PHYKOS
Phycological Society, India

Pseudokirchneriella subcapitata, a prospective micro-alga in mediating silver nanoparticle synthesis

Darsana R[1], Chandrasehar G[2], Deepa V[2#], Chitrikha T[2]

[1]Spices Board, Quality Evaluation Laboratory, Regional Office, Mumbai 400 703, Maharashtra, India
[2]International Institute of Biotechnology and Toxicology, Padappai 601 301, Kancheepuram District, Tamil Nadu, India
[#]Syngene International Ltd., Biocon Park , Plot 2&3, Bommasandra Industrial Estate - Phase-IV Bommasandra-Jigani Link Road, Bangalore 560 099, India

Abstract
In the present study, the fresh water green alga, *Pseudokirchneriella subcapitata* was used for the first time in mediating the synthesis of silver nanoparticles. Cell free extract and whole algal sample were treated with various concentrations of silver nitrate solution. Structural and morphological properties of the synthesized nanoparticles were characterized using UV-Vis spectrophotometry, FTIR, and TEM. The treated whole algal sample showed a gradual change in the color due to the formation of silver nanoparticles. The UV-Vis spectroscopic analysis of whole algal sample showed an absorption peak in the range 420-484nm indicating the presence of nano particles. The synthesized nano particles appeared spherical in shape as observed using TEM analysis with size ranging from 10 to 90 nm. FTIR analysis showed that the reduction of silver nitrate has been carried out due to the presence of polysaccharides and C –O group present in the algal sample.
Keywords: *Pseudokirchneriella subcapitata*, Silver nitrate, Cell free extract, Whole alga sample, Silver nanoparticle biosynthesis, Characterization

Introduction

The application value of silver nanoparticles has reached a gamut of biomedical, therapeutic, electrochemical and cosmetic industrial products besides food and crop production (Mahdi *et al*., 2011; Kajori and Padma, 2012; Jayashree *et al*., 2013; Arun *et al*., 2013). The employment of plants, algae, fungi, yeasts and bacteria in the fabrication of silver nanoparticle is of late a nascent approach in nanotechnology (Arun *et al*., 2013; Shankar *et al*., 2004 Chandran *et al* 2006; Huang *et al*., 2007; Sadowski *et al*., 2008; Klaus *et al*.,1999; Barwal et al., 2011). The green synthesis of nanoparticles, wherein a biological organism or its derivative is employed as a contraption which functions at the nano level using non toxic and environmentally benign procedures to synthesize nanoparticles (Thakkar *et al*., 2010; Xie et al., 2007a). Scientific findings have affirmed the importance of microalgae in nanoscale particle synthesis in recent times (Mahdi *et al*., 2011; Kumar *et al*., 2012;Jayashree *et al*., 2013)

Pseudokirchneriella subcapitata, a fresh water green alga, commonly used in ecotoxicology studies is taken up to find out the potential of the alga in mediating silver nanoparticle synthesis.

Materials and methods

The alga, *P. subcapitata* (formerly known *Selenastrum capricornutum*) culture was obtained from SAG: Collection of Algal Cultures, Strain No. 61. 81 SAG, Institute of Plant physiology, University of Göttingen, Germany. The culture is maintained in the Department of Ecotoxicology, International Institute of Biotechnology And Toxicology (IIBAT) as per the methods stated in the Organization for Economic Cooperation and Development (OECD) Guideline No. 201 adopted on 23rd March, 2006. The OECD TG 201 medium (pH of 8.1 ± 0.1) is used for maintenance and growth cabinet provided with white fluorescent lamps yielding continuous light illumination of 4440 – 8880 lux light intensity and temperature in the range of 22 ± 2°C.

Cell free extract (CFE) and whole algal sample (WAS) were prepared. Logarithmic phase culture was chosen for the study. For the preparation of CFE, 1.5g (wet weight) algal biomass was added to 30 ml of sterile water in an Erlenmeyer flask and maintained at 100°C for 30 min. The boiled mixture was cooled and centrifuged at 5000 rpm for 15 min. The supernatant stored at 4°C for further use. For WAS, the culture was centrifuged at 4000 rpm, 4°C for 20 min. The collected biomass was washed five times with sterile water to remove impurities and used after re-suspending with 47.5 ml of sterile water (Jayashree *et al*., 2013).

Silver nitrate ($AgNO_3$) (Merck) concentrations of 0.01, 0.025, 0.05 and 0.1M were prepared. 1 ml from each concentration was treated with 19ml of CFE and 19ml of WAS. Separate flasks holding 20 ml of CFE and WAS without the addition of $AgNO_3$ was taken as controls and the flasks incubated under dark at 100-120 rpm, 28°C for 72 hours. Cell count and microscopic observation of the treated algal samples were noted using an improved Neubaur

Counting Chamber at 0, 24, 48 and 72 hours. Periodically, colour changes was observed and after completion of 72 hours the two batches of treated samples were centrifuged and the pellets stored at -20°C.

