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Factors Affecting the Geometry of Silver Nanoparticles Synthesis in *Chrysosporium Tropicum* and *Fusarium Oxysporum*

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Abstract: Problem statement: Biosynthesis of nanoparticles using fungal cells is a novel approach to develop biotechnological possess such as bioleaching and bioremediation. In the present study, an effort was made to investigate the effect of physio-chemical parameters on the silver nanoparticle formation with the fungus *Chrysosporium tropicum* Carmichael and *Fusarium oxysporum* Schltdl. **Approach:** The possibilities to manipulating the geometry of silver nanoparticles by altering the key growth parameters such as pH, temperature, concentrations and time have been explored. The effect of $AgNO_3$ with the cell free extract of fungi and time, temperature, pH with the formation of silver has also been investigated. The presence of nanosilver has been carried out with the Micro-scan reader and has been confirmed by X-Rays Diffractometer (XRD). The micrographs of the silver nanoparticles have been evaluated through the Transmission Electron Microscope (TEM) and confirmed by Scanning Electron Microscope (SEM). The effect of concentrations with response to time, temperature and pH has studied with the help of Micro-scan reader and their microstructure analyzed by TEM and SEM. **Results:** It was observed that fungus *C. tropicum* and *F. oxysporum* Schltdl significantly activate the extra-cellular production of silver nanoparticles. The different sized and spherical shaped nanoparticles have been formed in different strains. With the increase in concentration, the absorbance increased with response to time (24-120h) and temperature. Significantly, the pH was found decreasing with the increase of absorbance. **Conclusion:** We presume that these changes initiate new geometry of nanosilver in the cell free solutions. These different shaped, sized and geometry of nanoparticles can be used in the field of medicine for drug formation and diseases diagnosis.

Key words: *Chrysosporium tropicum*, *Fusarium oxysporum* Schltdl, silver nanoparticles, physio-chemical parameters

INTRODUCTION

Recently, nano science has taken up the cause of a new dimension. Nanotechnology is involving the production, manipulation and use of materials managing in size less than a micron to an individual atom. Although nano materials can also be synthesized using chemical approaches. The biological method preferred for various reasons. It is now possible to include the use of fungi, bacteria and other biological materials. Silver is a soft, white, lustrous transition metal, it has the highest electrical conductivity of any element and the highest thermal conductivity of any metal. The metal occurs naturally in its pure, free form (native silver), as an alloy with gold and other metals and in minerals such as argentite and chlorargyrite. Most silver are produced as a by-product of copper, gold, lead and zinc refining. Nano-silver is pure de-ionized water with silver (Ag) in suspension. Approximately 80% of the silver is in the form of metallic silver nano-particles. The remaining silver is in ionic form. Though similar to colloidal silver, generally, a colloid is a suspension of particles of from 10 nm to 1 micron in diameter and the silver particles in Nano-Silver are less than 2 nm in diameter and therefore too small to be considered in "colloidal" suspension. At this size they are still metallic, but smaller ones turn into insulators. Their equilibrium structure changes to icosahedral symmetry, or they are even hollow or planner, depending on size. The present paper intends to explain the origin of this special behavior of nanomaterials. Unicellular and multicellular organisms are known to produce inorganic materials either intra- or extra-cellular (Kumar *et al.,* 2003; Peto

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et al., 2002; Sastry *et al.*, 2004). There is an enormous interest in the synthesis of nanomaterials due to their unusual optical (Krolikowska *et al.*, 2003), chemical (Kumar *et al.*, 2003), electronic (Peto *et al.*, 2002), biological (Glotzer and Anderson, 2010, Jones *et al.*, 2010; Sako, 2006) properties.

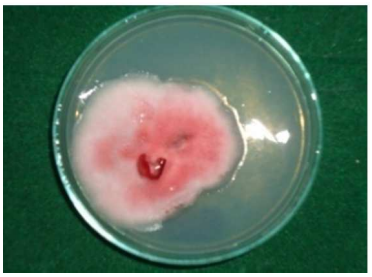
To our surprise, the roles of fungi which have so far been used are *Colletotrichum* sp. (Shankar *et al.*, 2003), *Trichothecium* sp. (Ahmed *et al.*, 2005). Nanoparticles with well defined dimensions can be obtained by fungi. Metal nanoparticles exhibit unique electronic, magnetic, catalytic and optical properties that are different from those of bulk metals (Klaus-Joerger *et al*., 2001; Mandal *et al.*, 2005). These unique properties have the potential for use in diverse range of industrial applications. Their optical and electronic properties can be used in optics, electronics, medical diagnostics and treatments, sensors and coatings (Corti and Holliday, 2004; Daniel and Astruc, 2004). Numerous chemical methods, aimed at controlling the physical properties of the particles, have been reported in the literature (Daniel and Astruc, 2004; Burda *et al.*, 2005).

