CHAPTER - 6

BIOSORPTION OF SILVER NANOPARTICLES USING *ASPERGILLUS TERREUS* DEAD BIOMASS
6.1. Introduction

Silver and silver based compounds have a wide range of applications in various fields such as electronics, mirrors, photography, food and medicine or textile production and also as catalysts in chemical industries. Use of silver and silver containing products has been escalating in the recent days; however, it poses a greater problem to the environment due to the release of silver ions (Absalan et al., 2007; Benn et al., 2010; Geranio et al., 2009; Gottschalk and Nowack, 2011; Hagendorfer et al., 2010; Kaegi et al., 2010).

Nowadays, various products that are incorporated with different nanoparticles are increasingly entering into the market and their wide range of applications raises concern over health, safety and environmental protection (Wijnhoven et al., 2009; Whiteley et al., 2013). The toxicity effects of various forms of silver during speciation may cause (Panyala et al., 2008) significant problems to fish, algae and the usage of silver ions as bactericide (Wise et al., 2010; Navarro et al., 2008; Tappin et al., 2010; Ratte, 1999) possess severe toxicity leading to bio-uptake and reproductive failure in earthworms (Tourinho et al., 2012; Fabrega et al., 2011a; Scown et al., 2010; Marambio-Jones and Hoek, 2010; Fabrega et al., 2011b; Levard et al., 2012). Despite these attempts, there is very limited data available on the fate and behavior of manufactured NPs in the environment (Klaine et al., 2008).

However, from the environmental point of view, silver being a valuable metal, it is necessary to remove and recover it from wastewater sources like photographic processing waste (Pethkar and Paknikar, 2003). Most of the popular traditional methods like chemical absorption, oxidation–reduction and electrolysis were limited due to their technological and economical properties (Chen and Lim, 2002; Pollet et al., 2000; Ajiwe et al., 2000; Adani et al., 2005; Othman et al., 2006).

Different microbial cells have the ability to tolerate various stressful situations in the presence of toxic metals for their survival. The ability of silver tolerating by microbes involves various mechanisms of resisting which includes efflux systems, altering metal solubility and toxicity, altering redox state of metals, extracellular precipitation of metals and the inability of metal transport function (Rouch et al., 1995; Silver, 1996; Beveridge et al., 1997). The various tasks like bioaccumulation, biomineralization, bioleaching,
microbial corrosion, bioremediation and very recently microbial fabrication of metal nanomaterials are based on the interactions of microbes with the metals.

Interestingly, microorganisms have the potential to become an alternative for the uptake and recovery of silver as they have the higher absorption capacity in aqueous environment (Zhang et al., 2007; Merroun et al., 2001). Among the different microorganisms, filamentous fungi possess some distinctive advantages compared to bacteria in tolerating and absorbing silver and silver related compounds. Moreover, the handling and culturing of large scale fungal cultivation is comparatively an easy task and their high tolerance towards the metals ions, extracellular binding capacity as well as intracellular uptake of metal abilities prove as a strong contender in the biosorption process (Dias et al., 2002).

The cell wall components of microorganisms play a major role in the biosorption and accumulation of various metal ions. The study on the mechanism of silver biosorption using dry biomass of *Myxococcus xanthus* (Merroun et al., 2001), *Corynebacterium* sp. (Zhang et al., 2005), *Saccharomyces cerevisiae* (Simmons and Singleton, 1996) showed that binding of silver metals was due to the presence of various intercellular and extracellular cell wall components of the microorganisms. Very few studies have been carried on the filamentous fungi for their metal tolerance ability and biosorption potential. The present chapter deals with the investigations of biosorption potential of *Aspergillus terreus* dead biomass for the removal of AgNPs from the aqueous solution.
6.2. Materials and methods

6.2.1. Strain selection

For the biosorption study of AgNPs from the aqueous solution, the strain, *Aspergillus terreus* BIOS PTK 6 was utilized.

6.2.1.1. Preparation of fungal biosorbent

The test fungus, *Aspergillus terreus* was inoculated in sterile potato dextrose broth and incubated at 27 °C for 4 to 7 days till the mat was grown profusely. The fungal mycelium was washed thoroughly with metal free distilled water to remove the traces of medium components. The mycelial mat was then treated with 0.5 N NaOH in a fresh conical flask and incubated in a water bath at 80 °C for 15 min. At the end of the incubation, the mat was thoroughly washed with metal free distilled water for about 6-7 times. The procedure was followed till the final pH of the content reaches 7 and the mat was dried in hot air oven for 24 h at 50 °C. Further, the dried dead fungal mat was cooled, powdered using mortar and pestle and then stored in air tight container until further use (Khambhaty *et al.*, 2009).

