

## SYNTHESIZE AND CHARACTERIZATION OF SILVER NANOPARTICLES USING MANGROVE HUMIC ACIDS AND THEIR ROLE AS CATALYST IN THE DEGRADATION OF ORGANIC DYES

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### ABSTRACT

The present study focuses on the isolation and characterisation of humic acid isolated from tropical mangrove sediments from the south west coast of India and their use in the formation of self-assembled humic –silver supramolecules. The silver nanoparticles (AgNPs) with higher stability were prepared in a completely green and cost effective manner using aqueous extract of humic acids synthesised using standard procedure IHSS 2010. The humic acids were extracted from the mangrove sediments collected from Mangalavanam bird sanctuary (Kochi) in April 2018. Silver nanoparticles were synthesised by the reduction of silver nitrate in the presence of humic acids (HA) which acted as capping agents. The characterization studies were carried out using by FT-IR, UV-Visible spectroscopy and HR-TEM. The high crystallinity of the biogenic AgNPs was confirmed from clear lattice fringes in the HR-TEM image, bright circular spots in the SAED pattern and tuning of dipole and quadrapole oscillation (silver nanoparticles size of 5 nm). The study also highlights the spectroscopic investigation on the catalytic efficacy of the biosynthesized AgNPs in the reduction reactions of hazardous organic dyes like Congo red and Methylene blue using sodium borohydride.

**KEY WORDS** : Nanoparticles, Mangroves, Humic acids, Catalytic efficiency

### INTRODUCTION

Recent advances in the field of green synthesis of nanoparticles using biological resources and its various applications have led to learn about unexploited resources. Organic soils contain high levels of humic substances and it is the decomposition product of plants, animals and microorganisms. Humic substances are complex molecules that exist naturally in sediments which often serve as a prominent reservoir of organic carbon. These are heterogeneous compounds having high molecular weight and are “supra molecular structures” (Fisher, 2017). The complex

formation is attributed to the presence of oxygen rich functional groups like carboxyl, carbonyl and hydroxyl groups as well as hydrophobic aliphatic and aromatic groups. Humic substances of different origins differ in composition and chemical structure. The polyelectrolyte nature and reducing property of humic acid is because of the polar functional groups such as phenols, hydroxyls, carbohydrate subunit and the presence of carboxylic acids (Bauer *et al.*, 2007; Prashob Peter *et al.*, 2014). Nanoparticles possess variable size and morphology and it shows different catalytic ability and can be commercialised as adsorbents on industrial scale.

The Green synthesis of metallic nanoparticles

provides innovative scientific solutions and reduces the negative effects of hazardous substances on human and the environment health. (Nadagouda *et al.*, 2008). Synthesis of silver nanoparticles by green synthesis has lot of advantages over other conventional methods which eliminates the use of toxic chemicals (Joseph *et al.*, 2014). Silver nanoparticles from various natural compounds are currently applied in pollution research, medicine, catalysis, optics, electronics and sensing. There is an increasing interest to develop cheap, non-toxic and environment friendly procedures for anisotropic nanoparticle synthesis from naturally occurring humic substances. In this study, the synthesis of silver nanoparticles was achieved by means of chemical reduction of silver ions by sodium borohydride in the presence of humic acid. Here Humic acted as a capping agent due to its chelating potential towards metals and metallic ions. The interaction of amphiphilic humic acids with metals results in the formation of self-assembled structures (Wandruszka *et al.*, 1997; Prashob Peter *et al.*, 2014).

Due to plasmonic properties, silver and gold nanoparticles are increasingly used in optical sensing, biomedicine and environmental applications (Lu and Lieber, 2007). Humic acids can be used to synthesize metal nanoparticles because of their self-assembling nature and reduction potential. Mud bank sediment was used to isolate humic acid, which was used in the synthesis of AgNPs (Prashob Peter *et al.*, 2014). Dyes are major group of synthetic organic compounds used in a variety of applications. Synthetic dyes such as Congo red and Methylene blue is widely used in chemical industries such as textile industries, paper and pulp mills etc. discharge into the water bodies. These are highly hazardous to the aquatic organisms. Recent studies revealed that the nanoparticles can act as catalyst on these synthetic dyes through catalytic degradation (Wilhelm *et al.*, 2007; Francis *et al.*, 2018). The catalytic degradation of synthetic dyes using AgNPs synthesised by using seaweeds was reported earlier (Princy and Anu Gopinath, 2018).