The use of fungal cells for the synthesis of metal nanoparticles, nanosized materials has recently emerged as a novel approach. Although the biosynthesis of nanomaterials is recent, the interactions between micro-organisms and metals have been well documented (Slawson *et al.*, 1990; Beveridge *et al.*, 1997; Savvaidis *et al.*, 1998; Malik, 2004). Ability of micro-organisms to extract and/or accumulate metals is being employed in commercial biotechnological processes such as bioleaching and bioremediation. Many microbes are known to produce inorganic nanostructures and metallic nanoparticles with properties similar to chemically-synthesized materials, while exercising better control over size, shape and composition of the particles. The objective of this study is to highlight the progress made at Dayalbagh on the biosynthesis of silver nanoparticles with new species of fungi. Manipulation of the size and shape of silver nanoparticles, by altering key growth parameters, have also been demonstrated.

MATERIALS AND METHODS

Fungal strains: Fungal strains *C. tropicum* Carmichael (2828) and *F. oxysporum* Schltdl (2480) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology Chandigarh, India. These strains were routinely maintained in our laboratory on Sauboraud's Dextrose Agar and Potato Dextrose Agar (PDA) medium at 27±2°C (Fig. 1a and b).

(a)



(b)

Fig.1: Culture of *C. tropicum* (a) and F.oxysporum (b) on PDA and SDA medium respectively

Fungal culture: These fungi were grown in different culture medium. *F.oxysporum* was grown in Potato Dextrose Broth (PDB) medium, containing potatoes infusion 200g, dextrose 20g and deionized water $1000mL^{-1}$. Whereas, *C. tropicum* was grown in Sauboraud's Dextrose Broth (SDB), containing dextrose 40g, peptone 10g and deionized water $1000mL^{-1}$. The fungi were grown in 250ml conical flask, each containing $100mL^{-1}$ of PDB and SDB medium. The media were autoclaved at 20 psi for 20 min. The fungus colonies grown on PDA plates were transferred to broth using inoculation needle. The broth, inoculated with *F. oxysporum* and *C. tropicum* was incubated at 27±2°C for 7 days.

Bioreduction of $AgNO_3$: After incubation, the biomass was separated from the medium by filtration through whatman-1 filter paper and washed thrice in sterile distilled water to remove any nutrient media that might interact with the silver ions. Approximately 10g of *F. Oxysporum* and *C. tropicum* biomass was transferred to a 250 ml conical flask containing 100 ml of distilled water and incubated for 72h at 27±2°C and then the aqueous solution components were separated by filtration. To these solutions (liquid fungal), $AgNO_3$ (10^{-3}M) was added and kept for 72h at 27±2°C. Periodically, aliquots of the reaction solutions were removed and their absorption was measured in a Micro-Scan reader model no. MICROSCAN MS5608A. Then the solution was converted in powder for X-Rays Diffractometer (XRD) measurements. The micrographs

of silver nanoparticles were obtained by Philips CM-10 Transmission electron microscope and conformed by Scanning electron microscope.