The bioreduction of $AgNO_3$ ions in solution was monitored by (Ultraviolet visible) UV- Vis spectral analysis. Samples of aliquots (3ml) were periodically collected. The UV-Vis spectra of the treated CFE and WAS were measured in 10 mm -optical-path-length quartz cuvettes with an UV-1601 Shimadzu, Version 2.20 spectrophotometer at a resolution of 1 nm between 200 and 800 nm with a scanning speed of 1,856 nm/min.

The images of algal mediated Silver nanoparticles (AgNPs) were obtained using transmission electron microscopy (TEM) analysis. TEM images were obtained from a Hitachi HS -8 TEM operating at an accelerated voltage of 120 kV. Samples were prepared by placing a drop of aqueous CFE and WAS on the carbon-coated copper grid and dried under infrared lamp prior to examination.

Fourier Transform Infrared Spectroscopy (FTIR) spectroscopy was carried out to identify the likely interactions involving silver and biomolecules. It was used to examine the changes in the chemical composition of the treated samples. The bioreduced solution of $AgNO_3$ was centrifuged for 15 min at 15,000 rpm, and the pellet was washed with deionized water to remove any free proteins or enzymes. The purified suspension of the CFE and WAS was then freeze-dried (Singh *et al*., 2013). The freeze-dried samples of CFE and WAS were ground with Potassium bromide (KBr) pellets, and scanned in Shimadzu FTIR Spectroscopy (FTIR 8400S) to obtain the FTIR spectra. The spectrum was obtained in the transmission mode in the range of 4000 – 400 cm^{-1} at a resolution of 4 cm^{-1} with 40 scans per sample.

Results

The CFE treated with different concentrations of $AgNO_3$ showed a decreasing pattern of cell count with increasing incubation time. CFE has lesser cell counts compared with WAS. This could be attributed to the heat treatment done to obtain cell-free supernatant. The WAS showed increase in cell counts during the 0 – 72 hour study. Moreover the cells when observed under the Improved Neubaur Counting chamber appeared to be normal, green colored and sickle shaped without any abnormalities. This may be taken as an indication that $AgNO_3$ has not affected the normal shape or color of the fresh water algae. Further studies are warranted to confirm the effect of $AgNO_3$ on *P. subcapitata*.

The CFE and WAS after treatment with 0.01, 0.025, 0.05 and 0.1M of $AgNO_3$ showed observable colour change during the 72 h study. The colour changed from pale green to pale brown for the treated CFE and green to brown in the case of WAS sample. The treated CFE and WAS were centrifuged and pellets stored at - 4°C till further use. Periodical absorbance measurement presented interesting observations in the case of $AgNO_3$-treated WAS unlike the CFE which did not show any absorption spectra in the appropriate range. For the WAS treated with 0.01, 0.025, 0.05 and 0.1M of $AgNO_3$, absorption spectra were detected in the range 420-484 nm during the study indicating the presence of synthesized nanoparticles (Fig 1). The algal mediated nanoparticles obtained after treatment with different concentrations of $AgNO_3$ observed using TEM showed spherical shaped nano particles (Fig 2, 3, 4, 5). FTIR analysis was done to identify the potential biomolecules responsible for the reduction of silver ions to silver nanoparticles. The FTIR analysis of the treated WAS showed strong bands indicating the possible interaction of biomolecules in capping and stabilization of AgNPs (Fig 6, Fig 8, Fig 9, and Fig 10). FTIR analysis was also done for the control i.e. the untreated WAS and silver nitrate respectively (Fig 7 and Fig 11).

