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SYNTHESIS AND STRUCTURAL CHARACTERIZATION OF POLYDISPERSE SILVER AND MULTISHAPED GOLD NANOPARTICLES USING *FUSARIUM OXYSPORUM* MTCC 284

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Metal nanoparticles are exploited in different fields of biology, biomedicine and other areas of science. The developments of biological systems for synthesizing such metal nanoparticles are quite interesting. In this paper, the fungus *Fusarium oxysporum* MTCC 284 has been tested for the synthesis of metal (silver and gold) nanoparticles. The fungus was exposed separately to aqueous silver nitrate and chloroaurate ions. In both cases, rapid reduction of metal ions was observed resulting in the formation of stable silver and gold nanoparticles of variable sizes and shapes. The sizes of silver nanoparticles were found to be ~ 20-40 nm. Aggregation was observed in the case of gold nanoparticles and the sizes were in the range of 5 – 20 nm with maximum of 60 nm. The crystal structure of the particles were characterized by X-ray diffraction pattern and found that both metal nanoparticles exhibited face centered cubic phase.

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1. Introduction

Nanoscience and nanotechnology are emerging areas of research that focus on (i) the development of synthetic methods and surface analysis tools for building structures and materials at nanoscales (ii) to understand the changes in chemical and physical properties due to miniaturization and (iii) the use of such properties in the development of novel and functional materials and devices [17 a & b]. In recent years, noble metal nanoparticles have been the subject of focused research due to their unique electronic, optical, mechanical, magnetic and chemical properties that are different from those of bulk materials [13]. These special and unique properties could be attributed to their small sizes and large specific surface area. For these reasons metallic nanoparticles have been found many applications in different fields as catalysis, electronics, photonics, etc. The most common method used to prepare metallic nanoparticles is the chemical reduction of their respective salts. However, other techniques such as UV irradiation, lithography, laser ablation, ultrasonic fields, aerosol technologies, and photochemical reduction of gold have also been widely used and investigated [11, 12, 16, 22, 26]. While these methods may successfully produce pure and well–defined nanoparticles, they remain expensive and sometimes involve the use of hazardous chemicals.

Therefore, nowadays researchers are looking for biological systems for producing metal nanoparticles which is inexpensive, biocompatible, non-toxic, safe and environment friendly. It is well known that many unicellular organisms such as bacteria and algae are capable of synthesizing inorganic materials, both intra and extracellularly [25]. Some of the examples include magnetotatic bacteria, which synthesize magnetite nanoparticles, [5], diatoms and radiolarians that synthesize

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siliceous materials [10 a & d] and S-layer bacteria that produce gypsum and calcium carbonate as surface layers [24]. The accumulation of inorganic particles in microbes has been reported by several authors, gold in Precambrian algal blooms [28], gold in algal cells [9], gold in bacteria [2], CdS in yeast [18 a & d]. Recently, the synthesis of nanoparticles of variable morphology using leaves of different plants, sprouts, roots, stems of live alfalfa plants have also been demonstrated [7a,b and 23 a-d].

Various *Fusarium oxysporum* strains were used by Absar Ahmad and his coworkers for the production of various metal nanoparticles [1, 3, 14 and 27] and the mechanisms have been described. In the present work, the fungus *Fusarium oxysporum* [MTCC 284] was used for the production of silver and gold nanoparticles. The possible mechanism for nanoparticles production and its further characterization by UV-visible spectrophotometry, High Resolution Transmission Electron Microscopy (HR-TEM), Fourier Transform Infra-red Spectroscopy (FTIR) and X-Ray Diffraction (XRD) have been discussed. The fungus used in this study is found to be different from various other *Fusarium oxysporum* strains so for used in this field.

2. Materials and methods

2.1 Growth conditions

Fusarium oxysporum MTCC 284 was obtained from Microbial Type Culture Collection (MTCC), Chandigarh. The fungal culture was routinely subcultured in potato dextrose agar (PDA) medium and maintained at 4^{o}C.

