

Biogenesis of Silver Nanoparticles Feasible for Industrial Application Using Yeast-fungi Secreted Enzymes and Proteins

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SACCHAROMYCES *cerevisiae*, *Rhodotorula glutinis* and *Geotrichum candidum* were selected for biogenesis of silver nanoparticles (AgNPs) using yeast reductases. Extracellular proteins act as stabilizers for so formed AgNPs. Factors affecting biosynthesis; biomass concentration, AgNO₃ concentration and the ratio of AgNO₃ to biomass concentration on. The Optimum conditions for biosynthesis of AgNPs could be attained using biomass of *Saccharomyces cerevisiae* (3 mL/100 mL): .9 mM AgNO₃; pH 12: 25 °C and 24 h incubation). Under these conditions, the maximum concentration of well stabilized AgNPs obtained was 900 ppm with a mean diameter range of 1.5–12 nm. Such solution is unequivocally feasible for industrial applications.

Keywords: Silver nano particles, Yeast fungi, Reductase enzymes, Optimization, Textile treatment.

Introduction

The nano-scale silver will play roles in understanding and ability to manipulate biological processes which will be the central theme to present biomedical and biological issues that need a nanoscience or nanotechnology approach[1]. The extremely small size of nano-particles relative to their volume allows them to easily interact with other particles and increases their antibacterial efficiency[2].

Metal nanoparticles of desired size and shape have been obtained successfully using living organisms from simple unicellular organisms to highly complex eukaryotes. Several microorganisms have been found to be capable of synthesizing intra and/or extra cellular inorganic nanocomposites[3].

Prokaryotes as nanoparticle synthesizers, can be easily modified using genetic engineering techniques for over expression of specific enzymes, apart from the ease of handling. The use of eukaryotes, especially fungi, is potentially exciting since they secrete copious amounts of proteins, thus increasing productivity, and their easy usage in laboratory works is a suitable option in production of metallic nanoparticles

among other microorganisms [4-8]. Moreover, the process can be easily scaled up, economically viable with the possibility of easily covering large surface areas by manipulation of growth parameters[9].

Biogenesis of metal nanoparticles depended on the type and behavior of microorganism. Researchers have been working on extracellular biosynthesis of metal nanoparticles by bacteria, actinomycetes, yeast, fungi and other biological sources[10].

Several methods could be used to optimize biotechnological processes in the advance of bioprocess engineering [11-13]. Methodology is a collection of techniques that are developed to find out optimal conditions of input factors which maximize/minimize the output variables [14,15].

The aim of recent study is to determine factors affecting biogenesis of silver nanoparticles (AgNPs) by *Saccharomyces cerevisiae* (*Baker's yeast*), *Rhodotorula glutinis* and *Geotrichum candidum*, to obtain the optimum conditions for the preparation of AgNPs solution feasible for industrial application such as biomass concentration, AgNO₃ concentration and the ratio of AgNO₃ to biomass concentration on the production of AgNPs.

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Materials and Method

Microorganism and culture conditions

Saccharomyces cerevisiae (Baker's yeast), *Rhodotorula glutinis* and *Geotrichum candidum*. used in this study were obtained from Regional Center for Mycology and Biotechnology, AL-Azhar University, Nasr city, Cairo. strains were Test maintained at 30°C on Czapek Dox Agar.

Culture media

Czapek Dox Agar medium (CDA) (Maintenance medium)

Maintenance medium is composed of (g/L): sodium nitrate 2, magnesium sulfate 0.5, potassium chloride 0.5, potassium dihydrogen phosphate 1, ferrous sulfate 0.1, sucrose 20 and agar 20. pH was adjusted at 6.5-7.

Fermentation media

The fermentation medium was Czapek Dox medium devoid of agar. pH was adjusted to be 6.5-7. Ingredients were dissolved in fresh distilled water, and applied to 250 mL Erlenmeyer conical flasks each containing 50 mL, then sterilized at 121°C for 20 min.

Fermentation process

Inoculation was performed by 2 mL of 7 days old vegetative growth in two replicates of 250 mL Erlenmeyer conical flasks containing 50 mL of the fermentation medium. Incubation was done on a shaker incubator at 30±2 °C and 150-200 rpm. First and second samples were removed after 72hr and 96hr, respectively, for the bio- synthesis of silver nanoparticles.

Preparation of silver nanoparticles

After fermentation process the biomass was recovered by decantation-centrifugation and washed extensively with fresh distilled water. The harvested biomass then re-suspended in 100 mL fresh distilled water at pH 12. 0.1m mol of silver nitrate was added in the presence of cells. After 30 min, the solution was filtered to remove cells then subjected to U.V-Vis spectrophotometer after 24 hr. Reduction of metal ions was detected by visual inspection of the solution, as well as, by UV-Vis spectra, and transmission electron microscopy (TEM).

As a farther step, the colloidal solution formed was checked for the presence of excess silver ion (Ag^+) using dilute NaCl solution, in order to be sure of complete reduction. When no white precipitate is observed, this means the complete transformation of (Ag^+) to (Ag^0).

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Characterization of silver nano-particles

Ultra violet-visible (UV-Vis) spectra

Ultra violet-visible (UV-Vis) spectra have been proved to be quite sensitive to the formation of silver colloids because silver nanoparticles exhibit an intense absorption peak due to the surface plasmon excitation (it describes the collective excitation of conduction electrons in a metal). Silver nanoparticles were recorded by spectrophotometer from 300 to 700nm. The filtrate without adding silver nitrate was used as a blank.

Transmission Electron Microscope (TEM)

Particle size and shape were detected by JEOL-JEM-1200. Specimens for TEM measurements were prepared by dissolving a drop of colloid solution on a 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the silver nanoparticles was determined from the diameter of 100 nanoparticles found in several arbitrary chosen areas in enlarged microphotographs.

Results and Discussion

Current study attempts to achieve well stabilized AgNPs colloidal solution with higher concentration feasible for industrial applications. This, indeed, was done through investigations into biomass concentration, AgNO_3 concentration, and ratio of AgNO_3 to biomass concentration as detailed under.

Investigations into biomass concentration.

Geotrichum candidum

Biomass suspension produced from 0.5, 1.0 and 2.0 mL of vegetative growth of *Geotrichum candidum* as inoculum size were exposed to 0.1 mM AgNO_3 for 72 hr.

Figure 1, showed the effect of biomass concentration on stability of biogenic nano-silver produced by *Geotrichum candidum*. pH of solutions was adjusted to 8, 10, 11 and 12 followed by addition of 0.1 mM AgNO_3 . Vessels were kept for 30 minutes, then cells were removed from the reaction medium. Vessels were kept under ambient conditions ~ 25° C for 24 hr.

Data presented in Fig.1 indicated the extreme stability of Ag^0 nanoparticles produced by using biomass concentration 1.0 mL.