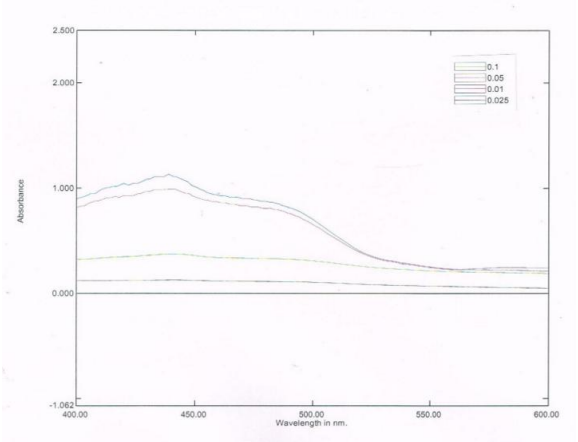
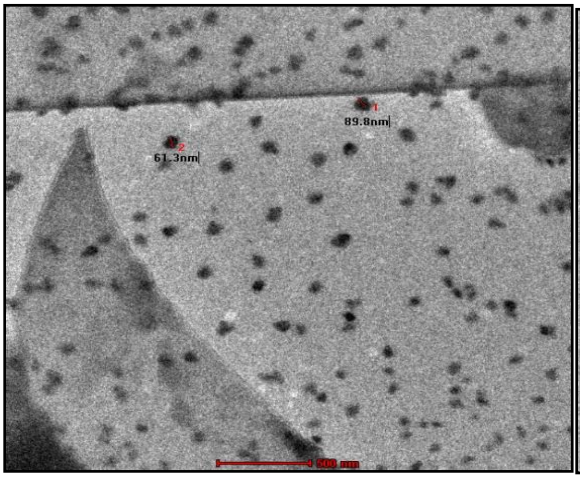


Figure 1. Absorption spectra of WAS treated with $AgNO_3$ at 72 hours.



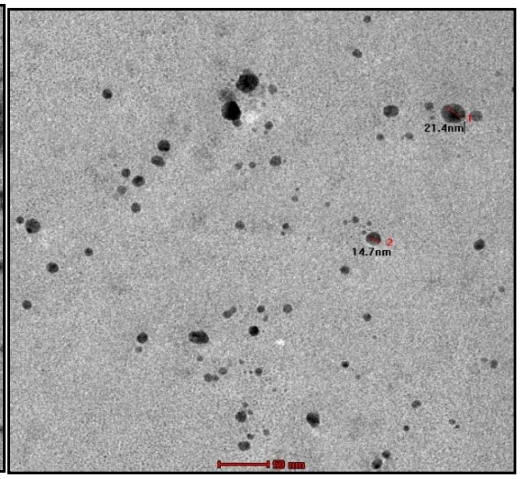
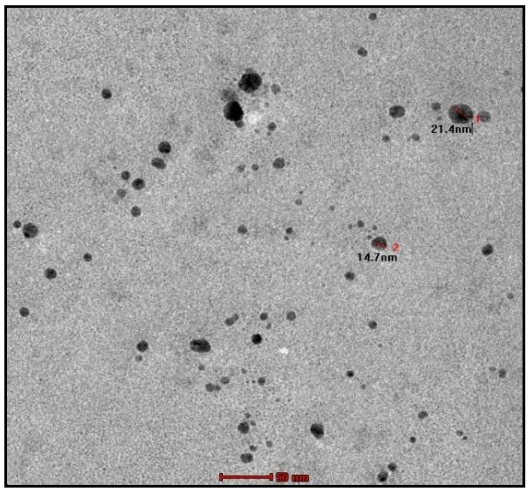


Fig 2 Fig 3

Figure 2. TEM image of Silver nanoparticles obtained after treatment of WAS with 0.01M $AgNO_3$

Figure 3. TEM image of Silver nanoparticles obtained after treatment of WAS with 0.025M $AgNO_3$



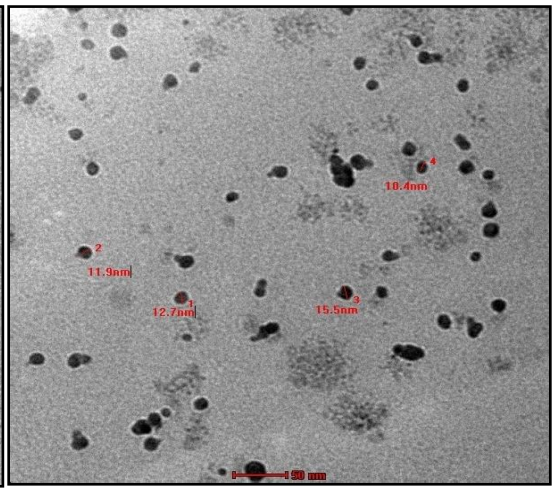


Fig 4 Fig 5

Figure 4. TEM image of Silver nanoparticles obtained after treatment of WAS with 0.05M $AgNO_3$

Figure 5. TEM image of Silver nanoparticles obtained after treatment of WAS with 0. 1M $AgNO_3$

