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Silver nanoparticles applications (AgNPS) in aquaculture

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Abstract
Aquaculture around world has converted as an important industry supplier of high nutritional value food, employment and incomes in different localities around world. However, Aquaculture industry needs to attend different problems to optimize production systems, among which were found: diseases, nutrition and water pollution, which impact not only the cultured organisms, but also in the environment. Due to above, aquaculture industry is using different innovative technologies like metallic nanoparticles, but until now all studies were incipient with test that only were proved *in vitro*, but lack of toxicology studies at cultured specie or habitat. That's why the goal of this review is to show a general view with obtained results with silver nanoparticles in aquaculture application, analyzing not only the disadvantages, but also their problems and perspectives related with their use.

Keywords: Aquaculture, nanoparticles, nanotechnology, silver

1. Introduction

The term 'nanotechnology' was introduced by Norio Taniguchi in 1974, from the Tokyo University research, who pointed out the possibility of handling material at the nanometric level, with dimensions between 1 and 100 nm. [1]. In this size range, particles show physical and chemical new properties that can be used in different science areas like medicine, food, textile, chemical, and energetic industry, to mention some [2]. Currently, metal nanoparticles are beginning to be used like silver (Ag), gold (Au) and copper (Cu) with biological, optical, and magnetic interesting properties, within which silver nanoparticles (AgNPs), is the most studied because of their showed antimicrobial potential to bacteria, virus and fungus. Due this, AgNPs nanoparticles has attracted the aquaculture sector, because their high development in Mexico and because it is continuously affected by infection process that produce economic losses [3]. That is why different researchers showed that nanotechnology has wide applications to aquaculture industry in water treatment, pond sterilization, nano-food particles for fishes, and aquatic diseases control [4]. In Asiatic countries it has increase the use of nanoparticles in fishes' culture, but in Mexico, studies made of this topic are incipient. First reported studies of AgNPs, was focused in infected shrimp with white spot virus (WSSV) and *Vibrio parahaemolyticus* bacteria, which provoke early dead syndrome (EDS), with positive results of 80% of survival until now [5]. Because nanotechnology is biotechnology with recent application in Mexico, the necessity to increase the knowledge about their aquaculture application at worldwide level appear, emphasizing their obtained benefits, challenges, and long-term prospects.

2. Nanotechnology and Nanoparticles

When nanotechnology and nanoparticles concepts are described, reference must be made to particle size, because its works to nanometric scale [6]. The "nano" prefix show a scale (billion parts of something) and was characterized to be essential multidisciplinary field cohesive exclusivity with the scale matter which it works [7]. In this size range, particles show new physical and chemical properties that can be used in different science and productive fields, because their lower dimensions and their increase number of superficial atoms, which confers them specific biological, optical, magnetic, and electric properties.

The physical properties of these particles are different to those observed in normal size solid or macroscopic with same chemical composition. Nowadays it has begun to be use, as formal experimental techniques which allow to make, characterize, and manipulate nanoparticles from different metals like gold, silver, platinum, and palladium [8]. AgNPs were the most particles studied because their antimicrobial and antifungal properties that present, for what they are considered as infection treatment tools to several types of microorganisms [9].

3. AgNPs synthesis

To obtain AgNPs, synthesis techniques are used by biological, physical, and chemicals methods. Chemical method was more employed to make the synthesis [10]. This chemical method is based principally with reduction of metal salt to zerovalent atom, which act as nucleation center, allowing agglomerates formation which growth go on with more atom aggregation, and formed bigger size particles with polyhedral forms increasingly complex [11]. However, it is necessary to stabilize the particles with an envelope of molecules or stabilizing agents (generally polymers), to inhibit agglomeration process [12]. Principally chemical reduction method was their reproducibility, and their possibility to obtain mono disperse colloids with a narrow distribution of particle size. After synthesis, AgNPs characterization was necessary, because their morphological and physical-chemical particles properties had a significant impact in their biological applications [13].

