Charecterization of bacterial culture on ZnO and Pb (NO3)2 nanopasrticles

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Abstract

The most abundant organisms in our biosphere are bacteria as Escherichia coli. Slight climate changes can potentially be disastrous to the life processes of bacteria; this can result in the prolific advantage for the production of nanoparticles. On the other hand synthesis of metal nanoparticles by eukaryotic cells such as fungi Aspergillus niger is reported. A. niger have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate. Mycelia provide a much higher surface area than bacteria and this area could be used to support the interaction of metal ions and fungal reducing agent thus enhancing the reduction of metal nanoparticles. The bio reduction of NPs was monitored by ultraviolet-visible spectroscopy, and the nanoparticles obtained were characterized by electron microscopy. In bacterial culture ZnO and Pb(NO3)2 NPs have sharp absorbance with the highest peak at 300nm and 250nm respectively. On the other hand, in fungal culture ZnO and Pb(NO3)2 NPs have highest absorbance peak at 230nm and 240nm respectively. The synthesized NPs (fungal biomass) were almost spherical in shape and some of them were aggregated ranging in size from 30-70nm and 10-50nm stabilized in the solution. Furthermore, the antimicrobial potential of zinc and lead nanoparticles was systematically evaluated. The synthesized nanoparticles could efficiently inhibit various pathogenic organisms, P. aeruginosa and S. aureus. The bactericidal effect of zinc and lead nanoparticles were compared based on diameter of inhibition zone in agar diffusion assay, disc method tests and minimum inhibitory concentration (MIC).

Keywords: Atomic force microscopy, Powder X-ray diffraction, ultraviolet-visible spectroscopy, Nanoparticles using fungus, Nanoparticles using bacteria, Optical density.

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Introduction

Nano materials characterization is done through utilizing a variety of various techniques such as AFM (atomic force microscopy), electron microscopy of TEM and SEM, DLS (dynamic light scattering), XRD (powder X-ray diffraction), FTIR (Fourier transform infrared spectroscopy), Ultraviolet-visible spectroscopy, dual polarization mainly drawn through materials science. Two Common techniques of Characterization are Optical characterization (ultraviolet-visible spectroscopy) and Microscopic characterization (TEM, SEM) are presented in the study. The overall composition of synthesized particles is studied by conventional techniques: Electron Microscopy (transmission electron microscopy, scanning electron microscope) and Optical microscopy (UV-Visible spectroscopy). UV-Vis spectroscopy is a very useful technique which allows estimation of nanoparticles size, concentration, and aggregation level. Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The average particle diameter, their size distribution, and charge affect the physical stability and the in vivo distribution of the nanoparticles. Electron microscopy techniques are very useful in ascertaining the overall shape of polymeric nanoparticles, which may determine their toxicity.

Optical Microscopy

UV-Visible spectroscopy

For UV-Vis Spectrophotometric Measurements the reaction mixture was subjected. It is a very useful and reliable technique for the primary characterization of synthesized nanoparticles which is also used to monitor the synthesis and stability [1]. According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiations determines when light of certain frequency passed through the samples. This spectrophotometer analyses records the intensity of absorbance or optical density (O.D) as a function of wavelength. Absorption is directly proportional to the concentration of the absorbing species (Beer's law). Metal nanoparticles have unique optical properties which make them strongly interact with specific wavelengths of light. In addition, UV-Vis spectroscopy is fast, easy, simple, sensitive; selective for different types of NPs, needs only a short period time for measurement, and finally a calibration is not required for particle characterization of colloidal suspensions. In metal nanoparticles the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band, occurring due to the collective oscillation of electrons of metal nanoparticles in resonance with the light wave. The absorption of metal nanoparticles depends on the particle size, dielectric medium, and chemical surroundings. Observation of this peak—assigned to a surface Plasmon is well documented for various metal nanoparticles with sizes ranging from 2 to 100 nm [2].

When the dimensions of materials are reduced to nanoscale, they demonstrate unique properties which are far variant from those of their bulk counterparts. For example, their optical and electronic properties change, their chemical properties can be increased or decreased and mechanical / structural stabilities are changed

dramatically. These features make nanoparticles attractive for unique sensing applications, and also at that time cause complications in their characterization processes. Therefore, the challenge exists in finding the actual characterization techniques which have the optimum efficiency for studying the properties of nanomaterials generated by these techniques. An increased number of techniques can be employed for nanoparticle characterization. In addition, applicability of these techniques for investigating different types of nanomaterials and their relevance to sensor technology has also been described. Nanoparticle characterization is of extreme significance to establish control and understanding of nanoparticle production and applications.

