in the available test system.

Hence, the policy makers and the regulatory bodies should provide the guidance document to safe uses, the validated protocols, and the nanoparticle disposal. The understanding of the nanoscience and nanotechnology safe application in water quality management and agri-food will help in the "nano-agri-technology" sustainable growth. Regulatory and government authorities (regulatory agencies and certification bodies) as well as health, environmental, and safety councils (such as environmental health services), scientific authorities, and non-governmental organizations, all over the world, detected the nanotechnologies' risk assessment importance (Vyom et al. 2012) and have given their own view, suggestion, and guidance (RS/RAE 2004; USEPA 2007; SCCP 2007; Scenihr 2009; Dhawan et al. 2011). The same nanomaterial, with a different length, diameter, surface modification, and crystal structure, will have different toxicities (Fig. 20.14).

20.8.1 *Effects of Nanotechnologies on Human Health*

There are four pathways where nanoparticle may enter the body of human: swallowing, inhalation, skin absorption, and deliberate injection during medical processes (or release from implants). Due to the fact that nanoparticle diameter is extremely small, once these nanoparticles have entered the human body, nanoparticles have a high mobility degree. So they can pass the blood-brain barrier in some cases.

The potential nanoparticles danger to human and animals do not allow neglect. University of Missouri (USA) researchers detected in their recent study nanoparticle residue in fruits can enter the human body. So, it also can get into the liver, spleen, heart, brain, etc., vitals, through lymphatic and blood system. It was demonstrated that the residue of nanoparticle is very difficult to be removed by common rinsing methods. Therefore, they appeal that in food wrap papers, nanotechnologies should be carefully used.

20.8.2 *Effects of Nanotechnologies on Fish Health and Aquatic Environment*

The nanoparticle toxicity has focused in mammalian models on the respiratory exposure and the human health implications (Handy and Shaw 2007). With the nanotechnology's rapid development, there is an increasing risk for human and environmental exposure to the nanotechnology-based materials and products. Water resources are particularly vulnerable by nanotechnologies' direct and indirect contamination, and the nanotechnologies' environmental implication and potential toxicity to aquatic organisms must be evaluated (Wang et al. 2008). TiO_2 nanoparticle suspension stability in water had been investigated (Hao et al. 2009). One hundred and 200 mg/L TiO_2 nanoparticle resulted in statistically significant reduction in superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities and significant increase in levels of lipid peroxidation (LPO) in tissues, suggesting that fish exposed to TiO_2 nanoparticles two concentrations suffered from the oxidative stress.

A comprehensive toxicity assessment included the modified acute (72 h) as well as the chronic (21 days) toxicity tests, and accumulation analysis of TiO_2 nanoparticles using a model organism such as *Daphnia magna* was conducted (Zhu et al. 2010a, b). The results showed that TiO_2 nanoparticle within the traditional 48-h exposure time exerted minimal toxicity to daphnia, but when the time of the exposure was extended to 72 h TiO_2 nanoparticle caused high toxicity. This demonstrates that the duration of the exposure may be a contributing factor in mediated toxicity of nanoparticle. Moreover, upon TiO_2 nanoparticles' chronic exposure for 21 days, daphnia displayed severe mortality and growth retardation in addition to reproductive defects. TiO_2 nanoparticle and quantum dot potential toxicity using the unicellular green alga "*Chlamydomonas reinhardtii*" were assessed (Wang et al. 2008).

The growth kinetics showed that inhibition of growth occurred in the first two to three days of cultivation in TiO_2 nanoparticle or quantum dots' presence. Moreover, quantum dots were more toxic than TiO_2 nanoparticle to Chlamydomonas cells under experimental conditions. These results indicate a potential risk of TiO_2 nanoparticle released to the aquatic environment. The different aqueous nanotechnology suspensions, such as nZnO, $nTiO_2$, C_{60}, nAl_2O_3, single-walled carbon nanotubes, and multi-walled carbon nanotubes, can inhibit the algae growth *Scenedesmus obliquus* (*S. obliquus*) as well as prevent movements of *D. magna* and can lead to death. However, several nanomaterial toxicities are not similar to each other (Wang et al. 2008).

According to six types of nanomaterial EC values on *S. obliquus* growth at 96 h, the toxicity order was as follows: "nZnO > C60; TiO_2, multi-walled carbon nanotubes and single-walled carbon nanotubes > nAl_2O_3". While according to the EC values on *D. magna* movement inhibition at 48 h, the toxicities order of the nanomaterials aqueous suspensions of six types were as follows: "nZnO > single-walled carbon nanotubes > C60; multi-walled carbon nanotubes > $nTiO_2$ > nAl_2O_3".

