

Enhanced Antimicrobial Effect of Yeast Mediated Silver Nanoparticles Synthesized From Baker's Yeast

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Abstract

In recent science Nanotechnology is a burning field for the researchers. To meet the requirements and growing technological demand, there is a need to develop an eco-friendly approach. In the present effort, the baker's yeast (*Saccharomyces cerevisiae*) has been taken in order to assess its potential as putative candidate fungal genera for the transformation of silver nanoparticles. Silver nanoparticles were successfully synthesized from *Saccharomyces cerevisiae* by green synthesis method. The formation of silver nanoparticles and the concentration of yeast extract required to produce yeast mediated silver nanoparticles with no aggregation was found out by UV-Visible spectroscopic analysis. The detailed characterization of the Ag NPs was carried out using Scanning Electron Microscopy (SEM), and FTIR. From the UV-visible spectroscopy, the maximum absorption peak was found at 440 nm. From the SEM images, it is confirmed that the sample contains spherical silver nanoparticles at a range of 10 to 60 nm. The silver nanoparticles are crystalline in nature, which was confirmed by the FT-IR peak at 518 cm⁻¹ corresponding to the Ag vibration present in crystalline structure. The water filtration system depicted 5 log reduction for AgNPs [99.99% reduction]. The antibacterial activity of silver nanoparticles was determined by well diffusion method, and found that silver nanoparticles have significant antibacterial activity against *E. coli* with an inhibition zone of 2.1cm. The MIC test was performed to test the inhibitory concentration of AgNPs against the pathogens and was found to be 40 µg ml-1 for *E.coli* and comparatively higher for other microorganisms.

Keywords: Nanoparticles, Water filtration, Log reduction, Minimum inhibitory concentration.

1. INTRODUCTION

Silver nanoparticles are one of the promising products in the nanotechnology industry. The development of consistent processes for the synthesis of silver nanoparticles is an important aspect of current nanotechnology research. Silver nanoparticles can be synthesized by several physical, chemical and biological methods. One of such promising process is green synthesis [3]. However, for the past few years, various rapid chemical methods have been replaced by green synthesis because of avoiding toxicity of the process and increased quality. Cost-effective filter materials coated with silver nanoparticles is an alternative technology that could

assist the developing countries. Silver ion (Ag+) has long been known as a potential antimicrobial agent and is used in wound dressings to prevent infections in burn patients, to blindness in newborns, for severe chronic osteomyelitis and urinary infection, to control *Legionella* bacteria in hospitals and to improve the performance of drinking-water filters. It can bind to bacterial cells and enzymes (proteins) at multiple sites, damaging them and preventing them from performing their functions and result, to cells death through penetration at specific bacterial DNA and RNA [5]. Silver in the form of nanoparticles that release silver ions more

effectively has a better bactericidal activity due to its high surface-area-to-volume ratio [2, 15]. As a result, researchers have considered silver nanoparticles for drinking-water treatment due to its strong and broad spectrum of antimicrobial activities. With the advancement of material development, silver nanoparticles can be easily deposited on solid materials for the deactivation of microorganisms in water treatment [14]. In the case of drinking-water treatment, various forms of silver nanoparticles coated on materials/substrates have been used. In this paper we studied the inactivation of bacteria during percolation of water through the filtration sheet and also the antimicrobial effect of yeast mediated silver nanoparticles against pathogens.

2. EXPERIMENTAL PROCEDURE

2.1 Biosynthesis of Yeast Mediated Silver Nanoparticles (AgNP)

Saccharomyces cerevisiae were grown as suspension culture in carbon and nitrogen source for 36 hours. From this culture 25 ml was filtered and centrifuged four times by 30% ethyl alcohol and allowed to grow for another 24 hours till straw colour was seen. The above sample was centrifuged for 36 hours at 200 rpm in order to prepare a cell free extract. Again centrifuged at 10000 rpm for 10 minutes and the supernatant were collected. This was then lyophilized to get a powder form. 20 ml of 0.025 M AgNO₃ was added to the culture solution and colour change to silver grey was observed [5].

2.2 Study of Antibacterial Effect in Water Filtration System

Silver nanoparticles are deposited by in-situ reduction of silver nitrate on the cellulose fibres of an absorbent blotting paper sheet or filter paper. These AgNP papers were tested for the antibacterial effect by plate count method. The log reduction value was also determined before and after treatment [12].

