Study of Mechanisms Used by Algae to Decrease The Silver Toxicity in Aquatic Environment

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Abstract

In the study SEM, EDS, TEM and UV-vis analysis were used to investigate the biosorption, bioaccumulation and bioprecipitation/bioreduction of silver by freshwater green alga Parachlorella kessleri *and to shed light on the reasons of biological silver nanoparticle production. When dead biomass of* P. kessleri *was used for silver removal, majority of silver (75%) was removed within 2 min. Biosorption was probably the main mechanisms responsible for Ag+ ions removal from aqueous solutions. However, when behaviour of living biomass in the presence of silver ions was studied, the decrease of silver concentration was slower (68% within 24 hours) with subsequent increase of silver concentration in the solution and extracellular formation of silver nanoparticles. The formed AgNPs exhibited a lower toxicity against tested organisms. Algal cells probably used the formation of nanoparticles combined with rapid biosorption as detoxification mechanisms against silver toxicity. Bioaccumulation inside the cells played only a minor role in the detoxification process.*

Keywords: silver, nanoparticle, detoxification, alga

Introduction

Although silver has a great reputation for its use in jewellery and coins, its primary use is industrial. Majority of silver, about 35% is used in electrical applications. Except of utilization in industry followed by utilization in jewellery and coins, silver is used in many various fields (Deschatre et al., 2015). However, nowadays we can see increasing utilization of silver in the form of silver nanoparticles because of their unique characteristics resulting in their application in various technologies such as solar energy absorption, catalyses of chemical reaction, surface enhancement Raman spectroscopy (SERS), biosensors, and many others. Silver's antibacterial qualities have applications that reach far beyond the medical world. Washing machines, refrigerators, air conditioners, air purifiers and vacuum cleaners all rely upon silver nanoparticles to sterilize up to 650 types of bacteria (Cantuaria et al., 2014). The total silver physical demand stood at about 33 thousand tons in 2014 and its supply was by 4.9% lower. Silver is known to be released to the environment through its industrial applications (Das 2010). Annually a minimum of 150 tons of produced silver enters the aquatic environment (Salunkhe et al., 2011).

Ionic silver is known as highly toxic to aquatic organisms, however recent studies have shown that the nano form of silver is more toxic and can cause damage in new ways. Even if the nanoparticle itself is not especially toxic, it increases the effectiveness of delivering silver ions to locations where they can cause toxicity (Fabrega et al., 2011). In the present time, considerable amount of nanosilver is used in various commercial products despite the fact that only very little is known about the environmental effects of their widespread use. On the other hand there is increasing amount of evidence that different organisms, their extracts or even exhausted cultivation media can be used for nanoparticles production. So it is visible that probably large group of biological molecules can be responsible for the silver reduction and nanoparticle formation. What means that organisms have already met with nanoparticles and they probably own mechanisms leading to nanoparticle toxicity elimination. So the aim of our work was to contribute to the study of silver nanoparticles fate in the environment and mechanisms which allow aquatic organisms to cope with the toxicity of silver ions. We have investigated three basic mechanisms – biosorption, bioaccumulation and bioreduction and their contribution to the silver removal or transformation in the presence of freshwater alga *Parachlorella kessleri*. These algae are commonly present in various aquatic environments so it is very probable that they have met with the silver ions and can be the part of biochemical routes resulting in its detoxification in the environment. We have compared the range of silver biosorption, bioaccumulation and bioprecipitation and their contribution to silver removal from environment and studied the differences in the toxicity of Ag+ ions and AgNP produced by algae so we would be able to contribute to the understanding of reasons of massive silver nanoparticle production in the biological environment.

As it is known biosorption is a process independent of metabolism, fast and reversible. Its mechanisms are generally based on physico-chemical interactions between metal ions and the functional groups present on the cell surface. Biosorption is mostly described in non-living cells however it can take place in living cells being the first, fast and reversible adsorption step operating within a much slower and complex overall bioaccumulation mechanism (Fomina, Gadd, 2014). Bioaccumulation is a process of intracellular metal accumulation taking part mostly in living organisms and depends on a variety of physical, chemical and biological mechanisms. It is slow process dependent on cell metabolism (Chojnacka, 2010, Kadukova, 2016). Bioprecipitation can