Rhodotorula glutinis

Biomass suspension produced from 0.5 mL, 1 mL, and 2 mL of vegetative growth of

Rhodotorula glutinis were incubated on a shaker incubator at 30 ± 2 °C at 150 rpm for 72 hr to 0.1 mM AgNO_3 respectively for 24 hr.

Figure 2, shows the effect of biomass concentration on stability of biogenic nano-silver upon exposure of 100 mL of biomass suspension produced from 0.5 mL, 1 mL, and 2 mL inoculum of *Rhodotorula glutinis* incubated on a shaker incubator at 30 ± 2 °C at 150 rpm for 72 hr to 0.1 mM AgNO_3 respectively for 24 hr.

Figure 2, indicated the extreme stability of the Ag^0 nanoparticles attained at using the biomass concentration of 1 mL.

Saccharomyces cerevisiae

Biomass suspension produced from 0.5 mL, 1 mL, and 2 mL of vegetative cells of *Saccharomyces cerevisiae* were incubated on a shaker incubator at 30 ± 2 °C at 150 rpm for 72 hr to 0.1 mM AgNO_3 respectively for 24 hr.

Figure 3, shows the effect of the biomass concentration on stability of silver nanoparticles. pH of the biomass solution was adjusted to 8, 10, 11 and 12 using diluted solutions of NaOH (0.1N) followed by addition of 0.1 mM of AgNO_3 . Vessels were kept for

30 minutes, then cells were removed from the reaction medium. Vessels were kept under ambient conditions ~ 25 °C for 24 hr.

Data presented in Fig. 3 indicated the extreme stability of the Ag^0 nanoparticles attained upon using biomass concentration of 1.0 mL.

Effect of silver nitrate concentration on bio-synthesis of silver nanoparticles

Geotrichum candidum

The effect of silver nitrate concentration on bio-synthesis of silver nanoparticles was studied by exposing biomass suspension produced from 1 mL of *Geotrichum candidum* inoculation size incubated on a shaker incubator at 30 ± 2 °C at 150 rpm for 72 hr to 0.1, 0.2, 0.3, and 0.4 mM of AgNO_3 respectively for 24 hr.

Figure 4, represents the U.V-Vis spectra for the AgNps solution prepared by *Geotrichum candidum* by variation of silver nitrate concentration to 0.1, 0.2, 0.3, and 0.4 mM of AgNO_3 respectively.

Figure 4, indicated that the best results obtained for silver nanoparticles bio-synthesis was by using 0.3 mM of AgNO_3 .

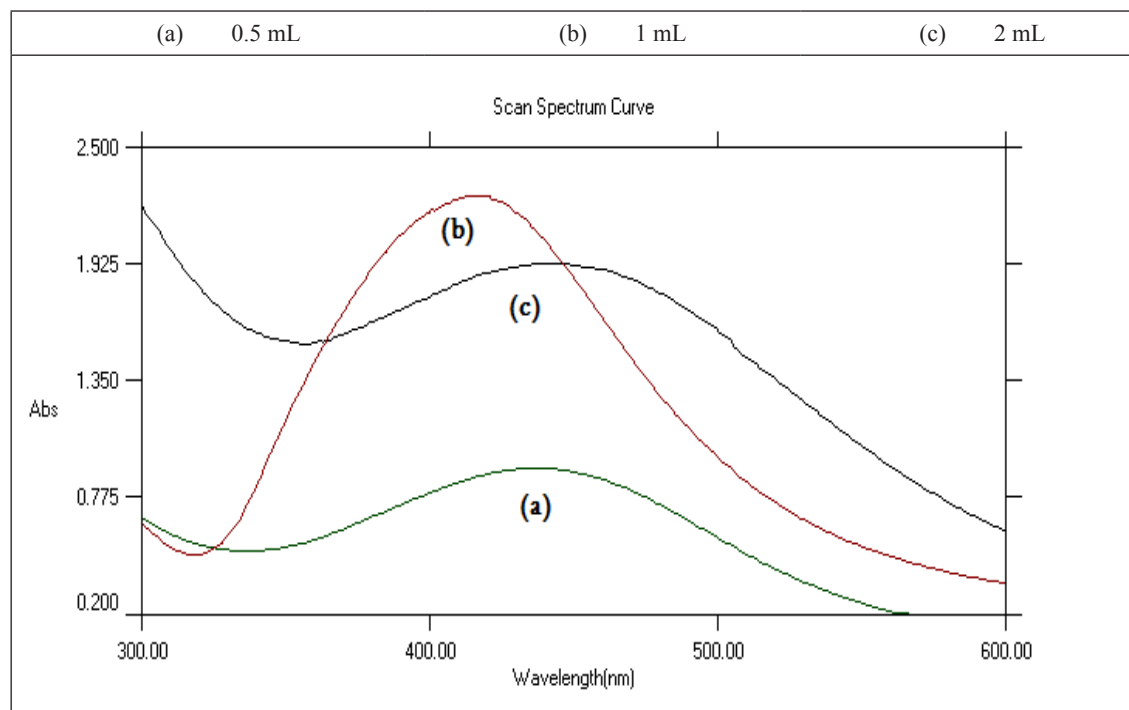


Fig. 1. UV-Vis spectra recorded as a function of biomass concentration of *Geotrichum candidum* after 72 hr incubation.

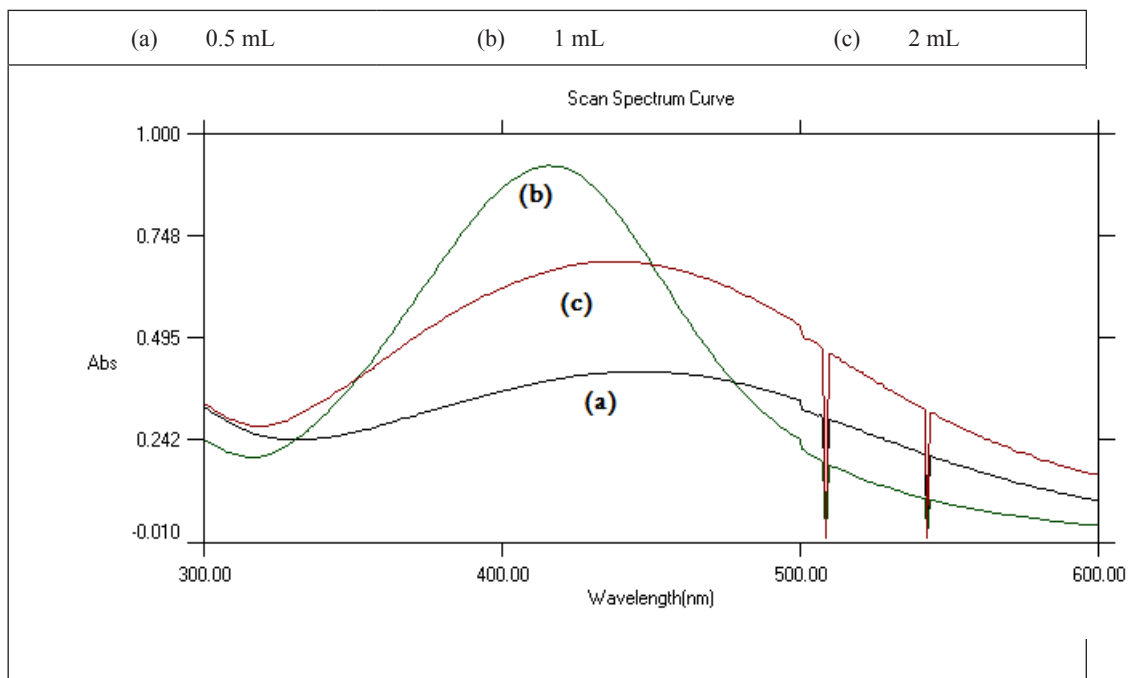


Fig. 2. UV-Vis spectra recorded as a function of biomass concentration of *Rhodotorula glutinis* after 72 hr incubation.

