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CONTROL OVER THE BIOLOGICAL SYNTHESIS OF Ag NANOPARTICLES BY SELECTION OF THE SPECIFIC ALGAL SPECIES

The application of green synthesis in the nano-science and technology is of great importance in the area of the preparation of various materials. In this work, three selected algal species *Parachlorella kessleri*, *Dictyosphaerium chlorelloides* and *Desmodesmus quadricauda* were successfully used for the preparation of silver nanoparticles (AgNPs). Presence of AgNPs was confirmed by UV-vis spectroscopy and transmission electron microscopy. AgNPs produced by *P. kessleri* had narrow size distribution and average sizes of 7.6 nm. However, nanoparticle production lasted for long time. Nanoparticle formation by *D. chlorelloides* was the fastest, although, their average sizes were 23.4 nm with broad size distribution. Nanoparticles produced by *D. quadricauda* had average sizes 23.9 nm but they were the least stable, aggregated and precipitated from solutions within 3 days. These results confirmed that the size distribution and mean diameter of the nanoparticles, crucial for various applications, can be controlled by the organism selection.

Keywords: nanoparticles, silver, algae, *Parachlorella kessleri, Dictyosphaerium chlorelloides, Desmodesmus quadricauda*

1. Introduction

Exceptional properties of nanoparticles, primarily attributed to the quantum size effect are confronted by their environmentally unfriendly synthesis method [1]. In recent years, great efforts have been made in the field of bionanotechnology to develop eco-friendly and sustainable greener processes [2].

Among heavy metal nanoparticles, silver nanoparticles have received major attention due to their unique characteristics (chemical stability, catalytic activity or electrical conductivity) resulting in their application in various technologies [3]. The preparation of nanoparticles with narrow particle distribution is crucial for utilization of AgNPs in many applications [4]. This has motivated different activities in searching for new synthesis routes that allow better control of shape and size during nanoparticle formation [5].

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 [6] or dry algal biomass [7-9]. However, biosynthesis of metal nanoparticles by living algae has not been frequently reported. Chakraborty at paper [10] used living blue-green algae *Lyngbya majuscule*, *Spirulina subsalsa* and green alga *Rhizoclonium hieroglyphicum* 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 at paper [11] used living cells of *Chlamydomonas reinhardtii* for production of stable bimetallic Ag-Au nanoparticles.

The main aim of the present research was to study if the selection of specific algal species can influence the production of nanoparticles (rate of production, rate of aggregation) or major nanoparticle characteristics such as size, size distribution or shape. For the preparation of silver nanoparticles three selected living algal species *Parachlorella kessleri*, *Dictyosphaerium chlorelloides* and *Desmodesmus quadricauda* were used.

2. Experimental methods

Algae *Parachlorella kessleri,* (syn. *Chlorella kessleri*) strain LARG/1, *Dictyosphaerium chlorelloides* strain Kováčik 1978/11 and *Desmodesmus quadricauda* (syn. *Scenedesmus quadricauda*) strain Greifswald/15 supplied by Institute of Botany, Czech Academy of Sciences were used for the experiments. The algal strains were cultivated on agar plates in Petri dishes (diameter 9 cm). Millieu Bristol nutrient solution with 2% agar added was used for cultivation. Algal strains were cultivated

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for 3 weeks under continuous light regime, at the temperature from 20 – 25°C.

Stock silver solution was prepared by dissolution of $AgNO_3$ p.a. (supplied by Mikrochem Company) in deionised water with final Ag^+ concentration of 1 g/l. The solution with final concentration of 50 mg/l (0.46 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 concentration 50 mg/l 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 procedure was repeated on the 3rd, 7th, 10th and 14th days.

The silver nanoparticle formation from silver ions was monitored by measuring the UV-vis spectra by UNICAM UV/vis Spectrometer UV4 (from CHROMSPEC Company).

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).

Differences in measured nanoparticle size produced by different algal clones were evaluated by Kruskal-Wallis analysis and analysis of variance, respectively. This was followed by multiple range analysis to ascertain differences between individual groups. The results of analyses were evaluated at 0.05 significance level using Statgraphics® statistical software.

