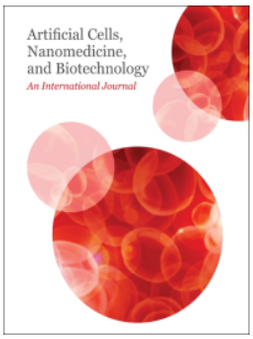

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Biosynthesis of silver nanoparticles using *Saccharomyces cerevisiae*

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Abstract

The objectives of this study were the biosynthesis of silver nanoparticles (NPs) by biotransformations using *Saccharomyces cerevisiae* and analysis of the sizes and shapes of the NPs produced. Dried and freshly cultured *S. cerevisiae* were used as the biocatalyst. Dried yeast synthesized few NPs, but freshly cultured yeast produced a large amount of them. Silver NPs were spherical, 2–20 nm in diameter, and the NPs with the size of 5.4 nm were the most frequent ones. NPs were seen inside the cells, within the cell membrane, attached to the cell membrane during the exocytosis, and outside of the cells.

Keywords: biosynthesis, biotransformation, nanoparticle synthesis, *Saccharomyces cerevisiae*, silver nanoparticles, synthesis location

Introduction

Nowadays, research in nano biotechnology is focused on developing eco-friendly processes for the synthesis of nanoparticles (NPs) with well-defined morphologies and characteristics (Iravani 2011, Iravani and Zolfaghari 2013, Korbekandi et al. 2009, 2012, 2013,, Iravani et al. 2014a, 2014b). Actually, there is a growing need to develop environmentally and economically friendly processes, which do not use toxic and expensive chemical materials in the synthesis protocols. Increasing pressure to develop green methods for nanoparticle synthesis has provoked researchers to shift to biological systems, including bacteria, yeasts, actinomycetes, algae, and plants). Biotechnological applications such as bioremediation of toxic metals have been employed for a long time (Aiking et al. 1982, Shankar et al. 2005, Mehra and Winge 1991, Beveridge and Murray 1980, Niemeyer 2005, Southam and Beveridge 1994). However, the possibility of using such organisms in the deliberate synthesis of NPs is a relatively new procedure.

Saccharomyces cerevisiae (baker's yeast) is a useful yeast which grows fast and its industrial use is simple. *S. cerevisiae* is probably the best studied yeast species, regarding physiology and genetics, and definitely the one with the biggest industrial significance, which can be considered as one of the best candidates for nanoparticle synthesis. Several publications reported the potential of baker's yeast biomass for bioremediation by biosorption of heavy metals, for example, cadmium and lead or by binding cadmium to glutathione (GSH) with the resulting cadmium–bisglutathionate complex and consecutive transport into vacuoles (Breierova et al. 2002, Goksungur et al. 2005). The potential of *S. cerevisiae* to synthesize metal NPs has been reported, as well. For instance, Lin et al. reported that gold ions were bound to the cell wall of dead cells of the yeast *S. cerevisiae* and then reduced *in situ*. In another study, Jha et al. (2009b) demonstrated that baker's yeast was able to produce spherical antimony trioxide (Sb_2O_3) NPs. Spherical amorphous iron phosphate nanoparticles with broad size distribution of 50–200 nm were formed within baker's yeast cells exposed to $FeCl_3$ solution (He et al. 2009). In other studies, the formation of almost spherical extracellular TiO_2 NPs (8–35), biosynthesized by *S. cerevisiae*, was reported (Jha et al. 2009a).

The objectives of this study were the biosynthesis of silver NPs by biotransformations using this yeast and analysis of the sizes and shapes of the NPs produced. Dried (commercially available), and freshly cultured *S. cerevisiae* were used as the biocatalyst.

Materials and methods

Culture and media

Freshly cultured S. cerevisiae.