2.3 Antimicrobial Potential of Silver Nanoparticles by Well Diffusion Method

The pathogenic microorganisms were swabbed on agar plates. The synthesized silver nanoparticles were diluted with distilled water. The three wells in the agar plate were loaded with silver nanoparticles solution, silver nitrate solution (1mM) and nutrient broth. The plates were incubated at 37°C [3]. The pathogenecity was tested against Cryptococcus gastricus, Escherichia coli, Trichophyton rubrum, Shigella flexneri, Fusarium oxysporum [9].

2.4 Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC), defined as the lowest concentration of material that inhibits the growth of an organism, was determined based on batch cultures containing varying concentration of nanoparticles in suspension (20–300µg ml⁻¹). Microbial growth was measured as increase in absorbance at 600 nm determined using a spectrophotometer [6]. All the experiments were carried out in triplicate [1, 2]. The nanoparticles were tested for bactericidal effect using all the microbial cultures selected for the study.

3. RESULTS AND DISCUSSION

3.1 Biosynthesis of Yeast Mediated AgNP

The nanoparticles were synthesized successfully from yeast using the method of bioreduction. This was confirmed by the sooty grey colour of the broth. This was obtained by incubating in dark. The bioreduction [15] is facilitated effectively under darkness.

Once on addition of silver nitrate solution the nitrate ions reduce the yeast biomolecules and hence form nanosized particles. Having done the incubation and the formation of grey colour the broth was centrifuged till a variant pellet was formed. This pellet has to be retained. The supernatant is used for extracellular enzyme studies. The pellet was dried and then lyophilized to get a powder form of

the nanoparticles. The nanoparticles were extracted extracellularly which was used for further analysis.

3.2 UV-Vis Analysis

The reduction of silver ions into silver nanoparticles during exposure to yeast was observed as color change which arises due to the Surface Plasmon Resonance phenomenon [4, 6]. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in resonance with light wave. The optical property of AgNPs was determined by UV-Visible spectrophotometer. After the addition of Silver nitrate to the yeast cell free extract, the spectrum was taken at 48Hrs between 300 nm to 600 nm. The AgNPs have sharp absorbance with highest peak at 440nm and progressively decreased while nanometer increased.

3.3 FTIR Analysis

The FTIR spectral analysis reveals the presence of three ring stretching vibration of Ag-NPs at 3350, 1636, 543 in the region of 500- 3500 cm⁻¹. The bond stretching[13] arises due to carbonyl stretch in the amide linkage of the protein has the stronger ability to bind silver so that the protein could most possibly form a coat around Ag- NPs and it stabilize the aqueous synthetic medium [4,13]. The bond at 3350 for O-H stretching corresponds to carboxylic acid, 1636 cm⁻¹ for stretching C=C corresponds to aromatic amino groups. The band at 675 cm⁻¹ corresponds to C-H stretching of phenyl ring of substitution band, whereas the stretch for Ag-NPs was found around 518 cm⁻¹. This evidence suggests that the biomolecules could possibly perform the function for the formation of stable Ag-NPs in aqueous medium. It was well known that the protein can bind to silver nanoparticles through free amide groups.

3.4 SEM Analysis

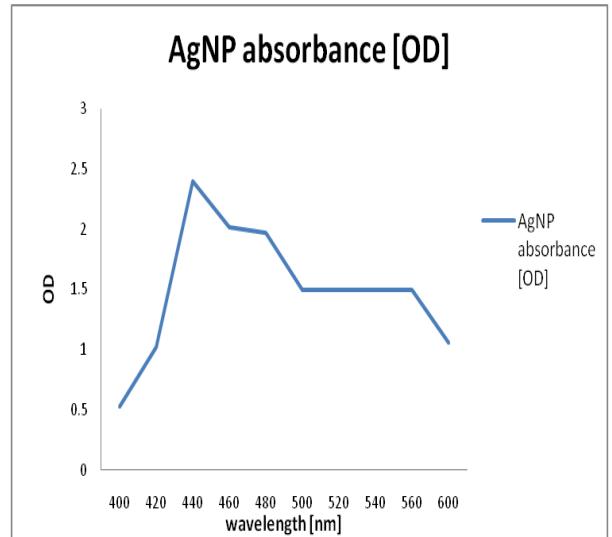


Figure 1 Absorbance of yeast mediated AgNP.