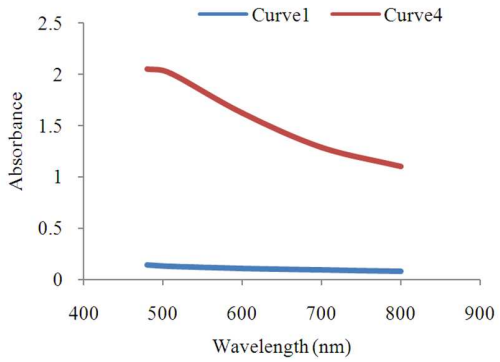
RESULTS

Micro-scan reader analysis: Fig. 2a shows a test tube containing *C. tropicum* suspension in deionized water before addition of $AgNO_3$ solution. The pale yellow color of the fungal suspension can clearly be seen in the figure. A picture of the test tube containing the fungal suspension after exposure to 10^{-3} M aqueous solution of $AgNO_3$ for 72h is shown (Fig. 2b). The dark brown colour clearly seen in the fungal suspension is indication of the synthesis of Ag nanoparticles. Fig. 2c shows the Micro-Scan Reader spectra recorded form fungal suspension of *C. tropicum* (curve 1) and after addition of 10^{-3} M aqueous solution of $AgNO_3$ for 72h (curve 4). Fig. 3a shows a conical flask with the *F. oxysporum* biomass which is a pale yellow in color before the addition of $AgNO_3$ solution. This changed to brownish color on completion of the reaction with Ag^+ ions for 72h (Fig. 3b). The appearance of a brownish color in solution containing the biomass was a clear indication of the formation of silver nanoparticles in the reaction mixture. Fig. 3c shows the Micro-Scan Reader spectra recorded form fungal suspension of *F. oxysporum* (curve 1) and after addition of 10^{-3} M aqueous solution of $AgNO_3$ for 72h (curve 4). The fungal biomass exposed to Ag^+ ions shows a distinct and fairly broad absorption band centered at 450 nm. The presence of the broad resonance indicates and aggregated structure of the silver particles in the biomass.

XRD analysis of Ag nanoparticles: The synthesized material after the reduction of $AgNO_3$ was characterized by X-ray diffractometer for the structural analysis (Figure 4). Figure 4-a depicts the XRD pattern of *C. tropicum* powered silver nanoparticles in the 2θ range 15-60248°. It exhibits a broad peak at 38°. The broadening of the peaks clearly indicates that the particles are in the nanoregime. Apart from these, many unidentified peaks at 22, 26, 30, 32, 34, 36 and 44° arises, possibly due to other chemical reactions or organic impurities presents in the sample. Figure 4-b depicts the XRD pattern of *F. oxysporum* powered silver nanoparticles in the 2θ range 20-60°. It exhibits a broad peak at 38.4°. The broadening of the peaks clearly indicates that the particles are in the nanoregime. Apart from these, many unidentified peaks at 16, 18, 21, 26, 30, 32, 35, 43, 45 and 52° arises, possibly due to other chemical reactions or organic impurities presents in the sample.

(a)

(b)



(c)

Fig. 2: Test tubes showing the fungal suspension. (a) Control (without AgNO3). (b) *C. tropicum* after immersion in 10-3 M aqueous AgNO3 solution on completion of the 72h reaction time. (c) C.tropicum Micro-Scan spectra recorded from fungal suspension before (curve 1) and after immersion in 10-4 M aqueous AgNO3 solution for 72h (curve 4).

TEM and SEM analysis of Ag nanoparticles: After reduction, silver nanoparticles were precipitated at the bottom of conical flask. This precipitate was washed out twice with double distilled water and then analyzed by employing TEM.