2.2 Biosynthesis of silver and gold nanoparticles using the fungus *F. Oxysporum* MTCC 284.

a) Intracellular synthesis of silver and gold nanoparticles:

Production of nanoparticles was done according to the method of Ahamed et al. [1]. The fungus was grown in 100 ml MGYP medium composed of malt extract (0.3%), glucose (1%), yeast extract (0.1%) and peptone at 25-28^{o}C under shaking condition for 3 days. The mycelium was harvested after 72 hrs and separated from the culture broth by filtration. The harvested cells were washed thrice with sterile distilled water. Then, the cells (10 g/100 ml) were suspended in 1 mm silver nitrate ($AgNO_3$) solution or 1 mm gold hypochlorate ($HAuCl_4$) solution and incubated in shaker (140 rpm) for 3 days at 30^{o}C. The mycelial suspension in the flask was monitored for any visible changes.

b) Extracellular production of Silver and Gold nanoparticles:

For extracellular productions, the fungal biomass was suspended at (1 g/ 10 ml) in sterile Milli Q water and incubated for 3 days. Then, the supernatant (fungal filtrate) was separated from the cells by centrifugation at 6000 rpm for 10 min. Equal volume of supernatant was mixed with equal amount of 1.0 mm of the metal solutions ($AgNO_3$ and $HAuCl_4$) and incubated for 3 days at 30^{o}C.

2.3 Characterization

2.3.1 UV-Vis Spectra Analysis

Bioreduction of silver and gold ions in solution was monitored by the collection of aliquots of the suspensions after 72 hr of incubation and subjecting them to UV-Visible spectrophotometric analysis. The optical characteristics were studied by scanning the sample from 200–800 nm in an UV-Visible spectrophotometer (ELICO SL-159).

2.3.2 HR-TEM Characterization of silver and gold nanoparticles

The fungal biomass after reaction settled spontaneously at the bottom of the flask within 30 min. The suspension (1 ml) above the biomass was collected and centrifuged at 6000 rpm for 10 min. The clear supernatant was discarded and the pellet was redispersed in 500 µl of sterile Milli Q water. This suspension was used for microscopic study. All HR-TEM images were recorded with a UHR polepiece transmission electron microscope JEOL 3010 (Jeol Ltd., Tokyo, Japan) operating at 200 kV with a point to point resolution of 0.12 nm which gives a lattice resolution of 0.14 nm. Samples were prepared by placing a drop of the aqueous nanoparticles solution on a 3-mm copper grid covered with carbon film. Images of the nanoparticles were recorded at different magnifications.

2.3.3 XRD Measurement

XRD analysis of the intracellular silver and gold nanoparticles was carried out according to the published protocol [6]. After 48 hr of reaction, the fungal biomass was collected by centrifugation (6000 rpm) for 10 min, dried in a hot air oven at 60° C overnight. Approximately 1.0 g of finely powdered sample was weighed and XRD analysis was performed on a PAN analytical - X'pert PRO and the measurements were carried out at 40 kV and 30 mA in CuK radiation.

2.3.4 Fourier Transformed Infrared Spectroscopy

The samples for FTIR spectrum analysis were prepared as described above in HR-TEM analysis. Then the suspension was mixed/triturated with 300 mg of KBr pellet and the analysis was done on FTIR, Schimadzu, Japan, at a resolution of $1cm^{-1}$.

3. Results and discussion

3.1 Visual Observation

Silver and gold nanoparticles were synthesized using the fungus *F. oxysporum* 284 according to the method described elsewhere. Fig. 1A shows the *F. oxysporum* biomass before and after treatment with 1.0 mM silver nitrate solution for the period of 72 hr. The change in color from white to brown indicates the formation of silver nanoparticles. Silver nanoparticles formation was also observed extracellularly (Fig. 1B) which shows that the Ag^+ ion reduction does take place extracellularly, possibly through the release of reducing agents by the fungus into solution.