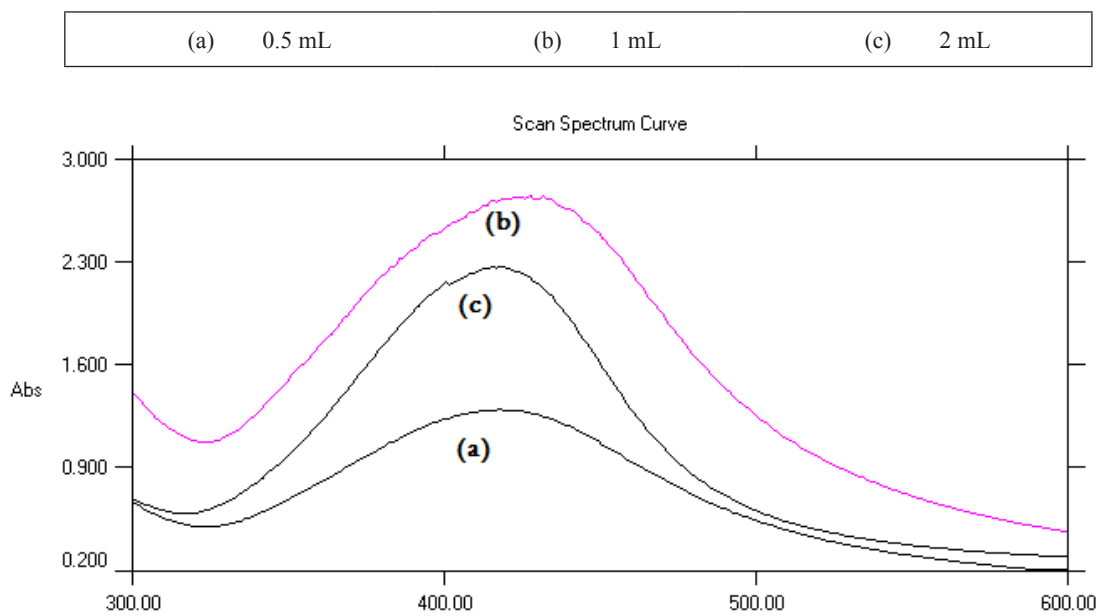


Fig. 3. UV-Vis spectra recorded as a function of biomass concentration of *Saccharomyces cerevisiae*.

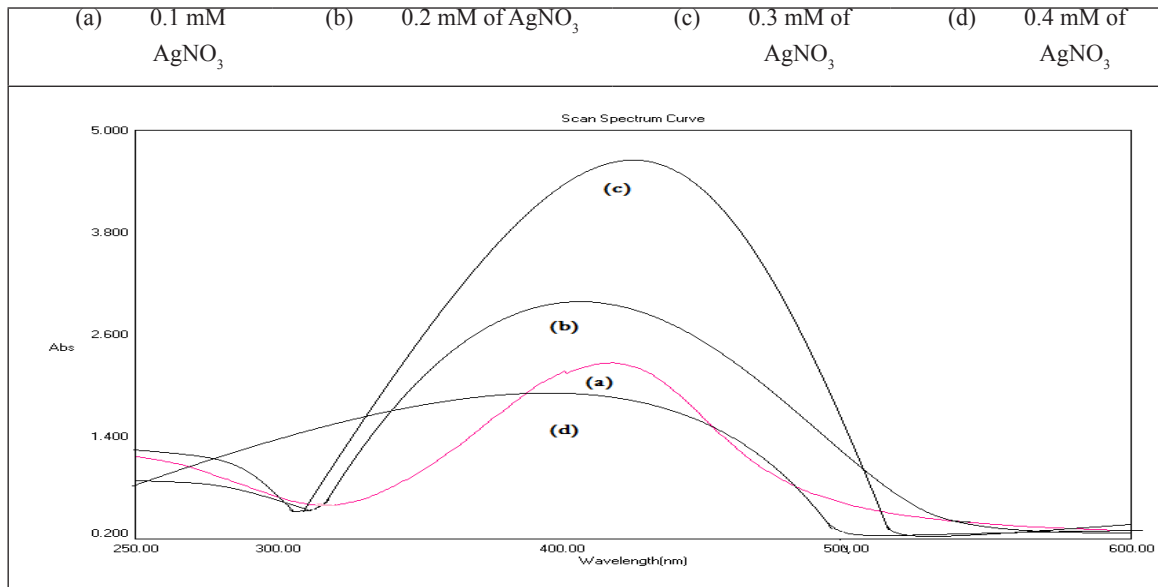


Fig. 4. UV-Vis spectra recorded as a function of silver nitrate concentration for *Geotrichum candidum*.

Figure (5a and b) showed TEM micrograph and particle size distribution, respectively, using 1.0 mL of *Geotrichum candidum* for 72 hr and 0.1 mM AgNO₃. Particle size range was 2.5-13 nm.

Figure (6a and b), showed TEM micrograph and particle size distribution, respectively, using 1 mL from 7 days old culture of *Geotrichum candidum*, incubation period was 72 hr and 0.3 mM AgNO₃ in the presence of cells. Cells were removed before reaction by 30 min.

Figures (6a and b) showed silver nanoparticles size range 8-30 nm which was obtained by *Geotrichum candidum*, using 0.3 mM silver nitrate.

Figures 5 and 6 indicated that particle sizes getting bigger with the increase of silver nitrate concentration.

Rhodotorula glutinis

The effect of silver nitrate concentration on bio-synthesis of silver nanoparticles was studied by exposing 100 mL of biomass suspension produced from 1 mL of *Rhodotorula glutinis* inoculation size incubated on a shaker incubator at 30±2 °C and 150 rpm for 72 hr to 0.1, 0.2, 0.3, and 0.4 mM of AgNO₃ respectively for 24 hr.

Figures (7a, b, c and d) represent U.V-Vis spectra for AgNPs solution prepared by *Rhodotorula glutinis* by using 0.1, 0.2, 0.3, and 0.4 mM of AgNO₃ respectively. The best result was obtained by using 0.3 mM of AgNO₃.