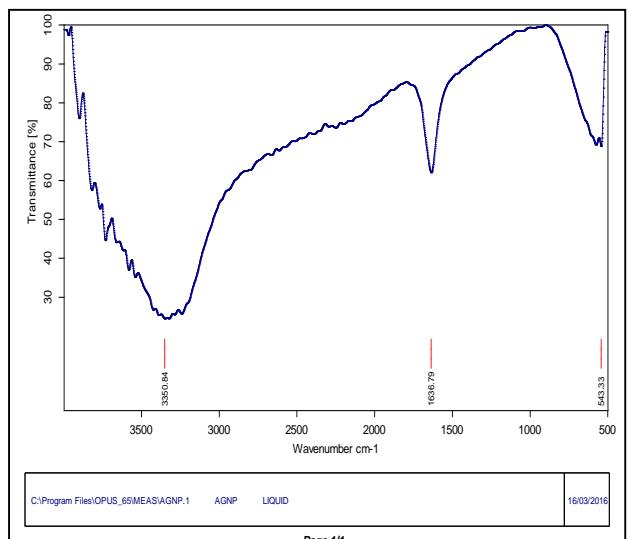


Figure 2. FTIR results for yeast mediated AgNP.

The electrostatic interactions such as hydrogen bond, bio-organic bond and capping molecules are the reason for biosynthesis of silver nanoparticles. The synthesized nanoparticles were mostly spherical with the size in the average range of 10 to 60 nm [7]. The difference in size is due to formation of nanoparticles at different times which may limit the nanoparticle size due to constraints related to the particles nucleating inside the organisms [8, 11]. The results presented are at single pH value. The figure shows the high density of yeast mediated AgNP.

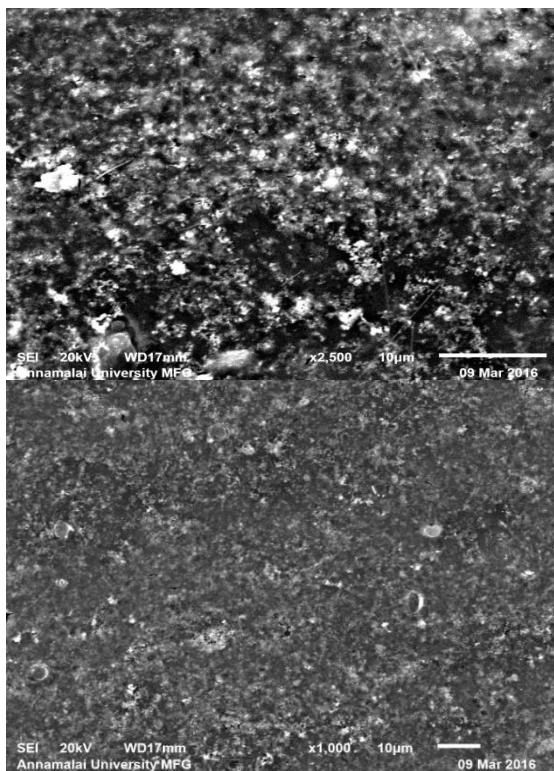


Figure 3. SEM image of biosynthesized yeast mediated AgNPs.

3.5 Water Filtration

The water purification analysis was compared between normal Watman No.1 filter paper and AgNP coated Watman No.1 filter paper. Tap water was used as the sample. After the filtration the filtrate was analysed for microbial counts. Plain filter paper filtrate showed significant microbial growth whereas AgNP coated filtrate showed no microbial growth at all.

3.6 Log Reduction

Cell counting cytometer was used to count the number of microorganisms

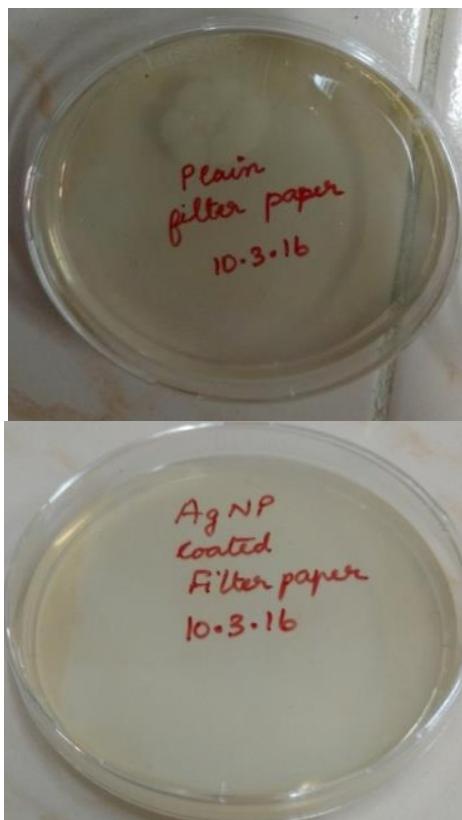


Figure 4. Microbial count before any treatment and after treatment.

before and after treatment. Also the log reduction values were calculated.