3. Results and discussion

Addition of green algal biomass to $AgNO_3$ solution led to appearance of yellow-brown colour solutions after 24 hours indicating the formation of silver nanoparticles. Solution colour is the result of the radiation absorption in the visible region of the electromagnetic spectrum (380-450 nm) due to the localised surface plasmon of silver nanoparticles [12].

The presence of Ag nanoparticles in solution was confirmed by the UV-vis spectroscopy. Position and number of the surface plasmon resonance (SPR) bands in absorption spectrum depend on the particle shape – one band with a maximum at app. 400 nm for spherical nanoparticles [13].

The absorbance spectra (Fig. 1b) showed the slowest increase in the absorbance of AgNP solution when *P. kessleri* was used, with the maximum on the 10th and 14th days indicating the slowest nanoparticle formation. The SPR bands were symmetrical suggesting that the nanoparticle size and shape distribution is narrow (Fig. 1a).

The SPR bands symmetry started to decrease only on the 7th day indicating the formation of different size particles with anisotropy in the shape. The SPR bands shifted to higher wavelengths during the experiments in both cases. The significant shift of maximum absorbance (λ_{max}) from 419.5 to 450 nm was observed for *P. kessleri* strain. This shift of λ_{max} to higher wavelength values has been associated to an increase in size of AgNPs.

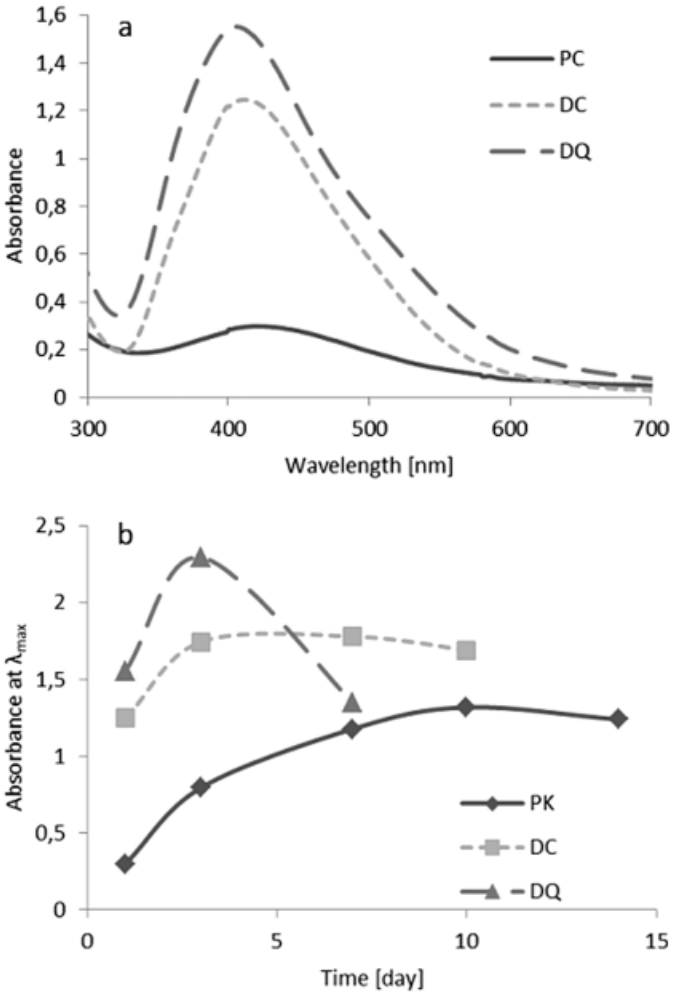

Fig. 1. The UV-vis absorption spectra measured for the AgNPs solution prepared by *P. kessleri* (PK), *D. chlorelloides* (DC) and *D. quadricauda* (DQ) after 24 hours (a) and absorbance variations at λ_{max} as a function of time during nanoparticle preparation (b)

From all three studied algal species the highest absorbance was observed in the nanoparticle solution when alga *D. chlorelloides* was used. The absorbance intensity did not change significantly after the 3rd day indicating that all Ag^+ ions were turned to nanoparticles [14]. The decrease of λ_{max} may be attributed to the decrease of the nanoparticle size but according to [15] it is possible that nanoparticles undergone intraparticle ripening after complete consumption of the monomer and might become more spherical-like in shape without changing their size.

