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Application of by-products and waste in the synthesis of nanosilver particles

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ABSTRACT

Extracts from strawberry and raspberry leaves, carrot pomace, and spent grains, were tested as bioreducing agents for the synthesis of nanosilver particles (AgNP). Based on UV-vis spectra, the leaf extracts produced the most AgNP, carrot pomace was less effective, and spent grains did not produce AgNP. The dynamic light scattering method revealed that AgNP ranged from 1 to 92nm in size, and that over 80% of the particles were about 10nm. Energy dispersive X-ray spectroscopy showed that elements that typically stabilize nanoparticles were present. The well diffusion method (nutrient agar medium) indicated that AgNP synthesized with raspberry leaf extract exerted strong bacteriostatic and bactericidal activity against Gram-negative bacteria and weaker activity against Gram-positive bacteria. Although further analysis is needed to determine the mechanism of their synthesis, the results of this study show that plant-extract based synthesis can produce nanoparticles with controlled size and morphology.

INTRODUCTION

Nanoparticles are characterized by a size of 1 to 100nm in at least one dimension. These nanomaterials possess properties that differ from the characteristics of macromolecules. Noble metal nanoparticles, mainly silver nanoparticles (AgNP), have been reported to possess antifungal, antiviral, anti-angiogenesis and anti-inflammatory activities. Owing to their antimicrobial activity, AgNP are very often used in medicine (nanomedicine) and in this application, their activity against pathogens is extremely important because it could overcome the problem of bacterial resistance to traditional antibiotics. The antimicrobial activity of AgNP may be due to the ability of nanoparticles to influence quorum sensing, cell-to-cell chemical communications that are important in biofilm formation and infections. However, there is some evidence that AgNP inhibits the growth of microorganisms, but leaves the cells intact and metabolically active .

Biological methods for AgNP synthesis, such as the use of extracts from plant materials, have attracted attention recently. Such biological methods offer a number of advantages: they are cheap and non-toxic, and the low reaction rate during bioreduction with plant extracts makes it easier to control nanoparticle formation. The mechanism of biological reduction of Ag^+ to Ag^0 is not fully understood, although non-enzymatic and enzymatic reactions are involved, and in many cases the proteins from plant extracts stabilize or cap the nanoparticles. Various plant compounds may be involved in bioreduction: polyphenols, flavonoids, alkaloids and terpenoids.

The aim of this study was to establish the conditions for effective and environmentally friendly ("green") synthesis of nanosilver particles (AgNP) and to characterize selected properties of AgNP.

MATERIAL AND METHODS

The synthesis of AgNP was performed using food industry waste-product spent grains, carrot pomace and extracts from

strawberry (*Fragaria×ananassa*) and raspberry (*Rubus idaeus*) leaves. Silver(I) nitrate ($AgNO_3$) (BioXtra, >99% purity, Sigma-Aldrich) and high purity Milli-Q water (Millipore system) was used in all experiments.

Synthesis of nanosilver

Spent grains and carrot pomace were obtained from local factories (BK Brewery, Olsztyn, and Tymbark SA, Olsztynek), respectively. After they were obtained from the factories the spent grains and carrot pomace were not modified in any way before the extract was prepared. The strawberry and raspberry leaves were obtained from the Department of Horticulture experimental station (University of Warmia and Mazury in Olsztyn, Poland). Before extract preparation, they were washed in de-ionized water and cut into 1·1cm pieces. All extracts were prepared by heating 20g of the plant material in 100cm^3 of de-ionized water at 100°C for 5min. The cooled extract was separated from the insoluble fraction by filtration through Whatman No. 1 filter paper.

Synthesis of nanosilver particles was performed by adding 10cm^3 of extract to 50cm^3 of a 1, 3 or 5mM aqueous solution of $AgNO_3$. The bioreduction was carried out in darkness in 300cm^3 Erlenmeyer flasks placed in an incubation shaker at 30°C and 300rpm.