Log reduction= $\log_{10}[A/B]$ where, A is the number of microorganisms before treatment and B is the number of microorganisms after treatment. The number of microorganisms after treatment with AgNP coated filter paper was almost zero. Thus it attained the maximum reduction of nearly 5. Whereas the log reduction value of filtration through plain filter paper was 1.27. Hence we can also find the percent reduction using the following formula: $P = [1-10^{-L}]*100$, where, P is the percent reduction and L is the log reduction.

Water filtration studies revealed that log reduction values of AgNP coated filter paper was 5 when compared to 1.27 for plain filter paper which is depicted in the above graph. A value of more than 2 log reduction is desirable to conclude that the treatment was effective.

Table 1. Log reduction values and reduction in percent.

	Log Reduction Values	Percent reduction%
Plain filter paper	1.27	94.6
AgNP coated filter paper	5	99.99

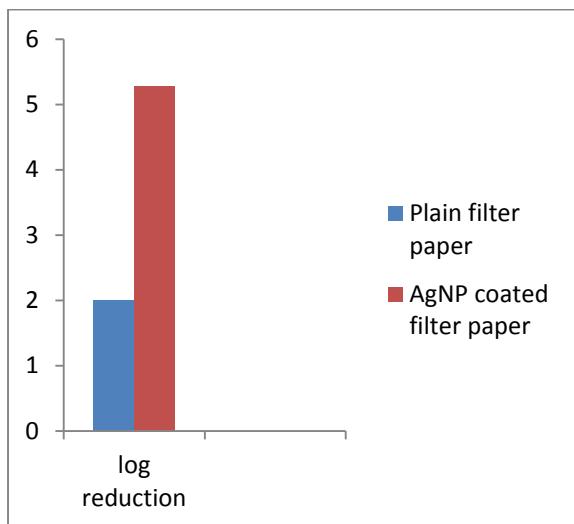


Figure 5. Comparison of Log reduction values.

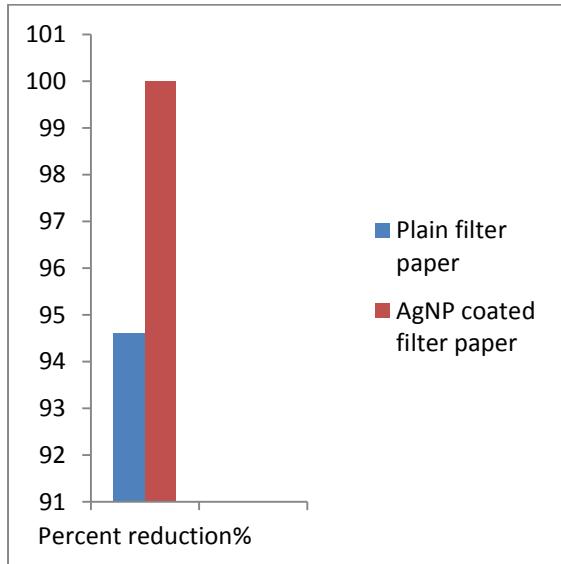


Figure 6. Comparison of Percent reduction values.

The above graph indicates that the silver nanoparticles increased the filtration efficacy i.e. 99.99%. Due to antimicrobial effects of metallic silver, silver

nanoparticles play a crucial role in water disinfection [12].

3.7 Antimicrobial Activity of *Saccharomyces Cerevisiae* Pure Culture And Biosynthesized Silver Nanoparticles

It's proven that yeast possesses some antimicrobial activity. Now this activity is enhanced by coupling with silver nanoparticles and thus we attain maximum inhibition of pathogens. The antibacterial activity of silver nanoparticles was compared for various microorganisms using the diameter of inhibition zone in disk diffusion test. The diameter of inhibition zone (DIZ) reflects magnitude of susceptibility of the microorganism. The strains susceptible to disinfectants exhibit larger DIZ, whereas resistant strains exhibit smaller DIZ [13].

The MIC representing the antimicrobial activity of nanoparticles dispersed in batch cultures is summarized in Table 2. Representative growth profile of microbial strains in the presence of varying concentrations of silver nanoparticles are depicted in Fig 13, 14, 15, 16, 17.

The relatively low MIC is possibly due to suspension of the cells in distilled water compared to suspension in nutrient media as employed in our study. For studies conducted on agar plates, the MIC of silver nanoparticles for *E. coli* was reported as $75 \mu\text{g ml}^{-1}$ [10]. In batch studies with *E. coli* and colloidal silver nanoparticle (size range 2–25 nm), MIC was reported to be in the range of $3\text{--}25 \mu\text{g ml}^{-1}$ for initial bacterial concentration $10^5\text{--}10^6 \text{CFU ml}^{-1}$.