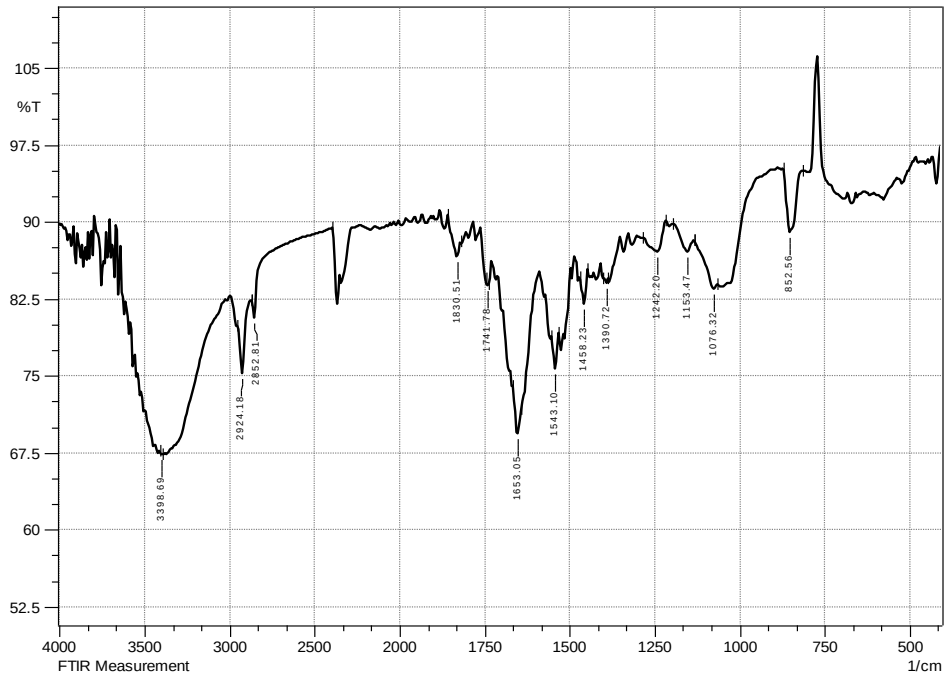


Figure 6. FTIR analysis of Silver nanoparticles obtained after treatment of WAS with 0.01M $AgNO_3$