So, the present study focuses on the preparation and characterisation AgNPs from humic acids isolated from mangrove sediments of Kerala, south west coast of India. The catalytic efficiency of the synthesized AgNPs was optimized for the degradation of hazardous organic dyes like Congo red and Methylene blue using Sodium Borohydride as the reducing agent.

## MATERIALS AND METHODS

### Description of the study area

Mangrove sediments were collected from Mangalavanam bird sanctuary (mangrove forest) situated in Kerala, south –west coast of India. The study area is shown in Figure 1. It is an ecologically sensitive area of 2.74 hectares situated in the centre of Kochi located between 9° 59' 133" N latitude and 76° 16' 26" E longitude. This mangrove forest region attract a number of exotic and rare varieties of migratory birds and the system has lot of organic inputs. The dominant mangrove species existing in this area are *Avicenniaofficialis*, *Acrostichumaureum*, *Rhizophoramucronata* and *Acanthus ilicifolius*. These are protected mangroves in the closed area which is connected to Kochi backwaters. It is often regarded as the "Green lung of Kochi" (Anuradha *et al.*, 2011).

Sediment samples were collected using an acid washed metallic spatula and stored in glass bottles and stored at -20 °C till analysed. The samples were freeze dried and preserved and this was used for the isolation of humic acids.

### Extraction and characterization of humic acids

The IHSS 2010 method was used for humic acid extraction. Sieved sediment samples were air dried and washed with 1000ml 1N HCl and the settled sediment was then treated with 1N NaOH. This alkaline sample was shaken for 24 hours and the pH was adjusted to 2 by adding 6M HCl. The precipitate was freeze dried and used for further analysis. The humic acids were characterized using UV-Visible, FT-IR, <sup>1</sup>HNMR spectroscopic methods followed by elemental analysis. The elemental compositions(C, H, N, S) of HA s was analysed on an ash-free basis with the help of the CHNS Elemental analyser of (ElementarVario ELIII). The oxygen percentage was calculated using the formula 100-(C%+H%+N%+S %). The E4/E6 ratio was determined using UV-Visible spectroscopic analysis (Thermo Fischer Model No 117). Humic acids (2 mg) was dissolved in 10ml, 0.05 N Sodium bicarbonate of P<sup>H</sup>8 and the optical densities at 465 and 665 nm was observed and then the E4/E6 ratio was determined (Oskar Purmalis *et al.*, 2013, Chen *et al.*, 1977).

Fourier Transform Infrared (FTIR) spectra of the samples were determined using Avatar 370 FTIR spectrophotometer. The dried HA sample was mixed with 400mg spectroscopic grade KBr and

samples were loaded into the instrument.  $^1\text{H}$ NMR spectra was assessed by using TMS and the signals were determined with Bruker Advance 400 MHz NMR spectrometer fitted with QNP  $^1\text{H}$  probe.

### Preparation of Silver nanoparticles

For the preparation of silver nanoparticles, the aqueous humic acid was mixed with 1mM  $\text{AgNO}_3$  solution, shaken well and kept at room temperature in the dark condition. The formation of silver nanoparticles is indicated by the appearance of reddish brown colour. The AgNPs thus obtained were characterized using Thermo scientific Evolution 201 UV-Visible spectrophotometer operating at a resolution of 1 nm in the wavelength range of 300-700 nm. Further characterization of AgNPs was done using FT-IR, HR-TEM analysis. The shape and distribution of HA-AgNPs was determined by using HR-TEM (JOEL 3010) analyzer.