According to the studies carried out with different concentrations of silver nanoparticles and the corresponding absorbance we represent the following growth profile. The controls indicated the microbial growth profile in the absence of nanoparticles.

The antimicrobial studies carried out against several pathogens revealed that yeast mediated silver nanoparticles were highly significant against *E. coli*.

Table 2 The zone of inhibition of the silver nanoparticles and silver nitrate against pathogenic microorganisms.

Name Of The Organism	Diameter Of The Zone Of Yeast Pure Culture [cm]	Diameter Of The Zone Of AgNO ₃ [cm]	Diameter Of The Zone Of Yeast Mediate d AgNPs [cm]	MIC
Esherichia coli	1.7	1.4	2.1	40
Shigella flexneri.	0.2	0.9	1.5	220
Cryptococcus gastricus	1.5	1.1	1.8	120
Trichophyton rubrum	0.8	0.7	0.8	180
Fusarium oxysporum	1.0	0.8	1.0	140



Figure 7. Inhibition zone of *Escherichia coli*.

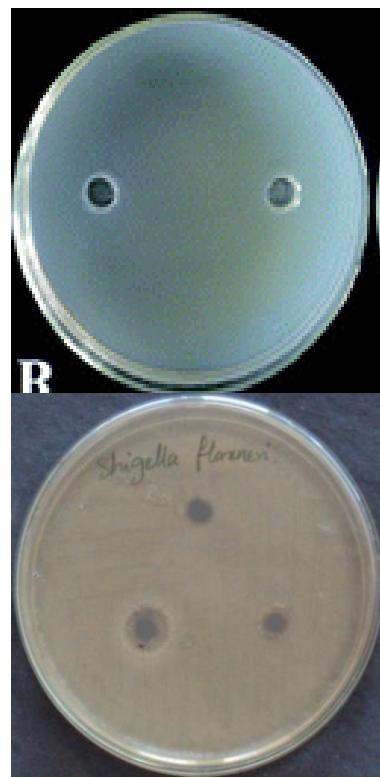


Figure 8. Inhibition zone of *Shigella flexneri*.



Figure 9. Inhibition zone of *Cryptococcus gastricus*.

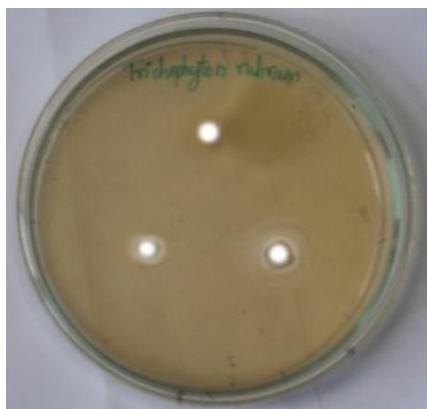


Figure 10. Inhibition zone of *Trichophyton rubrum*.



Figure 11. Inhibition zone of *Fusarium oxysporum*.

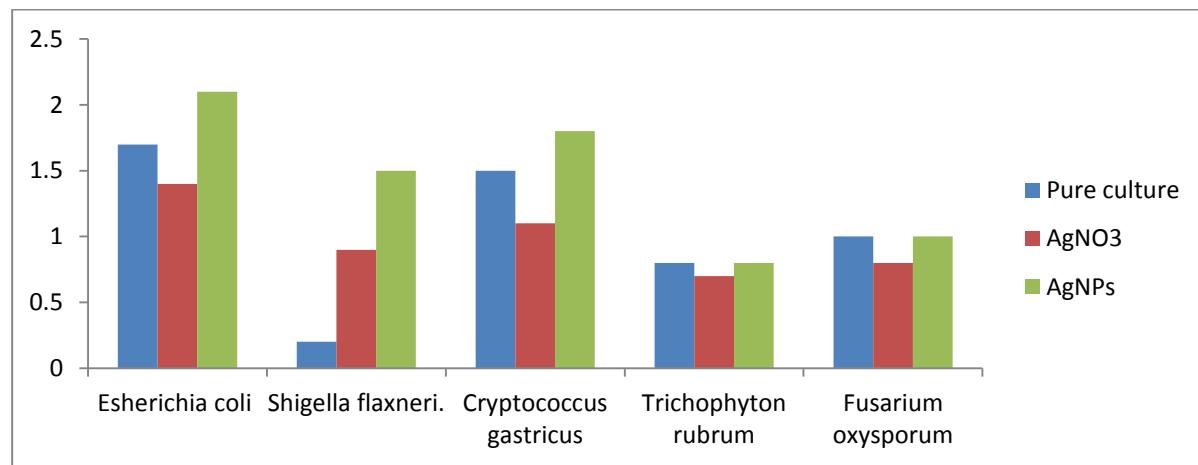


Figure 12. The zone of inhibition against pathogenic organisms.