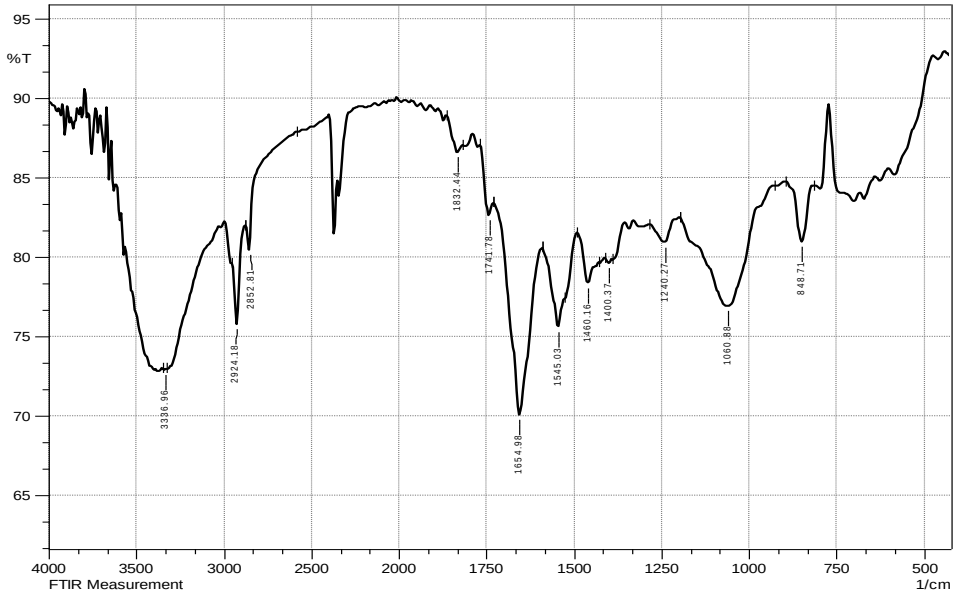


Figure 7. FTIR analysis of untreated WAS

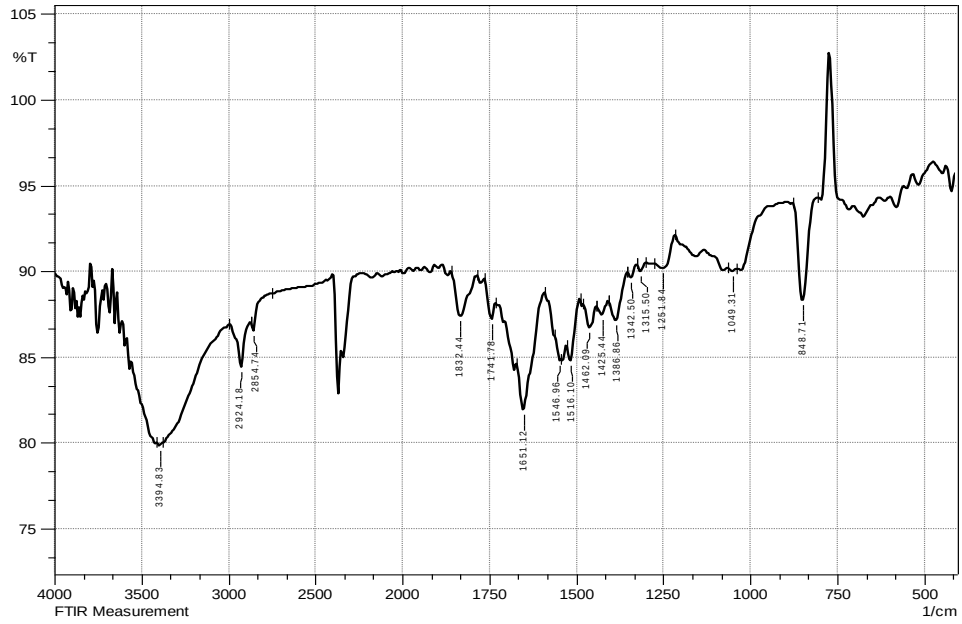


Figure 8. FTIR analysis of Silver nanoparticles obtained after treatment of WAS with 0.025M $AgNO_3$

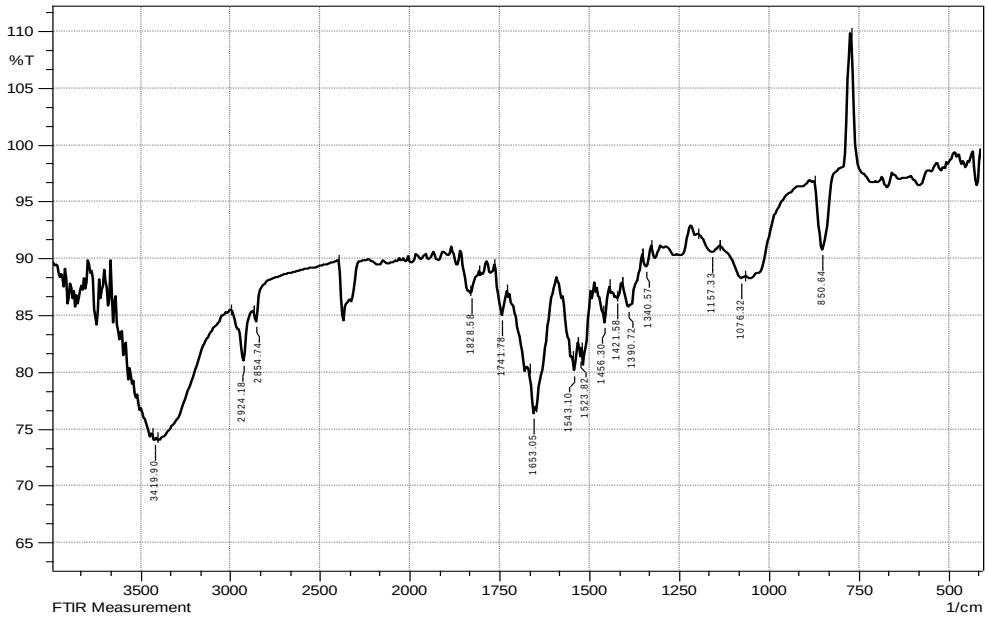


Figure 9. FTIR analysis of Silver nanoparticles obtained after treatment of WAS with 0.05M $AgNO_3$