### Catalytic activity

The catalytic efficacy of the biosynthesized AgNPs at optimum conditions was studied for the degradation of hazardous dyes like Congo red and Methylene blue with the application of  $\text{NaBH}_4$  by following the protocol formulated by Gangula *et al.*, 2011. For this 2mM aqueous dye and 0.03 M of freshly prepared  $\text{NaBH}_4$  solutions were added to the biosynthesized AgNPs and the degradation was monitored periodically using UV-Visible spectrometer (Thermo Fischer Model No 117).

## RESULTS AND DISCUSSION

### Characterization of Humic acids

The humic acids were characterized by using UV-Visible spectral studies, Fourier-Transform Infrared Spectroscopy (FT-IR), Proton Nuclear Magnetic Resonance ( $^1\text{H}$ NMR) spectroscopic techniques and CHNS analysis for the elemental content.

### Elemental analysis and E4/E6 ratio

The elemental compositions such as C, H, N, S and O of extracted humic acids were obtained and the ratios such as H/C %, N/C % and O/C % were calculated (Table 1). In this study, the humic acids extracted from mangrove sediments showed higher hydrogen content i. e. 9.76 % depicting higher aliphaticity in their structure. The Carbon content was 54.03% and the sulphur content attached to the humic acid was 2.6%.

The percentages of H/C, O/C and N/C ratios were calculated as 0.18, 0.53, 0.08 respectively and it indicated the structure and molecular shape of the HS (Rigobello *et al.*, 2017). An increase in hydrogen content indicates the predominance of aliphatic carbons ( $\text{CH}_2$ ) than aromatic carbons ( $\text{C}=\text{C}$ ) (Traina *et al.*, 1990). The presence of excess organic sulphur content in humic acids depicts the presence of sulphate-reducing conditions (Christenson, 1989). The oxygen content in humic acids was 29.01% which can be attributed to the polar functional group attached to the humic acid. The isolated humic acid showed lower H/C atomic ratio (0.18%), which signifies more aromatic fractions. The O/C atomic ratio is associated with the carbohydrate and carboxylic acid content (aromatic and aliphatic) as well as degree of oxidation of the HS. The lower values of this ratio (0.53%) suggest a higher degree of humification due to reduction in carbohydrate content in the O-containing structures (polysaccharides). The occurrence of low H/C ratio is indicative of large amount of unsaturated structures. The low N/C (0.085%) ratio indicates the presence of lignin mineralization.

The E4/E6 ratio (ratio of the absorbance at 465nm and at 665 nm) has been widely used to study aromaticity and degree of humification. In this study the E4/E6 ratio obtained was 11.5 showing high degree of aromaticity/aliphaticity). The E4/E6 ratio (condensation degrees) help to determine the

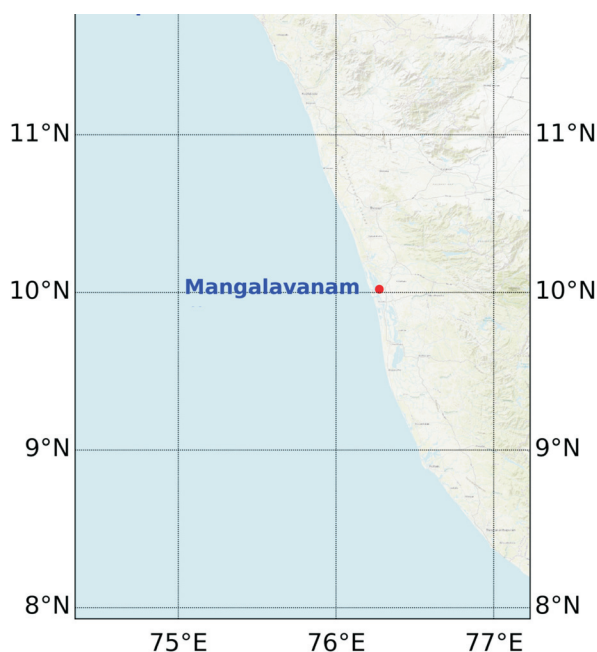
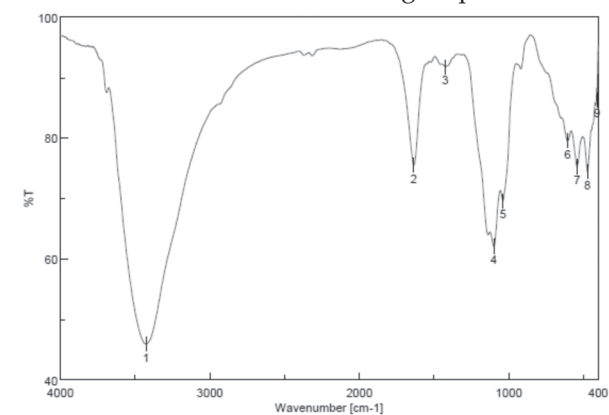