From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

4. CONCLUSION

Silver nanoparticles were successfully synthesized by biological method from *Saccharomyces cerevisiae*. The Surface

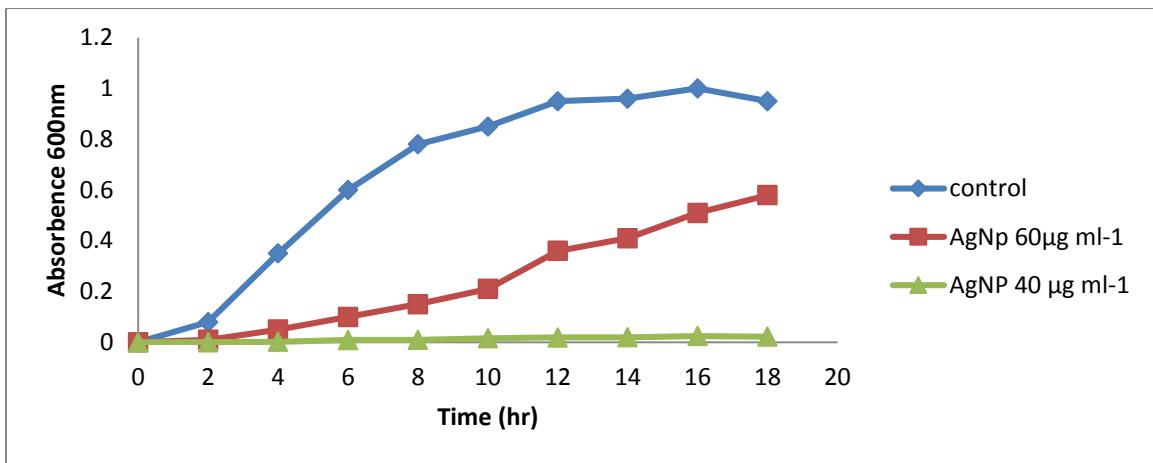


Figure 13. Representative batch growth profile in presence of varying concentration of silver nanoparticles for *E.coli*.

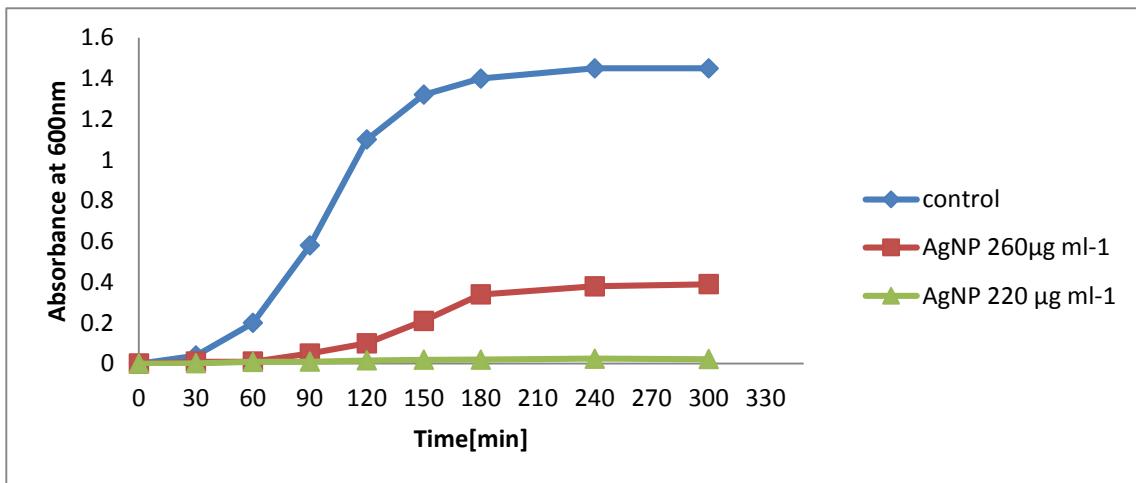


Figure 14. Representative batch growth profile in presence of varying concentration of silver nanoparticles for *Shigella flexneri*.

