Research Article

Green Synthesis of Silver Nanoparticles from the Opuntia ficus-indica Fruit and Its Activity against Treated Wastewater Microorganisms

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Wastewater can be reused after a treatment process and compliance with high quality standards that guarantee its safe use. The wastewater treatment plant of the Autonomous University of Aguascalientes (AUA), like others, uses primary, secondary, and tertiary processes. The tertiary process followed is chlorination and is used to eliminate microorganisms from the secondary process. Although water of acceptable quality is obtained with chlorine, there is evidence that toxic substances are generated when reacting with organic matter, so alternatives to the use of chlorination have been analyzed. In the present study, silver nanoparticles were synthesized from the aqueous extract of the Opuntia ficus-indica fruit peel (OfAgNPs), by reducing a 2 mM solution of AgNO₃. OfAgNPs were characterized by UV-visible spectroscopy, scanning electron microscopy, energy-dispersive X-ray spectroscopy, atomic absorption spectroscopy, and dynamic light scattering, in addition to his electrophoretic mobility. The OfAgNPs are spherical, with an average particle size distribution of $64.28 \pm 11.82$ nm, relatively stable at room temperature, negatively charged ($-25.1 \pm 0.03$ mV), and composed of 61.29% silver. The activity of OfAgNPs was evaluated in water from the effluent of the AUA treatment plant, before and after chlorination, and inhibition of bacteria Escherichia coli var 1, Enterobacter aerogenes var 1, Citrobacter freudii var 2, atypical E. coli, and aerobic mesophilic microorganism was tested.

1. Introduction

Nanotechnology is the science of the design and production of materials at nanoscale (1-100 nm), also called nanomaterials, characterized by having different physical, chemical, and biological properties than those of larger scales [1]. Within these nanomaterials are nanoparticles (NPs), which measure less than 100 nm [2]. Silver NPs (AgNPs) have been employed in various important applications in human health such as antibacterial, antifungal, antiviral, and anticancer [3–6].

Different NPs synthesis methods have been developed in which its physicochemical properties depend directly, such as size, surface charge, agglomeration, degree of dilution, and elemental composition [7, 8]. The synthesis of AgNPs based on chemical reduction is the most popular, simple, and high performance [9], where a metallic precursor (metallic salts) reacts with reducing agents such as sodium citrate, ascorbic acid, alcohol, and borohydride, which are toxic for the environment [10]. Furthermore, chemical synthesis processes can increase the toxicity of NPs due to its ability to absorb substances [10–12]. One of the alternatives is green synthesis in which chemical reducing agents and stabilizers are replaced by proteins, vitamins, alkaloids, carbohydrates, and antioxidants, which are obtained from living organisms such as bacteria, algae, fungi, yeasts, and plants [10–13]. The advantages of this technique are the following: (a) the
reduction of costs during synthesis, (b) processes are simpler, and (c) the resulting product is compatible for pharmaceutical applications and for biomedical use [10, 11, 14]. Some of the plants that have been reported for green synthesis are Azadirachta indica [15], Boerhaavia diffusa [16], Malva parviflora [17], Olea chrysophylla, Lavandula dentata [18], Pelargonium beenides [19], among others.

On the other hand, wastewater is defined as water from discharges of urban, industrial, services, agricultural, livestock, and domestic uses, and it is defined as treated wastewater after it has undergone any or all of the physical, chemical, and biological processes that make it suitable for reuse [20], generally for domestic use and irrigation water. Through physical process, solid waste is separated from water; with biological process, for example, activated sludge, a consortium of microorganisms removes organic matter; and with the chemical process, the microorganisms present are eliminated, for example, by chlorination [21, 22]. A disadvantage of the chlorination process is that when chlorine reacts in the environment, it generates by-products with genotoxic, mutagenic, and carcinogenic effect [23]. For this reason, alternatives to the use of chlorine in the tertiary process are being investigated.