4. Silver Nanoparticles Characteristics

For thousand years, silver was used as precious metal for human being as jewelry, utensils, coins, photography or explosive applications. Even, ancient civilizations, like Greeks, used silver to cook, and maintain water quality [14], because they observe that water storage in silver beakers remained clean with no microorganism inside it. Later, silver was used like antimicrobial agent whose use was diminished because innovation of wide range of antibiotics. However, the resistance caused by the abuse of several antibiotics in aquaculture, has resurged their application for infections control. The above, was the starting point to nanomaterials based on silver development [15]. The characteristics of these nanoparticles regarding shape and size, determined the active mechanism or effect on target cell, as following described:

Size: The AgNPs of low size can penetrate several biological cell membranes like bacteria cell walls, increasing their contact surface, and their possibility to freely penetrate it [16].

Shape: Antimicrobial efficiency of AgNPs was confirmed that is dependent to shape. Different authors [17], show, for example, that geometric or icosahedral shapes were more efficiently to bacteria control, than nanoparticles with truncated triangle shape.

Action Mechanisms: It was reported that AgNPs can act against bacteria through several mechanisms, and for that characteristics can avoid bacteria resistance, in contrast with antibiotics [18]. Interest for Ag use resurge by their bactericide effect in their ionic form (Ag^{+1}). Nanoparticles allow the release of Ag^{+} ions, which join to cell membrane protein causing a breakdown and consequently bacteria cells mortality. But also, they can join to cytochrome and nucleonic acids and causing damage and inhibiting cell division [19].

One proposed mechanism is nanotoxicity, usually triggered for oxidative stress induction by reactive oxygen species (ROS) formations [20]. The importance to ROS generation was the produced enzymes disturbance by respiratory chain through direct interactions with tioles groups in these enzymes or they super enzyme oxyradical like superoxide dismutase [21]. ROS levels can be controlled by cells antioxidative defenses to glutathione/glutathione disulfide (GSH/GSSG ratio) and ROS excess production can produce oxidative stress [22]. The formation of additional free radicals that can attack lipids from cells membrane, and lead to membrane and mitochondrial function ruptures or damage in DNA [23].

5. Nanoparticles use in aquaculture industry

According with Food and Agriculture organization (FAO) in 2014 [24], aquaculture production has increase in recent years. This was relative recent activity in comparison with fishing and farming activities. Aquaculture activity was constantly transformed to cope several problems like diseases provoked by bacteria and virus. Also, effluent pollution by production waste and excessive use of water source [25]. That’s why aquaculture industry needs to adopt innovative technologies to get over difficulties that limit their development.

Nanotechnology was a field that several aqua culturists are appealing [26]. While the nanoparticles can be utilized in different fields inside aquaculture industry, there are three principal areas which were benefit with their application: alimentation, filters to improvement water culture medium, effluents, and control of infectious diseases.

Silver nanoparticles to improve water quality in aquaculture: The intensive intervention which aquaculture practices produced, impact directly over the environment, because it needs higher water quantities, and this source every day is scarcer around the world. Also, not consumed food for fishes, excretion products, feces, chemical products, and antibiotics, generated higher quantities of waste during organism’s production and was released to environment around these production farming [27]. For a long time, the method most used to improve water quality was the continuous change of ponds water with new freshwater. However, water volume necessary to small or medium-large aquaculture systems can reach several hundred cubic meters per day [28, 29]. In this field, nanotechnology point to innovative solutions to water pollution remediation by disinfection using silver bactericide nanoparticles, for elimination of organic matter, and wastes, using membranes elaborated with nanoparticles compounds [30, 31]. In recent study [32], a review was made about nanoparticles used in water disinfection, avoid the presence of bacteria and virus pathogens, where it was reported positive results. However, this study show that is necessary to make a cost-benefit balance, because at this moment it can be an expensive technology. Another author [33], demonstrated that nanoparticles directly applied in water could affect the fish culture by bioaccumulation. It is necessary to make toxicity studies to determine their use in aquaculture. This situation was confirmed with other study where AgNPs was evaluated in three life stages of rainbow trout (alevin, fry, fingerlings), to 100, 32, 10, 3.2, 1, 0.32, 0.1 y 0.032 mg L^{-1} concentrations. Estimated values of CL_{50} to 96 hours were 0.25, 0.71 and 2.16 mg L^{-1} respectively [34]. These values show higher sensibility for first life stages. Also, it was observed a reduction of chloride and potassium in blood plasma depending concentration of doses and increase of cortisol and cholinesterase in juvenile stages. This only show that silver nanoparticles applied directly to aquatic habitat or as disinfection agent in aquaculture industry in organisms cultured for human food consumption cannot be accepted. To solve this problematic, in recent study [35], it was evaluated the use of filters equipment cover with silver nanoparticles to decrease the fungus infection in rainbow trout fertilized ovules. Different zeolite concentrations, cover with nanoparticles (0.5, 1.0 and 1.5% of AgNPs) were compared with no modified zeolite, as water filter media in semi-recirculating systems. The fertilized ovules were transferred to hatching systems, which they take in water from cover filters with nanoparticles. The filter efficiency was evaluated with survival rates since fertilization to complete absorption vitellus sac stage. Results showed that filters who have AgNPs at 5% increase survival rates 4.56% since fertilization to swimming phase compared with control test. These results did not show significant differences ($p<0.05$). Almost, additional application of activated carbon (as absorbent medium) joint with half-coated AgNPs in filters, provoke an increase of 11.24% in survival rates in alevine stage. Infections were not observed during incubation period in tests with filters cover with AgNPs. Thus, end results confirmed that directly use of AgNPs in filters was significant effective to avoid fungus infections in semi-recirculating systems of rainbow trout, without observing damages when directly inoculation was applied.

