In this chapter, the work presented in chapter 3, 4, 5, 6 & 7 is summarized. At the end, suggestions for future work are given.

8.1 SUMMARY

The green synthesis of Gold Nanoparticles was studied for six different mold strains. Six microbial strains were acquired from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. Mold Species *Aspergillus flavus* NCIM650**,** *Phoma exigua* NCIM1237*, Aspergillus niger* NCIM 616*, Aspergillus niger* NCIM 1025 **,** *Trichoderma reesei* NCIM 1186 and *Trichoderma reesei* NCIM 992 were included. Mold biosynthesis of nanoparticles was found to be a reliable phenomenon. In almost every growth plots of these molds is showing optimum growth of 70 hours.

Mold biosynthesis of nanoparticles was found to be a reliable phenomenon. The role of the electro-chemical behavior of the mold strains in Gold Nanoparticle formation was taken in account. This phenomenon can prove to be useful in the screening of potential candidates for microbial reduction of metal salts. *Aspergellus niger* NCIM 616 and *Aspergillus niger* NCIM 1025 showed better mediator releasing capacities. *Aspergillus fla*vus NCIM 650 and *Phoma exigua* NCIM 1237 also gave satisfactory results but they released mediators under test conditions only. Trichoderma reesei NCIM 1186 showed an almost absence of peak (very little mediator release). During the comparative growth study of two different mold strains i.e. without and with gold chloride solution, highest growth occurs in *Aspergillus niger*. So, *Aspergillus niger* is better among six. Of the two strains *Aspergillus niger NCIM* 616 and *Aspergillus niger NCIM* 1025, *Aspergillus niger* NCIM 616 is better. During the growth study, lowest growth occurs in *Trichoderma reesei*. *Trichoderma reesei* is best among six. Lesser the growth, lesser will be the toxic effect of the salt. Of the two strains

Trichoderma reesei NCIM 1186 and *Trichoderma reesei* NCIM 992, *Trichoderma reesei* NCIM 992 is better.

UV-Visible spectral analysis and Transmission Electron Microscopy confirmed the formation of spherical gold nanoparticles ranging from 10 nm (as in case of *Aspergellus* sp.) to 50 nm (as in case of *Trichoderma reesei* NCIM 1186). Among the six strains, gold nanoparticles fabricated by *Aspergellus niger* NCIM 616 gave quite promising results under optimum conditions. The free radical scavenging property as measured by DPPH assay and hydrogen peroxide assay showed that the mold mediated gold nanoparticles can play the role of effective antioxidants.

The use of *Aspergillus niger* will offer several advantageous since it is considered as a non pathogenic organism and has a fast growing rate. Mold strains having potential for nanoparticles synthesis and have capacity of metallic ion reduction. In my work, I had reported a fast and easy method of production of mono and bimetallic Ag/Au nanoparticles using *Aspergillus niger* as well as *Trichoderma reesei*, which acts as good reducing agents.

Production of nanoparticles had been successfully achieved using *Aspergillus niger.* The change of biomass colour from colour less to dark purple, yellow or maroon within 8 hours of incubation period. It shows that it was intracellular nanoparticle synthesis [Barua *et al.* (2015)].

UV-Vis Spectroscopic studies of blank Czapek Dox revealed no characteristic peak near the 540 nm range indicating no extracellular synthesis (as per no change in media colour) and only intracellular nanoparticle formation.

Cyclic Voltammeter studies of nanoparticles shows that the mold biomass has a good reducing property and the mold biomass as a whole supports the intracellular synthesis. X-ray differaction study showed the gold nanoparticles of size ranging from maximum ~20.2nm to 10.22nm, silver nanoparticles of size ranging from maximum ~30.85nm to 4.97

nm and bimetallic nanoparticles of size ranging from maximum ~39.71nm to 19.27nm were produced during synthesis. From particle size analysis, it was found to be clear that the results of particle size nanaoparticles is same as XRD analysis.

From SEM analysis it was found that the gold nanoparticles had irregular spherical shape and morphology. TEM images clarify that the nanoparticles formed are characterized by uniform distribution with irregular spherical shape.

In the present study, silver and bimetallic (gold-silver) nanoparticles were synthesized using *Trichoderma reesei* NCIM 992. The synthesized nanoparticles were confirmed using SEM - EDS, XRD and their stability was analyzed by FTIR. Production of bimetallic silver and gold nanoparticles were successfully achieved using *Trichoderma reesei* and the change of biomass color from colorless to dark purple within 48 hours of incubation period indicated it to be intracellular bimetallic nanoparticle synthesis. Characteristic peaks near the 420 nm - 540 nm range were revealed by UV-vis Spectroscopy studies. No extracellular synthesis (as per no change in media color) was observed. The stabilization of the nanoparticles by the carbonyl group of amino acid residues and peptides of proteins was confirmed by the FTIR spectroscopy studies.

Nanoparticles have been used in medicine for diagnosis and treatment of various diseases. The usage of nanoparticles is safe to consumer health and environment. Nanoparticles have attracted much attention in treatment of multi-drug resistant bacterial infections and as tropical application in wound healing [Nunez (2009)].

According to the Fold number percentage, the bimetallic nanoparticles showed the maximum synergistic effect in comparison to Gold & Silver nanoparticles. Bimetallic nanoparticles have the maximum capability to enhance the anti-bacterial activities in comparison to others.

8.2 Future Prospects of This Work

The present study explore the potentialities of few mold strains for the reduction of metal salt. Similarly, during scale-up of biological nanoparticle production processes, the initial screening of microorganisms can be done through the electro-chemical analysis and studies on growth kinetics. Also, beneficial bimetallic nanoparticles in combination of two different noble metals can be produced using this approach. Enzymes responsible for Bioreduction can be identified and enzyme immobization techniques can be utilized for better future perspectives. Thus, the biological synthesized metal nanoparticles have exciting possibilities in nano medicine and drug delivery.

The antimicrobial properties of the synthesized bimetallic nanoparticles in combination with antibiotic were found to be promising for treating infections caused by pathogenic bacteria. Though, the mechanism of action of this combinatorial drug is yet not deciphered. Future prospects of this work would involve extensive research on the exact mechanism involved and other relevant clinical applications.