Silver nitrate ($AgNO_3$, 99.99%) was purchased from Sigma-Aldrich (Steinheim, Germany). *S. cerevisiae* was purchased from a local market. The seed culture was developed by inoculating a single colony of *S. cerevisiae* into a growth medium (10 mL) containing peptone (3 g/L), yeast extract (3 g/L), and dextrose (10 g/L). The culture was incubated at 37°C for 24 h at 200 rpm after adjusting the pH to 7.0. The inoculum (2% v/v) was transferred to the production medium of the same composition and the culture was grown for 48 h at 37°C and 200 rpm. After sufficient growth, the cell mass was harvested by centrifugation (3080 *g*, 20 min, Gallenkamp centrifuge 200).

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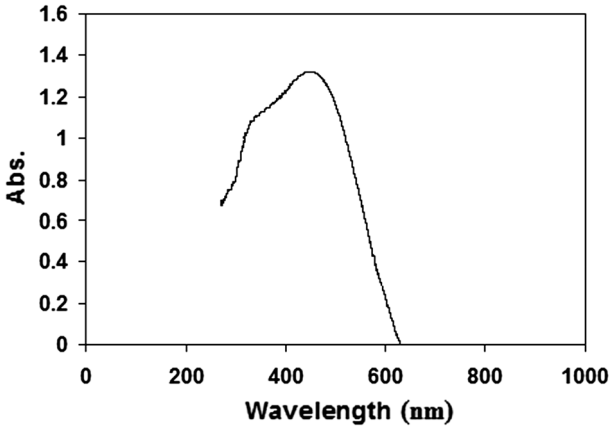

Figure 1. UV-Vis absorption spectrum of the colloidal silver produced. The spectrum was obtained 72 h after the start of $AgNO_3$ (1 mM) reduction using *S. cerevisiae.*

The reaction mixture contained the following ingredients (final concentrations): $AgNO_3$ (1 mM) as the biotransformation substrate, freshly cultured *S. cerevisiae* as the biocatalyst, glucose (56 mM) as the electron donor, and phosphate buffer (pH = 7, 100 mM). The aforementioned ingredients were added in appropriate volumes into Duran® bottles (100 ml) and were incubated (80 rpm, 25°C). Samples (1.5 ml × 3) were taken from the reaction mixtures at different times, and the absorbance (430 nm) of the colloidal suspensions of silver NPs (hydrosols) was read freshly (without freezing).

Dried S. cerevisiae

In another experiment, we used commercially available dried *S. cerevisiae* (0.1 g mL^{-1}) as the biocatalyst with the same reaction mixture and incubation conditions.

Characterization and analysis

UV-Vis analysis. Absorption spectra were measured on a Shimadzu (UVmini-1240) spectrophotometer.

Transmission electron microscopy analysis

Transmission electron microscopy (TEM) was performed on selected samples to investigate the process of formation of silver NPs, and study their sizes and shapes. Samples for TEM were prepared by drop-coating the silver nanoparticle suspensions onto carbon-coated copper grids. Micrographs were obtained using an EM 10C ZEISS® (Germany) transmission electron microscope.

Dynamic light scattering analysis

Furthermore, particle size distribution of NPs was analyzed using a Nano-Zeta Sizer (Nano ZS, ZEN 3600, Malvern Nano®, UK).

Results and discussion

Visual inspection and UV-Vis spectral analysis

When the *S. cerevisiae* biomass was exposed to silver ions ($AgNO_3$, 1 mM), the color of the reaction mixture turned to

