ENVIRONMENTAL BIOTECHNOLOGY 11 (1) 2015, 10-13; doi:10.14799/ebms249

Phytoremediation techniques in wastewater treatment

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Key words: macrophytes, pollutants, remediation

Received in February 2015. Published in October 2015.

ABSTRACT
Phytoremediation is a process of waste utilization and conversion by plants which includes such techniques as: phytoextraction, phytodegradation, phytovolatilization, rhizofiltration (phytofiltration) and phytostabilization. This review provides definitions and brief description of phytoremediation techniques and gives examples of their successful use.

INTRODUCTION

Pollution of the natural environment by heavy metals, pesticides, fertilizers, products of fuel combustion, etc. (Karadede and Unlü 2000; Riding et al. 2013) may pose a threat to humans, animals and plants (Lu et al. 2010; Zemleduch and Tomaszewska 2007). Traditional methods of the environment reclamation are usually expensive and can sometimes cause secondary pollution. Biological methods that use plants for treatment of polluted environment may provide an alternative to traditional techniques (Cunnigham and Berti 1993; Macek et al. 2000; Siwek 2008).

Phytoremediation is a rapidly developing method that uses plants to reduce, degrade, assimilate and metabolize environmental pollutants such as heavy metals, hydrocarbons, pesticides, etc. (Frick et al. 1999; Fu et al. 2003; Różański 1998; Susarla et al. 2002). The aim of our work was to provide a brief review of the literature concerning modern phytoremediation techniques that can be used in wastewater treatment processes.

PHYTOREMEDIATION TECHNIQUES

Phytoremediation is one of the biological methods that can be used in remediation of polluted sites *in situ*. There are various techniques of phytoremediation which are applicable in wastewater treatment, in surface water and groundwater purification, in the removal of excessive nutritive substances from water reservoirs, and in reclamation of soil polluted as a result of environmental disasters (Dordio and Carvalho 2013).

Phytoextraction
This technique allows removal of contaminants from the soil, groundwater or surface water by plants that have a high capacity for accumulation of toxic substances (Susarla et al. 2002). The plants used in this method are tolerant of high concentrations of heavy metals or organic compounds. They should also grow fast and produce large amounts of biomass. There are two categories of phytoextraction: continuous and induced processes. The continuous phytoextraction uses plants that accumulate high levels of toxic contaminants throughout their whole life cycle. The induced phytoextraction involves the

use of chelators in a particular phase of plant growth, which increases the accumulation of toxins in plants tissues (Mukhopadhyay and Maiti 2010; Starck 2005). Monferrán et al. (2012) investigated tolerance of *Potamogeton pusillus* towards Cr and Cu, the process of bioaccumulation and the capacity of removal of those metals from aqueous solution. The authors showed that roots and leaves of *P. pusillus* accumulate larger amounts of Cu and Cr than the stems. They conclude that it can be recommended for the use in phytoextraction of heavy metals from contaminated waters.

Phytodegradation

This method uses plants which produce enzymes that catalyze the degradation reactions of xenobiotics (Alkio et al. 2005). Phytodegradation may occur both in the plant or outside it, when the plant produces enzymes which are secreted into the soil of the root zone (Burns et al. 2013). This technique is used for the treatment of soil, river sediments and sludges as well as ground and surface water (Edwards and Dixon 2004; Małecka and Tomaszewska 2005). Zazouli et al. (2014) investigated the ability of *Azolla filiculoides* to remove bisphenol A (BPA) from aqueous solutions. *A. filiculoides* was cultured in a solution that contained 5, 10, 25 and 50 ppm BPA. The BPA degradation depended on the amount of *A. filiculoides* biomass and BPA concentration. The removal efficiency was more than 90% when BPA concentration was 5ppm and the amount of biomass was 0.9g. The authors concluded that *A. filiculoides* removes BPA from aqueous solution by the breakdown of contaminants through the plant metabolic processes or by the degradation of the surrounding contaminants by the enzymes produced and secreted by the plant.