Fig. 1. Map showing the Study area

rigidity of the aromatic rings in humic acids (aromaticity), molecular weight, acidic nature and particle size (Chen *et al.*, 1977; Uyguner and Bekbolet, 2004). This ratio is characteristic of humic acids of varied origin and is a humification indicator. The E4/E6 ratio i.e., a decrease in absorptivity with an increase in wavelength obtained here is influenced by the varying content of aliphatic and aromatic fragments present in the humic acids isolated. From this study, the E4/E6 ratio of the humic acid sample is 11.5 nm which are in the higher side of the range (3-5.9) (Chen *et al.*, 1977). This indicates the presence of aromatic core replenished with aliphatic content and higher humification. (Yavmetdinov *et al.*, 2003).

#### Fourier –transform Infrared Spectroscopy (FT-IR) for the Characterization of isolated Humic Acid

The FT-IR spectrum indicated the existence of polar functional groups attached to humic acid. The FT-IR spectrums of humic acid isolated from the sediment sample are shown in figure 2. broad The presence of a broad peak at  $3426\text{ cm}^{-1}$  corresponds to  $-\text{OH}$  stretching frequency mentioning the type of bond as that of alcohol, carboxylic acids and phenol and minor part of N-H stretching vibrations of amide/amine (Samioes *et al.*, 2007; Nuzzo *et al.*, 2013). Here a well dissolved sharp peak is obtained at  $1634\text{ cm}^{-1}$  (Fig. 2) which corresponds to the stretching vibration of C=C aromatic bonds, conjugated carbonyl groups (quinone), carboxylic asymmetric stretching and amide groups (Vergnoux *et al.*, 2011). The peak  $1423\text{ cm}^{-1}$  (Fig. 2) indicates OH deformation and C-O stretching of phenol OH



| Result of Peak Picking |          |           | [Comment] |          |           |
|------------------------|----------|-----------|-----------|----------|-----------|
| No.                    | Position | Intensity | No.       | Position | Intensity |
| 1                      | 3426.89  | 45.9393   | 2         | 1634.38  | 75.5292   |
| 3                      | 1423.21  | 91.7753   | 4         | 1099.23  | 62.0803   |
| 5                      | 1038.48  | 69.6174   | 6         | 806.503  | 79.549    |
| 7                      | 541.899  | 75.2805   | 8         | 472.474  | 74.4253   |
| 9                      | 407.871  | 86.3746   |           |          |           |

Fig. 2. FTIR spectrum of humic acid isolated from the study area

(Enev *et al.*, 2014). The region of peaks  $1038\text{ cm}^{-1}$  and  $1099\text{ cm}^{-1}$  could be attributed to the C-O stretching of phenol, alcohol or OH deformation of COOH (Gracia *et al.*, 2016). Thus, the FTIR study showed the presence of hydroxyl, methyl, methylene, carbonyl, carboxyl, phenol, alcohol and amide groups in the humic substances obtained from the sampling site.

#### Proton Nuclear Magnetic Resonance ( $^1\text{H}$ NMR) studies of the isolated humic Acid

NMR studies (Fig. 3) indicate the polyelectrolytic and amphiphilic nature of humic acid, revealing the presence of oxygenated functional groups (Conte *et al.*, 2006 ; Prasob Peter *et al.*, 2012).