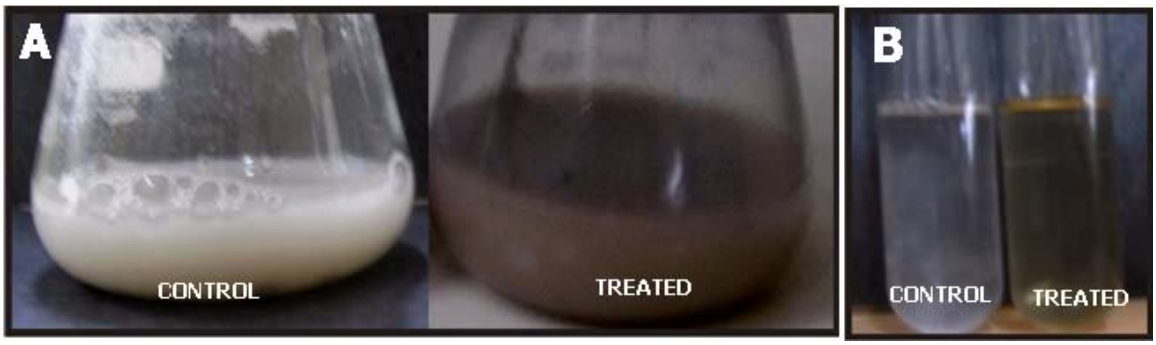

Fig 1A F. oxysporum 284 biomass before (control) and after treatment (treated) with 1mM silver nitrate solution for the period of 72 hrs. Fig 1B Extracellular production of silver nanoparticles.

In the case of fungal mycelial treatment with $HAuCl_4$, characteristic pink colour was observed for gold nanoparticles which indicated that the fungus reduces the gold hypochlorite to gold nanoparticles (Fig. 2). Respective color formations (from colorless to brown for silver and pink for gold nanoparticles) were due to the surface plasmon vibrations in the nanoparticles [8, 20, 21].

Fig 2 F. oxysporum 284 biomass before (control) and after treatment (treated) with 1mM gold hypochlorate solution for the period of 72 hrs.

3.2 UV-Visible Spectrometry:

The UV-Vis spectra recorded after 72 hr of reaction of *Fusarium oxysporum* 284 with silver nitrate/gold hypochlorite was represented in Figs.3 and 4. The strong surface plasmon resonance centered at ca.410-415 nm was observed for silver nanoparticles and at 540 nm for gold nanoparticles (Fig 3). Silver nanoparticles synthesized extracellularly had an absorption peak of 400-410 nm and the intensity of the peak was found increasing as the progress of the reaction continues which explains an increase in the number of particles. An absorption band at 270 nm was observed which indicated the presence of tryptophan and tyrosine residues in proteins (Fig 4). The result obtained is similar to the observations made by Duran et al., (2005) [3] where *F. oxysporum* 07SD strain has been used for synthesizing silver nanoparticles by intra- and extracellular methods. It was found that the surface plasmon resonance centered between 415 nm and 420 nm.

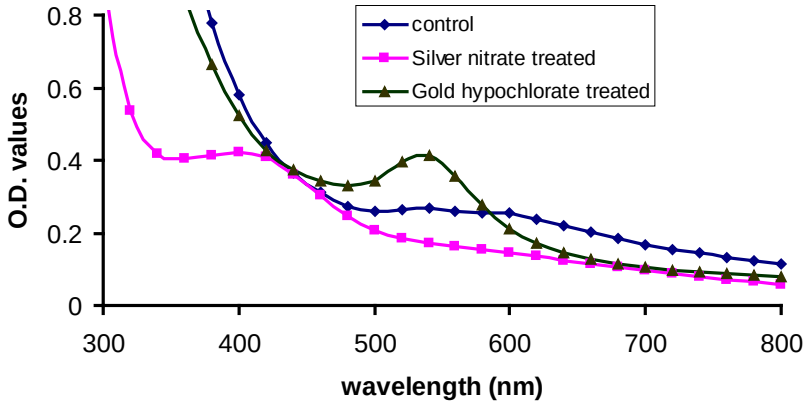

Fig 3 UV-visible spectra recorded after 72 hrs of reaction of an aqueous solution of 1mM silver nitrate and gold hypo chlorate with the fungal biomass.