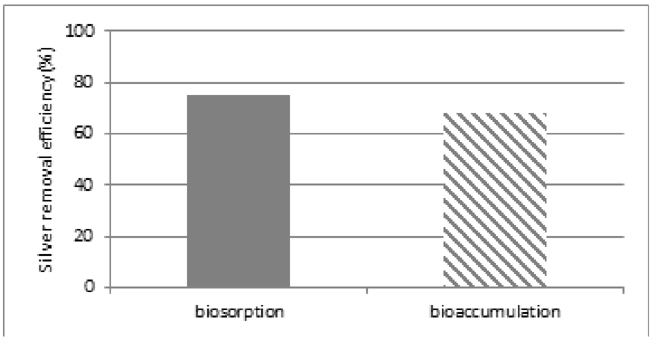

Fig. 1. Efficiency of silver removal by non-living (biosorption) and living (bioaccumulation) biomass of *P. kessleri*
Rys. 1. Efektywność usuwania srebra przez nieżywą (biosorpcję) i żywą (bioakumulację) biomasę P. kessleri

take place as metabolisms independent as well as metabolism dependent process resulting in production of precipitates in the cell environment or on the cell surface (Gadd, 2009).

Materials and methods

Biosorption experiments were performed using the same procedure as in previous studies with *P. kessleri* (Kadukova and Vircikova, 2005, Kadukova, 2016). Dried algal biomass (2 g/L) was placed in contact with $AgNO_3$ solution (80 mg/L) at room temperature and stirred magnetically for kinetic experiments. Equilibrium studies were conducted with initial silver concentrations in the range 5–850 mg/L. The pH values of solutions were adjusted to 5.0 (optimum value) by adding 0.1 M H_2SO_4 and 0.1 M NaOH solutions as appropriate. Three replicates were used in the study for both kinetic and equilibrium studies.

Samples were withdrawn at pre-determined time intervals (0, 2, 4, 6, 8, 10, 30, 60, 90, 120, 240, 480 and 1440 min, 2 and 4 days) for kinetic experiments and after 24 h for equilibrium experiments. The pH and redox potential were measured at the time of sample withdrawal using a GRYF 208L pH metre. Removed liquid samples were filtered through a membrane filter to determine the metal concentration using atomic absorption spectroscopy (Perkin Elmer model 3100 spectrophotometer). Sorption capacity q (mg/g) was calculated using the general equation which can be found elsewhere.

Bioaccumulation experiments were carried out similarly just living biomass of *P. kessleri* obtained after biomass filtration and consequent washing was used in the experiment. The volume of silver solution was adjusted so that the final cell concentration corresponded to 2 g/L of dry biomass. Solutions with the same concentrations as in the biosorption experiments were used for equilibrium studies and with the concentration 40 mg/L for kinetic studies. The amount of Ag accumulated by *P. kessleri* was calculated on a dry weight basis following the equation used for calculation of metal uptake by biosorption. To determine nanoparticle production UV–vis spectra of samples were recorded using an UNICAM UV4 UV/vis, dual beam spectrophotometer from 200 to 800 nm, operating at a resolution of 1 nm with quartz cells. As blanks, deionised water was used.

Results and discussion

The differences in silver ions biosorption and bioaccumulation are visible in the Fig. 1. When dead algal biomass was used 75% of silver was sorbed onto its surface within only 2 minutes. But in the presence of living cells the process was different. After addition of living biomass, the silver concentration showed an initial rapid decrease and remained roughly constant up to 24 h. A total decrease of silver concentration by 68% was observed. However, after 24 h the silver concentration in solution gradually increased up to 14 days. At the same time silver nanoparticles were produced in the solution. However, during the biosorption study reduction of silver into nanoparticles was not observed (Kadukova, 2016).

According to our previous studies (Kadukova et al., 2015, Kadukova, 2016) silver nanoparticles produced by alga *Parachlorella kessleri* were found to be less toxic not only for alga itself but also for other tested organisms in comparison with ionic silver. Thus, it is possible that nanoparticle production can be a part of detoxifying mechanism that alleviates the silver ion toxicity. The process of silver detoxification can take part in two steps:

1. The rapid binding of silver ions to the living algal biomass shortly after the biomass addition can be attributed to a metabolism-independent passive adsorption to the functional groups located on the cell surface
2. It is followed by active reduction either on the cell surface or in the solution later on.

The majority of silver taken up by the biomass was released back into solution. Although a gradual increase of total silver concentration was measured, the concentration of free silver ions remained constant for the whole process, suggesting that silver released back into solution was not only desorbed but probably reduced into metallic form (Kadukova, 2016).

According to the results, probably only a very small part of the silver ions (about 2%) was accumulated inside the cells. The majority (almost 50%) of the silver was reduced and nanoparticles were formed. Approximately 30% of silver ions remained in the solution.