6.2.2. Determination of silver nanoparticles concentration

The concentration of AgNPs in the aqueous solution at the end of each experiment was measured using ICP-AES. Briefly, at the end of each experiment, the solution was centrifuged at 8000 rpm for 5 min and the supernatant was analyzed for the residual AgNPs concentration using ICP-AES. All tests were carried out in triplicates and a blank sample, replacing the biosorbent with deionized water, was also measured (Li *et al.*, 2011).

6.2.3. Determination of biosorption percentage (Salvadori *et al.*, 2014)

The biosorption efficiency ($R$) of AgNPs removal was determined using the following equation,

$$R = \frac{(C_i - C_e)}{C_i} \times 100$$

Where,

- $C_i$ is the initial metal concentration
- $C_e$ is the equilibrium metal concentration
The metal uptake capacity of the biosorbent \((q_e)\) was calculated using the following equation,

\[
q_e = \frac{V \times (C_i - C_e)}{M}
\]

Where,

\(q_e\) (mg g\(^{-1}\)) is the biosorption capacity of the biosorbent at any time

\(M\) (g) is the biomass dose used

\(V\) (L) is the volume of the test solution

6.2.4. Metal biosorption experiments (Das et al., 2010)

The biosorption process was influenced to a great extent by various parameters such as pH, temperature, incubation time, agitation speed and initial biomass concentration, etc. These conditions vary depending upon the biomass and also the metal ions. The parameters \(viz\) pH, temperature, incubation time, initial biomass concentration and agitation rate were optimized for the effective biosorption of AgNPs and the optimized parameters were appropriately incorporated in the subsequent experiments. The parameters used for the optimization studies are as given below.

a) pH
b) Temperature
c) Incubation time
d) Biomass concentration
e) Agitation rate

6.2.4.1. Effect of pH on biosorption

To study the effect of pH on AgNPs biosorption, different flasks containing 50 mL of mycosynthesized AgNPs was taken and the pH was adjusted from 3 to 10 using 0.1 N NaOH and 0.1 N HCl solutions. As AgNPs lead to precipitation when it was adjusted at pH 1 and 2 and hence these conditions were not considered. AgNPs and biomass concentration were maintained at 0.1 mg/ mL and 1 mg/ mL, respectively, throughout the experiment at all the pH conditions ranging from 3 to 10. The flasks were then incubated at 37 °C for 5 h at 120 rpm in a rotary shaker incubator. At the end of the incubation period, the solutions were centrifuged at 8000 rpm for 5 min and the residual AgNPs concentration was analyzed by ICP-AES.
6.2.4.2. Effect of temperature on biosorption

To study the effect of temperature on biosorption of AgNPs, 50 mL of biologically synthesized AgNPs (0.1 mg/mL) and biomass (0.1 mg/mL) were incubated at different temperatures such as 20, 30, 40, 50, and 60 °C for 5 h in a shaker incubator at 120 rpm. After incubation, the solutions were centrifuged at 8000 rpm for 5 min and the residual AgNPs concentration was analyzed using ICP-AES.

6.2.4.3. Effect of incubation time on biosorption

In order to investigate the effect of incubation time on AgNPs biosorption, the flasks containing 50 mL of biologically synthesized AgNPs (0.1 mg/mL) and biomass (0.1 mg/mL) were incubated for 50, 100, 150, 200, 250 and 300 min. At the end of each incubation time, the solutions were centrifuged at 8000 rpm for 5 min and the residual AgNPs concentration was analyzed by ICP-AES.

6.2.4.4. Effect of biomass concentration on biosorption

To study the effect of biomass concentration on AgNPs biosorption, the flasks containing 50 mL biologically synthesized AgNPs (0.1 mg/mL) and biomass concentration ranging from 1 to 10 mg/mL were incubated at optimized temperature and incubation time. At the end of the incubation, the residual AgNPs concentration was analyzed by ICP-AES after centrifuging the solution at 8000 rpm for 5 min.

6.2.4.5. Effect of agitation on biosorption

In order to determine the effect of agitation rate on AgNPs biosorption, the experimental setup was agitated at different speed. The effect of agitation rate on AgNPs biosorption was determined by incubating 50 mL of biologically synthesized AgNPs (0.1 mg/mL) and biomass (0.1 mg/mL) in the shaker incubator at various agitation speeds of 50, 75, 100, 125, 150 and 175 rpm. At the end of the incubation period, the residual AgNPs concentration was analyzed by ICP-AES after centrifuging the solution at 8000 rpm for 5 min.
6.2.5. Characterization of biosorbents

6.2.5.1. Fourier transform infrared spectroscopic analysis

To investigate the functional groups involved in the biosorption process, the samples were subjected to Fourier transform infrared spectroscopic analysis (FTIR). The dead mycelial biomass was taken as control whereas the biomass treated with AgNPs was considered as test. The respective samples were pelletized using KBr (potassium bromide) and then subjected to FTIR analysis (Model 470 Shimadzu) (Salunkhe et al., 2011).