On the other hand, other authors [36], have mentioned that it was developed some nanoparticles bio-filters to eliminate ammoniac, chemical compound, which in high water concentrations, is nocuous for many aquatic organisms. This result allows to assure that nanoscale powder (ultrafine nature), also can be used to eliminate polluted substances in water. These powders can be used as effective tool to clean organic compounds, so that can be converted to simple and non-toxic compounds.

In other study [37], nanotubes were elaborated with carbon and cyclodextrin impregnated with AgNPs, to purify water samples where it was reported *E. coli* and *V. cholearae* presence. These samples were submitted to nanotubes test and observed result at final of experiment, was a decrease of colony forming units (CFU).

Use of nanoparticles in aquatic nutrition: Traditionally, alimentation in fish culture was supported with inert diets in pellets. These pellets were formulated from fish nutritional requirements, like lipids, proteins, carbohydrates, minerals, and vitamins. A new idea was that nanoparticles can be applied in foods to improve the proportion increase of nutrient and minerals which pass through directly in fish digestive tissue [38].

It was demonstrated that nanoparticles functions as nutrient and nutraceuticals carrier vehicles, allow encapsulation and controlled release of nutritious materials, and protection of nutritious products against pathogen microorganisms, forestall final product decomposition [39]. Some researchers [40], showed that in the fish food processing industry was obtained better results in pellets (physical and chemical aspects, nutritional quality of ingredients and pellets) when nanotechnology was applied in their production. It was reported [41], that when Nano compounds were used to incorporate vitamin C in rainbow trout food, their active permanency increase until 20 days after incorporation in comparison with control diet, which lost their active permanency the first three days. Also, was demonstrated that Nano compound protect vitamin C to acid and enzymatic conditions of rainbow trout intestinal tract, because its observed the continuous release of vitamin C on intestinal epithelium and, with this, allowing the increase of fish non-specific immune system. However, in other studies [42], the silver and copper nanoparticles effect were evaluated (500 mg kg^{-1} of food), for 14 days, on intestinal microbial community of fish *Danio rerio*, by transmitted electronic microcopy, and gel electrophoresis with denaturalized gradient (DGGE); this study showed that intestinal epithelium was not affected.

Use of nanoparticles to disease control: One of the applications of silver nanoparticles was disease control due to their bactericide capacity [43]. In Aquaculture case, one of the bigger challenges was infection disease control caused by virus, bacteria, fungus and parasites. Traditionally, the antimicrobials were used to oppose bacterial infections in aquaculture. However, excessive application of these compounds provoked resistant strains, making the treatments not very successful [44]. Previous study about resistant strains [45], in fish farmers in 25 countries, was determined that tetracycline was the antibiotic most used. Also, different isolated bacteria from tilapia, show wide spectrum antibiotic resistance like tetracycline, erythromycin, and streptomycin. The resistant strains were: *Aeromonas salmonicida*, *Photobacterium damselae*, *Yersinia ruckeri*, *Listeria* sp, *Vibrio* sp, *Pseudomonas* sp and *Edwardsiella* sp.