Phytovolatization

Plants used in this technique absorb contaminants from the soil or water, metabolize them and then release them to the atmosphere as a volatile and less toxic form. This mechanism is mainly used for the purification of water and soil contaminated with selenium (Se), mercury (Hg) or arsenic (As) and organic compounds such as trichloroethylene, benzene, nitrobenzene, phenol, antrazine (Burken and Schnoor 1999; Gonćalves et al. 2007; Komives and Gullner 2005; Stroiński 1999; Wang et al. 2009). Tu et al. (2004) determined the location of As reduction and thiol formation in Chinese Brake fern (*Pteris vittata* L.). The researchers investigated the ability of different parts of the excised plants (pinnae, fronds and roots) to absorb As under phosphorus (P) influence. The plants accumulated As in the order of: pinnae > fronds > roots. In the presence of P, the order of phytoavailability of As species was As(III) > monomethylarsonic acid (MMA) > As(V) for pinnae and roots and As(V) > As(III) ~ = MMA for excised fronds. Arsenic was reduced intensively in the plant aerial parts and both As reduction and oxidation occurred in the roots, resulting in high concentrations of As(III) in the fronds and As(V) in the roots. Thiol formation was triggered by the plant absorption of As species, especially MMA. Plant As detoxification, aided by P, occurred through suppression of As(V) absorption, enhancement of As(V) reduction and decrease of thiol formation. It is concluded that excised Chinese Brake fern can efficiently reduce As(V) into As(III) and synthesize thiols in the aerial parts, resulting in As hyperaccumulation.

Rhizofiltration (Phytofiltration)

This method is used for the treatment of surface wastewater produced by industry and agriculture. The wastewater is sprinkled on the surface of roots or the plants are submerged in the treated water. For this reason, the plants used in this method should be highly tolerant to toxic compounds, resistant to low oxygen concentration, and should have an extensive root system, which grows rapidly and produces large amounts of biomass (Srivastava et al. 2014). Rhizofiltration is used for the removal of heavy metals, particularly lead (Pb) and radioactive elements (Gajewska et al. 2006; Marecik et al. 2006). Pratas et al. (2014) investigated the capacity of three plant species (*Callitriche stagnalis*, *Potamogeton natans* and *P. pectinatus*) grown in the laboratory phytofiltration system to reduce the uranium (U) concentration in contaminated water. They observed rapid uptake of U resulting in a reduction of its concentration in water of up to 85.5%. The amounts of U absorbed by *C. stagnalis* ranged from 0.98 to 1567mg·kg^{-1}, by *P. natans* from 3.46 to 271mg·kg^{-1}, and by *P. pectinatus* from 2.63 to 1588mg·kg^{-1}. The authors conclude that selected plant species might be successfully used for remediation of waters contaminated with U.

Phytostabilization

In this process the roots of plants are used in the soil remediation process. Phytostabilization prevents the movement of contaminants to groundwater and their migration to the surface soil and further with the rainwater runoff. Plants used for phytostabilization should have the following characteristics: highly developed root system that enables adsorption, absorption, and accumulation of contaminants in the tissues and their conversion within the rhizosphere to less soluble compounds (Segura and Ramos 2013). In addition, the plants should have low accumulation capacity for pollutants in their aboveground parts and high tolerance to varying pH, salinity and soil moisture (Cunnigham and Berti 1993). In 2014 Plechońska and Klink conducted a study on the phytoremediation abilities of red canary grass (*Phalaris arundinace*) towards trace metals (Zn, Fe, Mn, Pb, Cu, Ni, Cd, Co and Cr) accumulated in water and sediments. The results showed different concentrations of trace metals in various organs of the plant: highest concentrations were in roots and lowest in leaves. The authors concluded that limited translocation of trace metals absorbed by the red canary grass makes this plant a potential species for phytostabilization of sediments contaminated by metal (especially of Co and Cd) (Plechońska and Klink 2014).

WATER PLANTS USED FOR PHYTOREMEDIATION

Aquatic plants (macrophytes) are often used in phytoremediation. These plants are commonly found throughout Poland and constitute an essential component of wetlands (Dordio and Carvalho 2013). Also, the assessment of water purity is often based on the environmental characteristics of macrophyte populations. In natural and man-made filtering systems, macrophytes play an important role in the biochemical processes of water treatment. Their presence exerts a positive effect on the environment. Macrophytes assist in the stabilization of sludge, secure good conditions for water filtering and provide a location for the growth of microorganisms. Macrophytes have adapted to functioning in permanent contact with the surface water and groundwater. They have thin outer tissues, as well as aerenchyma, a specialized tissue that forms a system of canals and spaces through which air is distributed to the parts of a plant beneath the surface of the water. Macrophytes occur naturally, reducing the acquisition costs (Mielcarek and Krzemieniewski 2013).