6.2.5.2. Field emission scanning electron microscopic analysis

Scanning electron microscopic analysis was performed to observe cell surface variations during biosorption of AgNPs using fungal biosorbent. AgNPs treated mycelial biomass and untreated biomass were subjected to FESEM (Hitachi FE-SEM S-4800 EDX (HORIBA Emax) equipped with energy dispersive X-ray microanlyzer to view the microscopic surface image and EDX spectra of the biosorbent. The samples were mounted using specimen stubs with double sided adhesive tape and sputter coated with gold particles under vacuum to increase electron conduction and to improve the quality of the micrographs (Salunkhe et al., 2011). The elemental composition of AgNPs treated biosorbent was also detected by EDX equipped with FESEM.

6.2.6. Statistical analysis

All the experiments were performed in triplicate and the data were expressed as means ± standard deviation.
6.3. Results and discussion

The present study focused on the biosorption of AgNPs by Aspergillus terreus and the optimization of biosorption process for maximum metal removal from the aqueous solution and the results were presented.

In the present scenario, AgNPs have emerged as one of the rapidly growing areas in the field of nanotechnology due to their broad applications in the field of pharmaceutical, textile, home appliances and cosmetics etc. Recently, few studies have observed that there is a substantial release of silver from various consumer related products that consist of AgNPs which may possess an imminent threat to various forms of living system (Herzog et al., 2013; Rai et al., 2009; Oberdorster et al., 2007). In the current study, the ability of fungal based biosorbent was investigated for the removal of AgNPs in the aqueous environment. Various parameters such as pH, temperature, incubation time, initial biomass concentration and agitation were optimized to achieve maximum biosorption of AgNPs from the aqueous environment.

Biosorption is a rapid and reversible process in which binding of ions from aqueous solution is based on the different functional groups present on the biomass surface. The biosorption process is independent of cellular metabolism and can be used against any type of metal removal (Davis et al., 2003). Biosorption has several advantages compared to other bioremediation process, where the former can be performed in a wide range of pH from 1 to 9 whereas the latter requires specific pH depending upon the microbial growth. Similarly, temperature can also be employed as it is non-metabolic and desorption can be achieved within a short span of time. The major advantage of the biosorption process is cost-effective, where inexpensive agricultural waste and industrial wastes of bio origin such as residual biomass can also be employed (Kuyucak, 1990). Another important feature of the biosorption process includes biosorbent regeneration, recovery of metal ions and there is no need of any additional nutrient to perform this process (Kratochvil and Volesky, 1998).

6.3.1. Optimization studies of AgNPs biosorption

6.3.1.1. Effect of pH on biosorption

pH is one of the major factors which plays a key role in the biosorption of AgNPs from the aqueous solution. The hydrogen ion concentration strongly influences the site of
dissociation of biomass surface, in addition to affecting the solution chemistry of the metal ions such as redox reactions, hydrolysis, precipitation, speciation and the biosorption availability of the metal ions (Jianlong, 2002; Esposito et al., 2002).

The effect of pH on the biosorption of AgNPs has been described in Fig. 6.1. The maximum biosorption (57.33%) was observed at pH 4 with a maximum silver uptake of 48.51 mg g⁻¹ biosorbent. Based on these results, it is observed that the biosorption efficiency was found to be increased from pH 3 to 6 and then it was decreased with increasing pH of the aqueous solution (above pH 7). The lowest biosorption percentage was observed when pH was maintained at 10 with biosorption efficiency of 8.67% and silver uptake of 7.65 mg g⁻¹ of biosorbent.

Several findings have been reported that pH can affect the charge of the functional groups on the biomass surface which in turn affects the biosorption process. Based on the pH dependency of biosorption process, researchers have suggested that the binding of silver ions to the biosorbent may occur through an ion exchange type of phenomenon (Lin et al., 2002). Contrastingly, other researchers have concluded that the binding of metal ions to the biosorbent takes place predominantly through the functional groups such as amide, carboxyl and hydroxyl present on the cell wall of the biomass. In this present study, the maximum biosorption was prevailed at pH 4, consistent to the findings of Lin et al. (2005) who have also reported that the hydrogen ion concentration greater than 4 deprotonated the carboxyl group present in the cell wall and in turn enhances the uptake of silver ions.

When the pH was maintained at lower values, transfer of H⁺ ions from the functional group has indicated that the binding sites for the metal ions were occupied. On the other hand, during increase in pH, the H⁺ ions concentration decreases and leads the positively charged metal ions to bind with the surface of negatively charged biomass (Davis et al., 2003).