Very high absorbance was also observed in the nanoparticle solution produced by the *D. quadricauda* on the first experimental day. Small shoulder was present in the spectrum curve suggesting the presence of not only spherical nanoparticles. According to [16] the presence of the shoulder indicates broad distribution in size and shape related to particle crystallisation. Nanoparticles produced by alga *D. quadricauda* had the highest tendency to aggregate, the sharp decrease of absorbance

and production of dark precipitated on the vessel bottom were observed on the 3rd day.

The information gathered by the UV-vis spectra was complemented by transmission electron microscope (TEM) images. Figure 2 show representative TEM images recorded from the silver nanoparticles that were synthesized within 24 hours by treating the silver nitrate solution with three selected algal species.

TEM analyses confirmed formation of spherical silver nanoparticles. According to the results of TEM analyses it can be assumed that the size of nanoparticles is influenced by the rate of their production within the first 24 hours what can be directly connected with the used alga.

The fast absorbance increase measured for *D. chlorelloides* and *D. quadricauda* was connected with the production of large irregular nanoparticles. The average size of the produced nanoparticles by *D. chlorelloides* was 23.4 nm with the broad size distribution ranging from 10 to 43 nm and nanoparticle of average size 24 nm were produced by *D. quadricauda*. Similar was also size distribution ranging from 7-50 nm.

On the contrary, the process of nanoparticle production by *P. kessleri* was the slowest but formed nanoparticles were the smallest (7.6 nm in diameter) with narrow size distribution. The silver particles´ size histograms (right part of Fig. 2) show that the particles ranged in size from 3 to 13 nm.