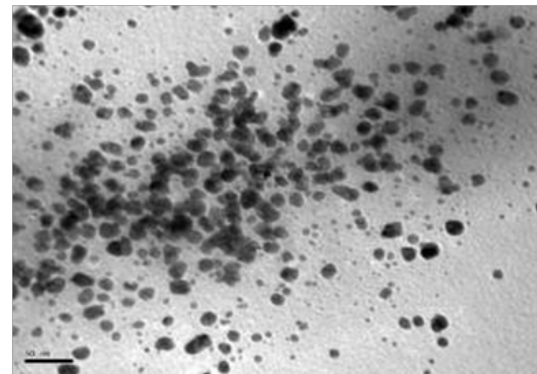


Fig.5a. TEM image of silver nanoparticles formed using 1.0 mM of AgNO₃ and 1.0 mL of *Geotrichum candidum*.

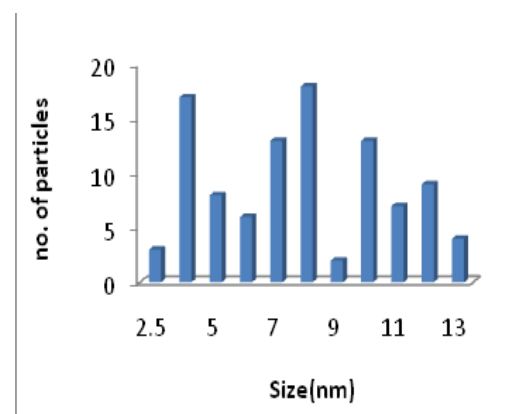


Fig.5b. Histogram illustrating particle size distribution of silver nanoparticles prepared using 1 mM silver nitrate and 1 mL biomass of *Geotrichum candidum*. The particles size range is between 2.5nm - 13 nm.

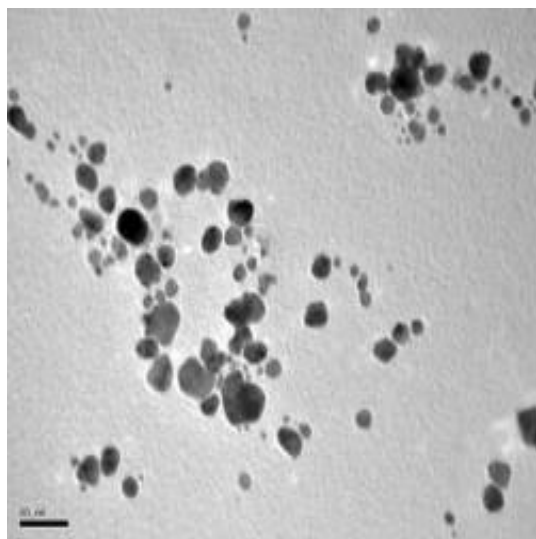


Fig. 6a. TEM image of silver nanoparticles formed using 3.0 mM of AgNO_3 and 1.0 mL of *Geotrichum candidum*.

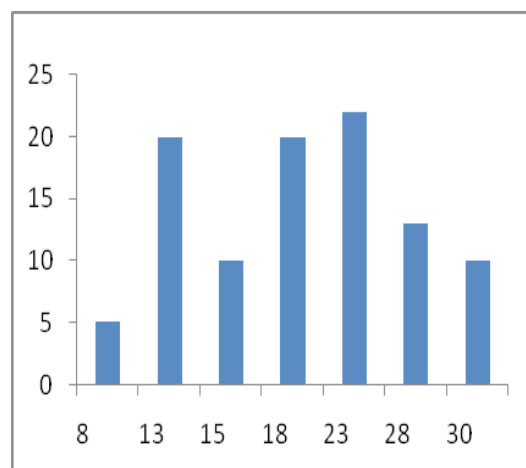


Fig. 6b. Histogram illustrating particle size distribution of silver nanoparticles prepared using 1 mM silver nitrate and 1 mL biomass of *Geotrichum candidum*. The particles size range is between 2.5nm - 13 nm.

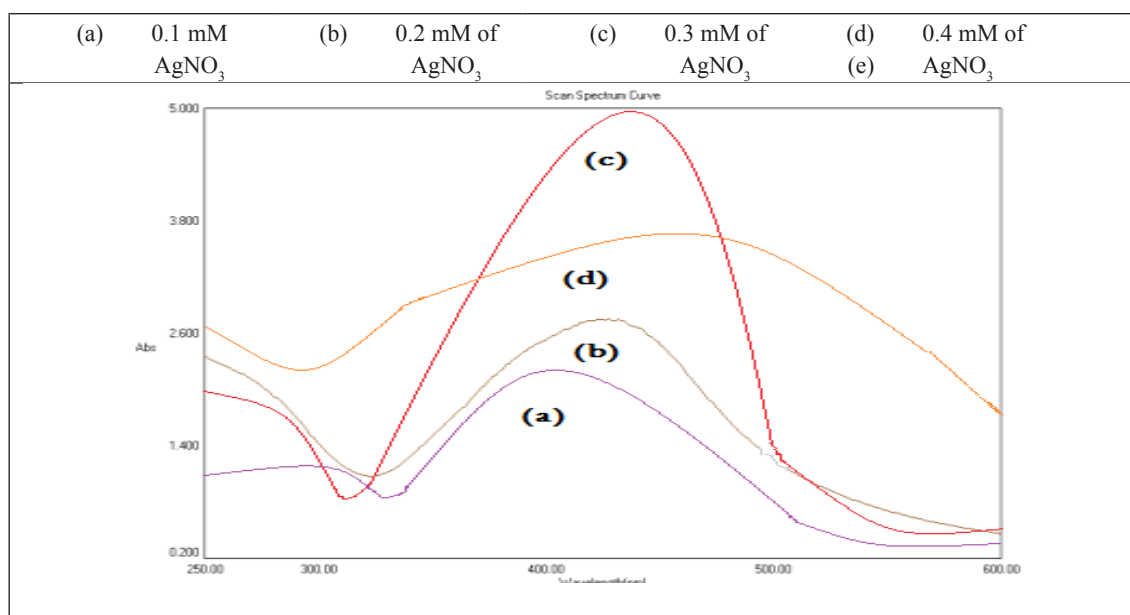


Fig. 7. UV-Vis spectra recorded as a function of silver nitrate concentration for *Rhodotorula glutinis*.

Figures (8 a and b) showed TEM micrograph and particle size distribution, respectively, using 1 mL of *Rhodotorula glutinis* for 72 hr and 0.1 mM AgNO_3 . Figures indicated that silver nanoparticles size range 4-14 nm was obtained by using 0.1 mM silver nitrate concentration.

Figures (9 a and b) showed TEM micrograph and particle size distribution, respectively, using 1.0 mL of *Rhodotorula glutinis* for 72 hr,

addition of 0.3 mM AgNO_3 . Figures indicated that silver nanoparticles size range 20-50 nm was obtained by using 0.3 mM silver nitrate concentration.