6.3.1.2. Effect of temperature on biosorption

The effect of temperature on the biosorption of AgNPs was studied at different temperatures such as 20, 30, 40, 50 and 60 °C. Similar to pH, temperature also plays a crucial role in the biosorption of metal ions from the aqueous solution. The influence of temperature on AgNPs biosorption was given in Fig. 6.2. The maximum removal of
55.45 mg of AgNPs per gram g$^{-1}$ of biosorbent (61% of biosorption efficiency) was detected when the temperature was maintained at 30 °C compared to 60 °C, which showed the least biosorption ability with 21.19 mg g$^{-1}$. Similarly, biosorption efficiency of AgNPs was found to be 54.31% and 45.20% when the temperature was maintained at 20 and 40 °C, respectively.

The results also indicated that the increase in temperature increased the adsorbed amount of AgNPs on the biosorbent surface. Hefne et al. (2010) have discussed that the change may occur due to the increase in the kinetic energy of silver ions which enhance the rate of collision between the fungal biomass and AgNPs, resulting in the increased adsorption of AgNPs over the surface of the fungal biomass. They have also confirmed that the adsorption processes of silver ions are endothermic which occur by virtue of redistribution of energy between the adsorbate and adsorbent and it is likely to increase the biosorption process spontaneously at normal and high temperatures due to greater thermodynamic parameters such as $\Delta H > 0$ and $\Delta S > 0$.

Similar findings were reported by Goyal et al. (2003) who have investigated the effect of temperature on biosorption of hexavalent chromium metal ions using S. cerevisiae. They have also found that increasing the temperature from 25 to 45 °C increases the metal uptaking ability of S. cerevisiae due to higher affinity or increasing binding sites for metal ions on the biomass surface. On the other hand, during very high temperature, the metal adsorption tend to be decreased due to the distortion of binding sites on the cell surface (Horsfall and Spiff, 2005; Sawalha et al., 2006). Sepehr et al. (2005) have also found that temperature has major effect on the fungal biosorption which influences the enzymatic systems as well as the metal solubility in the effluents and its adsorption rate. Yu et al. (2000) have also suggested that increase in temperature beyond a certain level may also affect the integrity of the cell membrane, development of ionization of chemical moieties of the cell wall of biomass and also the stability of metal complex depending on the binding sites.

6.3.1.3. Effect of incubation time on biosorption

The effect of incubation time on the biosorption of AgNPs was studied at various time intervals viz. 50, 100, 150, 200, 250 and 300 min. As shown in Fig. 6.3, the biosorption efficiency of A. terreus was found to be increased with the increase in incubation time from 50 to 200 min; later, the metal uptake seemed to be stationary.
Based on the results, optimum incubation time for the biosorption of AgNPs was found to be 200 min, where the uptake was 55.31 mg g\(^{-1}\) of biosorbent, though the contact time of 250 and 300 min showed a metal uptake of 55.62 and 56.04 mg g\(^{-1}\), where the metal adsorbed difference was found to be less significant, hence, 200 min was considered as the best incubation time. As already said, the increase in incubation time favored the metal removal wherein the AgNPs uptake was found to be 11.08, 25.47 and 39.05 mg g\(^{-1}\) of biosorbent when incubated for 50, 100 and 150 min, respectively.

Similarly, Li et al. (2011) have investigated the biosorption of sliver ions using Bacillus cereus biomass. They have reported that the maximum biosorption equilibrium was attained with 24 h incubation. In addition, they have also observed that biosorption of solid phase Ag\(^+\) concentration was found to be increased with the increase in contact time when compared with the aqueous phase. At the end of 600 min, the equilibrium capacity of B. cereus was 91.75 mg Ag\(^+\)/g of biomass.

Zhang et al. (2005) have reported that the increase in incubation time increases the biosorption of silver diamine using Corynebacterium strain SH09, which was isolated from Shanghang silver mine, Fujian, China. They have also found that Corynebacterium strain SH09 showed a good biosorption ability for [Ag (NH\(_3\))\(_2\)]\(^+\) with a maximum uptake of 350 (mg Ag) g per dried biomass. Martínez-Juárez et al. (2012) have studied the biosorption ability of mercury (II) using 14 different fungal biomasses and ascertained that the maximum biosorption of mercury was achieved at 24 h incubation with a highest removal rate of 95.4% when the dried biomass of Mucor rouxii IM-80 was maintained at pH 5.5.