The reduction of nanoparticles was monitored through measuring the UV-VIS spectrum. Ultraviolet-visible spectroscopy is a highly useful technique to study metals, semiconductors, and insulators in bulk, colloid, thin film and nano structures forms. UV–Visible spectroscopy is generally suitable for materials which are soluble in liquids. Also, UV–Visible diffuse reflectance spectroscopy (UV–Vis DRS) measurement is used for recording the spectrum of material; which is insoluble in liquid.

In the present study UV–Visible spectroscopy was used for monitoring the reduction of synthesized nanoparticles. The reaction mixture was subjected to UV-Vis Spectrophotometric Measurements. Ahmad et al 2003, According to this technique many molecules absorb visible or ultraviolet light. The percentage of transmittance light radiations determines when certain frequency light passed through the samples. This spectrophotometer analyses records the intensity of absorbance or optical density (O.D) as a wavelength function. Absorption is directly proportional to the absorbing species concentration (Beer's law). Measuring the particles reduction in the reaction medium after the time interval of 24hr and the light absorbance was recorded in UV-VIS spectrophotometer [3].

The preliminary characterization of the synthesized Pb and Zn nanoparticles was carried out through the use of Merck double beam UV-Visible spectrophotometer (UV-Vis, Systronics-2201). The absorbance spectra were recorded from 200-500 nm. To study the evolution of NPs, optical absorbance spectra were measured at regular intervals until constant optical density was reached.

For monitoring the reduction of nanoparticles through bacteria, optical density measurements from each test tube were taken after 24 hrs to record the bacterial growth from inoculation through late exponential phase using a spectrophotometer set at 550 nm. The bacterial cells growth rate interacting with the respective nanoparticles was determined through a plot of the log of optical density versus wavelength. For monitoring the reduction of nanoparticles through fungus, optical density measurements from each flask were taken every 24 hrs through late exponential phase using a spectrophotometer set at 260 nm. The fungal cells growth rate interacting with the respective nanoparticles was determined through a plot of the log of optical density versus wavelength.

UV-Vis Study

According to this study many molecules have ability to absorb ultraviolet / visible light. The percentage of transmittance light radiation determines when certain frequency's light passed through the samples. This spectrophotometer analysis records the intensity of absorption (A) or optical density (O.D) as a wavelength' function. Absorbance is directly proportional to the L (path length) and the c (concentration) of the absorbing species. Beer's Law states that:

$$A = € C L$$

Where € is a constant of proportionality, called the absorbtivity coefficient.

In the present study, two different salts (Pb(NO3)2 and ZnO) were used for the production of nanoparticles through the bacterial and fungal medium. The produced nanostructured materials were characterized through UV-Vis spectroscopy.

UV–Visible spectroscopy of synthesized nanoparticles using fungus (Aspergillus niger)

The reduction of Pb(NO3)2 and ZnO nanoparticles during exposure to fungus Aspergillus niger was observed through the result of color change from pale yellow to light brown. The brown coloration was a spectroscopic signature for the production of Pb(NO3)2 and ZnO NPs.

The phenomenon of Surface Plasmon Resonance is responsible for color changing. The metal oxide nanoparticles have free electrons that give the SPR (Surface Plasmon Resonance) absorption band, because of the combined vibration of electrons of metal oxide nanoparticles in resonance with a light wave. The optical property of Pb(NO3)2 and ZnO NPs was determined by UV-Visible spectrophotometer Figure 1. Once lead nitrate added to the fungus extract, the synthesized nanoparticle solution was scanned through Merck double beam UV-Visible spectrophotometer from 200-300 nm. The shape, size and distribution of nanomaterial affect the magnitude, peak and width of the spectrum band. In course of formation, as the colour of the extract changed, its absorbance was considerably recorded after 24 h, 48 h, 72 h and 96 h at regular time intervals. The Pb NPs has sharp absorbance with the highest peak at 240 nm which confirms lead in nanoscale range and after remains constant while nanometer increased. The characteristic of lead nitrate nanoparticles was observed in the supernatant solutions of fungal strains indicating the synthesis of lead nitrate nanoparticles. Figure shows the UV-visible graph of supernatant solutions of fungal strain Pb(NO3)2 respectively .