An important parameter to determine the quality of treated wastewater is measuring the load of indicator bacteria. This allows to indirectly calculate the presence of pathogenic microorganisms. Direct detection of pathogenic microorganisms can be a difficult and expensive process, with long analysis and culture isolation times. Furthermore, with indicator bacteria, it is possible to evaluate different physical and chemical disinfection treatments [24, 25]. The most widely used indicator microorganisms are coliforms (fecal and total) and aerobic mesophilic microorganisms [25, 26].

Opuntia ficus-indica, commonly called prickly pear, is an angiosperm and dicot plant that belongs to the Cactaceae family, whose center of origin is Mexico [27, 28]. Its cultivation satisfies the food needs by consuming its stems and fruits [29, 30]. Its fruit, called prickly pear, has been shown to be a good source of nutrients and antioxidants, and it has health benefits such as protective effects of the cardiovascular system, hepatoprotector, chemopreventive, antiproliferative, anticaner, and neuroprotective [27, 31]. However, its consumption also generates food waste, since the peel makes up 56.7% of the fruit, which represents a serious environmental problem due to the potential growth of microorganisms [32].

The objective of this work was to generate AgNPs from reducing and stabilizing agents present in the aqueous extract of the prickly peel and to verify its antibacterial activity in treated wastewater before and after the chlorination process.

2. Materials and Methods

2.1. Materials and Reagents. The reagents were 99% AgNO₃ (Karal), 2% HNO₃, and Silver Standard for Atomic Absorption Spectroscopy (PE Pure Atomic Spectroscopy Standard). Culture media are as follows: LB medium prepared with peptone (Bioxon), yeast extract (Bioxon), and NaCl (JT Baker); red phenol broth with lactose, MR-VP medium (DIBICO), Escherichia coli (EC) broth (Difco), methylene blue eosin agar (EMB), standard method agar, 2% bright green bile broth (BRILA), SIM medium (Bioxon), and Simmons citrate agar (MCD LAB). The water samples were taken from the effluent of the water treatment plant of the Autonomous University of Aguascalientes (AUA), after the biological treatment, before and after chlorination; the water samples were treated with 10% sodium thiosulfate to stop the effect of chlorination.

2.2. Preparation of the Prickly Pear Extract. The prickly pear peel was washed with sterile distilled water, 50 g weighed, ground with liquid nitrogen and mixed with 250 ml of sterile distilled water. The mixture was heated to 80°C in a water bath for 30 min, and the aqueous extract was recovered by filtration with Whatman No. 1 paper.

2.3. Green Synthesis of OfAgNPs. In a 250 ml flask, 10 ml of the filtered extract was mixed with 90 ml of 2 mM AgNO₃ and incubated at room temperature, protected from light, with constant shaking at 90 rpm, until it changed color from yellow to reddish brown. Later, it was centrifuged at 12,000 rpm for 5 min, the supernatant was decanted, and the precipitate was washed three times with sterile distilled water and once with 96% alcohol. The OfAgNPs were dried at 37°C for 24 h and pulverized and sonicated for 20 min for dispersion.

2.4. OfAgNPs Characterization

2.4.1. Obtaining the Resonance Plasmon. The colloids obtained were scanned in a wavelength range of 300-800 nm in the Thermo scientific UV-Visible Genesis 10s UV-Vis spectrophotometer.

2.4.2. Scanning Electronic Microscopy (SEM). The dried samples were placed in the 1 cm × 1 cm sticky graphite disc and were covered with the gold plating (Dentom Vacuum). A current flow was introduced at an estimated time of 80-150 s. The sample was introduced into the scanning electron microscope chamber, and the diameter of the OfAgNPs was measured from the micrographs with the Image J2 program [33]. Three hundred NPs were measured to obtain the size distribution histogram and the coefficient of variation (CV) was determined from the following formula:

\[
CV = \frac{\text{standard deviation}}{\text{mean}} \times 100 \tag{1}
\]