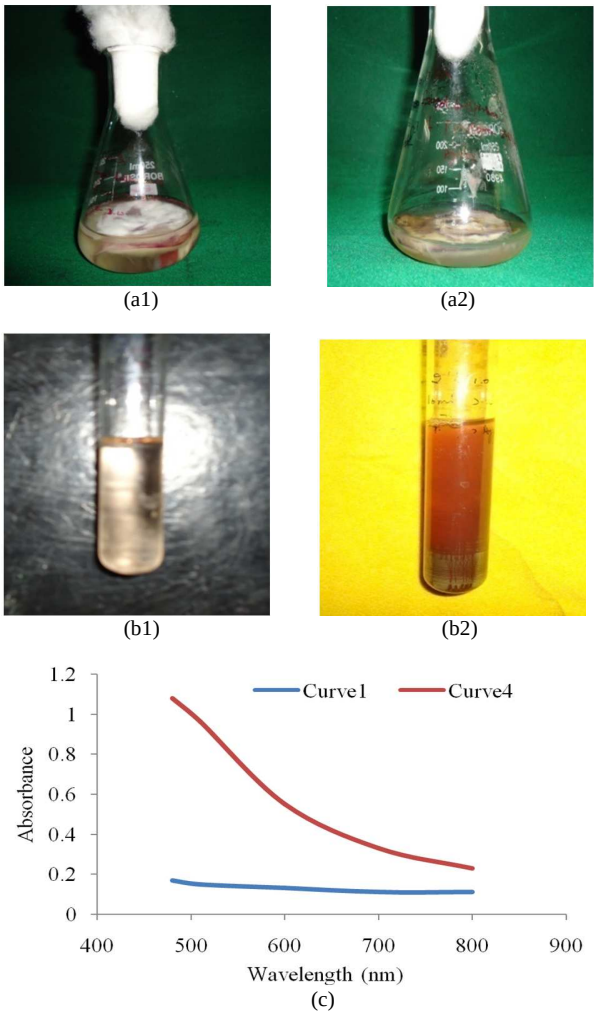


Fig. 3: Conical flasks showing the *F. oxysporum* biomass. (a1) control (without AgNO3 before filtration). (a2) test tube showing fungal suspension after filtration. (b1) after immersion in 10^{-4} M aqueous AgNO3 solution on completion of the 72 h reaction time (b2) test tube showing brownish color with Ag+ ion after filtration. (c) Micro-Scan spectra recorded from fungal suspension before (curve 1) and after immersion in 10^{-4} M aqueous AgNO3 solution for 72 h (curve 4).

The samples of silver nanoparticles synthesized using *C. tropicum* and *F. oxysporum* liquids were prepared by placing a drop of reaction mixture over copper grid and allowing water to evaporate. Figure 5-a shows typical TEM micrographs of *C. tropicum* silver nanoparticles. The 20-50 nm sized silver nanoparticles were observed. The SEM images are showing distinctly the high density silver nanoparticles synthesized by *C. tropicum* (Fig. 6-a). Figure 5-b shows typical TEM micrographs of *F. oxysporum* silver nanoparticles. The 20-50 nm sized silver nanoparticles were observed. The SEM images are showing distinctly the high density silver nanoparticles synthesized by *F. oxysporum* (Fig. 6-b) fungal species further confirmed the development of silver nanostructures.

Effect of Physico-chemical parameters: We could observe here only the effect of various physio-chemical parameters on the synthesis of silver nanoparticles and to determine their geometry in *C. tropicum* and *F. oxysporum*, which is significant.

Effect of Time: The fungal suspension of *C. tropicum* and *F. oxysporum* were incubated with $AgNO_3$ for 24, 48, 72, 96 and 120h respectively. After incubation the effect of time could be analyzed through the Micro-scan reader.

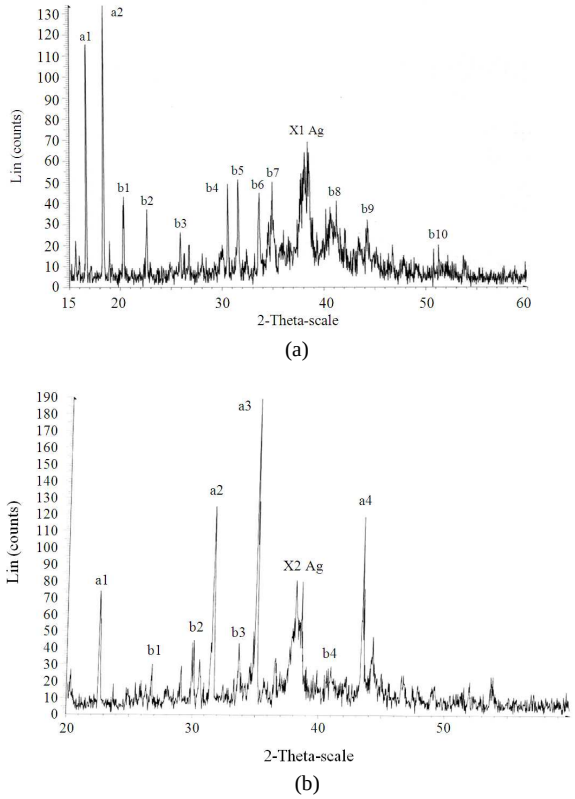


Fig. 4: XRD pattern in 2 theta scale with counts to depict system biology of *Chrysosporium tropicum* (a) Fusarium oxysporum (b) silver nanoparticle sample with concentrations profile of higher and lower metal ions concentration.

The effect of time against the absorbance can be seen, which increases with the time. The synthesis of silver nanoparticles by the fungus has thus been enhanced. The Micro-Scan spectrum of the *C. tropicum* (Fig. 7-a_1 and 7-a_2) and *F. oxysporum* (Fig. 8-a_1 and 8-a_2) reaction vessels at different times. The equation showing the relationship between the time and absorption at different wavelengths.