6.3.1.4. Effect of initial biomass concentration on biosorption

The effect of initial biosorbent concentration on AgNPs uptake by Aspergillus terreus dead biomass was illustrated in Fig. 6.4. Based on the results, it is clearly inferred that increasing the concentration of biosorbent from 1 - 10 mg/ mL increases the removal of silver ions due to the increased adsorption surface area and availability of free adsorption sites for metal binding. Though, the maximum biosorption efficiency (85.16%) was observed when the concentration of biomass was maintained at 10 mg/ mL, the biomass concentration of 1 mg/ mL showing biosorption efficiency of 61.51% and nanoparticles uptake of 55.80 mg g\(^{-1}\) of biosorbent was chosen as optimum concentration for further studies due to the biomass management for the metal uptake. From the results, it is clear that increase in biomass.
concentration (1 - 10 mg/ mL) increases the biosorption percentage of AgNPs from 61.51 to 85.16% and also decreases the loading capacity from 55.80 to 7.73 mg g⁻¹ of biosorbent.

As the biomass concentration increases, more and more absorption of metal ions on the surface of biosorbent occurs resulting in increased adsorption yield (Dönmez et al., 1999; Chojnacka et al., 2005); however in some cases, partial cell aggregation may occur at higher concentrations, resulting in the decrease of number of active sites (Esposito et al., 2001).

Biosorption of silver ions using an industrial strain of Saccharomyces cerevisiae was investigated by Simmons and Singleton (1996). Two types of biomass, the older (96 h old) and younger (24 h old), for the biosorption of silver ions was conducted based on their harvest time from the culture broth. The authors have determined the biosorption ability of both the biomasses with a concentration of 2 mg/ mL against the initial silver ions concentration of 0.1- 3.5 mM Ag⁺, where they have found a maximum biosorption efficiency of 0. 387 mM Ag⁺ g⁻¹ dry mass for 24 h old culture as opposed to 0.187 mM Ag⁺ g⁻¹ dry mass for 96 h old biomass.

Similar report was also shown by Sun et al. (2014), who have investigated the biosorption of Ag (I) ions from the aqueous solution using Bacillus licheniformis strain R08. They have optimized the biosorption with various parameters such as pH, temperature, contact time and initial biomass concentration; all these resulted in the maximum biosorption capacity of 73.6 mg g⁻¹ of biosorbent under optimized condition.

6.3.1.5. Effect of agitation rate on biosorption

The effect of agitation on the biosorption of AgNPs was studied by incubating the flasks with an agitation rate of 50, 75, 100, 125, 150 and 175 rpm for 6 h (Fig. 6.5). The agitation speed also plays a critical role during the biosorption process of heavy metal ions. The present results showed that biosorption of silver ions was found to be increased with the increase in agitation speed from 75 to 125 rpm and then it was decreased. The maximum biosorption of AgNPs was found to be 54.96 mg g⁻¹ of biosorbtent when the agitation speed was maintained at 125 rpm, followed by 50.56 mg g⁻¹ of biosorbent at 150 rpm. Lower uptake of AgNPs was observed when the agitation speed was maintained at 50 and 75 rpm with an uptake of 15.32 mg g⁻¹ and 28.88 mg g⁻¹ of biosorbent, respectively. In addition, the AgNPs uptake of 41.60 mg g⁻¹ and 44.94 mg g⁻¹ of
biosorbent was observed when the agitation speed was maintained at 100 and 175 rpm, respectively.

Similar kind of studies such as biosorption of Ag (I) using *Saccharomyces cerevisiae* was investigated by Chen and co-workers (2014). They have reported the maximum Ag (I) biosorption of 1.26 mM/g, when the agitation speed was maintained at 150 rpm along with 2 g/L of yeast biomass. Das *et al.* (2010) have reported the maximum Ag (I) biosorption of 46.7 mg g⁻¹ when the dead biomass of macrofungus, *Pleurotus platypus* was continuously agitated at 120 rpm. On the contrary, Simmons and Singleton (1996) have reported the maximum biosorption efficiency of silver ions (0.387 mmol Ag + g⁻¹ of dry biomass) when 24 h old yeast biomass was maintained at 200 rpm for 1 h at 27 °C.

Based on the results, Al-Qodah (2006) has concluded that the increase in agitation speed to a certain limit decreases the biosorption of heavy metals due to the increased turbulence. As a result, there could be decrease in external mass transfer resistance thickness over the adsorbent applied when the rate of agitation was increased. In addition, when the speed of agitation is increased, the rate of mixing also increases, resulting in an improper contact between the binding sites of biosorbent and the metal ions thereby reduces the biosorption efficiency (Ajaykumar *et al.*, 2009).