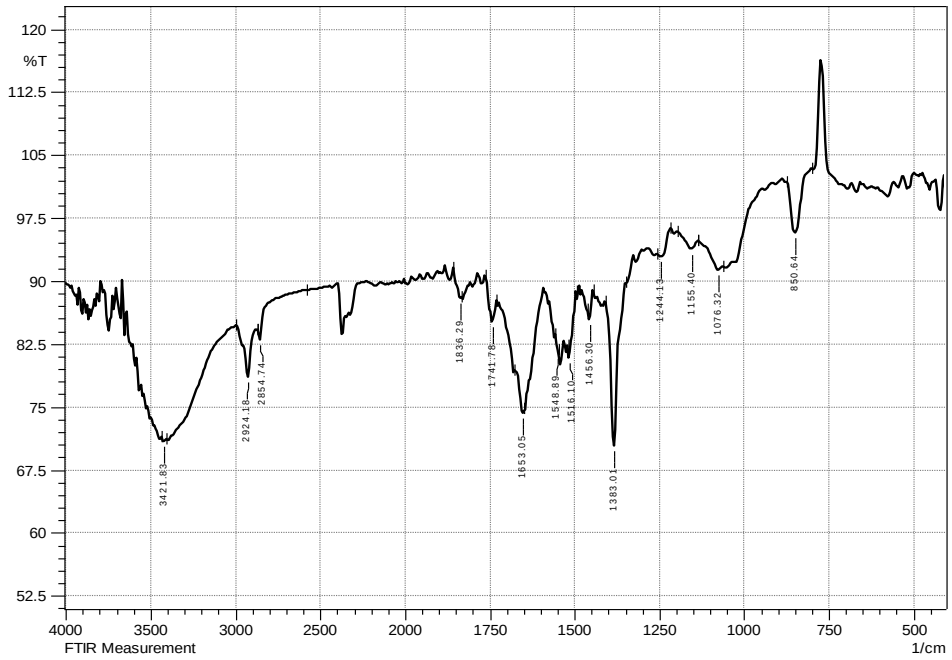


Figure 10. FTIR analysis of Silver nanoparticles obtained after treatment of WAS with 0. 1M $AgNO_3$

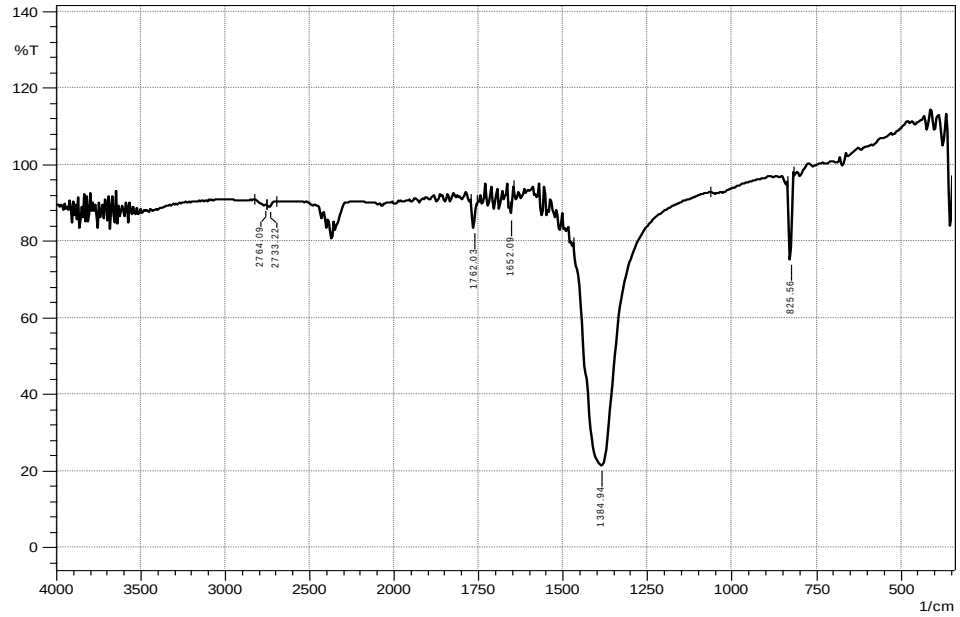


Figure 11. FTIR analysis of $AgNO_3$

Discussion

The colour change from pale green to pale brown is a maiden indication of biosynthesis of silver nanoparticles (AgNPs) (Jayashree *et al*., 2013; Kumar *et al*., 2012). Measurement of absorbance using UV- Vis Spectrophotometry is considered as one of the pilot authentications for biosynthesis of AgNPs. The phenomenon of surface plasmon resonance (SPR) is accountable for the robust scattering and absorption properties exhibited by AgNPs upon excitation at defined wavelengths. Depending upon the particle size and shape, AgNPs have color and the SPR peak wavelength can be adapted from 400nm – 530nm (Jayashree *et al*., 2013; Kuber and D'Souza, 2006; Henglein 1993; Kumar *et al*., 2012a).

The nanoparticles obtained from the WAS treated with 0.1M $AgNO_3$ ranged in size from 60 -90 nm. WAS treated with 0.025M (Fig 3), 0.05M (Fig 4) and 0.01M (Fig 2) $AgNO_3$ produced nanoparticles ranging in size from 15-30nm, 10-30 nm and 10-20nm respectively. This may be due to weak SPR vibrations produced by AgNPs at 420 nm (Sathishkumar *et al*., 2009).