$A \alpha T$

Here: A = Absorbance, T = time

Effect of concentration: We could observe the effect of different concentrations of solutions in production of silver nanoparticles (Table 1). After the incubation the absorption spectra were recorded with the Micro-scan reader.

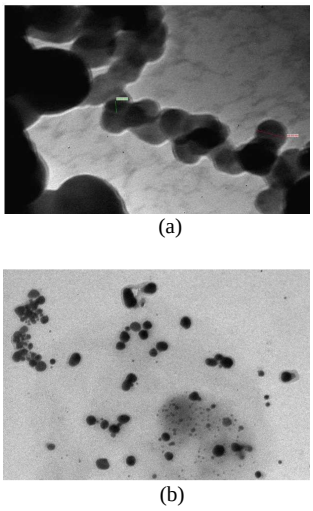


Fig. 5: TEM image of different size silver nanoparticles by *Chrysosporium tropicum* (a) and Fusarium oxysporum (b).

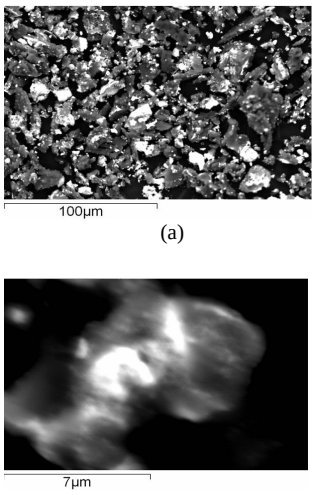


Fig. 6: SEM image of C .tropicum (a) and Fusarium oxysporum (b) synthesized silver nanoparticles with bright area at X650 magnification and at X9000 magnification respectively.

Table 1: Preparation of different concentration solution with their pH for different fungus species

Fungal $AgNO_3$ (g)	suspension (mL)	Concentration (ppm)	pH	
			C. tropicum	*F. oxysporum*
0.26	100	26	6.2	6.1
0.53	100	53	6.0	5.9
1.06	100	106	5.7	5.0
2.12	100	212	5.5	3.7