6.3.2. Characterization of biosorbent

6.3.2.1. Fourier Transform Infrared Spectroscopy

The FTIR spectroscopy is undoubtedly one of the most important tools which detects the vibration characteristics of functional groups present on the surfaces of biosorbent. In this present study, FTIR analysis was performed for AgNPs treated biosorbents in the range of 400–4000 cm⁻¹. Based on the optimization results, the biosorbents treated with AgNPs at different pH (3-10) were chosen for FTIR analysis because of their major role in the biosorption efficiency during the uptake of metal ions. In addition, the untreated biosorbent of *A. terreus* was also subjected to FTIR analysis to compare the treated biosorbent. The processed sample of each pH, from 3 to 10, was individually recorded in the range of 400–4000 cm⁻¹ and was shown in Figs. 6.6a and b.

The FTIR spectra of untreated and treated fungal biosorbent helped to study the nature of interactions between the functional groups of *A. terreus* and AgNPs. The FTIR
spectra of untreated biomass of *A. terreus* displayed a number of absorption peaks, indicating the complex nature of the biosorbent (Figs. 6.6a and b).

The FTIR spectra of untreated biomass exhibited broad bands centered at 3200 – 3600 cm\(^{-1}\) indicating the presence of –OH and –NH groups and also showed strong shift peaks in the range of 2800 to 3000 cm\(^{-1}\), which indicate the presence of C-H groups due to the existence of methyl and methylene groups (Bayramoğlu et al., 2006). The unloaded biomass also showed spectral band in the range between 1720 and 1630 cm\(^{-1}\), corresponding to the stretching vibration of C=O which is a characteristic feature of carbonyl group stretching from aldehydes and ketones; the spectral peaks at 1447.19 cm\(^{-1}\) and 1319.71 cm\(^{-1}\) indicated the stretching of C-O and –C-N stretching vibration of amino acid, respectively (Chen et al., 2006). The strong bend at 1806.08 cm\(^{-1}\) corresponding to C=O, carboxylic acid stretch was also exhibited in the unloaded fungal biomass. In addition, the spectral peaks of untreated biomass observed at 818.91 and 637.82 cm\(^{-1}\) were corresponded to the C-O and C–O–C stretch, respectively. The peaks at 1241.18 and 532.25 cm\(^{-1}\) were corresponded to the –SO\(_3\) stretch and C–I, respectively. The peak exhibited at 1529.64 cm\(^{-1}\) indicated the presence of N=O stretch.

To assess the changes occurred in the functional groups during the biosorption of silver ions, biosorbents treated with silver ions (pH 3 to 10) were studied using FTIR. The treated biosorbent exhibited several peaks similar to the spectral peaks observed in the control. However, few peaks with different intensity and shift in wavelength were observed compared to the untreated biosorbent. These results confirmed the role of functional groups along with metal ions during the biosorption process. The detailed information regarding the peak values and their corresponding functional groups were listed in Table 6.1a and b.

The results showed that few bands have been shifted during the biosorption process of silver ions using fungal biomass. Importantly, the band at 3420.67 cm\(^{-1}\) of untreated biosorbent was shifted to 3457.53, 3445.51, 3451.92, 3451.92, 3454.32, 3443.1, 3455.12 and 3463.69 cm\(^{-1}\) when the AgNPs treated biosorbent was maintained at pH 3, 4, 5, 6, 7, 8, 9 and 10, respectively; the peak was corresponded to the –OH and –NH groups of the fungal biosorbent. Similarly, major shift changes were observed at 637.82 cm\(^{-1}\) of unloaded biomass, which has been shifted to 635.41, 664.26, 678.68, 669.87, 634.61, 614.58, 638.62 and 636.21 cm\(^{-1}\) when the treated biosorbent was maintained at pH 3, 4, 5, 6, 7, 8, 9 and 10, respectively; this band was attributed to the C–O–C stretch. In addition, major shift in
functional groups were also observed corresponding to the C-I region (535.25 cm\(^{-1}\)) of untreated biomass compared with the treated biosorbent at different pH 3, 4, 5, 6, 7, 8, 9 and 10 showing 568.1, 568.91, 575.32, 568.91, 530.44, 529.64, 568.91 and 529.64 cm\(^{-1}\), respectively.

The present observations indicated the involvement of various functional groups during the biosorption of AgNPs from the aqueous solution. It is also clear that change in intensity and shift in the band under 700 cm\(^{-1}\) was very well observed due to the interaction of AgNPs with N-containing groups which may confirm the role of amino acids during the biosorption process.

