THE EFFECT OF CULTURE AGE AND INITIAL SILVER CONCENTRATION ON BIOSYNTHESIS OF Ag NANOPARTICLES

JANA KADUKOVÁ, OKSANA VELGOSOVÁ, ANNA MRAŽÍKOVÁ, RENÁTA MARCINČÁKOVÁ

Department of Material Science, Faculty of Metallurgy, Technical University of Kosice, Letna 9, 042 00 Kosice, Slovakia, (jana.kadukova@tuke.sk)

Abstract: Many organisms or their extracts have the ability to reduce Ag⁺ ions to Ag⁰ and stabilize them what results in nanoparticle formation in solution. The aim of the article was to study the influence of two selected parameters – initial silver concentration and culture age, on Ag nanoparticles production by green algae Parachlorella kessleri. The presence of Ag nanoparticles in the solution was confirmed by the UV-vis spectroscopy and TEM analyses. Typical curve with the peak at app. 420 nm was found for nanoparticles produced by algae. While culture age did not have any significant effect, the initial silver concentration had significant influence on nanoparticle production which influenced the rate of nanoparticle production, their amount, their size and stability, as well.

Key words: silver nanoparticles, Parachlorella kessleri, algae

1. Introduction

Utilisation of organisms in the field of nanoparticles production is gaining greater importance in the present time due to easy, non-toxic and environmentally friendly way of nanoparticle production (CASTRO et al., 2010). Biologically produced nanoparticles can be used the same way as nanoparticles produced by other synthetic ways in several fields such as non-linear optics, solar energy absorption (DAVENAS et al., 2008), biosensors (DUBAS and PIMPAN, 2008), sterilisation and disinfection (YAN and CHEN, 2003; RUPARELIA et al., 2008; MANEERUNG et al., 2008), bioimaging (SANGHI and VERMA, 2009), etc. Eukaryotic organisms seem to be very promising in biological production of nanoparticles due to their ability to release great amounts of enzymes into their environment. Extracellular formation in comparison with intracellular one represents easier and cheaper way of nanoparticle production (KORBEKANDI et al., 2009).

Nowadays, the ability of many organisms or even their extracts to reduce metal ions to metal particles and stabilize them what often results in nanoparticle formation in solution is known and studied in broad extent (GOVINDARAJU et al., 2008; SHARMA et al., 2009; FAYAZ et al., 2011; MUKUNTHAN et al., 2011; LOGESWARI et al., 2013; ROOPAN et al., 2013). The disadvantage of biological systems is production of nanoparticles with different shapes and wide size distribution. However, the preparation of nanoparticles with narrow particle distribution is crucial for utilization of silver nanoparticles (AgNPs) in many applications as their efficiency often depends on the surface area of silver nanoparticles (LIN et al., 2008). Particular emphasis has recently been placed on the control of shape, because in many cases it
allows properties to be fine-tuned with a great versatility that gives the particles a unique nature (XIANGQIAN et al., 2011).

In comparison with plant extracts the synthesis of nanoparticles using algae as a source has been unexplored and underexploited. There are few reports about biological synthesis of noble metal nanoparticles using algae, mostly based on the utilization of extracts from algae (XIE et al., 2007; JENA et al., 2013) or dry algal biomass (GOVINDARAJU et al., 2008; CASTRO et al., 2013; SHARMA et al., 2014). However, biosynthesis of metal nanoparticles by living algae has not been frequently reported. CHAKRABORTY et al. (2009) used living blue-green algae Lyngbya majuscula, Spirulina subsalsa and green alga Rhizoclonium hieroglypticum to study the biosorption and bioreduction of gold nanoparticles. They did not produce nanoparticles into the solution but desorbed them from the biomass surface. DAHOUMANE et al. (2014) used living cells of Chlamydomonas reinhardtii for production of stable bimetallic Ag-Au nanoparticles.

The aim of the article was to study extracellular production of silver nanoparticles using alga Parachlorella kessleri and the possibility to influence the nanoparticle formation process so that nanoparticles with the same shape and narrow size distribution would be produced.

2. Material and methods

2.1 Preparation of algal culture

Algae Parachlorella kessleri (syn. Chlorella kessleri) strain LARG/1, supplied by CCALA, no. 253. Locality: Russia, Moscow, Inst. General Genetics of Acad. Sci.USSR, Lab. Radiation Genetics were used for experiments. Algae were cultivated on agar plates in Petri dishes (diameter 9 cm). Millieu Bristol nutrient solution with 2% agar added (flake agar from Imuna Pharm, Šarišské Michaľany) was used for cultivation. Algal strains were cultivated under continuous light regime, at the temperature from 20 – 25°C.