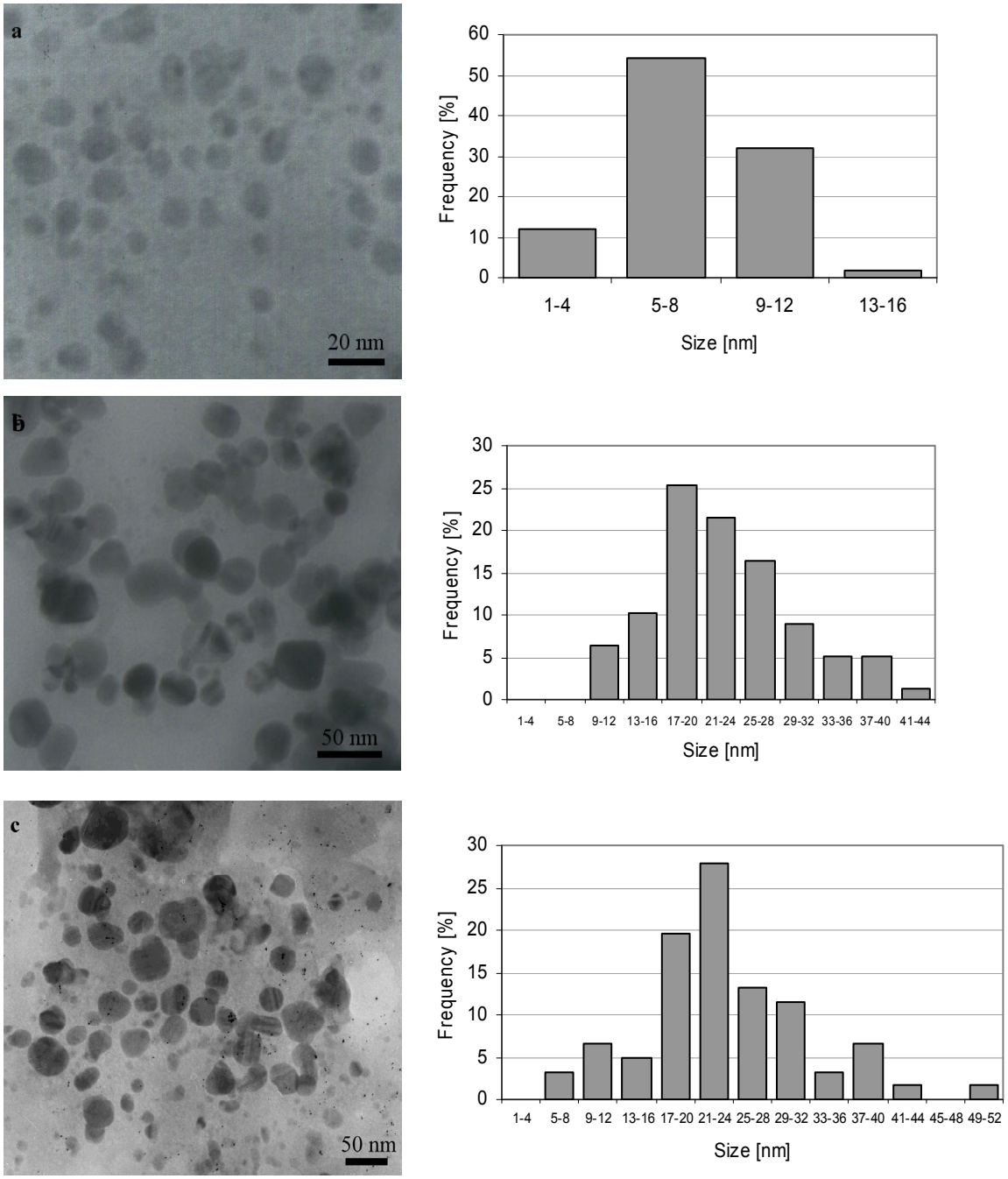

Fig. 2. TEM images and corresponding size distribution histograms built from their analyses for AgNPs synthesized with alga *Parachlorella kessleri* (a), *Dictyosphaerium chlorelloides* (b) and *Desmodesmus quadricauda* (c)

Statistically significant differences in average nanoparticle sizes were found between *P. kessleri* and two other used algal species *D. chlorelloides* and *D. quadricauda* (Fig. 3). The influence of particular species used for nanoparticle preparation to their size was found also for Au, Pt and Pd nanoparticles [10,17].

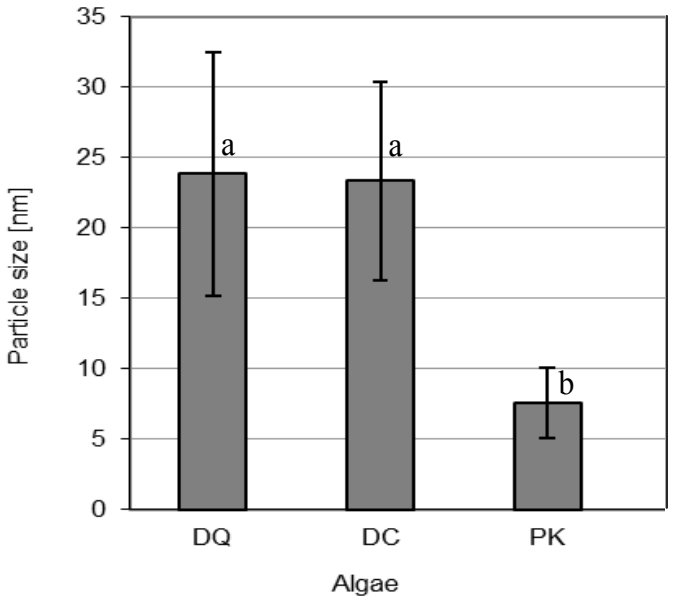

Fig. 3. Average sizes (±S.E.) of AgNPs produced by *P. kessleri* (PK), *D. chlorelloides* (DC) and *D. quadricauda* (DQ). Columns marked by the same letter are not significantly different at the $p < 0.05$ level based on the multiple range analysis

4. Conclusion

The present study demonstrates a facile, biosynthetic route for the fabrication of silver nanoparticles in a single step with all three studied algal species – *Parachlorella kessleri*, *Dictyosphaerium chlorelloides* and *Desmodesmus quadricauda*. Differences were found in the rate of synthesis and resulting size distribution and shape of the produced silver nanoparticles suggesting that the compounds released by algal cells had important influence on the nanoparticle size as well as shape. In conclusion, it has been confirmed that the size distribution and mean diameter of the nanoparticles can be controlled by the organism selection.

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