Generally in fungi, hydrogenases and nitrate reductases are involved in reducing the metal ions. In addition to enzymes, several napthoquinones and anthraquinones with excellent redox properties were reported in *F. oxysporum*, which could act as electron shuttle in metal reduction [3]. There are recent reports on the reduction of aqueous chlorate ions extracellularly using the fungus *F. oxysporum*, to generate extremely stable gold nanoparticles in water [14]. However, we observed that $HAuCl_4$ reduction occurs only by intracellularly and not by extracellularly in *F .oxysporum* 284.

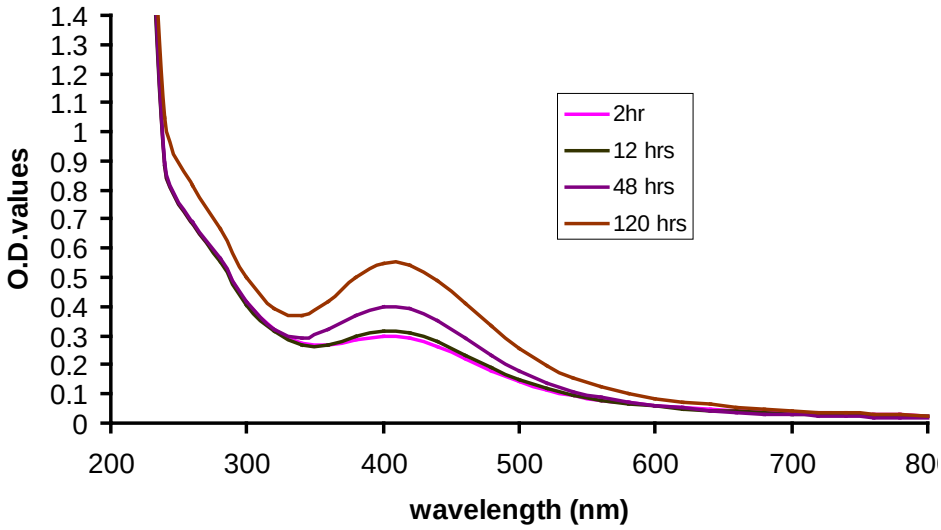

Fig 4 UV-visible spectra recorded as a function of time of reaction of an aqueous solution of 1mM silver nitrate with the fungal filtrate.

3.3 Structural Characterization:-

The structural features of silver and gold nanoparticles synthesized using *F. oxysporum* 284 are clearly visible in HR-TEM images. The silver nanoparticles are round in shape with sizes ranging between 20 and 40 nm, respectively. The gold nanoparticles were found to be spherical and further aggregated into larger irregular structures with no well defined morphologies (Fig. 6 (a) and (b). The sizes of smaller nanoparticles were between 5 nm and 20 nm with a maximum size of 60 nm. Cubo-octahedral shaped particles were also observed (Fig. 6b). Similarly in gold nanoparticles polydispersity and aggregations were observed [14]. Aggregation of gold nanoparticles may be due to the release of more amount of reducing enzyme(s) as compared to the capping protein by *F. oxysporum*. It is due to high toxicity of gold ions to the fungus than the silver ions [19].

Both the silver and gold nanoparticles exhibit contrast suggesting that they are multiply twinned particles (MTPs) (Figs. 5(e) and 6(a). The presence of MTPs has been observed in gold nanoparticles synthesized using Geranium leaf extracts [23c]. Stacking fault was observed in silver and gold nanoparticles (Figs. 5(d) and 6(d)). Such stacking fault has been observed by Zhong Lin Wang [30] in Au nanocrystals. According to him, it is a typical planar defect and usually produced by a distortion on the stacking sequence of atom planes. If an atom sequence in (111) plane is changed from A-B-C-A-B-C-A-B-C to A-B-C-A-B-A-B-C, it results in stacking fault. It is also created probably due the high strain energy in the volume.