Characterization of nanoparticle properties

The synthesized AgNP were first characterized with a UV-visible spectrophotometer (Lambda XLS, Perkin Elmer or DU640, Beckman) in the 350–800nm range (scan speed 120nm·min^{-1}). The size of the nanoparticles was determined by the dynamic light scattering method (Zetasizer ZS, Malvern). Microscopic observation of selected nanoparticles was performed using an atomic force microscope (Multimode 8 system, Brucker), a scanning electron microscope with a GEMINI column (Zeiss Ultra Plus, Brucker) and energy dispersive X-ray spectroscopy (Quantax 400, detector XFlash SVE III, 300kconts·s^{-1} input count rate, energy resolution 127eV, active area 30mm^2).

Determination of the antimicrobial properties of the nanoparticles

The well diffusion method was used to examine the activity of the AgNP against the following microorganisms: *Escherichia coli*, *Proteus vulgaris*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Staphylococcus aureu*s. The agar wells (about 15mm in diameter) were filled with reaction medium obtained after AgNP synthesis, or leaf extract and water as a control. Nutrient agar medium was used for the cultivation of bacteria at 37°C for 24h and, the diameter of the growth inhibition zone was measured.

Statistical analysis

The results of all experiments are presented as the mean of (at least) triplicate measurements. In all cases the standard deviation did not exceed 4% of the mean value.

RESULTS AND DISCUSSION

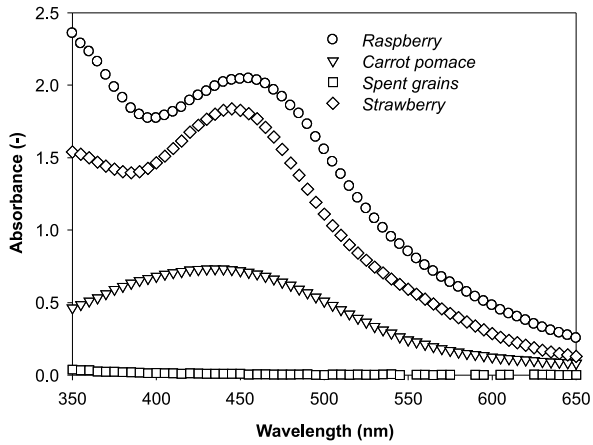

Figure 1. UV-visible light spectra of the products of 24h of bioreduction of Ag with various plant-waste extracts at 30°C. Absorbance in the range of 380-480nm indicates the presence of AgNP.

To determine if AgNP were produced by bioreduction with the plant-waste extract, UV spectrophotometry was performed. The specific absorbance of AgNP is at 380-480nm. After bioreduction with raspberry or strawberry leaf extracts, this absorption was strong (Figure 1). After bioreduction with carrot pomace extract, the absorption was weak, and after bioreduction with spent grains extract, it was not observed. These results indicate that raspberry and strawberry leaf extracts are better than the other extracts for AgNP production. Their superiority was confirmed by changes in the colour of the reaction medium from transparent (initial reaction mixture) to yellow, reddish, or dark brown (Figure 2). These changes are due to the excitation of the surface plasmon of AgNP. Based on these results, extracts from strawberry and raspberry leaves were selected for further experiments.

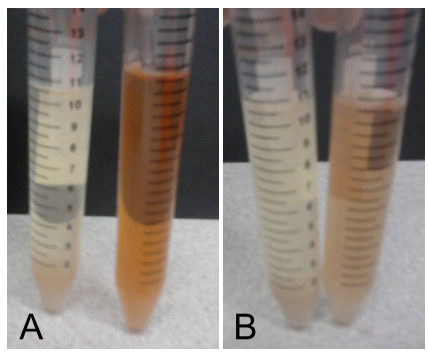

Figure 2. Reaction medium before (left) and after bioreduction (right) with (A) raspberry leaves and (B) carrot pomace extract.

Generally, four factors influence the biosynthesis of AgNP: pH, temperature, reaction time, and the ratio of plant-extract: silver-substrate (usually silver(I) nitrate). Typically, a plant extract-mediated bioreduction involves mixing the aqueous extract with an aqueous solution of the relevant metal salt. The reaction occurs at room temperature and is generally complete within a few minutes. The concentration of $AgNO_3$ is usually 1-5mM because of process economy and nanoparticle purity.