Macrophytes accumulate many pollutants by incorporating them into the structure of their cells. In addition, these plants have a natural ability to absorb and metabolize xenobiotics whilst adapting to harsh conditions in contaminated environment (Coleman et al. 1997).

ADVANTAGES AND DISADVANTAGES OF PHYTOREMEDIATION

Phytoremediation techniques are used extensively because they have a number of advantages. One of these is the reclamation of the polluted environment by direct use of this method *in situ* (Gerhardt et al. 2009). The use of plants for environmental cleanup may be more effective than traditional methods based on chemical extraction of xenobiotics because biological methods do not cause secondary pollution. The extensive root system of plants can protect the soil from erosion by improving soil structure and penetrating into the deep layers of the soil, increasing soil productivity and aeration. The roots of plants also provide a location for the growth of microorganisms (Salt et al. 1997; Siwek 2008).

Another advantage of phytoremediation is its low cost when compared with conventional techniques. Phytoremediation techniques do not require specialized equipment (Marecik et al. 2006) and are accepted by local communities (Różański 1998; Wójcik and Tomaszewska 2005).

However, phytoremediation has also limitations. A significant disadvantage of phytoremediation conducted by aquatic plants is their shallow root system which results in limited depth of soil treatment by the rhizosphere. Another disadvantage is the slow rate of the environmental cleanup process, which may even last more than ten years (Marecik et al. 2006). There are also drops in phytoremediation effectiveness during winter (when plant growth slows down or stops) and due to the damage to vegetation caused by weather conditions, plant diseases or pests (Pivetz 2001).

CONCLUSION

Phytoremediation techniques which use aquatic plants offer potential benefits for the reclamation of contaminated environments. Biological methods are applicable to a broad range of contaminants and provide techniques for cleaning up the environment by enhancing natural remediation processes. Carefully selected macrophytes may be successfully used for remediation of water contaminated by various pollutants.

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ENVIRONMENTAL BIOTECHNOLOGY 11 (1) 2015, 14-19; doi:10.14799/ebms257

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

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Key words: nanobiotechnology, nanoparticles, nanosilver, plant extracts, raspberry, strawberry

Received in March 2015. Published in August 2015.

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 100cm³ 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 10cm³ of extract to 50cm³ of a 1, 3 or 5mM aqueous solution of $AgNO_3$. The bioreduction was carried out in darkness in 300cm³ 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 aureus*. 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

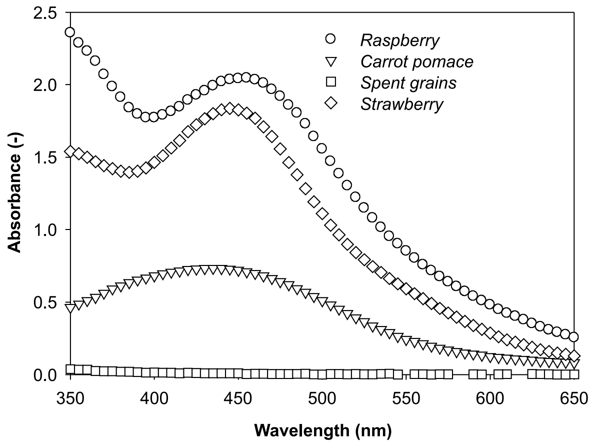

Figure 1. UV-visible light spectra of the products of 24h of bioreduction of Ag with various plant-waste extracts at 30°C. Absorbance in the range of 380-480nm indicates the presence of AgNP.

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.

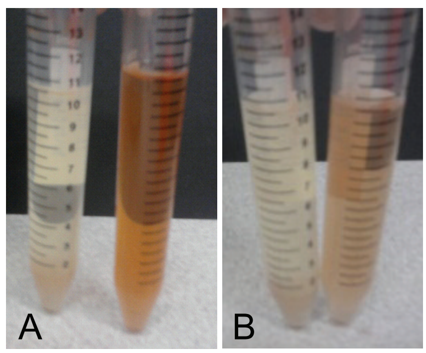

Figure 2. Reaction medium before (left) and after bioreduction (right) with (A) raspberry leaves and (B) carrot pomace extract.

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.