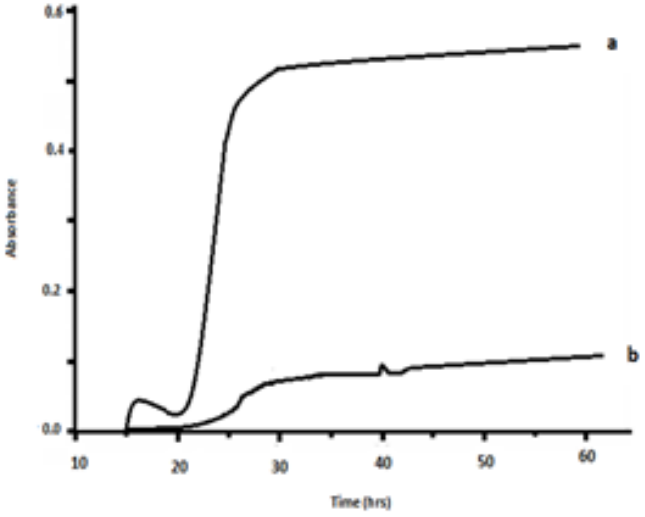

Figure 1. *Growth curve of fungus (A. Niger) in PDB (control).*

Once zinc oxide added to the fungal extract, the synthesized

nanoparticle solution was scanned through Merck double beam UV-Visible spectrophotometer from 200-300 nm. The shape, size distribution of nanomaterial affects the magnitude, peak and width of the spectrum band. In course of formation, as the colour of the content changed, its absorbance was considerably recorded after 24 h, 48 h, 72 h and 96 h at regular time intervals. The Zn NPs has sharp absorbance with the highest peak at 230nm which confirms zinc in nanoscale range and progressively decreased while nanometer increased. The characteristic of ZnO nanoparticles was observed in the supernatant solutions of fungal strains indicating the formation of ZnO nanoparticles. Figure shows the UV-visible graph of supernatant solutions of fungal strain ZnO respectively .

UV–Visible spectroscopy of synthesized nanoparticles using bacteria E.Coli

The reduction of Pb(NO3)2 and ZnO nanoparticles during exposure to bacteria E.Coli (DH5a) was observed as color change obtained from yellow to brown. The brown coloration is a spectroscopic signature for the production of Pb(NO3)2 and ZnO NPs. The phenomenon of Surface Plasmon Resonance is responsible for color changing. The optical property of synthesized Pb(NO3)2 and ZnO NPs was determined by UV-Visible spectrophotometer .

Once lead nitrate added to the bacterial culture, the synthesized nanoparticle solution was scanned from 200-500 nm using double beam UV-Visible spectrophotometer (Merck). The Pb NPs has sharp absorbance with the highest peak at 250 nm and progressively decreased while nanometer increased, characteristic of lead nitrate nanoparticles was observed in the supernatant solutions of bacterial strains indicating the synthesis of lead nitrate nanoparticles. Figure shows the UV-visible graph of supernatant solutions of fungal strain Pb(NO3)2 respectively .

Once zinc oxide added to the bacterial culture, the synthesized nanoparticle solution was scanned from 200-500 nm through double beam UV-Visible spectrophotometer (Merck). The ZnO NPs has sharp absorbance with the highest peak at 300 nm and slowly decreased while nanometer increased, characteristic of ZnO nanoparticles was observed in the supernatant solutions of bacterial strains indicating the synthesis of ZnO NPs. Figure shows the UV-visible graph of supernatant solutions of fungal strain ZnO respectively .

For this analysis, two different metal salts challenged with Escherichia coli and Aspergillus niger were characterized through UV–Visible spectroscopy for nanoparticles confirmation. The viable metal oxide nanoparticles synthesized through fungus showed better reduction result then bacteria, recorded from optical density after 24 h, 48 h, 72 h, 96 h at regular time intervals are shown in figures 7 and 8. As shown in Figure the ZnO and Pb(NO3)2 NPs synthesized through Aspergillus niger has sharp absorbance with the highest peak around 230nm which confirms zinc and lead in nanoscale range and progressively decreased while nanometer increased. The metal oxide nanoparticles' characteristic was clearly observed in the supernatant solutions of fungal strains indicating the synthesis of metal nanoparticles. There was a greater than 99% reduction in the growth of Aspergillus niger then bacteria during 24 hour of incubation. During a positive control terms, the obtained results agree with the results of flask shake methods.