2.4.3. Energy-Dispersive X-Ray Spectroscopy (EDXMA). The OfAgNPs solid sample was mounted in the vacuum chamber of the scanning electron microscope. The sample was observed at 12 mm distance between the lens and the focal plane, at an acceleration voltage of 20 kV, spot size of 42 on the SEM monitor. On the monitor of the EDS Rx analyzer, the image that appears on the screen was selected for quantitative and qualitative analysis. Four sites of interest were analyzed in a circular area of 144 mm². Each of the four sites was analyzed at 4000x magnification, 200 s to obtain element composition.
2.4.4. Atomic Absorption Spectroscopy. For digestion, 79.5 mg of powder containing the OAgNPs was dissolved with 15 ml of 1:1 HNO₃, heated to boiling, filtered through Whatman No. 1 paper, and heightened to a volume of 25 ml with distilled H₂O. Samples were analyzed in a Perkin Elmer 3110 kit, and a calibration curve with 1, 2.5, and 5 ppm was made from the silver standard. An atomic absorption hollow cathode lamp was used for silver at a wavelength of 328.1 nm.

2.4.5. Hydrodynamic Diameter and Zeta Potential. The hydrodynamic diameter of the OAgNPs was determined with the use of the Dynamic Light Scattering (DLS) technique in a Zetasizer ZS90 (Malvern) equipment. Starting from a cumulative adjustment up to order two of the correlation function of the intensity dispersed at 90°, as described in the literature [34]. Zeta potential was obtained from electrophoretic mobility using the Smoluchowski model [35] available in the team’s software. The analyses were performed on an OAgNPs colloid prepared in ultrapure water (Milli-Q) at a concentration of 100 mg/l. Measurements were taken in triplicate at times of 0, 72, 96, and 120 h.

2.5. Analysis of the Antibacterial Activity of OAgNPs in Wastewater Samples

2.5.1. Exposure of Wastewater Samples Treated with OAgNPs. Fifty ml samples of treated wastewater were collected in sterile containers. The samples were taken before and after chlorination (Figure 1). Two hundred µl of 10% sodium thiosulfate (Na₂S₂O₃) was added to each of the samples.

OAgNPs were added to 1 ml aliquots of these water samples with a 0.5 mg/ml concentration, from a stock of 5 mg/ml prepared in phosphate buffer (pH 7). To determine the minimum time of inhibition, the samples were incubated at room temperature with constant shaking and at times of 0.5, 1, 2, 4, and 6 h; it was plated by means of the plate extension technique in LB medium. The shortest time in which there was an absence of colonies determined the minimum inhibition time (0.5 h).

OAgNPs colloids were prepared with 0.5 and 1.0 mg/ml concentrations in a volume of 20 ml with water samples from the treatment plant; they were exposed for about 0.5 h with constant agitation at 90 rpm, and then, the microbiological analysis was performed.

2.5.2. Microbiological Analysis. The microbiological analysis was performed on the water samples that had the following treatments: (1) no treatment (control), (2) chlorination, (3) 0.5 mg/ml OAgNPs, (4) 1.0 mg/ml of OAgNPs, (5) chlorination with 0.5 mg/ml OAgNPs, and (6) chlorination with 1.0 mg/ml OAgNPs.

2.5.2.1 Total and Fecal Coliforms Determination. Total and fecal coliform microorganism determination was performed by the most probable number (MPN) method, which is divided into two phases: the presumptive and the confirmatory. Serial dilutions of water samples were made from the six treatments at 10⁻¹, 10⁻², 10⁻³, and 10⁻⁴. In the presumptive phase, 1 ml of each of the dilutions of the water samples was inoculated into the liquid phenol red broth liquid medium and incubated at 37°C in a period of 24-48 h. For the confirmatory phase of total coliforms, two roasts were taken from the positive tubes of the presumptive phase, from which they presented a change in coloration of the medium from red to yellow and the presence of gas. The BRILA broth was inoculated and incubated at 37°C for 24-48 h. For the confirmatory phase of fecal coliforms, two roasts were taken from the positive tubes of the confirmatory phase of total coliforms, which showed bacterial and gas growth, and the EC broth medium was inoculated and incubated at 44.5°C for a period of 24-48 h. The BRILA broth cultures that showed evident bacterial growth were inoculated in the EMB differential selective solid medium from which typical colonies were selected to then perform the biochemical tests for their identification (IMViC: Indole, Mobility, Voges Proskauer, Simmons Citrate) using the SIM semisolid medium, the MR-VP liquid medium and the Simmons citrate solid medium. All tests were performed in triplicate. Total and fecal coliforms are reported as the most probable number per 100 ml of sample (MPN/100 ml).