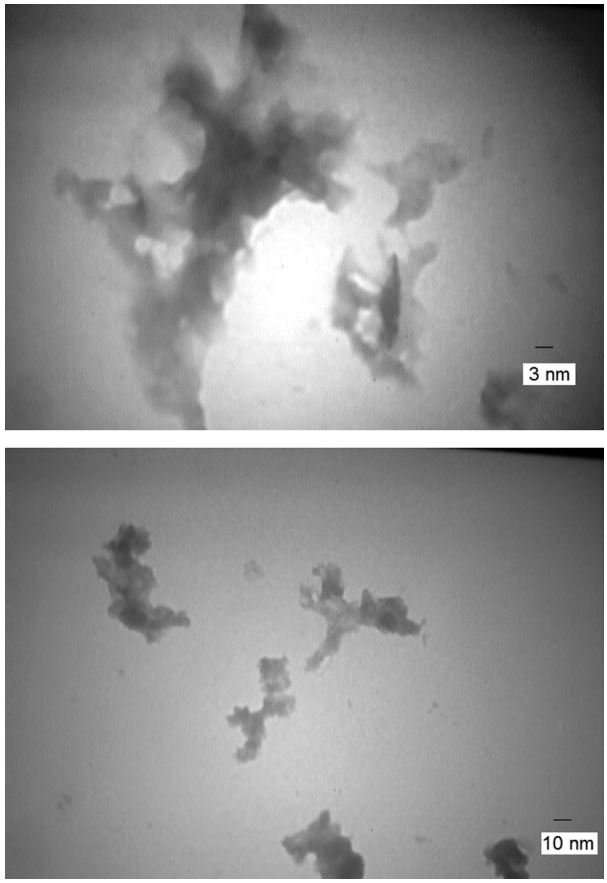

Figure 2. TEM micrographs of the NPs produced 72 h after the start of $AgNO_3$ (1 mM) reduction using dried commercially available *S. cerevisiae*- Magnification is 25,000.

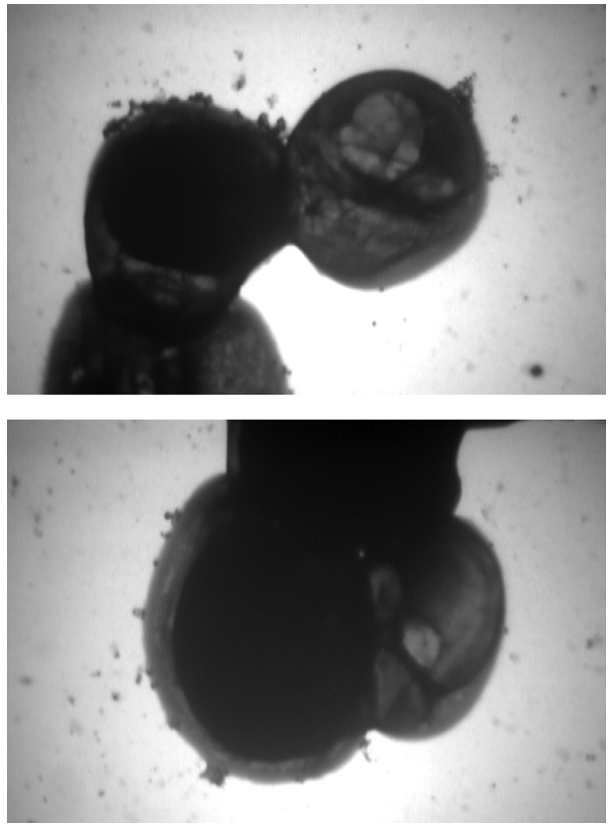

Figure 3. TEM micrographs of the NPs produced 24 h after the start of $AgNO_3$ (1 mM) reduction using freshly cultured *S. cerevisiae.* Magnification is 4000, and the micrograph is focused on the NPs outside of the yeast, and the ones attached to the membrane.

yellowish brown, and then dark brown, which was in agreement with the previous studies, and was considered as the production of colloidal suspension (hydrosol) of silver NPs. The appearance of dark brown seems to be due to excitation of surface plasmon resonance in the NPs (Balaji et al. 2009, Mukherjee et al. 2001).

The formation of silver NPs in the solution was confirmed by UV-Vis spectral analysis. Figure 1 shows the UV-Vis spectrum of reaction mixture containing *S. cerevisiae* biomass and silver nitrate (1 mM) incubated for 72 h. The λ_{max} was about 430 nm. Strong absorption at 430 nm confirms the formation of silver NPs in the solution. Parallel control experiments containing only *S. cerevisiae* biomass (without silver nitrate) and silver nitrate solution (without *S. cerevisiae* biomass) did not show any absorption at about 430 nm.