Pantidos et al., 2014, Aspergillus niger are relatively cheap to cultivate and have a high rate of growth compared to other biological systems. The ease of manipulation of fungus gives them advantages over bacteria (E. coli.), as the chassis of choice in the order of near-term bio-production of nanomaterials that require optimised formation through genetic engineering. Aspergillus niger have the advantage of producing very high yields of secreted proteins, which may increase nanoparticle synthesis rate. Many fungi contain mycelia which provide an extremely higher surface area compared to bacteria, this obtained area could be highly useful to support the metal salt interaction (ZnO and Pb(NO3)2) with fungal (Aspergillus niger) reducing agent thus this way will be enhanced the conversion of ions into metallic nanoparticles.

For further analysis used metal nanoparticles synthesized through Aspergillus niger, also have the advantage of ease of downstream processing when extracellular nanoparticles are produced, allowing for a more efficient way of extracting nanoparticles from them. Scalability, another factor for consideration in case of commercial production of nanoparticles, gives fungi the edge as the chassis of choice in order of long term development as they can be used more easily in large-scale reactors than bacteria [4].

Electron Microscopy

Transmission and Scanning electron microscopy (TEM and SEM), respectively, provide a way to observe nanoparticles directly, with the former method being better for morphological examination. Electron microscopy has a smaller size limit of detection, is a good validation of other methods, and affords structural information via electron diffraction, but staining is usually required, and one must be cognizant of the statistically small size and the effect that vacuum can have on the particles.

Transmission electron microscope (TEM)

Transmission electron microscopy (TEM) is another useful tool with high resolution for the structural and morphological analysis. It is ideally suitable for investigating nanomaterials as very high resolution is possible. It is similar to the optical microscopy, except electromagnetic radiations as well as optical lenses; electromagnets are applied to focus an electron beam on the specimen [5]. Electron microscopes have much greater resolving power than optical microscopes, and can obtain much higher magnifications up to 0.1 nm.

TEM is unique for providing a real space image on the atomic distribution in the nanocrystal and on its surface also. TEM also gives information about atomic resolution lattice images as well as chemical information at a spatial resolution of fem nanometers. Conventional TEM uses only the transmitted beams or some of the forward scattered beams to create a diffraction contrast image while high resolution transmission electron microscopy (HRTEM) uses the transmitted and the scattered beams to create an interference image.

At the top of the microscope an "Electron source" is attached; which emits the electrons that travel through vacuum in the column of the microscope. By propelling electrons at a thin sample, and detecting those transmitted through it, one is able to obtain a map of the local densities of the sample, as well as diffraction information when there are ordered structures such as crystals involved. An image is magnified and focused onto an imaging device, such as fluorescent screen or to be detected by a sensor such as a CCD camera. At lower magnification, TEM image contrast is because of the absorption of electrons by the material. Alternative modes of TEM are allowed to observe modulation in chemical identity, electronic structure,

and crystal orientation. In TEM, the set of magnification lenses can serve to magnify the image of planes. There are three image modes: low–magnification mode, high–magnification mode, and selected area diffraction mode, corresponding to intermediate magnification. The diffraction modes include selected area electron diffraction (SAED) and convergent beam electron diffraction (CBED). Electron diffraction is used to identify a structure known ahead of time through X–ray diffraction (SAED diffraction, nanodiffraction, and microdiffraction) or to determine the crystal lattice and the symmetry class (microdiffraction) or the symmetry class and the point group (CBED) [6]. In the TEM only thin samples, which allow a fraction of the incident electron beam to go through the sample can be studied. When an accelerated beam of electrons impinges upon a sample, a rich variety of interactions takes place. The specimen for TEM analysis has to be extremely thin (0.1 to 10μm) for the highly absorbable electrons to penetrate the solid and form an image. The sample preparation is an essential part of microscopy and there are many techniques (and variations) that can be used. The most commonly used methods for final thinning includes ion milling, reactive ion technique, chemical polishing, electro polishing, tripod polishing, ultramicrotomy, etc. Materials that have dimensions small enough to be electron transparent, such as powders can be quickly prepared by the deposition of a dilute sample containing the specimen onto support grids or film. Powder samples can be ultrasonicated to well disperse the particles. TEM image of the samples were recorded on a Tecnai F30 field emission transmission electron microscope operating at 300 kV.