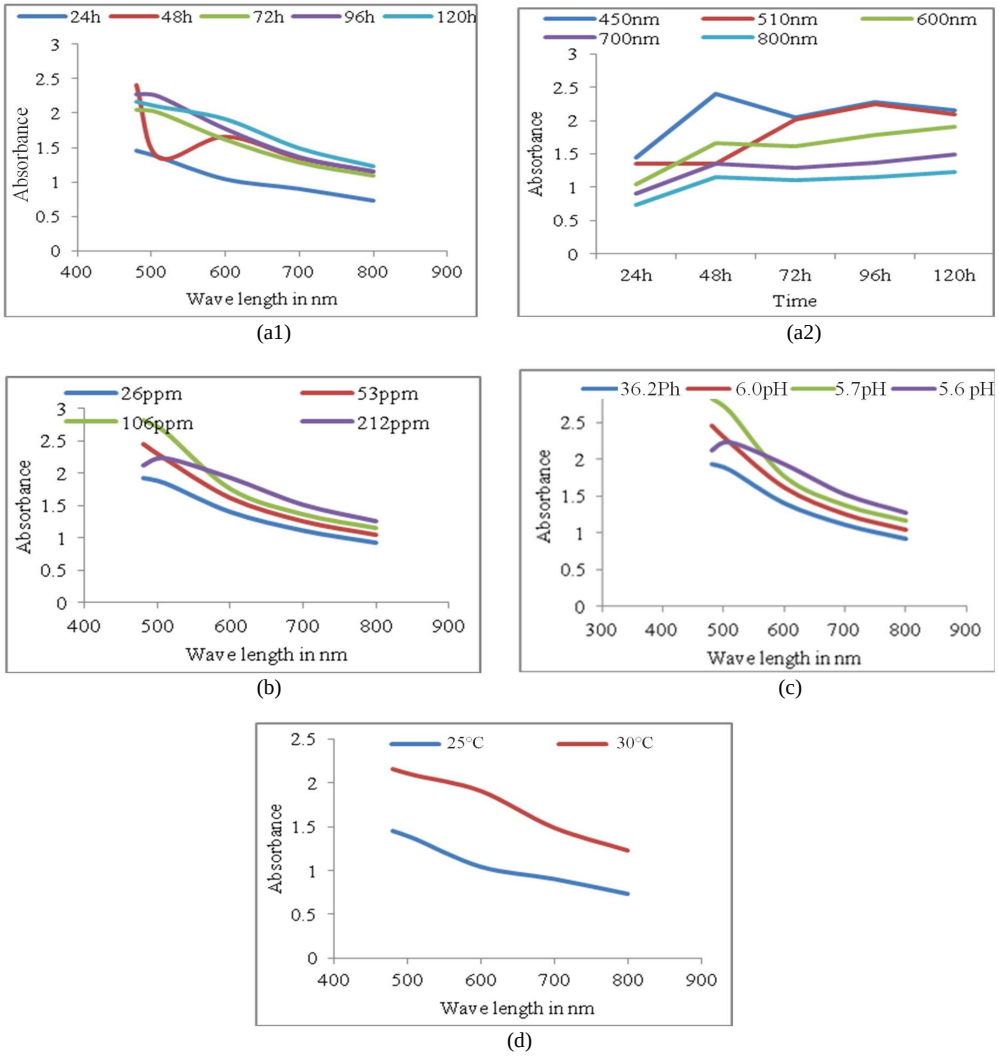


Fig. 7: Micro-Scan reader spectrum recorded as a function of different parameters of reaction in an aqueous solution of 10-3 M AgNO3 with the liquid fungal *Chrysosporium tropicum*. (a1) spectrum as a function of time of reaction. (a2) Wavelength. (b) Concentrations. (c) pH. (d) Temperature.

Fig. 7b and Fig. 8b shows the Micro-scan spectra of increased concentrations of *C. tropicum* and *F. oxysporum*. This indicates the increase in absorption with increase in the concentrations. The production of silver nanoparticles also increased with increased in concentrations. The equation showed a relationship between absorbance and concentration according to Beer's law.

$A \alpha C$

C = Concentration

Effect of pH: At same concentrations used for *C. tropicum* and *F. oxysporum* the solution has exhibited different pH (Table 1). Particle formed at different pH were predominantly spherical in shape, with the majority of the particles having 20-50nm in diameter. The effect of pH of *C. tropicum* and *F. oxysporum* liquid on the absorption is recorded through Micro-scan reader (Fig. 7c, Fig. 8c). This showed the increase in absorption while a decrease in pH. The result indicated the production of bigger particles with decrease in pH. The equation expresses a relationship between absorbance and pH.