TEM analysis

Dried yeasts synthesized few silver NPs (Figure 2), but freshly cultured yeast produced a large amount of them (Figure 3). TEM demonstrated the localization of the NPs inside and outside of the cells, the mean particle size, and the size distribution. The TEM micrographs showed that individual silver NPs, as well as a number of aggregates with almost spherical shapes, were produced with a diameter of 2–20 nm (Figure 4). A corona could be seen around the NPs (Figure 5). Silver NPs were seen inside the cells, within the cell membrane, attached to the cell membrane during the exocytosis, and outside of the cells (Figure 6). By monitoring the shape, size, and aggregation of the silver NPs produced by TEM until 72 h, no considerable changes were observed. It might be interpreted that the NPs are stable until this period of time.

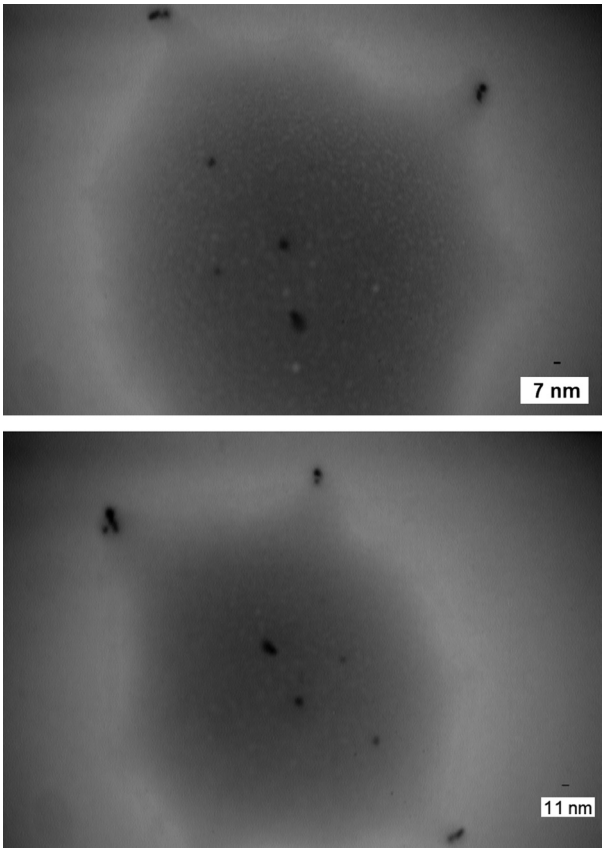

Figure 4. TEM micrographs of the NPs produced 24 h after the start of $AgNO_3$ (1 mM) reduction using freshly cultured *S. cerevisiae*. Magnification is 25,000, and the micrograph is focused on the location of the NPs inside the yeast.

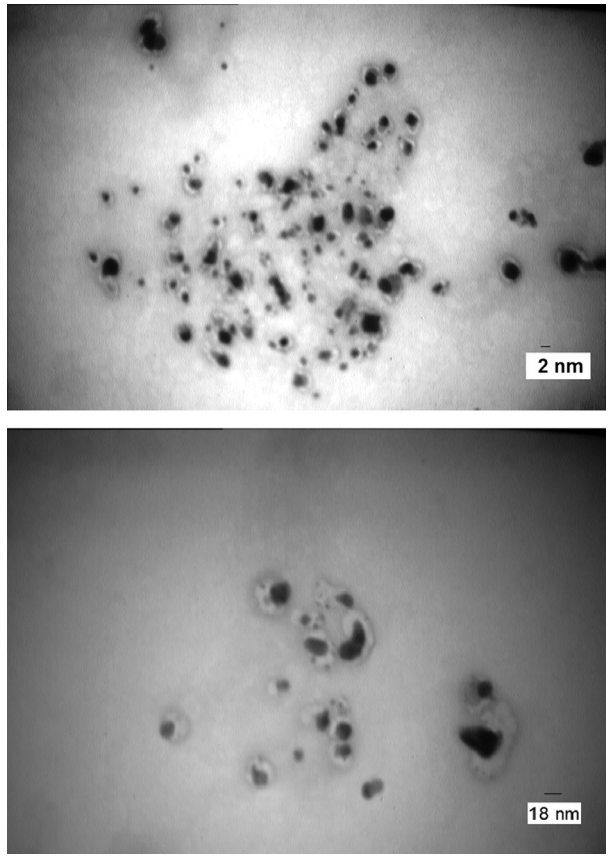

Figure 5. TEM micrographs of the NPs produced 5 h after the start of $AgNO_3$ (1 mM) reduction using freshly cultured *S. cerevisiae*. Magnification is 63,000, and the micrograph is focused on the corona or film around the NPs.