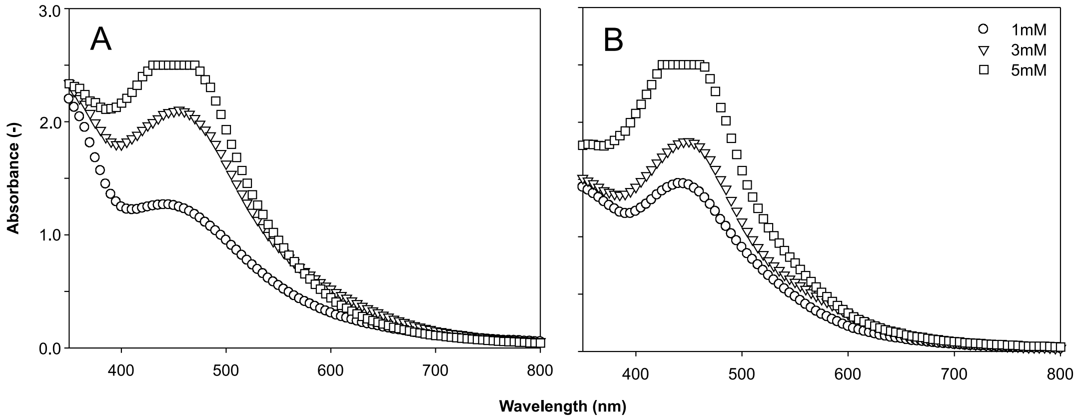

Figure 3. AgNP UV-vis spectra after bioreduction of different concentrations of $AgNO_3$ by extracts from leaves of (A) raspberry and (B) strawberry.

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.

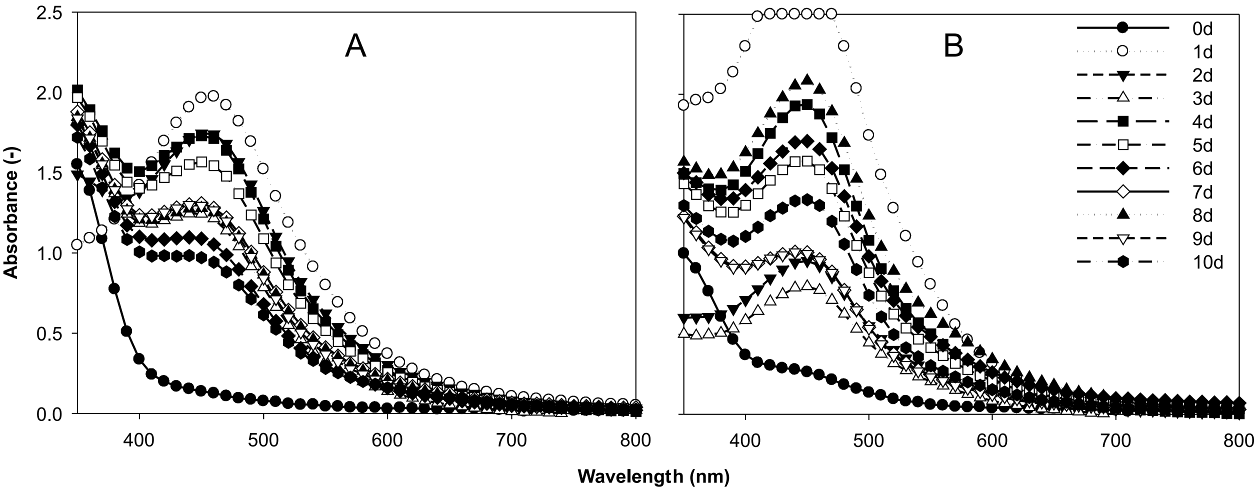

Figure 4. Influence of reaction time on AgNP synthesis by extracts from leaves of (A) raspberry and (B) strawberry (5mM $AgNO_3$).

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.