The particles size range was between 20 nm – 50 nm. Figures (8 and 9) indicated that particle sizes getting bigger with the increase of silver nitrate concentration.

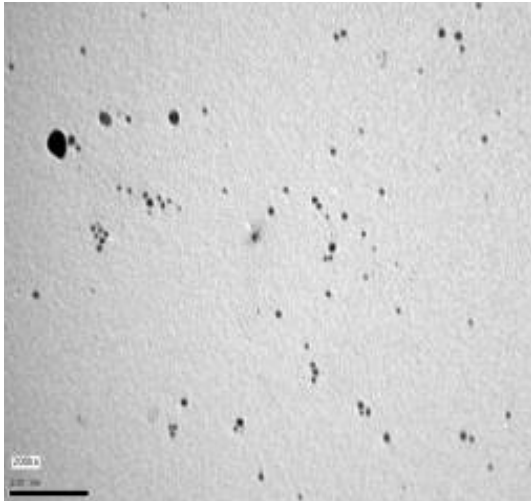


Fig. 8a. TEM image of silver nanoparticles formed using 1 mM of AgNO₃ and 1.0 mL of *Rhodotorula glutinis*.

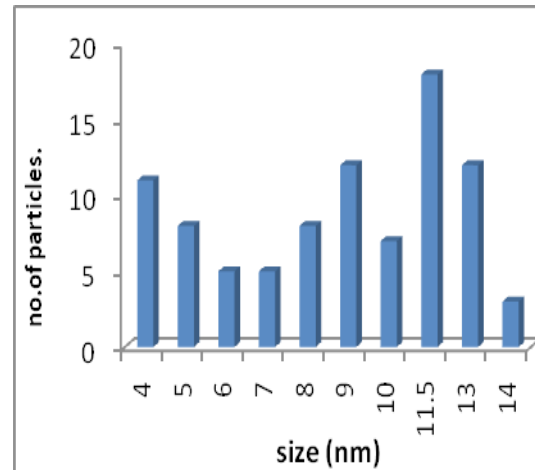


Fig.8b. Histogram illustrating particle size distribution of silver nanoparticles prepared using 3 mM silver nitrate and 1.0 mL of *Rhodotorula glutinis*. The particle size range is between 4nm - 14 nm.

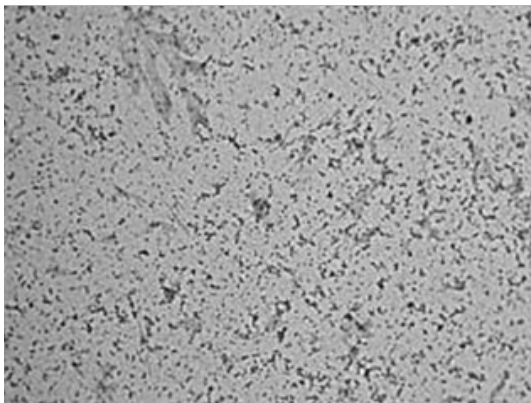


Fig. 9a. TEM image of silver nanoparticles formed using 3 mM of AgNO₃ and 1.0 mL of *Rhodotorula glutinis*.

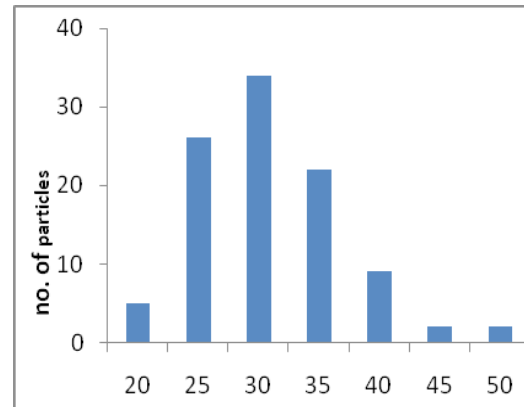


Fig. 9b. Histogram illustrating particle size distribution of silver nanoparticles prepared using 3.0 mM silver nitrate and 1.0 mL of *Rhodotorula glutinis*.

Saccharomyces cerevisiae

The effect of silver nitrate concentration on bio-synthesis of silver nanoparticles was studied by exposing 100 mL of biomass suspension produced from 1 mL of *Saccharomyces cerevisiae* (Baker's yeast) inoculation size incubated on a shaker incubator at 30±2 °C at 150 rpm for 72 hr to 0.1, 0.2, 0.3, and 0.4 mM of AgNO₃ respectively for 24 hr.

Figures (10. a, b, c and d) represent U.V-Vis spectra of AgNPs prepared using *Saccharomyces cerevisiae* by applying 0.1, 0.2, 0.3, and 0.4 mM of AgNO₃ respectively.

The figures illustrate that the best results obtained for the silver nanoparticles bio-synthesis were by using 0.3.0 mM of AgNO₃.

Figures (11 a and b) showed TEM micrograph and particle size distribution, respectively, using 1 mL of *Saccharomyces cerevisiae* and 0.1 mM AgNO₃ incubated for 72 hr. Figures (11 a and b) showed that silver nanoparticles size range 1.5-12 nm was obtained by using 0.1 mM silver nitrate\concentration.

Figures (12 a and b) showed TEM micrograph and particle size distribution, respectively, using 1 mL of *Saccharomyces cerevisiae* and 0.3 mM AgNO₃ incubated for 72 hr.

Figures also showed that silver nanoparticles size range 1-8 nm was obtained by *Saccharomyces cerevisiae* upon using 0.1 mM silver nitrate.

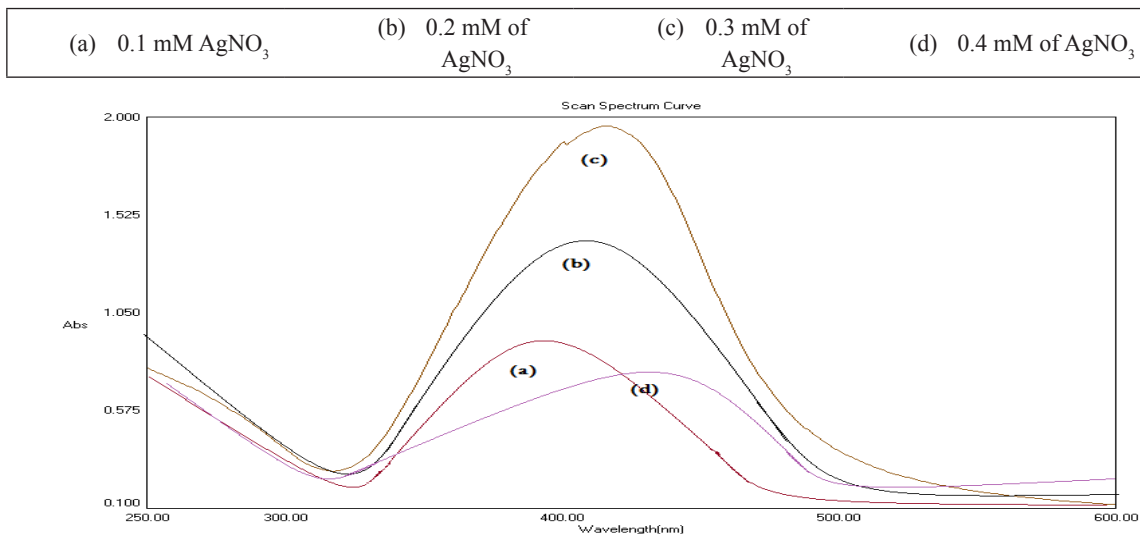


Fig. 10. UV-Vis spectra recorded as a function of silver nitrate concentration for yeast strain *Saccharomyces cerevisiae*.