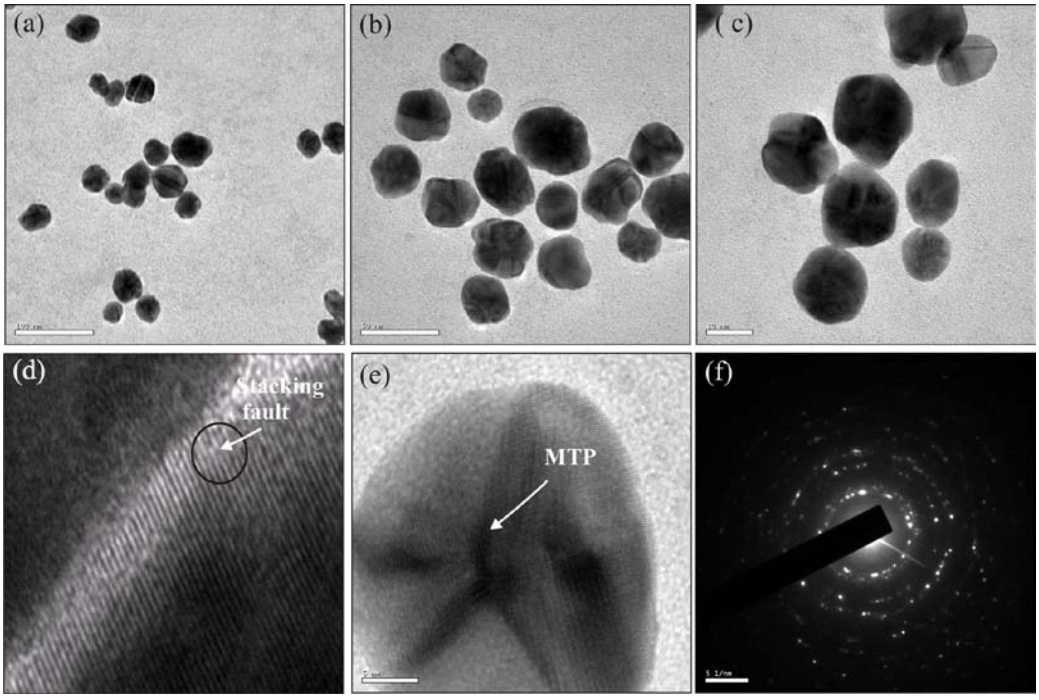

Fig 5 TEM image of silver nanoparticles synthesized by Fusarium oxysporum 284.

An edge dislocation is clearly observed in TEM image of gold nanoparticles which is indicated by a circle (Fig. 6(e)). Such defects are commonly seen at interfaces to accommodate the accumulated strain created by lattice mismatches.

3.4 FT-IR Spectrum analysis:-

The silver and gold nanoparticles synthesized using *F. oxysporum* 284 were subjected to FT-IR analysis to find out the bioactive compounds synthesized by the fungus and associated with the nanoparticles. A number of bands were observed in the region 350 – 3650 cm^{-1} centered at 1641, 2089, 3399 cm^{-1} in the case of gold nanoparticles whereas 1641, 2090, 3431 cm^{-1} peaks were observed in the case of silver nanoparticles (Fig. 7).

A strong sharp band centered at 1641 cm^{-1} represents amide I band and arise due to carbonyl stretch vibrations in the amide linkage of proteins secreted by the fungus [1]. This shows that the proteins can bind to the nanoparticles either through free amino groups or cysteine residues in the proteins and in turn confer stability to the nanoparticles by surrounding on its surface.