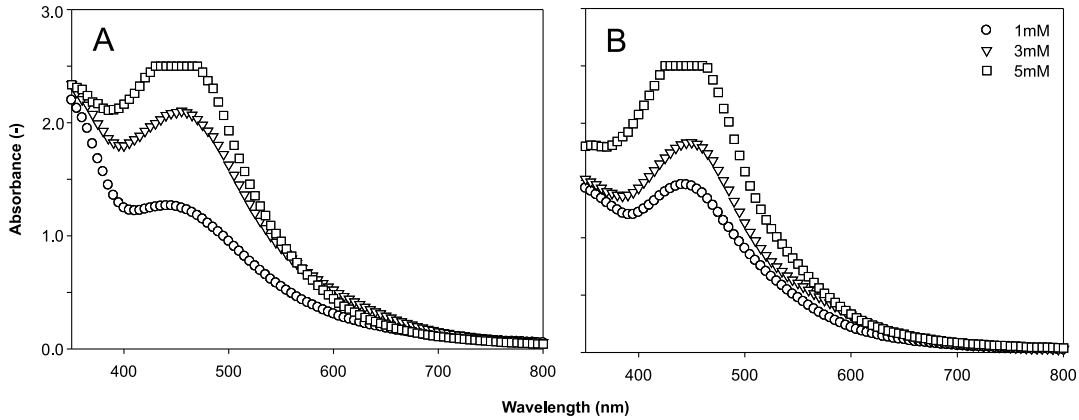

Figure 3. AgNP UV-vis spectra after bioreduction of different concentrations of $AgNO_3$ by extracts from leaves of (A) raspberry and (B) strawberry.

In the next step of the study, the influence of substrate concentration and reaction time on the biosynthesis of nanosilver was examined. With both raspberry and strawberry leaf extracts, the greatest absorbance for AgNP was recorded when a 5mM $AgNO_3$ solution was used as a substrate (Figure 3), indicating that AgNP production was greatest with this $AgNO_3$ concentration. Absorbance for AgNP was greatest after 1d (24h) reaction, after which it fluctuated but was always lower up to day 10 (240h) (Figure 4). The decrease in the absorption value after 24h of the reaction could be explained by spontaneous agglomeration of the nanoparticles and a mechanism of synthesis and growth kinetics during biosynthesis.