DLS analysis

Two peaks were seen in dynamic light scattering (DLS) analysis of the colloidal solution. The poly-dispersity index (PDI) was 1.0. The aggregates with the diameter of about 102 nm were the most frequent ones and smallest single NPs with diameter of 5.4 were about 14% of the NPs (Figure 7).

Mechanistic aspects

It might be interpreted that firstly, silver ions are absorbed into the cells, then are reduced by the enzymes present within the cytoplasm or in the cell membrane, and the NPs are synthesized intracellularly (Figure 8). It seems that the NPs are within vesicles and isolated from the cytoplasm, maybe due to their toxicity, and excreted through cell membrane by exocytosis and inside the same vesicles or coronas (Figures 3–6 and 8). As can be seen (Figure 8), a number of yeast cells containing silver NPs are disintegrated and NPs are released from them.

The previous researchers, using FTIR analysis, mentioned the involvement of proteins in the capping process of NPs synthesized by yeasts, and the existence of a

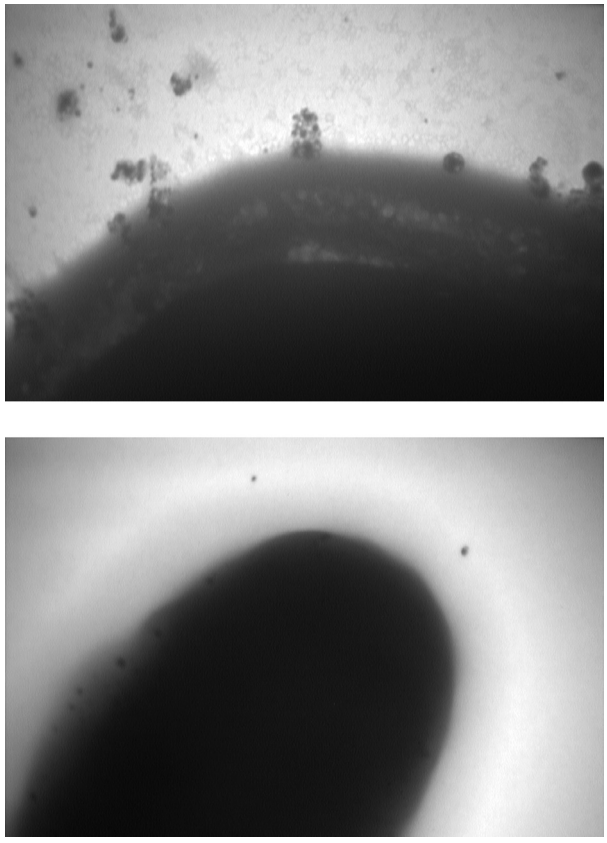

Figure 6. TEM micrographs of the NPs produced 24 h after the start of $AgNO_3$ (1 mM) reduction using freshly cultured *S. cerevisiae*. The micrograph is focused on the location of the NPs in the membrane, and the steps in the secretion of the NPs out of the yeast.

protective coat on the nanoparticle surface produced by fungi (Kaler et al. 2013). Moreover, some authors believe that biosynthesis of the NPs might be a result of the tautomerization of membrane-bound (as well as cytosolic) quinones or the pH sensitive oxidoreductases (e.g., mono or di-oxygeneses and mixed function oxidases/oxygeneses) (Jha et al. 2009b). Metallothioneins were regarded as the only molecules involved in maintaining metal ion homeostasis in *S. cerevisiae*, by binding excess heavy metal ions by thiolate coordination. Recent discoveries reveal the ability of baker's yeast to synthesize phytochelatins mediated by two vacuolar serine carboxypeptidases (Wünschmann et al. 2007). However, more efforts are still required to understand the effect of parameters regarding the effects of various biomolecules and enzymes for biosynthesis of silver NPs using *S. cerevisiae*.