$$A \alpha \frac{1}{PH}$$

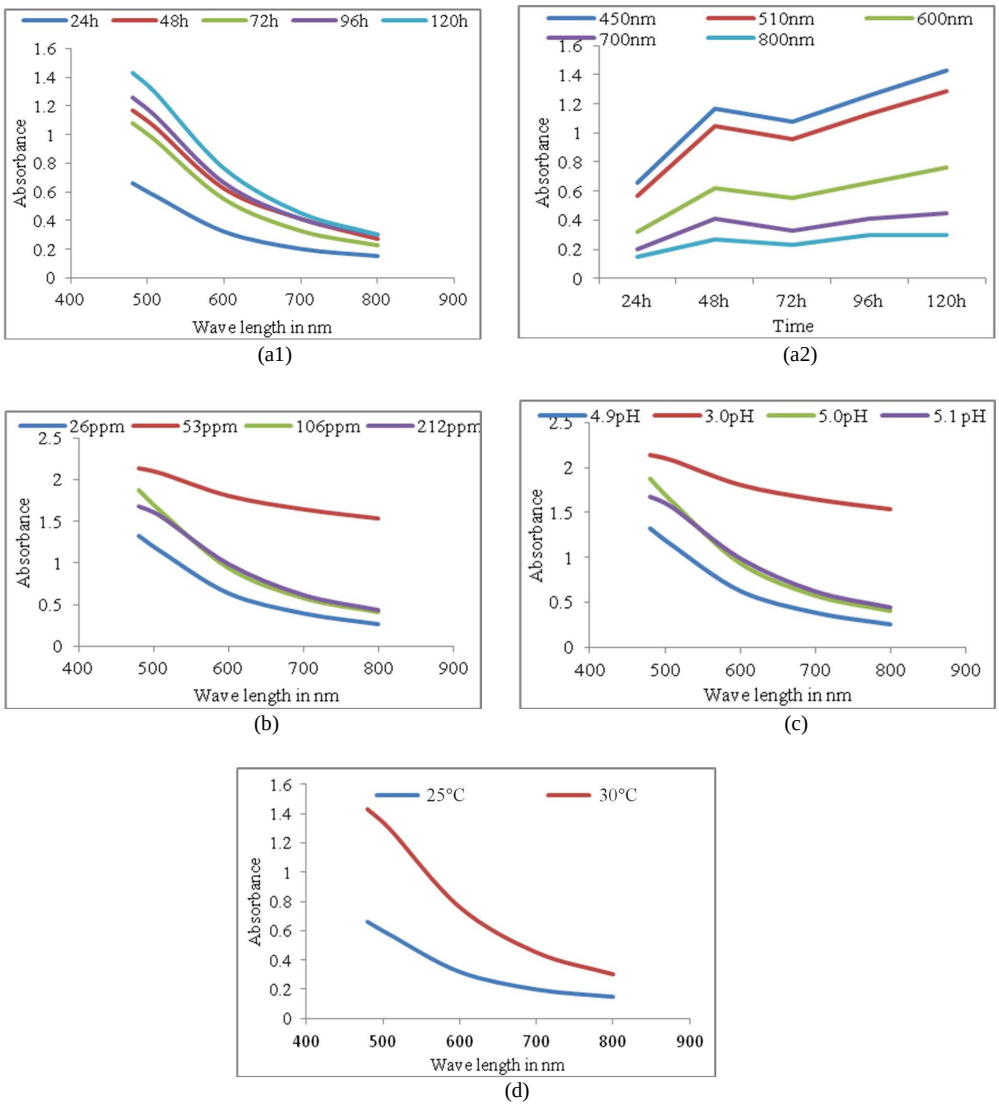


Fig. 8: Micro-Scan reader spectrum recorded as a function of different parameters of reaction in an aqueous solution of 10-4 M AgNO3 with the liquid fungal Fusarium oxysporum. (a1) spectrum as a function of time of reaction. (a2) Wavelength. (b) Concentrations. (c) pH. (d) Temperature.

Effect of temperature: The behavior of silver nanoparticles over a range of temperature was determined by exposing *C. tropicum* and *F. oxysporum* liquids to $AgNO_3$ at temperatures of 25C-30°C. The rate of formation of silver nanoparticle was related to the incubation temperature and a increase in temperature allowed particle growth at a faster rate. The effect of temperature was then recorded through the Micro-scan reader. As we increase the temperature (25C-30°C) the production and absorption of *C. tropicum* and *F. oxysporum* silver nanoparticles is also increased (Fig. 7d, Fig. 8d). At a lower temperature (25°C) the majority of silver nanoparticles were smaller. Further incubation at higher concentration (30°C) the smaller particles decreased and formation of larger particles. It exhibited well defined shapes of silver nanoparticles. Thus there is a distinct, direct relationship between absorbance and temperature of the solution. The equation reveled the relationship between absorbance and temperature.

$A \alpha t$

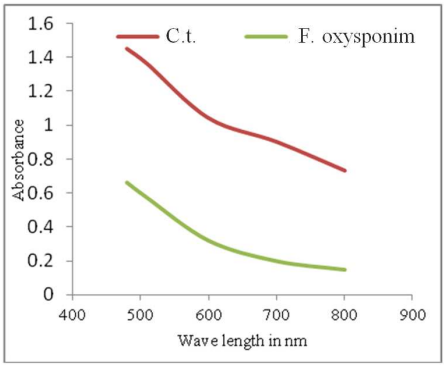


Fig. 9: A comparative relationships between *C. tropicum* and *F. oxysporum* in synthesis of silver nanoparticles