Scanning electron microscope (SEM)

The scanning electron microscope uses a beam of high-energy electrons to produce a variety of signals at the surface of specimens used. The signals show information about the sample including chemical composition, and crystalline structure, external morphology (texture) and orientation of materials which make up the sample.

SEM analysis is normally considered to be non-destructive because the X-rays generated do not lead to loss of volume of the sample, so it becomes possible to repeatedly analyze the same materials [7]. A scanning electron microscope is a kind of electron microscope which images a sample by scanning it using a high-energy electron beam. The electrons then interact with the atoms making up the sample, thus producing signals which reveal information about the sample's composition, surface topography and other properties such as electrical conductivity.

Various types of signals produced by a SEM include back-scattered electrons (BSE), secondary electrons, characteristic X-rays, specimen current, light (cathodoluminescence) and transmitted electrons. Back-scattered electrons (BSE) are the electrons which are rejected by elastic scattering from the sample. Because the intensity of the BSE signal is related to the atomic number of the specimen, BSE images can provide information about the different elements distribution in the sample very accurately. Characteristic X-rays are released when the electron beam removes an electron from the inner shell of the sample, thus causing a higher energy electron to occupy the shell and hence release energy in the form of X-rays. These characteristic X-rays are in turn used to find out the composition of the material and also measure the presence of elements in the sample as well as the level of impurities. Magnification in a scanning electron microscope technique can be controlled over a range of about 6 orders of magnitude from approximately 10 to 500,000 times. Assuming that the display screen has a fixed size, higher magnification is obtained by reducing the raster size of the specimen, and vice versa. Magnification is hence controlled by the voltage supplied to the x, y detector plates or the current supplied to the scanning coils and not by objective lens power.

TEM (Transmission electron microscopy) is another useful tool with high resolution for the structural and morphological analysis. It is ideally suitable for investigating nanomaterials as very high resolution is possible. TEM is unique for providing a real space image on atomic distribution in the nanocrystal and also on its surface. TEM also gives information about atomic resolution lattice images as well as chemical information at fem nanometers spatial resolution.

In case of Transmission electron microscopy only thin samples that allow an incident electron beam fraction to pass through the sample / object can be studied. When an accelerated electron beam impinges upon a sample, a rich variety of interactions occurs. The specimen for TEM analysis has to be highly thin (0.1 to 10μm) for the greatly absorbable electrons to pass through the solid and produce an image. The preparation of sample is an important part of microscopy and there are many techniques (and variations) that can be used. The most generally used methods for final thinning includes ion milling, reactive ion technique, chemical polishing, electro polishing, etc. [8]. Materials which have dimensions short enough to become electron transparent like as powders that can be quickly produced through the dilute sample deposition containing the specimen upon support film or grids.

Transmission Electron Microscopy used in this study was the instrument of JEOL JSM 1oocx. TEM shows the crystal structure and shape (if any) and also the size of the samples / particles. The grid for TEM analysis was prepared through fixing a drop of nanoparticles suspension containing Pb and Zn nanoparticles onto a copper grid which was carbon-coated and allowing the water evaporation inside vacuum dryer. Grid containing respective nanoparticles was scanned through a Transmission Electron Microscope.

SEM (Scanning electron microscopy) is a powerful technique for studying surface morphology of almost any material with a resolution down to about 3 nm. The image resolution given by Scanning electron microscopy depends not merely on the characteristics of electron probe, however also on to the interaction of electron probe with specimen. SEM uses a high-energy electrons beam to produce a signal variety at the surface of used specimens. The signals represent information about the particle including crystalline structure, chemical composition, external morphology which shows the texture and also orientation of materials that make up the particle / sample. The interaction of an incident electron beam with specimen produces secondary electron, with energies typically smaller than 50eV. The emission efficiency of the secondary electrons is directly related to surface geometry, surface chemical characteristics and chemical composition of a material. SEM analysis is generally considered to be a non-destructive due to the X-rays produced does not conduct to loss of the sample volume, so it becomes potential to analyze repeatedly the same materials. The X–ray radiation can be detected in a technique called energy dispersive X–ray spectroscopy (EDAX or EDS) that can be useful to identify specific elements. The SEM also offers the locations of selected point on the sample; which is applicable in qualitative or semi–quantitative identification of chemical compositions, crystal orientations and crystalline structure.