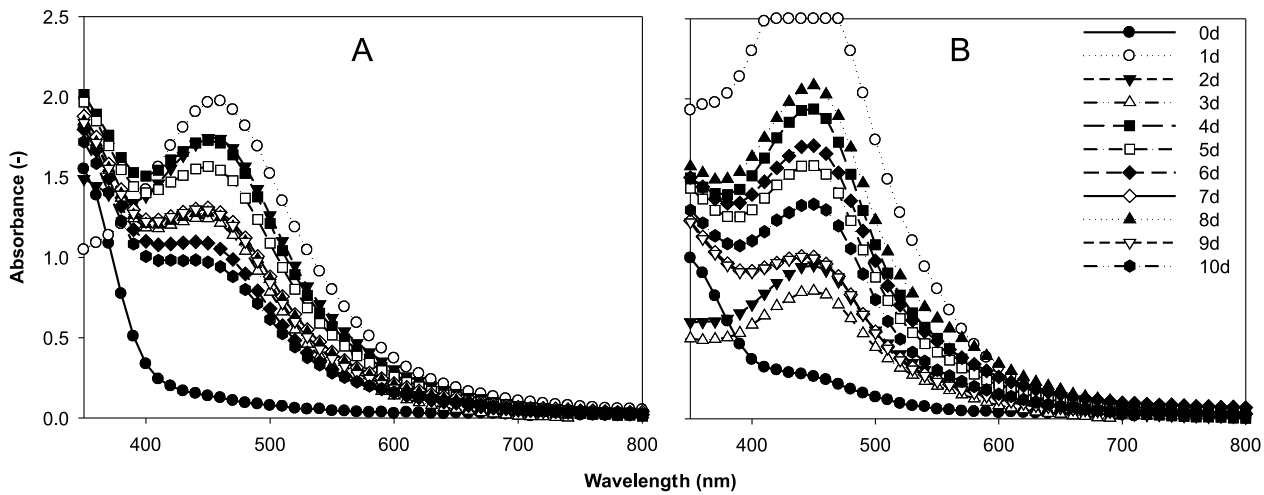

Figure 4. Influence of reaction time on AgNP synthesis by extracts from leaves of (A) raspberry and (B) strawberry (5mM $AgNO_3$).

The dynamic light scattering method revealed that the size of the AgNP synthesized with strawberry leaf extract was 10-14nm (peak area 92-95%) and 73-79nm (peak area 4-7%), and the size of AgNP synthesized with raspberry leaf extract was 12-14nm (92-100%) and 79-81nm (up to 7%). Atomic force microscopy and scanning electron microscopy

showed the uniform distribution of the particles (Figure 5, 6). Energy dispersive X-ray spectroscopy indicated, in addition to Ag^0, the presence of elements that typically stabilize nanoparticles (Figure 6, B1 and B2). The energy dispersive X-ray spectroscopy profile shows peaks indicating silver, oxygen and carbon; the latter two may have originated from the biomolecules in the extracts and been bound to the surface of AgNP. It has been reported that nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth and are thus stable in a solution up to 4 weeks after synthesis.