NMR signal in the range 0.82 ppm indicates the terminal methyl group of alkyl chain (Fransioso *et al.*, 2001; Graham *et al.*, 2002). Chemical shift value ranging from 1.12 to 1.48 indicates the presence of methyl groups of highly branched aliphatic structures (Fransioso *et al.*, 2001; Graham *et al.*, 2002). Chemical shift value at 2.18 ppm indicate the presence of aliphatic proton attached to carbon atom adjacent to highly electronegative atoms (carbon is next to C=O) and also the presence of unsaturated groups (Qi *et al.*, 2004; Fransioso *et al.*, 2001; Graham *et al.*, 2002). The resonance range at 3.8 indicates the presence of protons attached to alpha carbon to methyl group which may be  $-\text{CHOH}$  and  $\text{CH}_2\text{OH}$  functional groups (Yasuda *et al.*, 1999; Kingery *et al.*, 2000; Fransioso *et al.*, 2001). The chemical shift value in the range 7-7.3 ppm indicates the presence of aromatic OH group which can be of phenols and quinines (Jokica *et al.*, 2004). Chemical shift value at 8.244 ppm indicates the presence of hydrogen atom attached to C=O group. These results recognized the occurrence of deshielded protons and sterically hindered aromatic protons (Grasso *et al.*, 1990; Qi *et al.*, 2004).

#### Formation of AgNPs

The formation of Ag NPs was visually evident from the colour change of reaction mixture, i.e. within a few minutes a change in colour was observed from light yellow to stable brownish red colour and this indicated the formation of Ag NPs. The collective oscillation of free conduction electrons induced by an interacting electromagnetic field at the surface of NPs (Plasmon resonance (SPR) effect) gives the colour variation of Ag NP (Philip *et al.*, 2014). UV-Visible spectral analysis further confirmed the color variation. The formation of sharp peak at 420 nm



confirms the formation of AgNPs (Prashob Peter *et al.*, 2012; Princy and Anu Gopinath 2018).

### HR-TEM Characterization of AgNPs

Transmission Electron Microscopy (TEM) characterizes morphology of nanoparticles. TEM images showed that the biosynthesised nanoparticles were polydispersed and of spherical shape with an average size of  $17.24 \pm 6.5$  nm (Fig. 5). The face centred cubic crystal structure of the synthesised nanoparticles was confirmed from the clear lattice fringes in the High Resolution TEM images and XRD patterns.

### FTIR Characterization of AgNPs

The FT-IR spectrum of AgNPs (Fig. 6) shows a peak at  $3432\text{cm}^{-1}$  assigned to OH stretching of phenol and alcohol as well as hydrogen bonded N-H stretching (Sierra *et al.*, 2004). The asymmetrical or symmetrical C-H stretching bands were observed at  $2671\text{cm}^{-1}$  and  $2777\text{cm}^{-1}$  (Smid *et al.*, 2005). The well-developed band of C=O stretching of amide group or aromatic C=C stretching are observed in the region of  $1637\text{cm}^{-1}$  which attributed to the presence

of this particular peak (Smid *et al.*, 2005). The peak at  $1121.56\text{cm}^{-1}$  corresponds to  $\text{NH}_3^+$  rocking.

### Catalytic activity of HA-AgNPs in the reduction of Organic dyes

The high surface area to volume fraction and extremely small size of the nanoparticles contribute to its catalytic activity. The catalytic potential of AgNPs biosynthesised in optimum environment was used as a catalyst in the reduction of Congo red and Methylene blue using  $\text{NaBH}_4$  as reducing agent.

### Reduction of Congo red

Congo red is a non-biodegradable secondary diazo dye, applied in rubber, paper and textile industries. It is toxic in nature due to which degradation and eradication of its constituents is essential. The aqueous solution of Congo red is deep red in colour. Owing to transitions of the azogroup absorption peaks were obtained at 498 nm and 350 nm. (Farzaneh *et al.*, 2012; Princy and Anu Gopinath, 2018). Catalytic degradation of Congo red using  $\text{NaBH}_4$  yielded diphenyl and 4-aminonaphthalene-1-sulfonate.