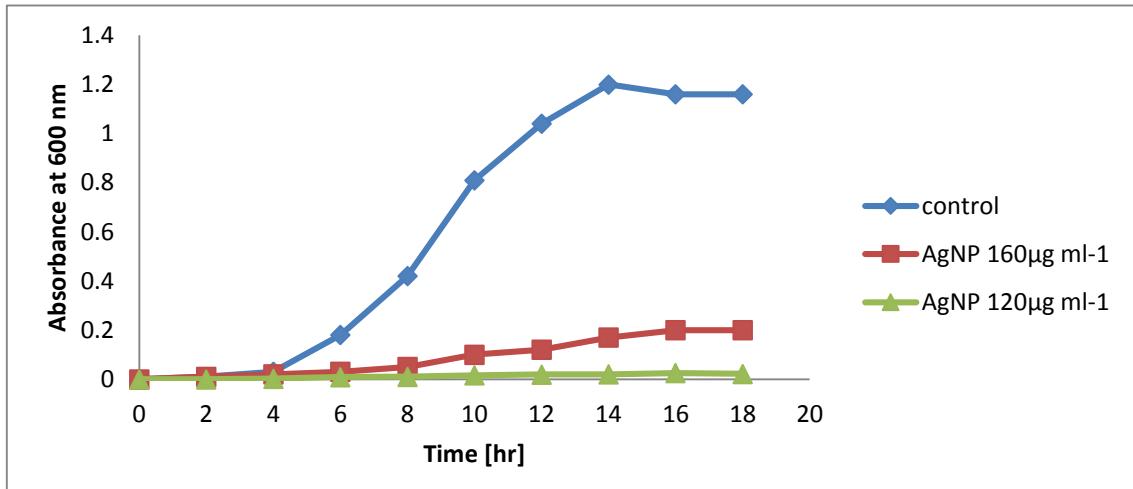


Figure 15. Representative batch growth profile in presence of varying concentration of silver nanoparticles for *Cryptococcus gastricus*.

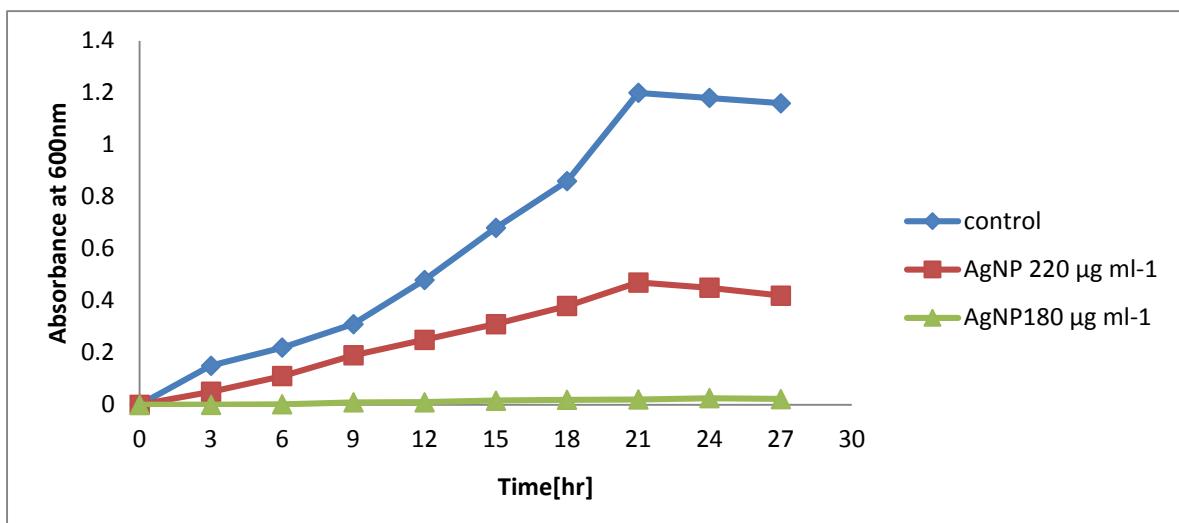


Figure 16. Representative batch growth profile in presence of varying concentration of silver nanoparticles for *Trichophyton rubrum*.