The effects of Ag nanoparticle's different particle sizes (61, 25, and 5 nm) on growth of ryegrass, biomass, and seedling height were investigated (Yin et al. 2011); the smaller the size of the nanoparticle, the stronger its toxicity was. Yang et al. (2010) investigated single-walled carbon nanotubes' different lengths of

(<l, 1–5, and 5 m) effects on the bacteria inhibition such as *Salmonella typhimurium,* and results showed that the single-walled carbon nanotubes' antibacterial ability increased with the increase in their lengths.

Khosravi-Katuli et al. (2017) summarized the various NPs information in addition to the target organisms of aquaculture interest and the main relative testing conditions. However, in the aquaculture industry there may be some concerns about development activities and research, for example, in situations where researchers were preparing new NMs containing feed formulations in the laboratory. The occupational exposure to NMs at the laboratory has been investigated (Demou et al. 2009; Tsai et al. 2009). The evidence suggests the routine procedures with manufactured NMs few grams, such as stirring and sonication, when normal precautions are applied in the laboratory (e.g., gloves, use of a ventilated fume cupboard, or a suitable dust mask).

It is worthy to mention that for routine research activities the normal precautions in the laboratory should be followed but personal protective clothing will not be needed, but of course, "researchers are required to carry out chemical risk assessments for NMs before they start work, as with any other laboratory procedure".

Surface inputs could be atmospheric deposition and coastal pollution. The seawater pH (typically pH 8) and, moreover, high ionic strength will enhance aggregation processes, so NPs may precipitate onto either coastal or deep ocean sediments (Fig. 20.16). However, with depth chemistry will change. Diatoms and

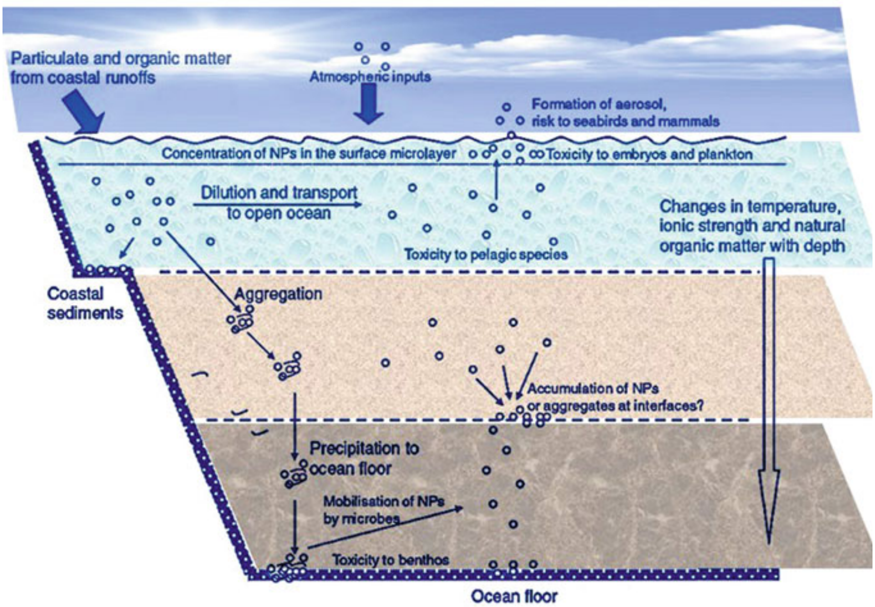

Fig. 20.16 Schematic diagram outlining the possible fate of nanoparticles (NPs) in the marine environment and the organisms at risk of exposure. Redrawn from Klaine et al. (2008)

microbes can remobilize the accumulated NPs on/in sediments. It is unclear whether NPs will accumulate in the ocean current mixing zones, there is a risk to organisms which feed at these interfaces. Some manufactured NPs may have surface activities that permit them to still disperse in saline conditions, and these NPs could accumulate because of surface tension effects and viscous properties at the ocean surface microlayer with consequent risks for planktonic organisms and larvae in the surface microlayer.

Ecotoxicity information so far suggests low acute toxicity by manufactured NMs to aquatic species, and so aquaculture systems and fisheries' immediate threats may be very small. However, on low-level exposure chronic studies are needed using realistic environmental scenarios to determine nanotechnology long-term impact on the environment, which must be balanced against nanotechnology benefits in water purification and environmental remediation technologies.

The NMs behavior and colloid chemistry suggest that NMs are likely to be precipitated in the water column and this will be particularly associated with sediments underneath fish cages. However, for the industry, this is not a new issue, and aquaculture systems under caged benthic environments are monitored for biodiversity and pollution (Carroll et al. 2003). Public engagement will be important to maintaining confidence in nanotechnology, especially with respect to the environment and food safety. Overall, nanotechnology benefits are worth pursuing in aquaculture so the hazard to wildlife should not act as a barrier to innovative, responsible "aqua-nanotechnology" development.

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