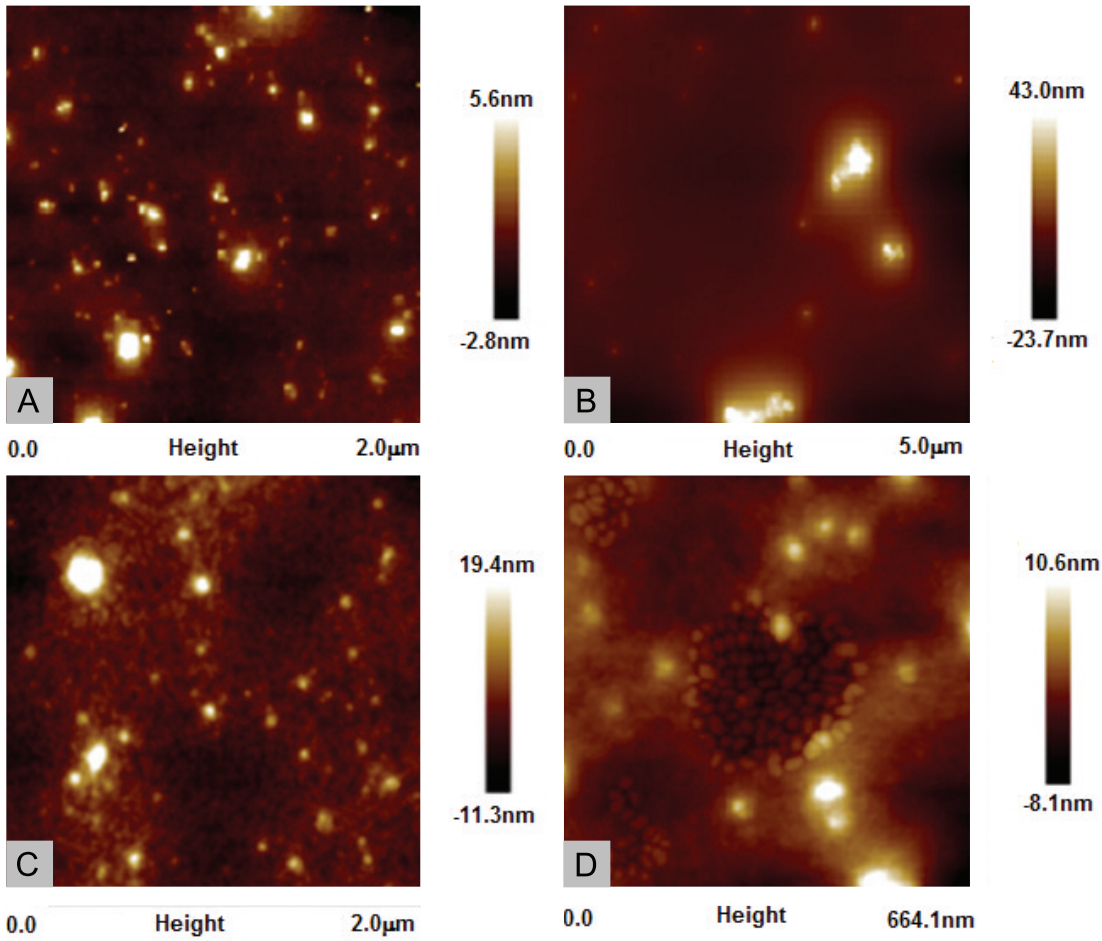

Figure 5. Atomic force microscope (AFM) images of AgNP synthesized by (A) carrot pomace, (B) strawberry or (C and D) raspberry leaf extract.

As mentioned earlier, AgNP find many applications because of their antimicrobial activity. AgNP synthesized by *Bacillus licheniformis* showed significant antiviral activity against the Bean Yellow Mosaic Virus. Also AgNP synthesized with *Allophylus cobbe* leaves extract have shown activity against Gram-negative and Gram-positive bacteria .

In the present study, the AgNP produced with the raspberry leaf extract exerted strong bacteriostatic and bactericidal activity against Gram-negative bacteria and no activity or very low activity against analyzed Gram-positive bacteria when used at a concentration of 6ppm (Figure 7). These results confirmed that AgNP have significantly less effect on the growth of Gram-positive bacteria, due to the cell walls of these bacteria .

It has been shown that the bactericidal activity of AgNP strongly depends not only on their concentration, but also on the size and shape of the particles. However, the shape of the particles used in the present experiments was uniform (Figure 6), so it was impossible to find a relationship between this characteristic and the antimicrobial activity of the AgNP.