2.5.2.2 Aerobic Mesophilic Microorganisms Determination. The aerobic mesophilic microorganisms were counted using the plate pouring technique on the agar medium for standard methods. One ml of the dilutions of each water treatment in 20 ml of agar medium for standard methods was added and incubated at 37°C for 24-48 h. After that time, the count of the colonies was carried out and the log inhibition (log10 N0/N) was calculated for each treatment; where N0 is the initial count, and N is the count after treatment [36]. The results were analyzed by means of a one-way ANOVA followed by a Tukey test (α < 0.05). The graphs were made in the GraphPad Prism 6.0 program [37], considering the average of the repetitions ± one standard deviation.

3. Results and Discussion

3.1. Green Synthesis and Characterization of OAgNPs. The synthesis of OAgNPs was initially verified with the change of coloration to dark brown in the colloidal solution (Figure 2(a)). This coloration change is characteristic of the AgNPs synthesis due to the reduction of silver nitrate in the presence of reducing agents from the extract of the prickly pear peel, which leads to an increase in color ranging from yellow to brown, as a result of the resonance of the superficial plasmon [38]. The color of the solution depends directly on the size and shape of the AgNPs [39]. The plasmon obtained showed the spectral characteristics of the AgNPs, with a peak in a wavelength of 435 nm and a width of 150 nm (Figure 2(b)). These characteristics allow to infer that the size of OAgNPs is between 60 and 80 nm as reported by Mullinger et al. [40, 41]. Similar results were obtained with the extract of Chrysanthemum indicul L. with a NPs size between 37.71 and 71.99 nm [42] and from the blackberry with a size between 12 and 50 nm [43], both with a maximum length of 435 nm. The optical properties of AgNPs result from the interaction with light, so the resonance plasmon depends on different factors such as shape, size, composition, refractive index of the metal, presence of adsorbed species, dielectric environment, electromagnetic mutual interactions, and the separation between them. These factors
determine that the absorption peak is in the range of 393-738 nm [13, 40, 44, 45]. SEM analysis showed that Ofs AgNPs have a spherical shape (Figure 3(a)), being the most common form. Other researchers synthesized AgNPs with Capsicum annuum L. [7] and Syzygium aromaticum extract [46] resulting in NPs with spherical morphology. In the distribution size histogram, it was observed that the average diameter is 64.28 ± 11.82 nm (Figure 3(b)), which coincides perfectly with the predictive results obtained from the resonance plasmon. AgNPs with a size of 25 nm were synthesized from the Boerhaavia diffusa extract [15] and 34 nm from Azadirachta indica [16]. The difference in size and shape between the biosynthesized AgNPs is due to temperature, pH, synthesis time, reducing agents present in the extract, and concentration of the metal precursor [47]. The coefficient of variation was 18.39, which proves that monodisperse nanoparticles exist in the colloid, and the OfAgNPs are mostly homogeneous in shape and size. These results are opposite to polydisperous biosynthesized NPs in the colloidal solution obtained from Albizia adiantifolia reported by Gengan et al. [48].