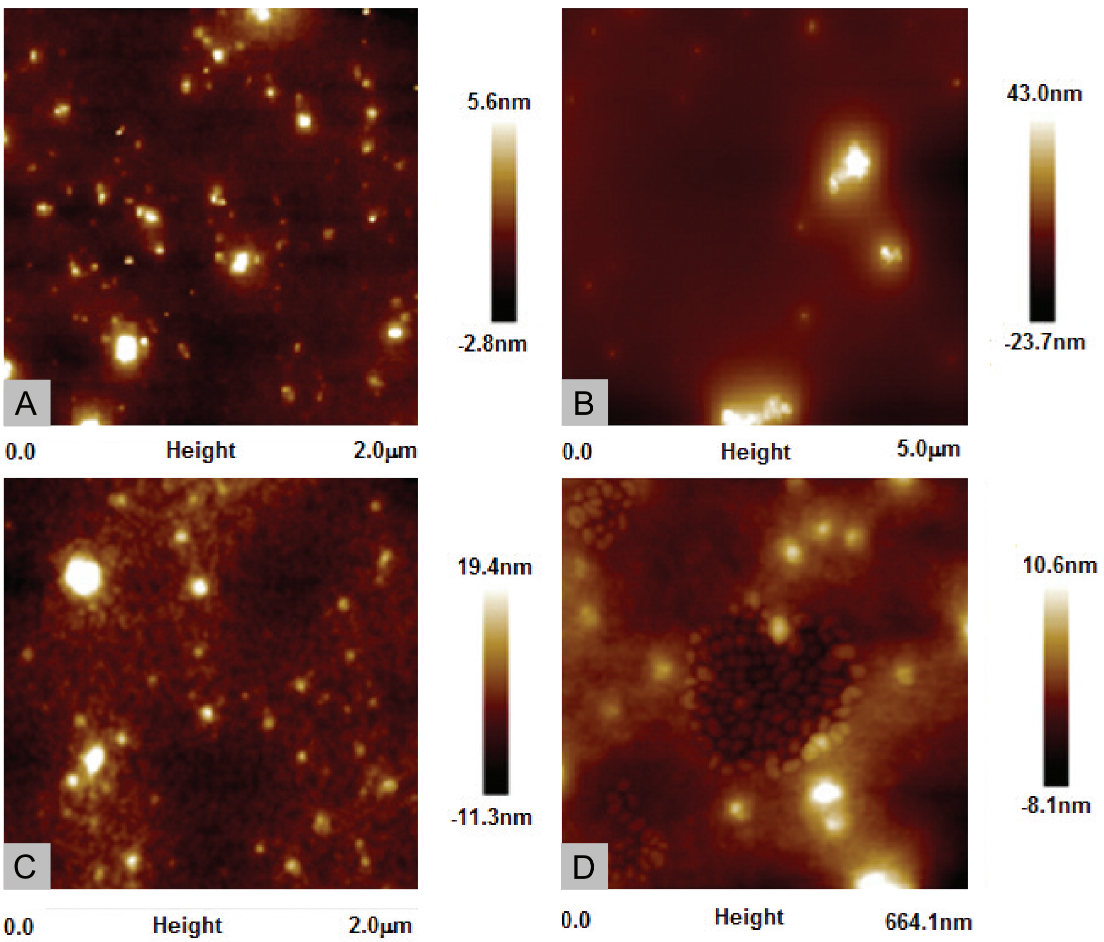

Figure 5. Atomic force microscope (AFM) images of AgNP synthesized by (A) carrot pomace, (B) strawberry or (C and D) raspberry leaf extract.

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.