2.2 Synthesis of silver nanoparticles

Stock silver solution was prepared by dissolution of AgNO₃ p.a. (from Mikrochem Company) in deionised water with final Ag⁺ ion concentration of 10 mM. The solution with required final concentration (in general 0.5 mM) was prepared by dilution of required volume of stock silver solution in deionised water.

Algae were added into Erlenmeyer flasks containing silver nitrate solution. All experiments were carried out in triplicates. The silver solution with Ag⁺ ion concentration 0.5 mM was prepared as a control sample and kept at the same conditions as experimental solutions. It was used as a reference solution for the absorbance measurement. After 24 hours (1st day) 3 ml of each solution was removed from the Erlenmeyer flasks and stored in the plastic tubes in a refrigerator. 1.0 ml of that solution was used to measure the absorbance. The samples were withdrawn and absorbance measured on the 3rd, 7th, 10th and 14th days.
To study the effect of culture age, algal cultures cultivated for 1, 2, 3 and 4 weeks on agar plates were used. To study the effect of initial silver concentration, the solutions with Ag⁺ ion concentrations of 0.5, 1 and 2 mM were used. For these experiments algae cultivated for 3 week were used.

2.3 UV-visible spectroscopy

The silver nanoparticle formation from silver ions was monitored by measuring the UV-vis spectra of the respective solutions in 10-mm optical-path-length quartz semimicrocuvettes. The spectrum was recorded on UNICAM UV/vis Spectrometer UV4 (from CHROMSPEC Company). The absorbance in the range of wavelengths between 200 nm and 800 nm was measured using wolfram (for measurement in vis range) and deuterium (for measurement in UV range) lamps.

2.4 Transmission electron microscopy (TEM)

The size and morphology of the nanoparticles were studied by means of a Transmission Electron Microscope (JEOL model JEM-2000FX microscope operated at an accelerating voltage of 200 kV). Samples for TEM analyses were prepared on carbon-coated copper grids. The films on the grids were allowed to dry in air prior analyses.

3. Results and discussion

A study on extracellular biosynthesis of silver nanoparticles by the vital culture of Parachlorella kessleri was carried out in this work. Visual observation of the experimental mixture after addition of algal cells into AgNO₃ solution showed a colour change from transparent to brown whereas no colour could be observed in control experiment without addition of cells. The appearance of brown colour of colloidal silver solution clearly indicates the formation of silver nanoparticles in the reaction mixture (PINTO et al., 2010). The formation of nanoparticles was confirmed by UV-vis spectroscopy. Typical spectrum is the result of the radiation absorption in the visible region of the electromagnetic spectrum due to the localised surface plasmon of silver nanoparticles (DUBAS and PIMPAN, 2008; BANKURA et al., 2012). The surface plasmon resonance (SPR) bands in absorption spectrum with the peak at app. 420 nm typical for spherical silver nanoparticles (PETIT et al., 1993) was found for nanoparticles produced by alga Parachlorella kessleri.

3.1 Influence of culture age

Alga Parachlorella kessleri has been grown on agar plates for different duration of time such as 1, 2, 3 and 4 weeks. The influence of the culture age on nanoparticle production was studied using the UV-vis spectral analysis of silver nanoparticles produced by selected algal cultures (Fig. 1A). The fact that all spectra are comprised of a single and well defined SPR bands is evidence that all colloids are composed of
small, round nanoparticles with relatively uniform size (DAHOUMANE et al., 2014). In the UV-vis absorption spectrum, SPR peaks located at 418, 420, 419 and 419 nm were observed for nanoparticles synthesized by 1, 2, 3 and 4 week-old algae, respectively. It is well documented that position of peaks, assigned to a surface plasmon, can be attributed to the nanoparticle size (PINTO et al., 2010). Based on the peak position of SPR bands shown in Fig. 1A it may be suggested that small and relatively uniform nanoparticles were produced.

To better understand the kinetics of production of the nanoparticles by algal cells, the formation of AgNPs in solution was monitored for 21 days using UV-vis spectroscopy. For each sample the maximum absorbance was plotted against time. As shown in Fig. 1B the same trend can be observed for all samples. Almost linear relationship between the maximum absorbance and time during the first 14 days was observed. The absorbance intensity did not change significantly between the 10th and 14th day indicating that all Ag\(^+\) ions were turned to nanoparticles (SHALIGRAM et al., 2009). From the 15th day the sharp decrease of maximum absorbance was recorded suggesting the aggregation and precipitation of nanoparticles. Dark silver precipitates were present in the bottom of the flasks on 21st day and solution colour changed into transparent confirming the precipitation of silver.