Since the mechanism of biological nanoparticle synthesis has not yet been fully understood, there are many different hypotheses for their production. In the presence of algae it is probable that the huge amount of hydroxyl groups in the algal cell wall act as reductors of silver cations forming, in consequence, carbonyl groups. Following equation can be suggested to describe the process:

$$Ag^+ + R - OH \rightarrow Ag^0 + R = O + H^+ \quad (1)$$

Conclusion

It is known that silver is very toxic for microorganisms including algae. Although silver is easily precipitated in the environment there is still a portion that can be sequestered by organisms and transformed into silver nanoparticles. In our study we have found that in the presence of living algal cells silver ions were probably sequestered on the algal surface and gradually transformed into nanoparticles which are according to preliminary tests less toxic to algal cells in comparison with ionic silver and so production of silver nanoparticles can represent the detoxication mechanisms decreasing the silver toxicity in the environment. However, in the presence of non-living biomass silver was rapidly taken up by the biomass and remained sorbed onto algal surface without its transformation onto silver nanoparticles.

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Literatura – References

1. CANTUARIA M.L., ALMEIDA NETO A.F., VIEIRA M.G.A.: Biosorption of silver by macrophyte Salvinia cucullata, Chemical Engineering Transactions, 38, 2014, 109-114
2. DAS, N.: Recovery of precious metals through biosorption – A review, Hydrometallurgy, 103, 2010, 180-189.
3. DESCHATRE, M., LESCOP, B., SIMON COLIN, C., GHILLEBAERT, F., GUEZENNEC, J., RIOUAL, S.: Characterization of exopolysaccharides after sorption of silver ions in aqueous solution, Journal of Environmental Chemical Engineering, 3, 2015, 210–216.
4. FABREGA, J., LUOMA, S.N., TYLER, CH.R., GALLOWAY, T.S., LEAD, J.R.: Silver nanoparticles: Behaviour and effects in the aquatic environment, Environment International, 37, 2011, 517-531.
5. FOMINA, M., GADD, G.M.: Biosorption: current perspectives on concept, definition and application, Bioresource Technology, 160, 2014, 3–14.
6. GADD, G.M.: Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment, Journal of Chemical Technology and Biotechnology, 2009, 84, 13–28.
7. CHOJNACKA, K.: Biosorption and bioaccumulation – the prospects for practical application. Environment International, 36, 2010, 299–307.
8. KADUKOVÁ, J., VELGOSOVÁ, O., MRAŽÍKOVÁ, A., MARCINČÁKOVÁ, R., TKÁČOVÁ, E.: Assessment of Biologically Synthesized Ag Nanoparticles Toxicity against E. coli, Staphylococcus aureus, Parachlorella kessleri and Sinapis alba, Nova Biotechnologica et Chimica, 14, 1, 2015, 69-77.
9. KADUKOVA, J., VIRCIKOVA, E.: Comparison of differences between copper bioaccumulation and biosorption, Environment International, 31, 2, 2005, 227-232.
10. KADUKOVA, J.: Surface Sorption and Nanoparticle Production as a Silver Detoxification Mechanism of the Freshwater Alga Parachlorella kessleri, Bioresource Technology, 216, 2016, 406-413.
11. SALUNKHE, R.B., PATIL, S.V., SALUNKE, B.K., PATIL, CH.D., SONAWANE, A.M.: Studies on Silver Accumulation and Nanoparticle Synthesis By Cochliobolus lunatus, Applied Biochemistry and Biotechnology, 165, 2011, 221–234.

Badanie mechanizmów stosowanych przez glony w celu zmniejszenia toksyczności srebra w środowisku wodnym

W badaniu wykorzystano analizę SEM, EDS, TEM i UV-vis do zbadania biosorpcji, bioakumulacji i bioprecypitacji/bioredukcji srebra przez słodkowodną zieloną algę Parachlorella kessleri *i rzucenia światła na przyczyny biologicznej produkcji nanocząstek srebra. Gdy do usunięcia srebra użyto martwej biomasy* P. kessleri*, większość srebra (75%) usunięto w ciągu 2 minut. Biosorpcja była prawdopodobnie głównym mechanizmem odpowiedzialnym za usuwanie jonów Ag+ z roztworów wodnych. Jednakże, gdy badano zachowanie żywej biomasy w obecności jonów srebra, spadek stężenia srebra był wolniejszy (68% w ciągu 24 godzin) z późniejszym wzrostem stężenia srebra w roztworze i zewnątrzkomórkowym tworzeniem nanocząstek srebra. Utworzone AgNP wykazywały mniejszą toksyczność wobec badanych organizmów. Komórki glonów prawdopodobnie wykorzystywały tworzenie nanocząstek połączonych z szybką biosorpcją jako mechanizmów detoksykacji przeciwko toksyczności srebra. Bioakumulacja wewnątrz komórek odgrywała tylko niewielką rolę w procesie detoksykacji.*

Słowa kluczowe: srebro, nanocząstka, detoksykacja, alga