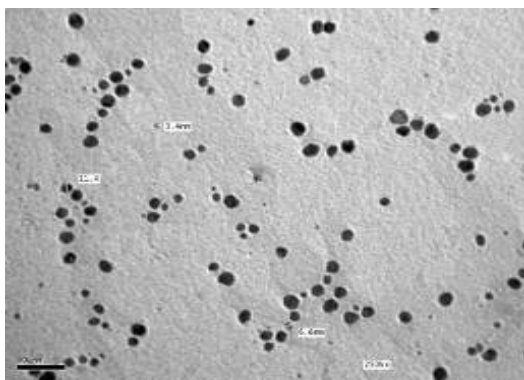


Fig.11a. TEM image of silver nanoparticles formed using 1.0 mM of AgNO₃ with 1 mL biomass for yeast strain *Saccharomyces cerevisiae*.

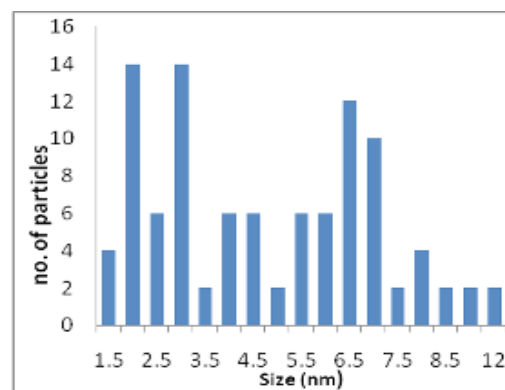


Fig.11b. Histogram illustrating the particle size distribution of silver nanoparticles prepared using 1mM silver nitrate with 1 mL biomass of yeast strain *Saccharomyces cerevisiae*. The particles size range was between 1.5nm - 12nm.

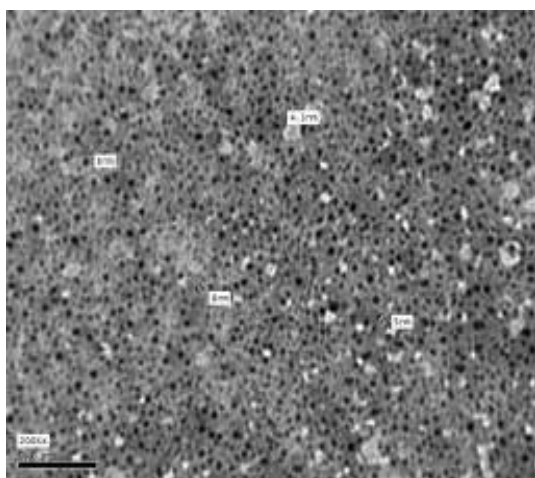


Fig. 12a. TEM image of silver nanoparticles formed using 0.3 mM of AgNO₃ with 1 mL of *Saccharomyces cerevisiae*.

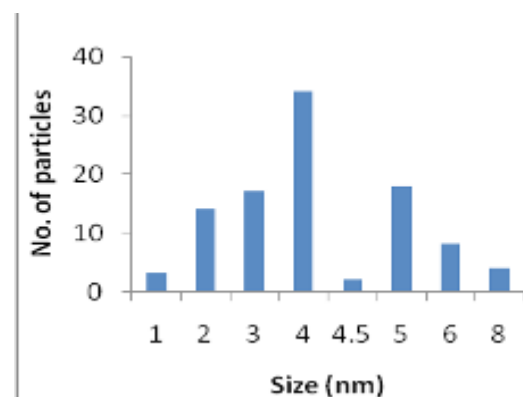


Fig.12b. Histogram illustrating particle size distribution of silver nanoparticles prepared by using 3.0 mM silver nitrate with 1 mL *Saccharomyces cerevisiae*. Particles size range is between 1nm - 8nm.

Figures (11, 12) for yeast strain *Saccharomyces cerevisiae* the particle sizes still in small range with the increase of silver nitrate concentration.

Study of AgNO₃ concentration Vs biomass concentration using Saccharomyces cerevisiae

Silver nanoparticles were prepared using the optimum culture conditions for *Saccharomyces cerevisiae* to prepare different concentrations of 100, 300, 600 and 900 ppm of silver nanoparticles.

Different concentrations of silver nanoparticles were obtained by variation in the ratio of yeast inoculum size to silver nitrate concentration. Silver nanoparticles preparation 1:1, 1:3, 2:6

and 3:9 (inoculum size : mM silver nitrate). The previously prepared nano-particles were characterized by UV-Vis spectroscopy and TEM.

Ultra violet-visible (UV-Vis) spectra.

Figures (13. a, b, c and d) represent the U.V-Vis spectra of AgNPs prepared by *Saccharomyces cerevisiae* by variation in the ratio of yeast inoculums size to silver nitrate concentration. The ratio was 1:1, 1:3, 2:6 and 3:9.

Figures (13. a, b, c, d) indicated that silver nanoparticles colloidal solutions prepared using these ratios are feasibly promising for the industrial application.

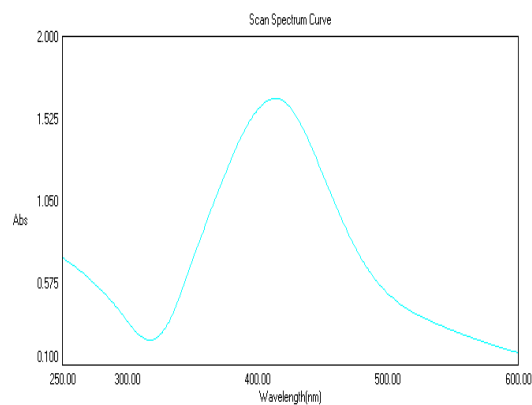


Fig.13a.U.V-Vis spectra for *Saccharomyces cerevisiae*, the ratio is 1:1 (mL inoculum size : 0,1 mM silver nitrate)

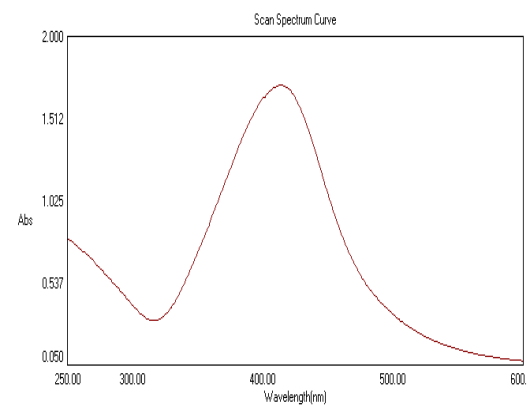


Fig. 13b. U.V-Vis spectra for yeast *Saccharomyces cerevisiae*. The ratio is 1:3 (1.0 mL inoculum size : 0.3 mM silver nitrate).