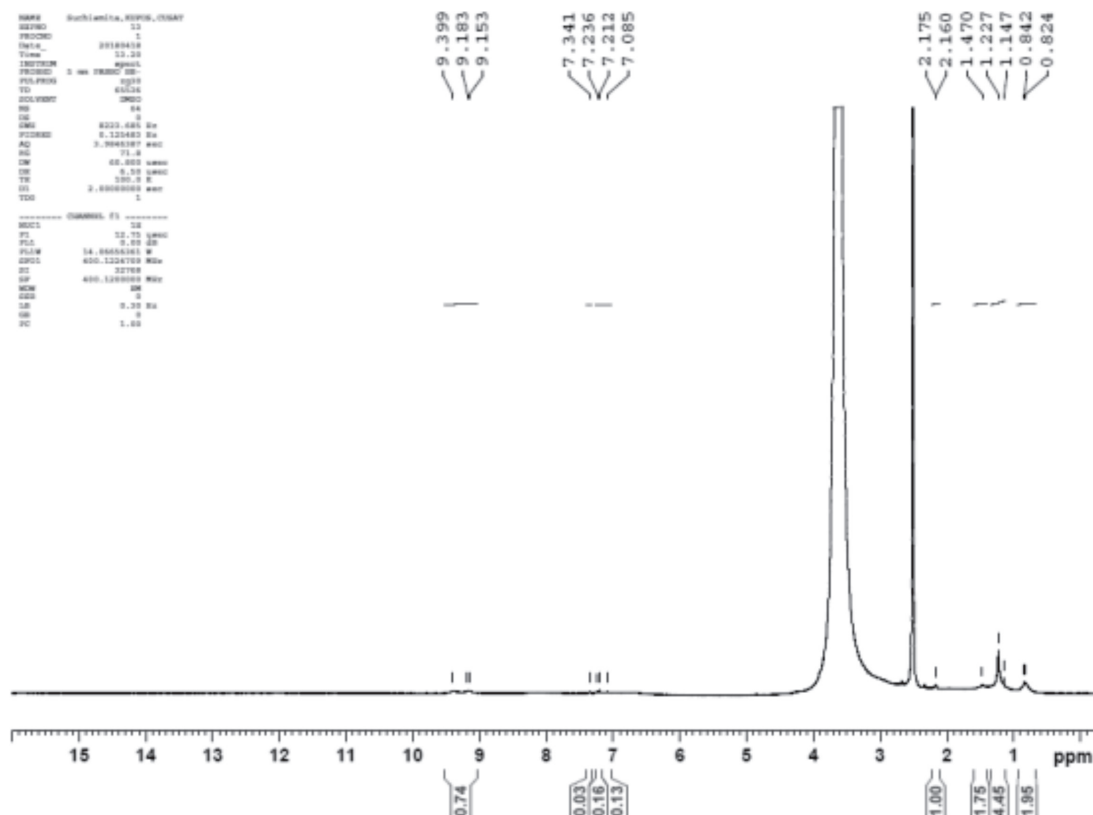


Fig. 3.  $^1\text{H}$ NMR spectrum of humic acid from the study area

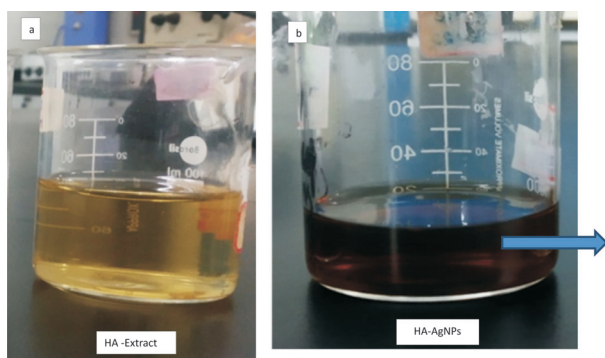


Fig. 4a. Humic acid Fig. 4b. Humic acid- AgNPs

The removal of Congo-red was experimentally demonstrated through a measured decrease in dye absorbance using UV-VIS spectrophotometer. The intensity of the peak at 498nm remained the same in the absence of anano catalyst indicating that Congo red reduction by  $\text{NaBH}_4$  is negligibly slow (Fig. 7a).