However, very few changes were observed in silver ions treated biosorbents compared with the untreated biomass, especially in the region between 1200 and 1800 cm\(^{-1}\). There was a little alteration in the peaks during the biosorption process, especially in the spectral peak 1806.08 cm\(^{-1}\) of control to 1808.49 cm\(^{-1}\) with a difference of 2.41. This was the major shift change compared to the other treated biosorbent. Similarly, 1711.53 cm\(^{-1}\) and 1712.33 cm\(^{-1}\) spectral peaks of treated biosorbent at pH 7 and 3 showed a change in the peak value of 9.62 and 8.82, respectively; this peak was corresponded to the C=O stretching from aldehydes and ketones (Bai and Abraham, 2002).

Our earlier results confirmed that the maximum biosorption of AgNPs occurred when the pH was maintained at 4. The bands at 1721.15, 1627.4, 1529.64, 1447.91, 1319.71 and 1241.18 cm\(^{-1}\) were shifted to 1714.74, 1616.18, 1531.25, 1445.51, 1318.91 and 1242.78 cm\(^{-1}\), respectively. This also suggests that biosorption of AgNPs could be due to the interaction of various functional groups such as amide, amino acids and carboxyl etc.

In addition, the biosorbent treated at pH 7, 3 and 5 showed shift in the peak values to 1488.71, 1329.6, 1245.99 cm\(^{-1}\) when compared to the untreated biosorbent, which indicates the C-O and C-N stretching vibrations of amino acid and \(–\)SO\(_3\) stretch, showing their involvement in the biosorption process. Similar results confirming the role of various functional groups during the biosorption of metal ions were also reported by various researchers (Li et al., 2008; Romero-González et al., 2003; Li et al., 2011).

Salvadori et al. (2013) have also reported the importance of FTIR analysis for the identification of possible functional groups which may interact with the dead biomass of Hypocrea lixii during the uptake of copper nanoparticles. The protein of the cell was
probably liberated during the autoclaving process and bound on the surface of the dead biomass. They have also reported that the amide groups of proteins present in the dead biomass have strong affinity to bind with metals during the biosorption process, similar to our findings.

Similarly, Zhang et al. (2005) have also studied the changes in the functional moiety of the dead biomass of Corynebacterium strain SH09 using FTIR spectroscopy before and after the biosorption of silver ions. Several functional groups such as amide, especially C=O, C-N and ionized carboxyl corresponding to the spectral peaks 1651 cm\(^{-1}\), 1537 cm\(^{-1}\) and 1385 cm\(^{-1}\), respectively, were shifted in metal ions treated biomass, thus indicating their association in the biosorption process. Based on these studies, it is apparent that the functional groups such as carboxyl, carbonyl and secondary amines present in the fungal cell biomass have played a major role in the biosorption of silver in nanoform. On the basis of FTIR spectral studies, various researchers have reported that shifting or group changes occurred in metal ions treated biosorbent during the biosorption of various heavy metals (Salunkhe et al., 2011; Yahaya et al., 2009; Kumar et al., 2011).

6.3.2.2. Scanning electron microscopic analysis

The surface morphology of the fungal biosorbent before and after treatment with AgNPs was studied by scanning electron microscopic analysis equipped with EDS. The SEM image of untreated biosorbent showed fungal mycelia with smooth surface without any protrusions (Fig. 6.7a). To study the morphological changes of the treated biosorbent, two different samples of processed biosorbent with AgNPs at pH 4 and 8 were chosen and the EDS spectrum was also recorded to confirm the presence of adsorbed AgNPs. The SEM images of both the treated biosorbent at pH 4 (Fig. 6.7b) and 8 (Fig. 6.7c) showed remarkable changes in the cell structure with partial disintegration of the cell wall. Very importantly, the SEM images of the treated biosorbent also revealed the presence of AgNPs over the surface of fungal biosorbent, which is evident for the successful metal uptake. The presence of silver peaks in the EDX spectrum also correlated with the SEM analysis and confirmed the biosorption of AgNPs. The SEM images of the treated biosorbent also depicted the rough texture with little protrusion due to the silver binding over the surface of the fungal biomass.

Similar findings were also reported by Chen et al. (2014) who have investigated the cellular surface changes of Saccharomyces cerevisiae during the biosorption of Ag (I) ions.
They have observed remarkable changes in the cell surface morphology, cell adhesion, formation and appearances of deposits, etc after Ag (I) uptake. In addition, AgNPs with uniform size were observed in biosorbent treated at pH 5 compared to pH 10, where partial aggregation of AgNPs was clearly observed due to the increased hydrogen ion concentration in the environment. The presence of C and O peaks along with silver peaks exhibited in EDX spectrum of treated biosorbent samples (Fig. 6.8) confirmed the presence of AgNPs and other peaks may be due to the presence of cell wall components present in the fungal biosorbent.