To explore other alternatives to avoid different diseases of aquatic organisms, some researchers studied the silver nanoparticles effect for diverse ways to control important pathogens from fishes, mollusks, and crustaceans [46, 47]. In recent study [48], it was evaluated the antimicrobial effect of silver nanoparticles in two fish pathogens: *Lactococcus garvieae* and *Streptococcus iniae.* The minimum inhibitory concentration (MIC), and minimum bactericide concentration (BC) were determined. According with the results, MIC have a range of 1.12 to 5 µg mL^{-1} for *L. garvieae* and 1.2 to 2.5 µg mL^{-1} to *S. iniae* isolated. The mean value of MIC was 2.59 and 2.1 for *L. garvieae* and for *S. iniae* respectively. The results showed that the strain *S. iniae* was more sensible to silver nanoparticles that *L. garvieae* strain. In other study [49], it was evaluated the antiparasitic effect of AgNPs against *Ichthyophthirius multifiliis,* a parasite causing the disease of White spot in freshwater fishes; determining that 10 and 5 ng L^{-1} of AgNPS shows antiparasitic effect *in vitro* and *in vivo* studies.

The use of AgNPs obtained from synthesis of natural products was applied with positive results [50], for example; when AgNPs was synthetized by *Camellia sinensis* to *Vibrio harveyi* control in infected *Feneropenaeus indicus* organisms. *In vivo* tests demonstrated that 10 µg mL^{-1} concentration inhibited bacterial growth in 70%. In other study [51] it was used *Bacillus subtilis*, a non-pathogen organism to synthetize a Nano compound, and it was evaluated their antimicrobial effect on *V. parahaemolyticus* and *V. harveyi*, in infected *Litopenaeus vannamei*. The results showed a survival of 1% in control group, but with Nano compounds was 90%. Also, was determined that when extract of bacteria and plants were used, was not observed a physiology alteration or toxic effect on studied shrimps.

It was reported [52, 53], that antiparasitic and antifungal effect of AgNPs encapsulated with starch and applied through immersion baths (20 minutes) with 10 ng of nanoparticles concentrations in *Carassius auratus*, infected with *Ichthyophthirius multifiliis* and *Aphanomyces invadans*. The results showed that fishes recover after three days without toxic effect to AgNPs application.
Some researchers [54] were investigating with AgNPs encapsulated with chitosan (75 µg mL^{-1}) *in vitro*, for antimicrobial effect against *Vibrio tapetis*. In 2017 [55], it was determined the minimum inhibitory concentration of one Nano compound of chitosan and AgNPs to evaluate *in vitro* the antifungal effect against *Fusarium oxysporum.* Later, *in vivo* tests were made with zebra fish infected by fungus. *In vitro* tests established that minimum inhibitory concentration of Nano compound was 38.69%, with a concentration of 250 µg mL^{-1}, and *in vivo*, using histological tests, was observed fungal mycelium deterioration storage in fish backs, concluding that fungus was affected by Nano compound contact.
Other studies, with this same topic, are shown in Table 1.

Table 1: Studies about silver nanoparticles used as pathogen control in aquaculture.