This is attributed to the substantial difference in

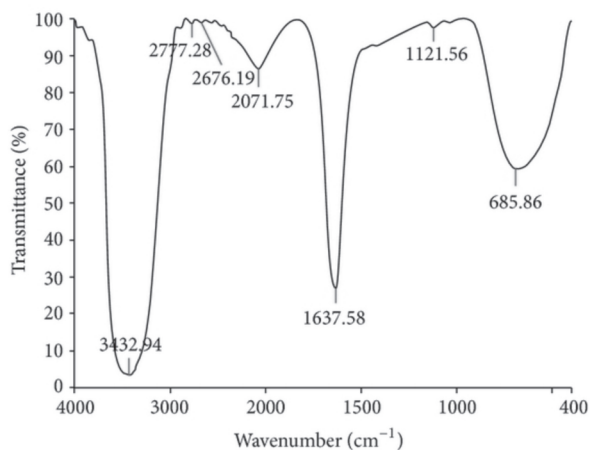


Fig. 6. FT- IR spectrum of AgNPs

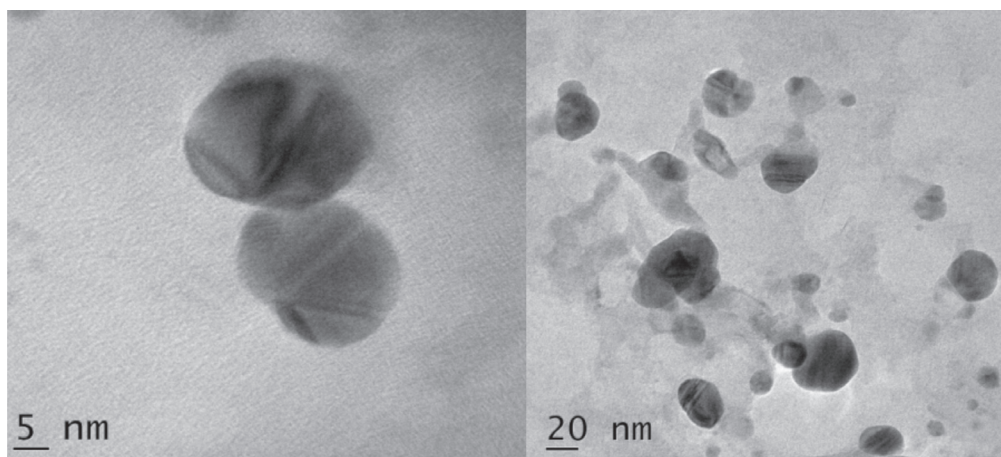


Fig. 5. HR TEM images of humic protected AgNPs with 5nm and 20 nm  
A representing spherical and polydisprsed nature respectively

redox potential between them. Thus this reaction is kinetically forbidden without a catalyst and could not be achieved merely with the reducing agent  $\text{NaBH}_4$ .

The degradation reaction began as soon as the Ag NPs were added as a catalyst. A new intense peak at 252 nm appears concomitantly which is due to the formation of colorless amino derivative as a result of the reduction of azo group ( $-\text{N}=\text{N}-$ ) of Congo red. (Francis *et al.*, 2018). The degradation was complete in 10 minutes (2mM catalyst) (Fig. 7a) and 4 minutes (1mM catalyst). (Fig. 7b). Evidence for the reaction was seen in the complete disappearance of the red color of the dye. Chemically the Congo red (toxic dye) is degraded to form primary amine (Princy and Anu Gopinath, 2018).