The morphology of the sample surface was examined by using the SEM (scanning electron microscope) images recorded through the use of scanning electron microscope (Model: FEI. Quanta, 250, The Netherlands), operated at 30 kV, and equipped with an energy dispersive X–ray spectrometry (EDS) system. Use of EDS was to determine elemental composition of the metal–doped Pb(NO3)2 and ZnO nanoparticles.Transmission electron microscope gives the direct imaging such as crystallographic structure and lattice imperfection of various types of materials on atomic scale. The image Figure 9 Shows that ZnO nanoparticle (synthesized through fungus A. niger) are nearly round or oval in shape. The particles size is in 30-70 nm range and the mean size of the NPs was obtained to be 59.60 nm. The image Figure 9 shows that Pb(NO3)2 nanoparticles (synthesized by fungus A. niger) are nearly spherical and rod in shape. The particles size is in 10-50 nm range. The size estimation of synthesized nanoparticles is characterized through transmission electron microscope Jeol Jsm 1oocx instrument (Jeol Ltd., 1400, Tokyo Japan). Thus all characterization results confirm the formation of ZnO (Zinc oxide) and Pb(NO3)2 (lead nitrate) nanoparticles.

SEM is used to analyze the morphology including shape, size as well as size distribution of materials. The synthesized nanoparticle with fungus (Aspergillus niger) is seen in SEM image was analysed and shown in Figure 10 by using scanning electron microscope (Model: FEI. Quanta, 250, The Netherlands). SEM analysis clearly shows the existence of the synthesized Zinc oxide (ZnO) and lead nitrate (Pb(NO3)2) nanoparticles. The ZnO NPs of size ranges 40-70 nm and Pb(NO3)2 NPs very small ranging 10-45 nm and some of few are aggregated were visualized as seen in Figure 10. Surface morphology of zinc oxide nanoparticles by SEM showed that zinc oxide nanoparticles adhere to the surface in a scaly pattern. It was observed that smaller sized particles were almost spherical in shape and some of those were aggregated.

Conclusion

Use of microbes for the production of metal oxide nanoparticles is a reliable and with eco-friendly protocol. The characterization of metal ions (lead nitrate and zinc oxide) exposed to microbial strain and the reduction of these metal ions to respective nanomaterials was confirmed by UV-Vis Spectrophotometer. After the addition of lead nitrate and zinc oxide to the bacterial culture, the synthesized nanoparticle solution was scanned through UV-Visible spectrophotometer. The ZnO NPs has sharp absorbance with the highest peak at 300nm and Pb(NO3)2 NPs has sharp absorbance with the highest peak at 250nm respectively.

Similarly, after the addition of zinc oxide and lead nitrate to the fungal culture, the synthesized nanoparticle solution was scanned UV-Visible spectrophotometer. The ZnO NPs has sharp absorbance with the highest peak at 230nm and Pb(NO3)2 NPs has sharp absorbance with the highest peak at 240nm respectively. The characteristic of metal oxide nanoparticles was more clearly observed in the supernatant solutions of fungal strains indicating the synthesis of NPs. It was observed that a greater reduction of metal oxide nanoparticles in the growth medium of fungus A. niger then bacteria E. coli during the 24 hour incubation, decrease in the intensity of radiation directly proportional to the concentration of the solution. A. niger have the advantage of producing very high yields of secreted proteins as well as an increased surface area, which may increase nanoparticle synthesis rate. Thus compared to bacteria, A. niger have significantly increase the productivity of biosynthetic approach of metal oxide nanoparticles.

Characterization of synthesized nanoparticles has been carried out through Uv-vis, TEM and SEM measurements. Zinc oxide and lead nitrate nanoparticles (synthesized through fungal growth medium) have particle size range at 30-70nm and 10-50nm, nearly round or spherical in shape. It is further added that the mechanism of metal oxide nanoparticle production through microbes is not clearly explored. However, in the present study it has been taken to understand the possible mechanism of metal and microbes interaction which may be due to structural specificity of the cell of microbes and how metal availability influences microbial resistance. Surface morphology of ZnO nanoparticles by SEM showed that zinc oxide nanoparticles adhere to the surface in a scaly pattern. It was also found that smaller sized particles were almost spherical in shape and some of them were aggregated.

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