WAS treated with 0.01M $AgNO_3$ solution showed peaks at; 3398.69 cm^{-1} which corresponds to O−H, $-NH_2$ group, 2924.18 cm^{-1} which corresponds to C−H stretch, 1741.78 cm^{-1} due to C═O stretch, 1653.05 cm^{-1} and 1543.10 due to the presence of amide, 1153.47 cm^{-1} and 1076.32 cm^{-1} due to C−O stretch (Fig 6). Similar peaks were observed for the other treated WAS as shown in Fig 8, 9 and 10. Peaks at 3394.83, 2924.18, 2854.74, 1832.44, 1741.78, 1651.12, 1546.96, 1516.10, 1462.09, 1425.44, 1386.86, 1342.50, 1315.50, 1251.84, 1049.31, and 848.71 cm^{-1} respectively were observed for WAS treated with 0.025M $AgNO_3$ (Fig 8.). WAS treated with 0.05M $AgNO_3$ revealed peaks at 3419.90, 2924.18, 2854.74, 1828.58, 1741.78, 1653.05, 1543.10, 1523.82, 1456.30, 1421.58, 1390.72, 1340.57, 1157.33, 1076.32 and 850.64 cm^{-1} respectively (Fig 9.). Peaks were observed at 3421.83, 2924.18, 2854.74, 1836.29, 1741.78, 1653.05, 1548.89, 1516.10, 1456.30, 1383.01, 1244.13, 1155.40, 1076.32 and 850.64 cm^{-1} respectively for WAS treated with 0.1M $AgNO_3$ solution (Fig 10.).

The peaks for untreated WAS were at 3336.96 cm^{-1} which corresponds to O−H stretch, 2924.18 cm^{-1} due to C−H stretch, 1741.78 cm^{-1} due to C═O stretch, 1654.98 cm^{-1} and 1545.03 cm^{-1} due to the presence of amide group, 1240.27 cm^{-1} due to polysaccharides, 1060.88 cm^{-1} due to C−O stretch and 848.71 cm^{-1} due to aromatic bending (Fig 7.). $AgNO_3$ showed major peaks at 2764.09 and 2733.22 cm^{-1} which corresponds to C−H stretch, 1384.94 cm^{-1} due to N═O stretch in $O-NO_2$ (Fig 11.).

The peak at 1384.94 cm^{-1} seen for $AgNO_3$ disappeared in the WAS treated with 0.01M, 0.025M and 0.05M $AgNO_3$. Absence of the peak at 1384.94 cm^{-1} for the treatments clearly indicates the reduction of $AgNO_3$ and formation of silver nanoparticles. Whereas for the WAS treated with 0.1M $AgNO_3$ peak was observed at 1383.01 cm^{-1} which corresponds to the appearance of $O-NO_2$ stretch. This can be due to the high concentration of $AgNO_3$ used. Thus the FTIR analysis reveals that the reduction of $AgNO_3$ to silver nanoparticles occurred due to the presence of polysaccharides and C−O group present in the WAS.

Purification of groundwater, surface water and industrial wastewater streams using nanomaterials is a subject of current curiosity. Dendrimers, zeolites, metal-containing nanoparticles, and carbonaceous nanomaterials are functional materials that are being evaluated for water purification. The broad range of physicochemical properties makes them particularly attractive as separation and reactive media for water purification. Already spherical aggregates of nanoparticles having similar size and shape to the resin beads are used in water purification as they are attractive as sorbents (Dhermendra *et al*., 2008). Microorganisms have been used to mediate nanoparticle synthesis, for example, the fungus *Aspergillus fumigatus produces* silver nanoparticles extracellularly (Kuber and D'Souza, 2006). There are reports of gold and silver nanoparticles production by other fungi and a number of bacterial species (Bhattacharya and Gupta, 2005). Silver (Ag) and silver compounds have been used as antimicrobial agents for coliform found in waste water (Jain and Pradeep, 2005). It is also found that the coliform bacteria treated with ultrasonic irradiation for short time period prior to silver nanoparticle treatment at low concentration, enhanced antibacterial effect.

Our preliminary study on the microalga, *P. subcapitata;* used for has shown that the alga has mediated the synthesis of silver nanoparticles. Future studies warrant the evaluation of the antimicrobial activity of silver nanoparticles produced using *Pseudokirchneriella subcapitata* against water- borne pathogens and their application in the area of water purification.

Acknowledgements

We are grateful to Dr. B. Vasantharaj David, Chairman, Scientific Advisory Board, IIBAT, for his valuable support during the study. We express our sincere thanks to the management, International Institute of Biotechnology and Toxicology (IIBAT) for giving the facilities in carrying out the work. Also, we extend our sincere thanks to Dr. Marimuthu Thiripura sundari (Department of analytical chemistry, IIBAT) and Dr. R. Rajarajeswari (QAU, IIBAT) for their valuable inputs during the study.