A weak broad band at 3431 cm^{-1} may represent the characteristic free hydroxyl group of any quinone compounds secreted by this fungus. Baker and Tatum [29] in 1997 isolated two novel anthraquinones (2-(1-Hydroxyethyl) -3,8-dihydroxy-6–methoxy or 3--(1-hydroxyethyl)-2,8-dihydroxy-6-methoxy anthraquinone) from stationary cultures of *F. oxysporum*, isolated from the diseased roots of blight-affected orange trees **(***Citrus sinensis* Osb.). The IR study of those anthraquinones showed an absorption band at 3420 cm^{-1}. This shows that the fungus used in this study may produce quinone like compounds where it could act as an electron shuttle to reduce the metal ions. [15].

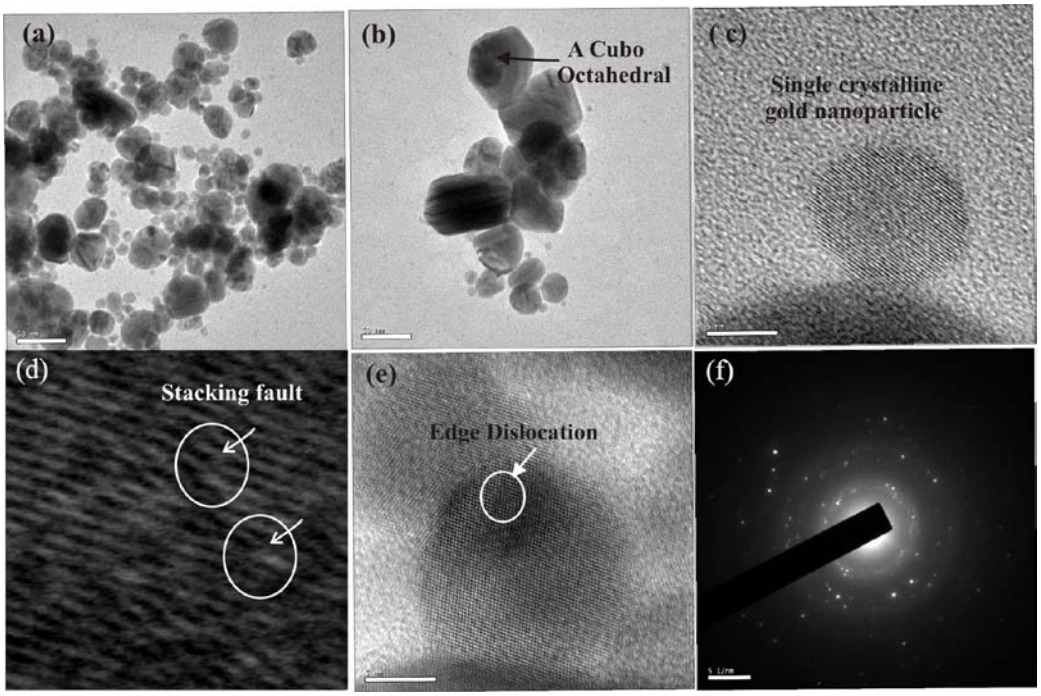

Fig 6 TEM image of gold nanoparticles.