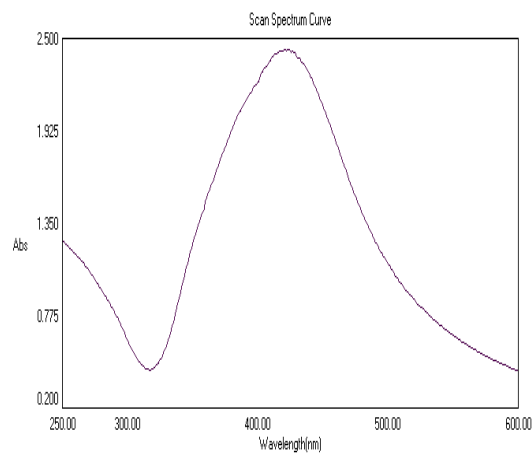


Fig. 13c. U.V-Vis spectra for yeast *Saccharomyces cerevisiae*. The ratio is 2:6 (2.0 mL inoculum size : 0.6 mM silver nitrate).

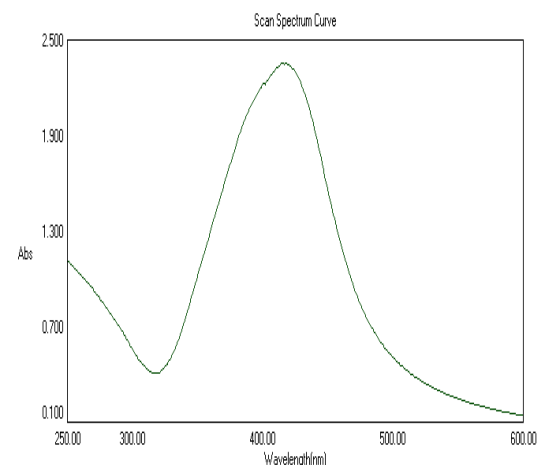


Fig. 13d. The U.V-Vis spectra for yeast *Saccharomyces cerevisiae* . The ratio is 3:9 (3.0 inoculum size : 0,9 mM silver nitrate).

Transmission Electron Microscope (TEM)

Figures (14 a and b) showed TEM micrograph and particle size distribution, respectively, using 1 mL of *Saccharomyces cerevisiae* and 0.1 mM AgNO₃ incubated for 72 hr in the presence of cells. Biomass removed before the reaction done (after 30 min).

Figures (14 a and b) showed silver nanoparticle size range 1.5-12 nm obtained by *Saccharomyces cerevisiae*, using biomass - silver nitrate ratio 1:1 (1.0 mL inoculums : 0.1 mM silver nitrate).

Figures (15 a and b) showed the TEM micrograph and particle size distribution, respectively, also showed silver nanoparticle size range 1 -8 nm prepared by *Saccharomyces*

cerevisiae using the ratio 1: 3 (1.0 mL inoculums : 0.3 mM silver nitrate).

Figures (16 a and b) showed t TEM micrograph and particle size distribution, respectively. Also figures illustrate silver nanoparticle size range 1 -7 nm which was obtained by *Saccharomyces cerevisiae* using biomass - silver nitrate ratio 2: 6 (2.0 mL inoculums : 0.6 mM silver nitrate).

Figures (17 a and b) showed TEM micrograph and particle size distribution, respectively. Also illustrate silver nanoparticle size range 2-15 nm which was obtained by *Saccharomyces cerevisiae* using the ratio 3: 9 (3.0 mL inoculums : 0.9 mM silver nitrate).

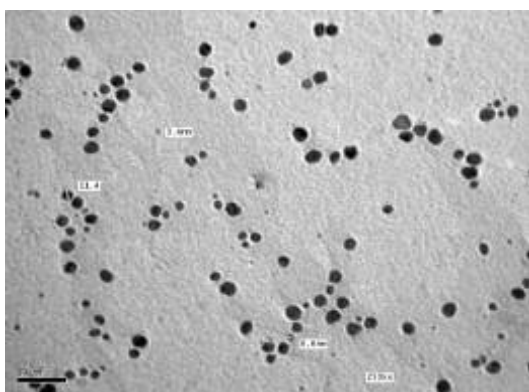


Fig. 14a. TEM image of silver nanoparticles using ratio 1:1 (1.0 mL inoculums: 0.1 mM silver nitrate).

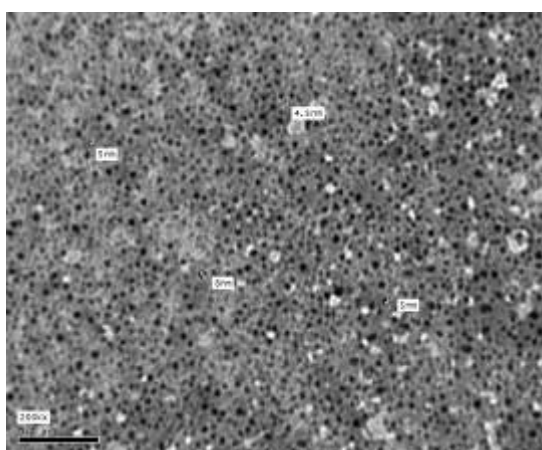


Fig. 15a. TEM image of silver nanoparticles formed using the ratio 1: 3 (1.0 mL inoculums: 0.3 mM silver nitrate).

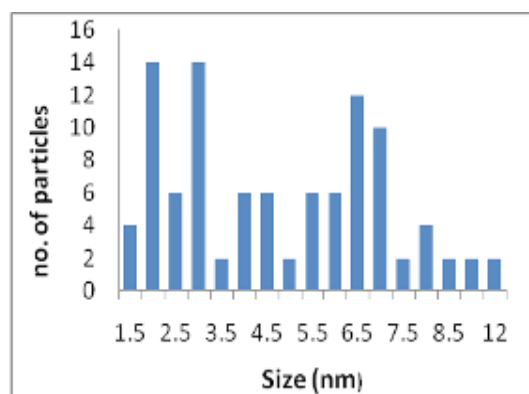


Fig.14b. Histogram showing the particle size and particle size distribution of silver nanoparticles prepared using the ratio 1:1 (1.0 mL inoculums: 0.1 mM silver nitrate). The particles size range is between 1.5nm - 12nm.