Conclusion

Silver NPs were successfully produced by using baker's yeast, and their sizes were much lower than in the results of previous research. Dried yeasts synthesized few NPs, but freshly cultured yeast produced a large amount of them. The optimum conditions for the reaction mixture were as follows (final concentrations): $AgNO_3$ (1 mM) as the biotransformation substrate, freshly cultured *S. cerevisiae* (0.1 g mL^{-1} wet biomass) as the biocatalyst, glucose (56 mM) as the electron donor, and phosphate buffer (pH = 7, 100 mM), which were incubated at 25°C and 80 rpm. The NPs produced were monodispersed, highly stable, and some

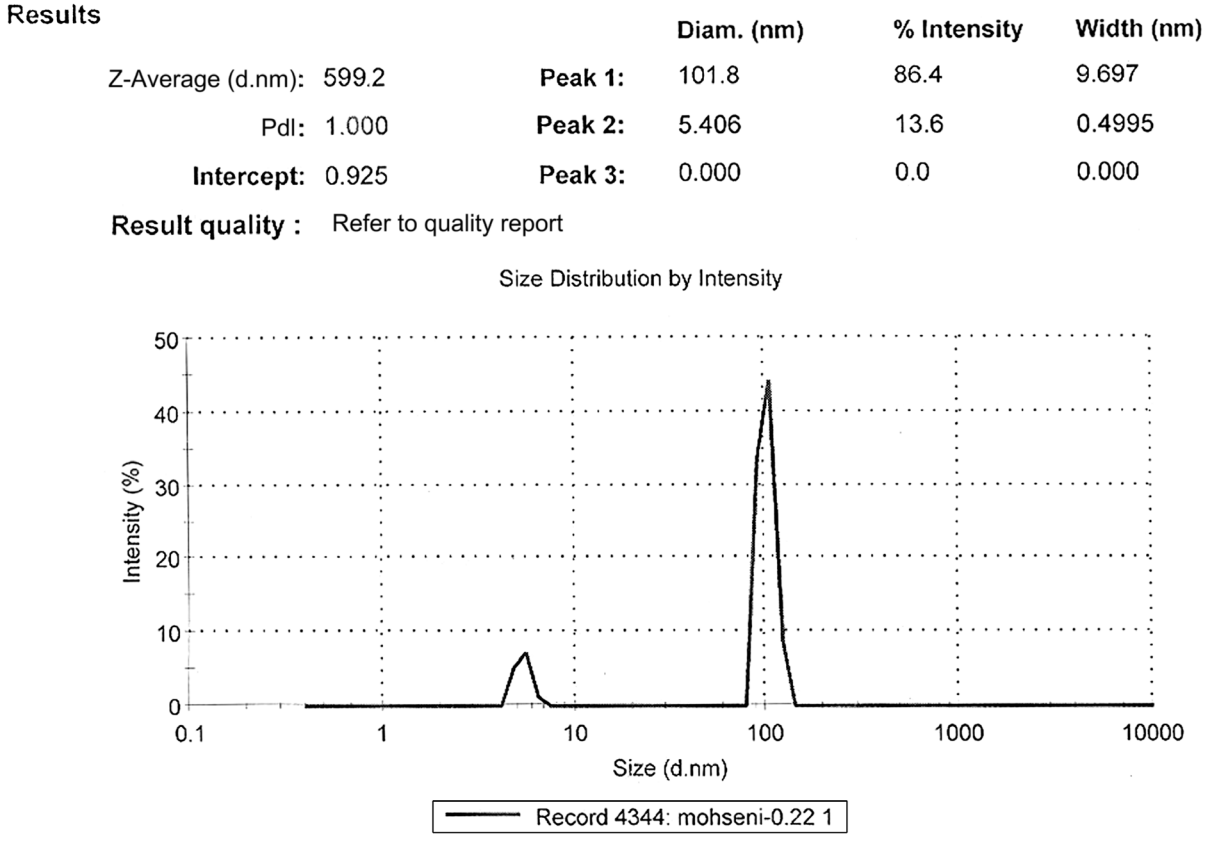

Figure 7. Particle size analysis of NPs produced after 48 h of biotransformation.