References

Arun, G, Ingale. and Chaudhari, A, N, 2013. Biogenic synthesis of nanoparticles and potential applications: An eco-friendly approach. *J Nanomed Nanotech* 4 : 2

Barwal, I. Ranjan, P. Kateriya, S. Yadav, S, C. 2011. Cellular oxido-reductive proteins of *Chlamydomonas reinhardtii* control the biosynthesis of silver nanoparticles. *J Nanobiotech* 9 : 56

Bhattacharya, D. and Gupta, R, K. 2005. Nanotechnology and potential of microorganisms. *Crit Rev Biotechnol* 25:199-204

Chandran, S, P. Chaudhary, M. Pasricha, R. Ahmad, A. Sastry, M. 2006. Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract. *Biotechnol Prog* 22: 577-583

Dhermendra, K, T. Behari, J. and Prasenjit, S. 2008. Application of Nanoparticles in Waste Water Treatment. *World Appl Sci J* 3 : 417-433

Henglein, A. 1993. Physicochemical properties of small metal particles in solution: “microelectrode” reactions, chemisorptions, composite metal particles, and the atom-to-metal transition. *J Phys Chem* 97 : 5457-5471

Huang, J. Li, Q. Sun, D. Lu, Y. Su, Y. *et al*., 2007. Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf. *Nanotechnol* 18 : 11-15

Jain, P. and Pradeep, T. 2005. Potential of silver nanoparticle-coated polyurethane foam as an antibacterial water filter. *Biotech Bioeng* 90: 59-63

Jayashree, J. Nilotpala, P. Bisnu, Prasad, D. Lala, Behari, S. Prasanna, Kumar, P. 2013. Biosynthesis and characterization of silver nanoparticles using microalgae *Chlorococcum Humicola* and its antibacterial activity. *Int J Nanomater Bios* 3: 1-8

Kajori D and Padma T, 2012. Mycobiosynthesis of metal nanoparticles. *Int J nanotech Nanosci* 1 : 1-10

Klaus, T. Joerger, R. Olsson, E. Granqvist, C, G. 1999. Silver based Crystalline nanoparticles, microbially fabricated. *Proc Natl Acad Sci U.S.A* 96 : 13611-13614

Kuber, C, B. D’Souza, S, F. 2006. Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids Surf B Interface* 47: 160-164

Kumar, P. Senthamil, Selvi, S. Govindaraju, M. 2012. Seaweed-mediated biosynthesis of silver nanoparticles using Gracilaria corticata for its antifungal activity against Candida spp. *Appl Nanosci* 3: 495–500

Kumar, P. Senthamilselvi, S. Lakshmipraba, A. Premkumar, K. Muthukumar, R. Visvanathan, P. Ganeshkumar, R, S. Govindaraju, M. 2012a. Efficacy of biosynthesized silver nanoparticles using *Acanthophora spicifera* to encumber biofilm formation. *Dig J Nanomater Bios* **7** : 511-522

Mahdi, M. Mohsen, B. Habib, Z. Siamak, A. Dariush, S. 2011. Potential of microalgae and lactobacilli in biosynthesis of silver nanoparticles. *B.I.* 1 (3): 149-152

Sadowski, Z. Maliszewska, I, H. Grochowalska, B. Polowczyk, I. Kozlecki, T. 2008. Synthesis of silver nanoparticles using microorganisms. *J Mater Sci*-Poland 26 : 419-424

Sathishkumar, M. Sneha, K. Won, S, W. Cho, C, W. Kim, S. Yun, Y, S. 2009. *Cinnamon zeylanicum* bark extract and powder mediated green synthesis of nano-crystalline silver particles and its bacterial activity. *Colloids Surf B Interface* 73: 332-338

Shankar, S, S. Rai, A. Ahmad, A. Sastry, M. 2004. Biosynthesis of silver and gold nanoparticles from extracts of different parts of the Geranium plant. *Appl Nanotechnol* 1: 69-77

Singh, M. Kalaivani, R. Manikandan, S. Sangeetha, N. Kumaraguru, A, K. 2013. Facile green synthesis of variable metallic gold nanoparticle using Padina gymnospora, a brown marine macroalga. *Appl Nanosci* 3: 145–151

Thakkar, K, N. Mhatre, S, S. Parikh, R, Y. 2010. Biological synthesis of metallic nanoparticles. *Nanomed: Nanotechnol Biol Med* 6 : 257-262

Xie, J. Lee, J, Y. Wang, D, I, C. Ting, Y, P. 2007a. Silver nanoplates: from Biological to Biomimetic Synthesis. *ACS nano* 1: 429-439