Author(s)	AgNPs characteristics	Microorganisms	Results
Dananjaya *et al*. 2016 [56]	AgNPs with chitosan	*Aliviibrio salmonicida*	MIC, 50 µg mL^{-1} and MCB, 100 µg mL^{-1}
Ravikumar *et al*. 2012 [57]	Commercial nanoparticles of Al_2O_3, Fe_3O_4, CeO_2, ZrO_2, MgO,	*Aeromonas hydrophila, Bacillus subtilis, Vibrio harveyi, V. parahaemolyticus* and *serratia sp.*	The CeO_2 Naps show higher antibacterial effect when 10 µg mL^{-1} concentration was used.
Soltani *et al.* 2009 [58]	Nanocid®	*Streptococcus iniae, Lactococcus garvieae, Yersinia ruckeri, Aeromonas hydrophila*	*S. iniae* MBC of 5 to 0.15 µg mL^{-1}, *L. garvieace* MBC of 10 µg mL^{-1} to 0.62 µg mL^{-1}, *A. hydrophila* MBC of 0.31 µg mL^{-1} to <0.15 µg mL^{-1}, *Y. ruckeri* MBCs of 2.5 to 0.62 µg mL^{-1}
Swain *et al.* 2014 [59]	Nanopartículas of CuO, ZnO, Ag, TiO_2	*Aeromonas hydrophila, Edwardsiella tarda, Pseudomonas aeruginosa, Flavobacterium branchiophilum, Vibrio spp Staphylococcus aureus, Bacillus cereus* and *Citrobacter spp.*	Show antibacterial effect in tested strains.
Mahanty *e. al*. 2013 [60]	Synthetized nanoparticles with leaves of *Mangifera indica, Eucalytus terticonis, Carica Papaya* and *Musa paradisiaca* plants	*Aeromona hydrophila*	Synthetized nanoparticles with *Carica papaya* show antimicrobial activity with 153.6 µg mL^{-1} concentration.
Vijay Kumar *et. al.*, 2014 [61]	Synthetized AgNPs with *Boerhaavia diffusa*	*A. hydrophila, P. fluorescens* and *F. branchiophilum.*	AgNPs concentration (50 µg mL^{-1}) was demonstrated higher zones of inhibition (15 mm) for *F. branchiophylum*, 14 mm for *A. hydrophilla* and (12 mm) for *P. fluorescen.*

Recently, it was development research about to vaccines generation to protect Asiatic carp using nanoparticles against *Listonella anguillarum* bacteria [62], and the white spot syndrome (WSSV), a virus which attack shrimps. This technique was able to provide to vaccine an additional protection barrier to avoid being inactivated for the metabolic machine of cultured species [63].
Otherwize [64], in 2016 it was evaluated the AgNPs application, using as reductor agent *Azadirachta indica* to evaluate the immune modular effect in infected fishes like *Cirrhinus mrigala* with *Aeromona hidrophila,* inoculating 20 µL concentration of AgNPs to fishes for 20 days. Functional activity of immunologic parameters like myeloperoxidase, phagocytic activity, anti-protease and lysozyme, increase significantly in fishes treated with AgNPs, obtaining against infection, survival rates of 73%.

6. Challenges and perspectives

At World level, in 2008, investments in nanotechnologies studies and research was of $700 thousand million US dollars. According to several forecasts, nanotechnology industry searches the quantity of $2.6 billion US dollars. Many countries will increase their investments and the investigation and development efforts in this field to improve the competitiveness [65]. Countries like China, India and United States of America were working with this.
Several studies have demonstrated that physical and chemical properties that AgNPs have, have helped to increase the efficiency of silver, principally in control disease area [66]. In aquaculture industry, nanoparticles were researched, and were added to water and food in commercial fish ponds to decrease the budget in water treatments, infection disease control, and environmental pollution [67]. However, most studies were made *in vitro*, and it is necessary to make the *in vivo* tests to know their efficiency in cultured organisms. At same time, toxicity and bioaccumulation tests were needing to be done on studied species.
Likewise, Nano compounds of chitosan and poly acid (lactic-co-glicolic) (PGLA) have several applications like nutrient, drugs, hormones vehicles, among others [68, 69]. Otherwise, the increasing use of AgNPs, enhance their release to environment and any advance in nanotechnology require, therefore, the environmental risk evaluations associated with these particles and their impact over biological diversity [70; 71]. Ecotoxicity studies about AgNPs exposition, would require of analytic technique which allow distinguish between silver nanoparticles and ionic silver dissolved under environmental conditions. Likewise, it needs the development of specific model for possible impact evaluation like environmental contaminant, determining exposed areas [72; 73], bioavailability [74], toxicity [75], and structure-activity relation [76].
At last, studies in this field allow the development of regulatory framework which allow the control and use of

these compounds, and their respective sanctions, when are bad-used, with no other purpose to restrict their impact in aquatic organisms and environment and consequently to human being.

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