Scanning electron microscopic analysis is one the most important and widely used characterization techniques to study the surface properties and morphology of the biosorbent. Salunkhe et al. (2011) have confirmed the presence of silver ions over the surface of the C. lunatus cell biomass during the biosorption process and this result was found to be consistent with our present findings. Similarly, several other researchers have also characterized the presence of metal ions over the surface of cell biomass using scanning electron microscopy (Salvadori et al., 2014; Kim et al., 2015; Mishra et al., 2014).

6.4. Summary

1. The biosorption ability A. terreus biomass was investigated for the removal of AgNPs from the aqueous solution.
2. Different parameters such as pH, temperature, incubation time, agitation speed and initial biomass concentration were optimized for the maximum biosorption of AgNPs.
3. Among different pH tested, the maximum biosorption efficiency (57.33%) was observed when the medium was maintained at pH 4 with AgNPs uptake of 48.51 mg g\(^{-1}\) biosorbent.
4. Among different temperatures tested, 30 °C supported the maximum removal of AgNPs (55.45 mg g\(^{-1}\) of biosorbent) and the biosorption efficiency was 61%.
5. Among different incubation time tested, the maximum biosorption of AgNPs occurred at 200 min with an uptake of 55.31 mg g\(^{-1}\) of biosorbent.
6. Among different biomass concentration tested, the biosorption efficiency of AgNPs was found to be increased with increasing concentration of biomass. The maximum biosorption efficiency of 85.16% was found when the biomass concentration was maintained at 10 mg/ mL.
7. Among different agitation speed tested, the maximum biosorption of AgNPs was recorded at 125 rpm with an uptake 54.96 mg g\(^{-1}\) of biosorbent.

8. The FTIR spectral analysis of untreated and AgNPs treated \textit{A. terreus} biomass showed shifting of certain bands and changes in vibration of chemical groups, thus confirming their role in biosorption of AgNPs.

9. The scanning electron microscopic analysis of untreated \textit{A. terreus} biomass showed fungal mycelia with smooth surface without any protrusions; whereas, the AgNPs treated biomass depicted remarkable changes in the cell structure with partial disintegration of the cell wall. In addition, the presence of AgNPs over the surface of fungal biosorbent evidenced the successful biosorption of AgNPs.

10. Further, the EDX spectrum of AgNPs treated biomass showed strong peaks at silver region, confirming the successful biosorption of AgNPs.
6.5. References


**Fig. 6.1: Effect of pH on AgNPs biosorption**

Biosorption of AgNPs at different pH; values represent mean ± standard deviation of three independent experiments; Line chart represents the biosorption efficiency and the bar diagram represents the biosorbent uptake efficiency.

**Fig. 6.2: Effect of incubation temperature on AgNPs biosorption**

Biosorption of AgNPs at different incubation temperature; values represent mean ± standard deviation of three independent experiments; Line chart represents the biosorption efficiency and the bar diagram represents the biosorbent uptake efficiency.
**Fig. 6.3:** Effect of incubation time on AgNPs biosorption

Biosorption of AgNPs at different incubation time; values represent mean ± standard deviation of three independent experiments; Line chart represents the biosorption efficiency and the bar diagram represents the biosorbent uptake efficiency.

**Fig. 6.4:** Effect of initial biomass concentrations on AgNPs biosorption

Biosorption of AgNPs at different initial biomass concentrations; values represent mean ± standard deviation of three independent experiments; Line chart represents the biosorption efficiency and the bar diagram represents the biosorbent uptake efficiency.
Fig. 6.5: Effect of agitation speed on AgNPs biosorption

Biosorption of AgNPs at different agitation speed; values represent mean ± standard deviation of three independent experiments; Line chart represents the biosorption efficiency and the bar diagram represents the biosorbent uptake efficiency.
AgNPs treated biosorbents of different pH (3 to 6) were analyzed using FTIR spectroscopy to study the functional groups involved in the biosorption process; untreated (control) biosorbent was also shown in the figure.
AgNPs treated biosorbents of different pH (7 to 10) were analyzed using FTIR spectroscopy to study the functional groups involved in the biosorption process; untreated (control) biosorbent was also shown in the figure.
Fig. 6.7a: Scanning electron micrograph of untreated biosorbent

Scanning electron microscopic image of untreated (control) biosorbent showing smooth surface without any protrusions.
Fig. 6.7b: Scanning electron micrograph of AgNPs treated biosorbent at pH 4

Scanning electron microscopic image of AgNPs treated biosorbent (pH - 4) showing rough surface; arrows indicate AgNPs.
Fig. 6.7c: Scanning electron micrograph of AgNPs treated biosorbent at pH 8

Scanning electron microscopic image of AgNPs treated biosorbent (pH - 10) showing rough surface; arrows indicate AgNPs.
EDX analysis of AgNPs treated biosorbent (pH 4) showing strong peak for silver.