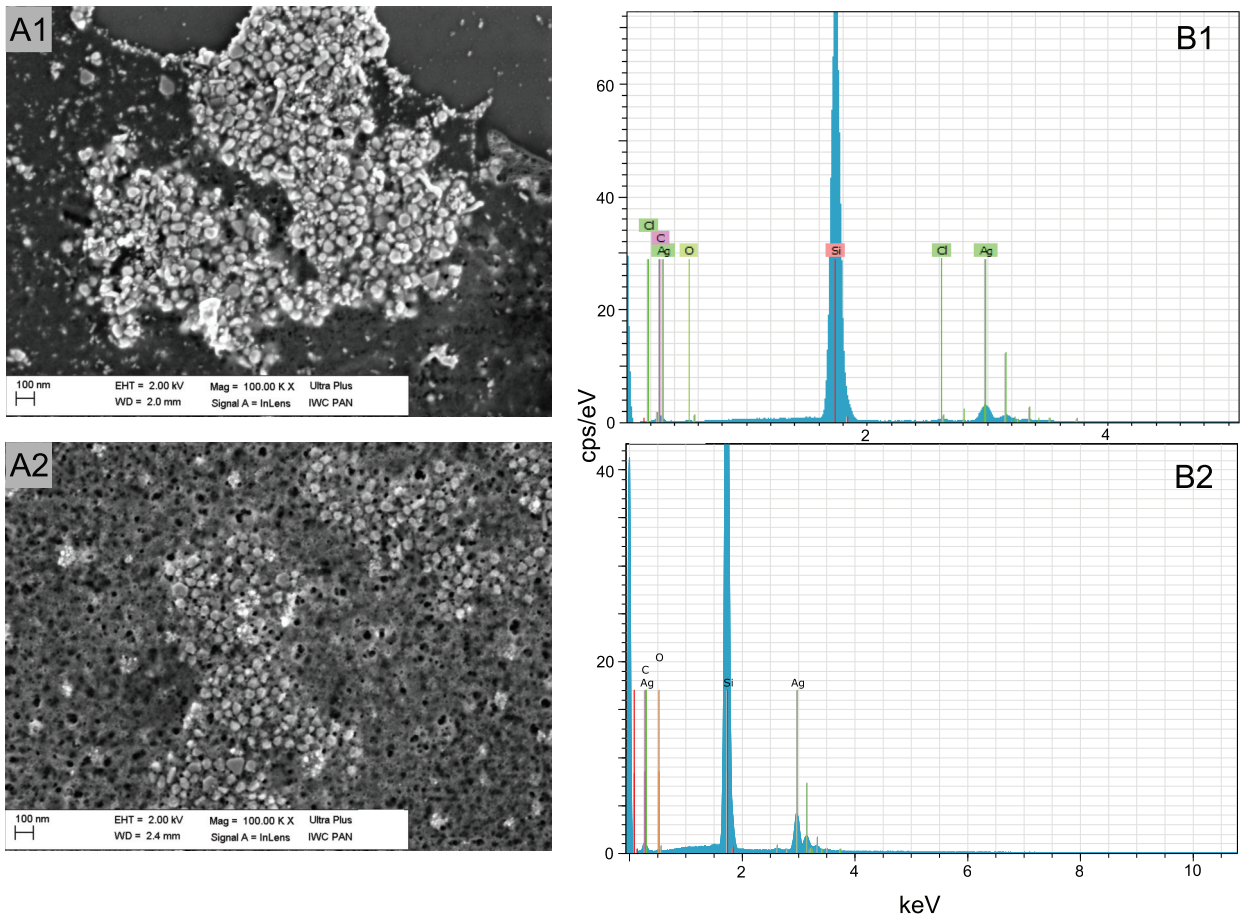

Figure 6. Scanning electron microscope image of AgNP (A) and energy dispersive X-ray spectroscopy elemental composition analysis (B) of AgNP obtained after bioreduction catalyzed by (1) raspberry or (2) strawberry leaf extract.

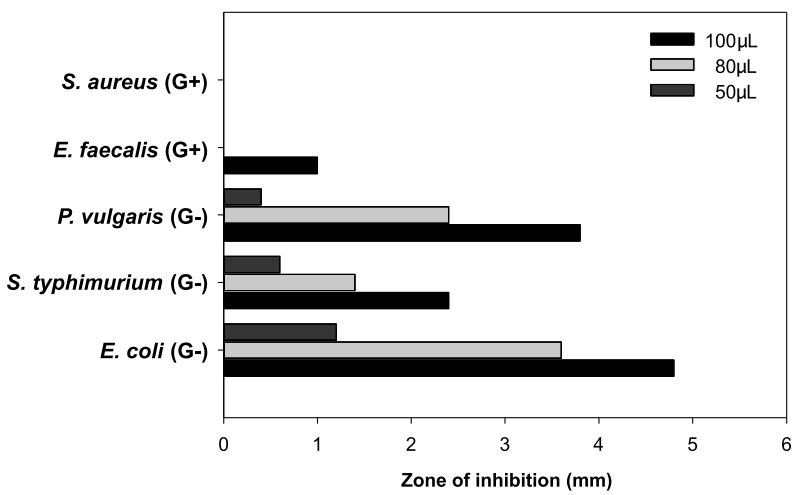

Figure 7. Inhibitory effect against test strains of AgNP synthesized with raspberry (*Rubus idaeus*) leaf extract (well diffusion method).

SUMMARY AND CONCLUSIONS

The use of waste and plant-materials in the synthesis of nanosilver (AgNP) is an attractive utilization of waste in biotechnology. The results of this study indicate that it is possible to bioreduce Ag^+ to Ag^0. This process should be analyzed in detail to study biomolecules and the chemical reactions that are involved, which will enable full control of the process for synthesis of nanoparticles possessing specific properties. Furthermore, the influence of nanoparticles on both living cells and the ecosystem should be examined

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