3.2 Influence of initial silver concentration

The possibility of controlling the reaction rate and particle size was further investigated by changing the initial Ag\(^+\) ion concentration. In order to study whether the concentration of silver ions plays an important role in the synthesis and size control of AgNPs, Ag\(^+\) ions concentrations of 0.5, 1 and 2 mM were used. The increase in the maximum absorbance measured after 24 hours of nanoparticle biosynthesis with the increase of the initial silver ion concentration was observed (Fig. 2A). The maximum synthesis of AgNPs occurred at 2 mM. The difference between maximum absorbance measured at concentration 1 mM and 2 mM, however, was not
significant what is different from the results obtained by GURUNATHAN et al. (2009). They found almost linear increase in the maximum absorbance up to concentration 5 mM. The AgNPs produced were characterised by UV-vis spectroscopy. The observation indicated that small, spherical nanoparticles were produced extracellularly (Fig. 2B).

Fig. 2. Effect of various concentrations of silver ions on AgNPs biosynthesis expressed by the dependence of maximum absorbance on initial Ag⁺ ion concentration (A) and UV-vis spectra of nanoparticles recorded after 24 hours of experiment.

The kinetics of production and the stability of silver nanoparticles can be observed in more detail in Fig. 3 where the maximum absorbance was plotted as a function of time.

Fig. 3. Maximum absorbance values of nanoparticles prepared at different initial silver concentration recorded at different time.

At silver ion concentration 0.5 mM linear increase of maximum absorbance with time was observed. At both higher studied concentrations (1 and 2 mM) the sharp increase of absorbance during the first 3 days was recorded then the decrease of maximum absorbance as well as formation of dark precipitates in the experimental flasks was observed suggesting the aggregation and precipitation of silver nanoparticles.
Fig. 4 shows that the sizes of AgNPs increase with increasing Ag$^+$ ion concentrations. This indicates that the size of AgNPs can be modulated by the concentration of AgNO$_3$. The reason of the increase in particle size with increasing AgNO$_3$ is not clear yet, however, it is obvious that silver ions, by their dispersive action, have a role in controlling the growth of AgNPs. On the contrary to our observations, GURUNATHAN et al. (2009) and SONG and KIM (2009) found that nanoparticle size decreased with the increase of Ag$^+$ ion concentration in the range of 1 – 5 mM. They speculated that AgNO$_3$ forms a coat on growing nanoparticles preventing their aggregation and resulting in formation of nanosized particles. In both studies mentioned above extracts from biomass were used in contrast to our experiments where algal cells were present in the experimental medium, thus, AgNO$_3$ could be adsorbed on the algal surface allowing broader aggregation of silver resulting in larger nanoparticles formation.

Surface plasmon resonance bands were observed at 414, 431 and 455 nm for the silver nanoparticles prepared at silver ion concentrations 0.5, 1 and 2 mM, respectively. According to literature the shift of $\lambda_{max}$ to higher wavelength values is associated with an increase in size of AgNPs (FRITSCHE et al., 1998), thus, this observation also confirms the increase of the nanoparticle size with increasing the initial silver ion concentration. This fact was finally confirmed by TEM analyses. The average nanoparticle size was found to be 9, 14 and 18 nm at initial silver concentration 0.5, 1 and 2 mM, respectively (Fig. 4).

![Graph showing average size of nanoparticles formed at different initial silver concentration.](image)

Fig. 4. Average size of nanoparticles formed at different initial silver concentration.

The representative TEM images of silver nanoparticles that were synthesised at different initial silver ion concentrations are shown in Fig. 5. Mono dispersed silver nanoparticles with uniform size and shape can be observed in left illustrations in Fig. 5. The silver particles’ size histograms (right illustrations in Fig. 5) show that the very small particles with average 9 nm were produced at silver ion concentration 0.5 mM.

Very narrow size distribution was found in this case in comparison with the both studied higher concentrations. More than 50% of nanoparticles were in the range of 6 – 10 nm. The presence of spherical silver nanoparticles relatively uniform in diameter in all three studied systems was confirmed by TEM images.
4. Conclusions

Size control during synthesis of particles is an important criterion in the area of silver nanoparticle biosynthesis. Depending on the size of nanoparticles, their application branch out. It has been demonstrated that the algae Parachlorella kessleri are capable of producing of silver nanoparticles extracellularly and the size and
stability of this nanoparticles can be controlled by the preparation conditions and nanoparticles with desirable characteristics can be produced. Although culture age did not affect the nanoparticle production, the initial silver concentration has influenced the process in great extent. The controlling particles size at different initial silver ion concentrations would play an important role during optimization of a process.

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References