The hydrodynamic diameter values have little variation over time; at time 0, it is 264 ± 0.015 nm, and at 120 h, it is 220 ± 0.018 nm (Figure 4(a)). These values exceed the nanometric scale and are also greater than the value obtained in the SEM measurements. The size differences between the SEM and the hydrodynamic diameter are because the SEM measures the physical size of the metallic NPs, while the DLS measures the size of the particle together with the biomolecules that are attached to the surface of the AgNPs [49]. Similarly, the AgNPs synthesized from leaves of Brassica rapa by Narayanan and Park [50] showed a larger hydrodynamic diameter size (39.5 nm) than the average size obtained in TEM (16.14 nm). The phytochemical compounds found in the prickly pear peel extract participate as reducing agents in the synthesis and in the coating of AgNPs, which represents an advantage, since it is not necessary to add chemicals stabilizer. Alkaloids, flavones, anthracenes, terpenoids, and flavonoids present in extracts of plant stabilize and coat metallic NPs in plant biosynthesis [51]. Besides, it has been reported that aqueous extract of prickly pear peel contains phenolic acids (quercentine, kaempferol), vitamins (k1, alpha-tocopherol, beta-tocopherol), and sterols (campesterol, lanosterol and stigmasterol) [27]. These compounds could be the ones that cover the OfAgNPs. The zeta potential values were at time 0 of −25.1 ± 0.03 mV and at 120 h of −24.2 ± 0.06 mV (Figure 4(b)), indicating that OfAgNPs have a negative charge and are relatively stable over time. These results coincide with those of Sufyani et al. [18] who synthesized AgNPs from Lavandula chrysophyla extract with negative charge and greater stability, having a zeta potential value of -14.3 mV, but not with those synthesized from the Olea chrysophylla extract with a low stability according to the zeta potential value of -0.877. The negative charge and the stability are provided by the molecules integrated into the surface during the synthesis process, which generate electrostatic or steric repulsion, giving them stability, avoiding agglomeration and therefore precipitation in solution [52].

The element analysis by EDXMA from OfAgNPs showed that the silver was found in the highest percentage with
Figure 3: Morphology and diameter of AgNPs. (a) SEM micrography, the AgNPs have a spherical morphology and are agglomerated due to the preparation of the sample. (b) Histogram of size distributions of the AgNPs, the diameter is distributed between 46.1 and 104.5 nm, with an average of $64.28 \pm 11.82$ nm ($n = 300$).

Figure 4: Graphs of the behavior of the hydrodynamic diameter and zeta potential of AgNPs over time in ultrapure water at room temperature. (a) Hydrodynamic diameter, the average value of the hydrodynamic diameter is $238 \pm 0.015$ nm. (b) Zeta potential, the values indicate that the AgNPs are relatively stable and that they have a negative charge with an average value of $-24.87 \pm 0.05$ mV. The measurements were made in triplicate, and the average with a standard deviation is shown.
61.29%. The presence of other elements was also identified such as carbon (15.40%), nitrogen (1.17%), oxygen (6.59%), magnesium (0.29%), chlorine (14.68%), and calcium (0.58%) (Figures 5(a)–5(c)). Calcium and magnesium are elements present in the prickly pear peel [29]; therefore, it was expected that they form part of the synthesized NPs. Dhand et al. [53] biosynthesized AgNPs from the Coffea arabica seeds with silver as the main element. Arokiyaraj et al. [42] obtained AgNPs using extract of Chrysanthemum indicum L. made up for silver, carbon, oxygen, and chlorine. These elements are part of the covering of the AgNPs. The distribution of the elements in the sample shows that the highest percentage is silver (Figure 5(a)). By atomic absorption spectroscopy, the silver yield was calculated, where it was observed that, for 100 g of synthesized material, there is 24.15 g of silver, leading to a percentage of 24.15%. This proves that the rest of the weight of the sample are phytochemical compounds from the extract.

### Table 1: Total and fecal coliforms in water samples from the AUA treatment plant, treated and untreated with different OfAgNPs concentrations.

| Tertiary treatment | Total coliforms (MPN/100 ml) | Faecal coliforms (MPN/100 ml) | Identified species |
|--------------------|-----------------------------|-----------------------------|--------------------|
| No treatment       | 430                         | 150                         | E. coli var 1, E. aerogenes var 1, C. freundii var 2, E. coli atypical |
| Chlorination       | 91                          | 0.03                        | E. coli var 1 |
| 0.5 mg/ml of OfAgNPs (0.120 mg of Ag⁺) | 0.03                  | 0.03                        | None |
| 1.0 mg/ml de OfAgNPs (0.241 mg of Ag⁺) | 0.03                  | 0.03                        | None |
| Chlorination + 0.5 mg/ml of OfAgNPs | 0.03                  | 0.03                        | None |
| Chlorination + 1.0 mg/ml of OfAgNPs | 0.03                  | 0.03                        | None |