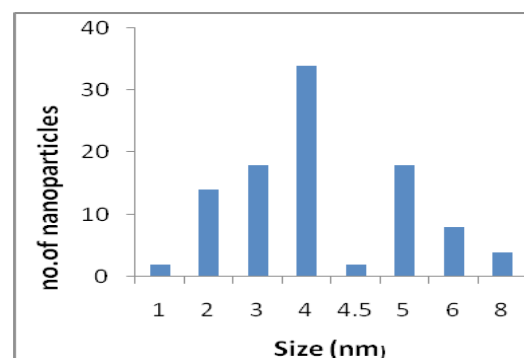


Fig. 15b. Histogram showing the particle size and particle size distribution of silver nanoparticles prepared using the ratio 1: 3 (1.0 mL inoculums: 0.3 mM silver nitrate). The particles size range is between 1nm - 8nm.

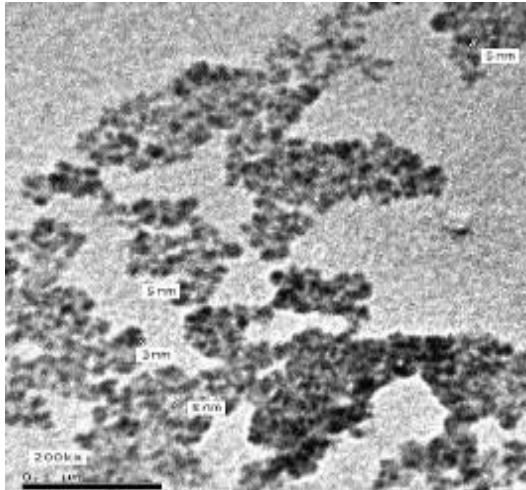


Fig. 16a. TEM image of silver nanoparticles formed using ratio 2: 6 (2.0 mL inoculums: 0.6 mM silver nitrate).

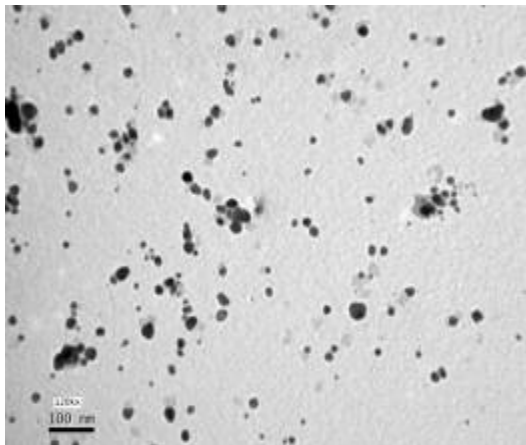


Fig. 17a. TEM image of silver nanoparticles formed using the ratio 3: 9 (3.0 mL inoculums: 0.9 mM silver nitrate).

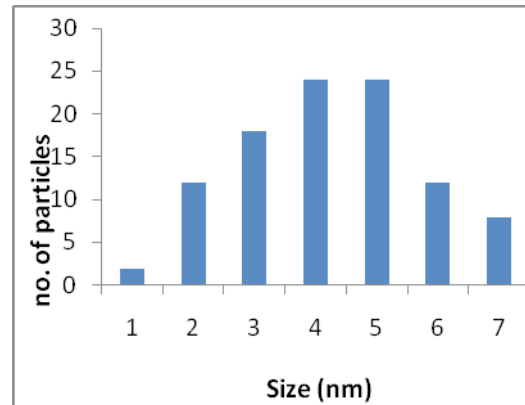


Fig.16b. Histogram showing the particle size and particle size distribution of silver nanoparticles prepared using ratio 2: 6 (2.0 mL inoculums: 0.6 mM silver nitrate). The particles size range is between 1nm - 7nm.

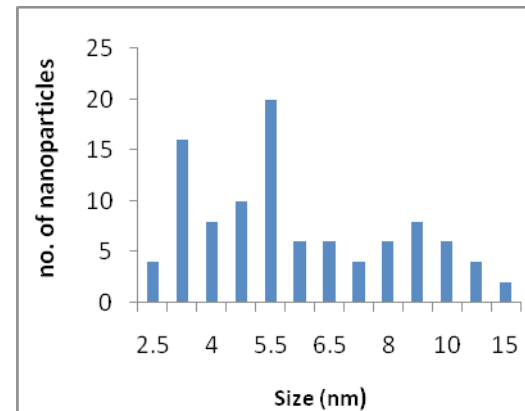


Fig. 17b. Histogram showing the particle size and particle size distribution of silver nanoparticles prepared using The particles size range is between 2.5nm - 15nm.

Application of AgNPs on cellulose based textiles

The so obtained silver nanoparticles as finishing agent were applied to cellulose based textiles [16]. Final evaluation of physical and antimicrobial properties of the treated fabrics was performed. Cotton fabrics and cotton/polyester blend fabrics having excellent antibacterial properties and can withstand repeated washing, could be obtained by treating the fabrics with 50 ppm AgNPs in the presence of binder [17, 18].

Antimicrobial activity was observed when silver nanoparticles were incorporated in cotton and cotton/polyester fabrics. This work demonstrates the possible use of bio-

logical synthesized silver nanoparticles and its incorporation in fabrics impart to sterilization of fabrics [17, 19].

Conclusion

Saccharomyces cerevisiae, *Rhodotorula glutinis* and *Geotrichum candidum* were tested for biosynthesis of silver nanoparticles (AgNPs). Factors affecting biosynthesis of AgNPs for industrial application were studied.

The maximum concentration of well stabilized AgNPs obtained was 900 ppm with a mean diameter range of 1.5–12 nm by *Saccharomyces cerevisiae*.

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التخليق الحيوي لجسيمات الفضة النانوية القابلة للتطبيق الصناعي باستخدام إنزيمات وبروتينات الخمائر الفطرية

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تم اختيار قدرة الخمائر سكرومييسز سيريفيسياي، رودوتورولا غلوتينيس و جيوتريتشوم كانديدوم على التخليق الحيوي لجسيمات الفضة النانوية باستخدام البروتينات المختزلة الخارج خلوية للخميرة حيث تعمل بمثابة مثبتات AgNO₃ لدقائق الفضة المكونة. العوامل التي تؤثر على الانتاج الحيوي هي. تركيز الكتلة الحيوية، تركيز إلى تركيز الكتلة الحيوية . ويمكن تحقيق الظروف المثلى للتخليق الحيوي لدقائق الفضة: AgNO₃ ونسبة درجة الحموضة: AgNO₃ باستخدام الكتلة الحيوية من سكارومييسز سيريفيسياي (3 مل / 100 مل): 9. ملي 12: 25 درجة مئوية و 24 ساعة حضانة). في ظل هذه الظروف، كان أقصى تركيز من لدقائق الفضة المستقرة جيدا تم الحصول عليها 900 جزء في المليون مع متوسط قطرها من 1.5-12 نانومتر. وهذا يمثل تركيزا مناسباً بالنسبة للتطبيقات الصناعية.