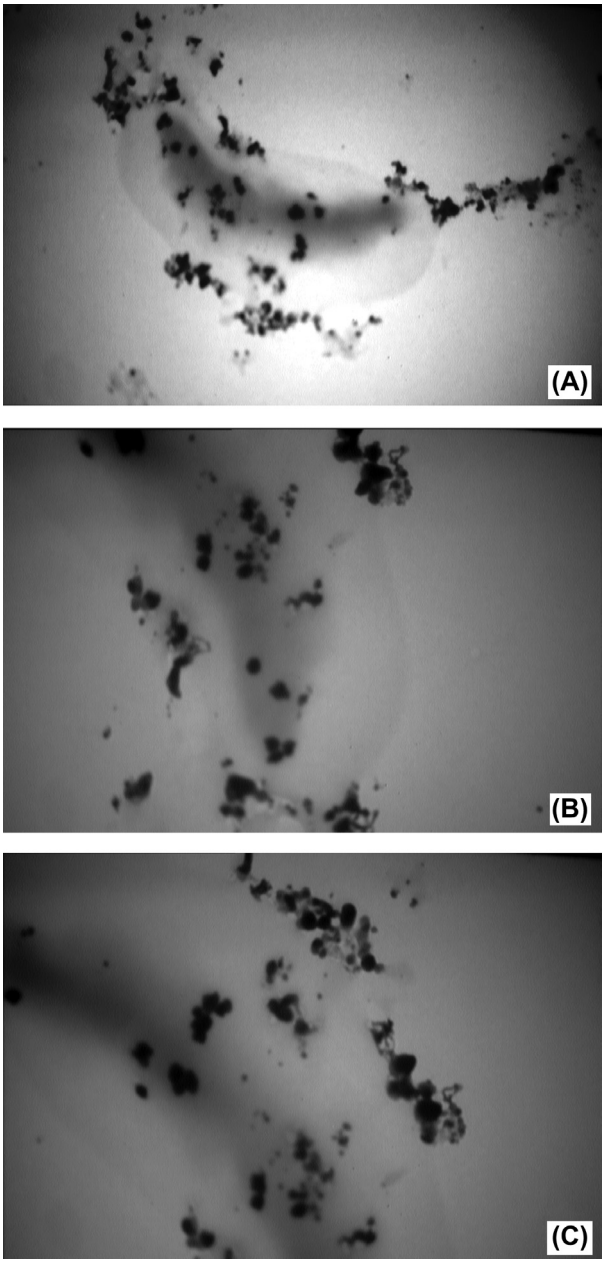

Figure 8. TEM micrographs of the NPs produced 24 h after the start of $AgNO_3$ (1 mM) reduction using freshly cultured *S. cerevisiae.* The micrograph is focused on disintegration of the yeast by NPs.

of them were aggregated outside of the cell. Results from the TEM and DLS analyses demonstrated that by increasing the time of reaction, the NPs were aggregated further. It was shown that the NPs are mainly synthesized intracellularly, which is in agreement with our previous work about the bacteria and fungi. Therefore the biomass should be used in the synthesis, instead of discarding it. The previous researchers used only the supernatant and reported that the synthesis is extracellular. It seems that discarding the cells is a waste of reduction potential of the biocatalyst. It is recommended to use both the cells and the supernatant for future studies using this biocatalyst or the other yeasts. This eco-friendly and green method of silver nanoparticle synthesis can potentially be applied in various products that directly come in contact with the human body, such as cosmetics, foods, and consumer goods, besides medical applications.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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