MPN: most probably number.
2, and an atypical E. coli. These bacteria generate diseases in humans: E. coli causes diarrhea, intestinal and urinary tract infections, and meningitis [54]; E. aerogenes causes infections in the urinary tract, gastrointestinal, and respiratory system [55], and C. freundii causes sepsis and meningitis in the neonatal period [56]. Furthermore, these microorganisms are considered indicators of the presence of pathogenic organisms [26].

In the microbiological analysis of chlorinated water from the treatment plant, an elimination of 79% of the total coliform bacteria was observed. The only coliform microorganism detected was E. coli var 1, indicating the presence of fecal contamination (Table 1). The water samples, both those that went through a chlorination process and those that did not, were treated with 0.5 and 1.0 mg/ml OfAgNPs for 0.5 h. These conditions were determined considering the minimum inhibition time previously calculated. A value of 0.03 MPN/100 ml was obtained for both total and fecal coliform (Table 1), indicating the absence of these bacteria, while in the samples that only were subjected to the chlorination process, a total coliform count of 91 MPN/100 ml was obtained, which shows their presence. From this parameter, it would allow the reuse of the residual water, since the Mexican standard indicates that in order to use it in public service with direct contact, a maximum allowable limit of 240 MPN/100 ml is required, and for public services with indirect or occasionally contact, a maximum limit of 1,000 MPN/100 ml is required [20].

According to the logarithmic inactivation results, the treatments that involve exposure to the OfAgNPs are significantly more effective than the chlorination in the inhibition of aerobic mesophilic microorganisms. The chlorination process caused 90% inhibition of aerobic mesophilic bacteria, while OfAgNPs treatments showed an inhibition greater than 99% (Figure 6). Furthermore, in these organisms, no significant differences were observed between the two concentrations of OfAgNPs evaluated in both water samples (chlorine-free and chlorinated) (Figure 6). These results showed that a treatment with 0.5 mg/ml of OfAgNPs for 0.5 h is sufficient to eliminate both coliforms and aerobic mesophilic microorganisms, proving their action against Gram(-) bacteria. It is important to highlight the availability of Ag⁺ in the synthesized material, because, according to the analysis of atomic absorption spectroscopy, it is equivalent to 0.120 mg and 0.241 mg of Ag⁺, in the samples of 0.5 and 1 mg/ml of OfAgNPs, respectively.

There is a direct relationship between the action mechanisms of AgNPs against bacteria and their characteristics, such as shape, size, surface area, agglomeration state, and surface charge [57]. The AgNPs cause cell damage because they interact directly with the membrane, and because silver ions released from AgNPs generate reactive oxygen species (ROS) which interact and damage biomolecules such as lipids, DNA, and proteins [57, 58]. Currently, biosynthesized AgNPs are recognized both for their activity against Gram (+) and Gram (-) bacteria, as well as for their low toxicity in plants and animals [58–60]. Due to these observations, the use of OfAgNPs as an alternative to chlorination used as a tertiary process in wastewater treatment plants is proposed.

4. Conclusions

AgNPs were biosynthesized from aqueous extract of prickly pear fruit peel and were designated as OfAgNPs. OfAgNPs had a spherical, negatively charged morphology and were relatively stable. In addition, the growth of coliform bacteria E. coli var 1, E. aerogenes var 1, C. freundii var 2, an atypical E. coli, and aerobic mesophilic microorganisms present in treated wastewater was inhibited with 0.5 mg/ml OfAgNPs for 0.5 h. OfAgNPs presented an antimicrobial activity higher than chlorination, so they could be used as a tertiary process for wastewater treatment.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

We declare that we do not have conflicts of interest.

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