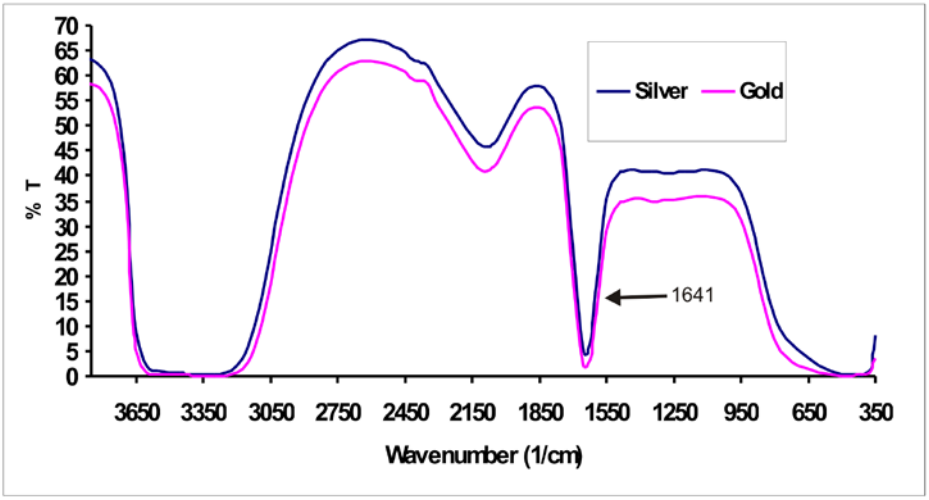

Fig 7 FT-IR of silver nanoparticles and gold nanoparticles synthesized using F. oxysporum 284 biomass

3.5 XRD Analysis

Fig. 8 shows the powder XRD pattern of silver and gold nanoparticles, respectively. The peak position at 37.8 in red peak pattern represents presence of silver and the value is consistent with the reflection lines of face – centered cubic silver (JCPDF 89-3722 and 04-0783). Similarly face centered cubic gold was observed in the XRD pattern of gold nanoparticles (blue). (JCPDF 65-2870 and 04-0784). The XRD patterns clearly show that both the nanoparticles are crystalline in nature.

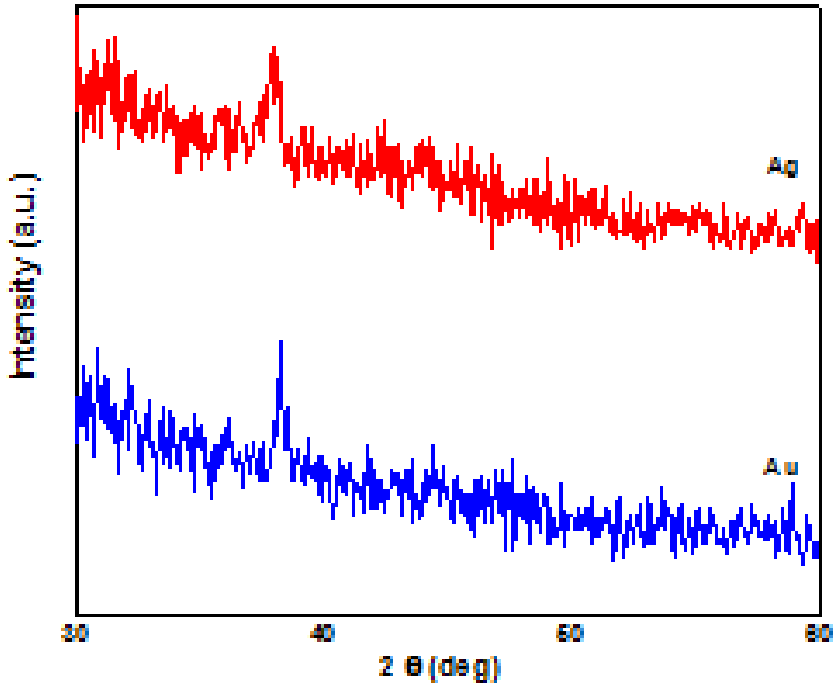

Fig. 8 XRD pattern of silver nanoparticles (red) and gold nanoparticles (blue).

4. Conclusion

We have shown that the fungus *F. oxysporum* 284 when incubated with silver and gold ions results in the formation of particles of nanoscale dimensions. From the TEM results, it was clear that the fungus produced polydisperse metal nanoparticles with multi-shapes. The difference in mechanism of reduction by this fungus is an additional advantage. It is shown that there is a possibility of production of quinone compounds by this fungus which might also involve in the reduction of metal ions [3]. This is greener approach of synthesis of nanoparticles using this fungus. We are now focusing on the production of monodisperse nanoparticles, which could find applications in disease diagnosis and drug delivery.

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