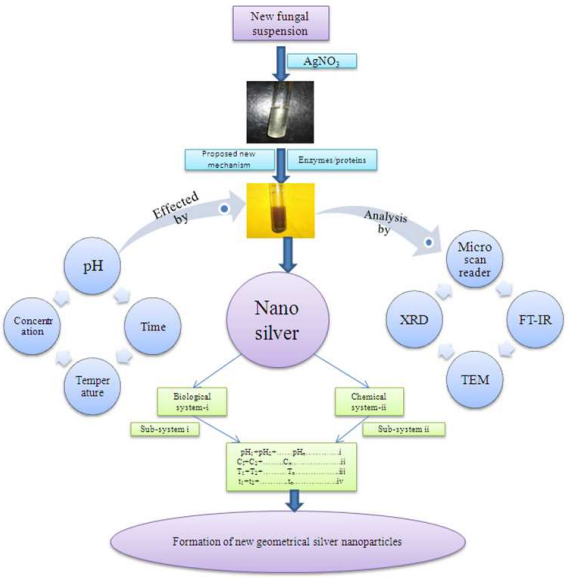


Fig. 10: Schematic presentation of silver nanoparticles formation

t = Temperature

A comparison on the effects of *C. tropicum* and *F. oxysporum* in synthesis of silver nanoparticles is presented (Fig. 9) which shows that the *C. tropicum* produce more nanoparticles than the *F. oxysporum*. A high absorbance was observed in *C. tropicum* and very low in *F. oxysporum*. The bioreduction of aqueous Ag^{+} ions by the fungi *C. tropicum* and *F. oxysporum* has been demonstrated.

The schematic presentation of silver nanoparticles formation has been shown (Fig.10). The results imply that exposure of whole cells to the silver solution during nanoparticles formation is not necessary. Identification of the active reducing proteins or enzymes involved in the process could potentially allow for a process in a cell-free environment, where the size and shape of the particles can be precisely controlled.

DISCUSSION

The extracellular synthesis of silver nanoparticles of various morphologies and sizes in the fungal cultures, *C. tropicum* and *F. oxysporum* has been recorded in our study. The rate of particle formation and therefore the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as the pH and temperature. In biological systems, cellular networks, which can often be thought of as assemblies of logic gates, underlie computation. To perform logic operations in such systems, researchers can engineer synthetic circuits in which biological substrates such as new fungal species are used as inputs, outputs and the information (nanosilver)-processing geometrical shapes of nanosilver (Bochong and Lingchong 2011). The intracellular synthesis of gold nanoparticles of various morphologies and sizes in two fungal cultures, V. luteoalbum and Isolate 6-3, has been investigated (Gericke and Pinches 2006). The rate of particle formation and therefore the size of the nanoparticles could, to an extent, be manipulated by controlling parameters such as the pH and temperature. The potential of nanocrystalline palladium particle production using Cinnamom zeylanicum Bark Extract (CBE) as the biomaterial have been studied (Sathishkumar *et al.*, 2009). They studied the effects of biomaterial dosage, pHand temperature on nanoparticle formation. These factors have a major effect on the size and shape of the nanoparticles. Transmission Electron Microscopy (TEM) observations confirmed the synthesis of nano-sized palladium particles. These are results which were performed on the bark extract, whereas in our study we have selected the fungal species. The role of reaction temperature in the formation and growth of silver nanoparticles through a synergetic reduction approach using two or three reducing agents have been studied (Jiang *et al.*, 2010). By this approach, the shape-/size-controlled silver nanoparticles (plates and spheres) can be generated under mild conditions.

The extracellular formation of silver nanoparticles on exposure of cell-free extract to silver ions with one new and other known fungi has been demonstrated for the first time. This may have the potential of nano silver

factory formation. Extracellular formation of silver nanoparticles would be advantageous as it would eliminate the need to recover the particles formed within the cells. The development of chemical procedures to control the morphology of nanoparticles is an ongoing area of research. A biological process with the ability to control the shape of the particles produced would therefore be an exciting prospect. However, the cellular mechanism leading to the biosynthesis of silver nanoparticles is not yet fully been understood however, a possibility has been discussed with system science approach. Further research therefore will focus on fundamental understanding of its mechanism on cellular and molecular levels, including isolation and identification of the intermediated compounds responsible for the reduction of silver ions Chitinase. A model automated nano silver factory of this kind in laboratory can thus be made to developed synthesizer of new silver nanoparticles.

CONCLUSION

In this study, we are now in a position to conclude that a new system biology approach has to be generated to study role of the fungal species in formation of nanosilver. The factors such as temperature and pH can be manipulated for initiating geometry of Ag nanoparticles formation. Be using different species one can have a new sub-system nano factory capable of producing different shaped and sized silver nanoparticles. This study can thus be employed for making nanoparticles of fungal origin. Nanobiology, nanomedicine, nanochemistry can take advantage of new particle synthesis. We suggest that research needs to be addressed not only from the nanotechnology and fungal microbiology point of view but by system biology biosynthetic pathways also. The size, surface, geometrical biogenic nanoparticles should be recognized as parts of the whole systems as sub-system of the whole.

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