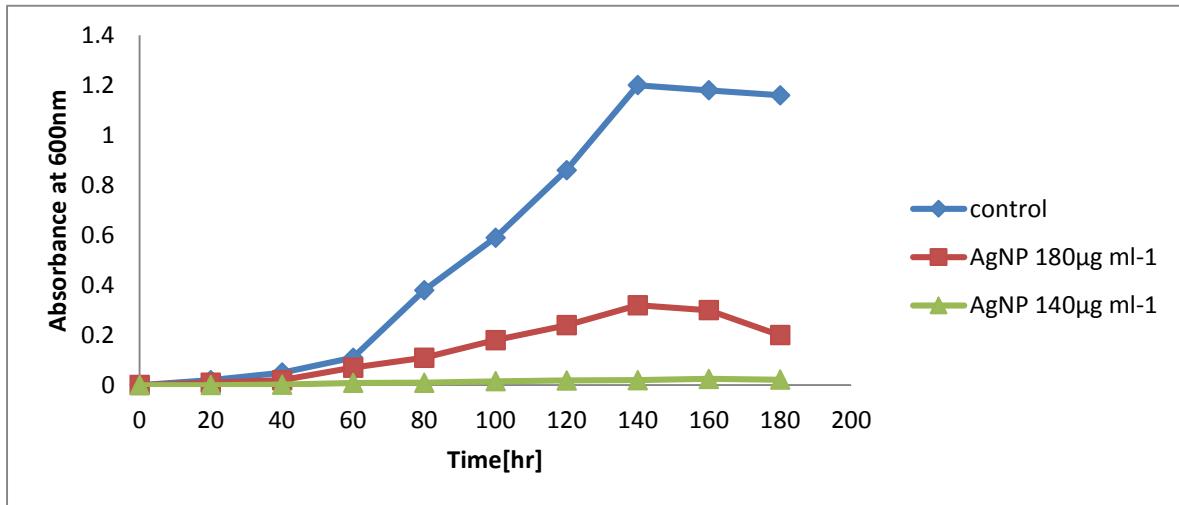


Figure 17. Representative batch growth profile in presence of varying concentration of silver nanoparticles for *Fusarium oxysporum*.

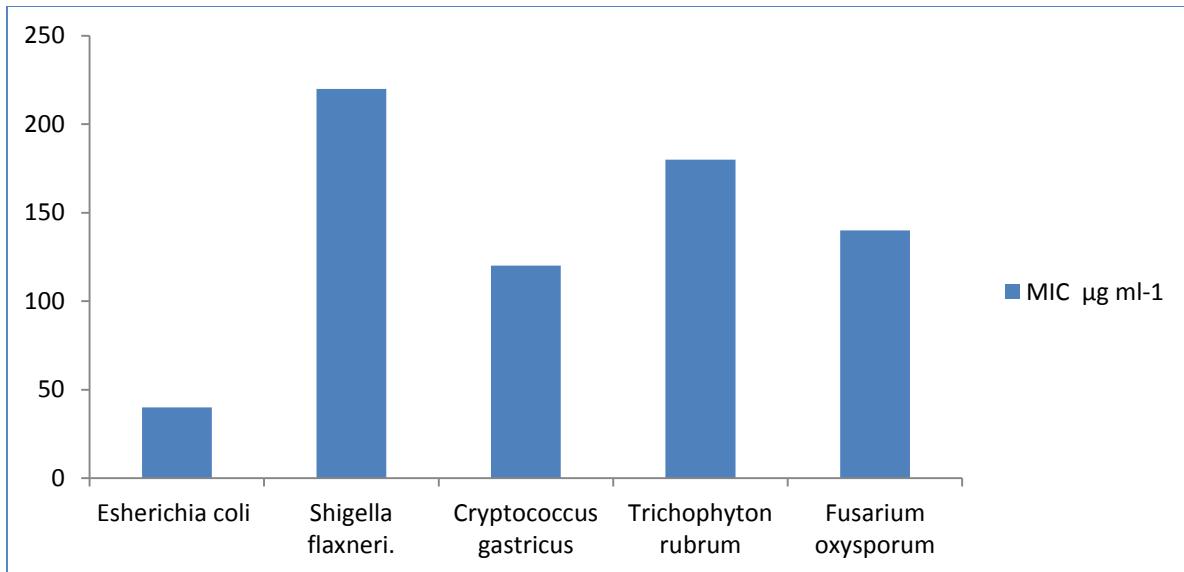


Figure 18. MIC ($\mu\text{g ml}^{-1}$) of silver nanoparticles for various microorganisms.

Plasmon Resonance (SPR) property of synthesized nanoparticle was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 440 nm. The morphological study of AgNPs using SEM suggests that the nanoparticles are spherical in shape with diameter around 10 nm to 60nm. The physiochemical properties of silver nanoparticles using FTIR conclude that the nanoparticle formed in the process is crystalline. The proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketones, aldehydes, etc., were

found from the characterization. The filtration studies conclude that there is maximum reduction of microbial count post filtration with AgNP coated filter paper with a percent reduction value of 99.99%. The antibacterial activity of silver nanoparticles concludes that the silver nanoparticles shows significant antibacterial activity against E. coli with an inhibition zone of 2.1cm whereas less activity against Cryptococcus gastricus, Trichophyton rubrum, Shigella flexneri and Fusarium oxysporum.

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