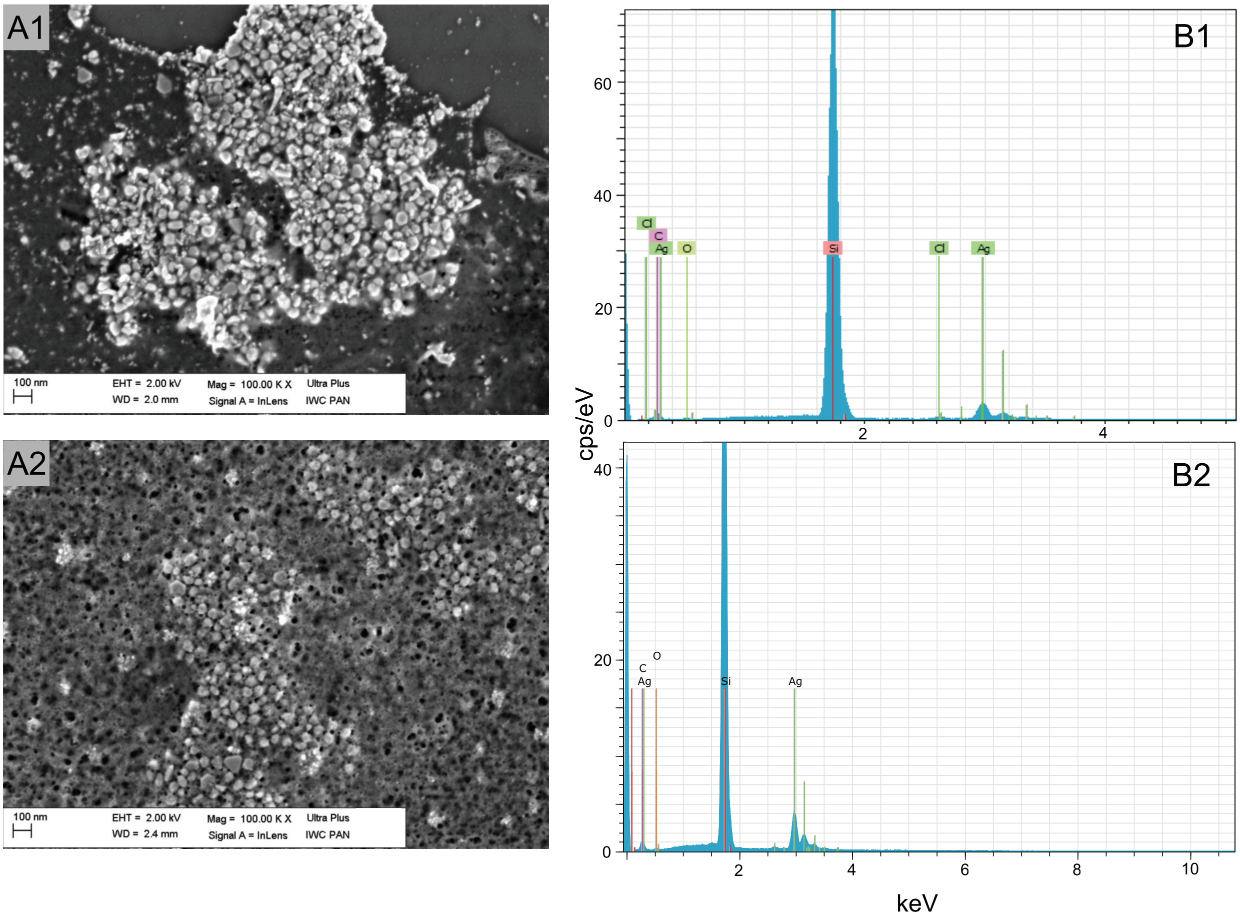

Figure 6. Scanning electron microscope image of AgNP (A) and energy dispersive X-ray spectroscopy elemental composition analysis (B) of AgNP obtained after bioreduction catalyzed by (1) raspberry or (2) strawberry leaf extract.

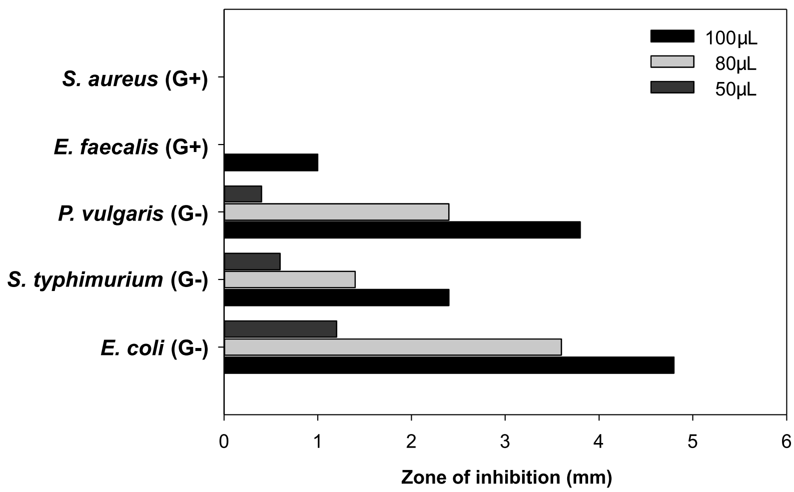

Figure 7. Inhibitory effect against test strains of AgNP synthesized with raspberry (*Rubus idaeus*) leaf extract (well diffusion method).

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

ACKNOWLEDGEMENTS

This work was supported by grants from the National Science Centre (Poland), project No. N N312 311339.

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