### Reduction of Methylene blue

Methylene blue, a cationic thiazine dye, has become a predominant dye in the textile industry. It is applied in several fields of chemistry and biology. It is commonly used as a redox indicator in analytical chemistry. It serves as a peroxide generator, an antimalarial agent and is used also in sulphide analysis (Small *et al.*, 2007). Accidental consumption leads to vomiting, nausea, diarrhoea and breathing problems (Kavitha *et al.*, 2007). The aqueous solution of Methylene blue is deep blue in colour and its UV-visible spectrum (Fig. 8a)

displays one clear band at around 662 nm that corresponds to the  $n \rightarrow \pi^*$  transitions (Joseph *et al.*, 2015). The presence of biosynthesized nanocatalysts causes the intensity of absorption at 662 nm to diminish progressively with time indicating a strong reduction of Methylene blue. The total

degradation of Methylene blue to leuco methylene blue was attained within 6 min for AgNPs (2mM) catalysed reaction (Fig.8a). Comparatively it took only 3 min for AgNPs (1mM) to catalyse the degradation (Fig.8b) which was indicated by the absolute disappearance of deep blue colour of their action mixture.

Biodegradation reaction depends upon the concentration of the catalyst. In both the reactions silver nanoparticles act as electron transfer mediator between the dye and  $\text{NaBH}_4$ . The catalytic reduction proceeds by an electron relay effect (Xu *et al.*, 2017; Joseph *et al.*, 2012). When reactants adsorb on the surface of the nanoparticles, the electrons from  $\text{BH}_4^-$  transfers to the dye component. Thus silver nanoparticles serve as an efficient catalyst in the reaction which is confirmed by the results obtained using UV-Visible spectroscopy.

## CONCLUSION

The application of green synthesis method is environmental friendly and safe. Hence the synthesis of silver nanoparticles using the humic acids isolated from mangrove sediments is a completely green synthetic method. The green synthesized silver nanoparticles were characterized using UV-Visible, FTIR and HR-TEM techniques. The formation of AgNPs was visually observed from the colour change from light yellow to stable brownish red colour. TEM images showed that the biosynthesised nanoparticles were polydispersed and of spherical shape with an average size of 17.24 nm. Because of the high complexity and the heterogeneity of the humic acid the exact structure could not be well defined but FTIR,  $^1\text{H}$ NMR studies proved the presence of hydroxyl, methyl,

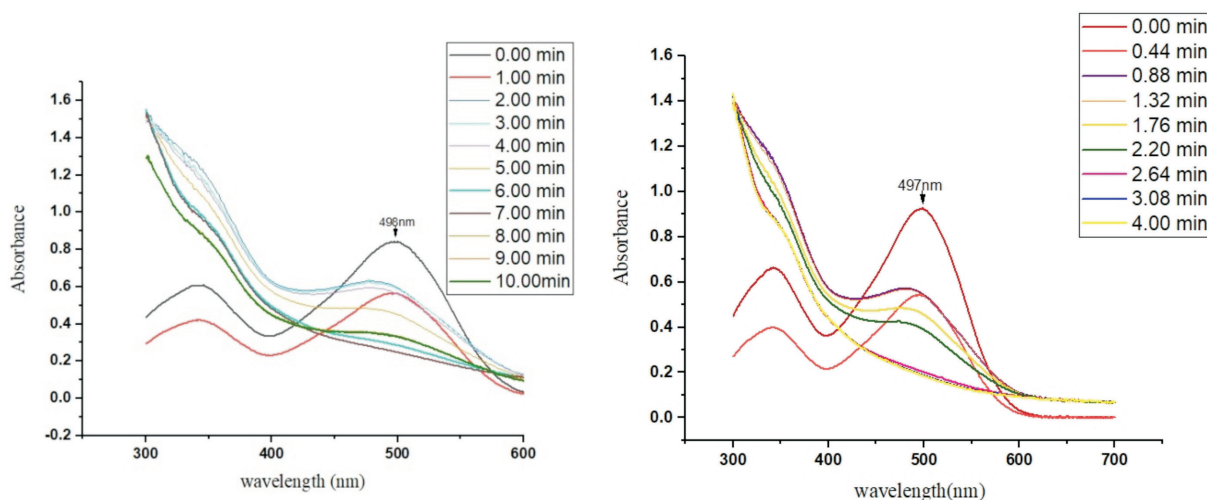


Fig. 7. Catalytic degradation of AgNPs with Congo red: a)2mM b) 1mM

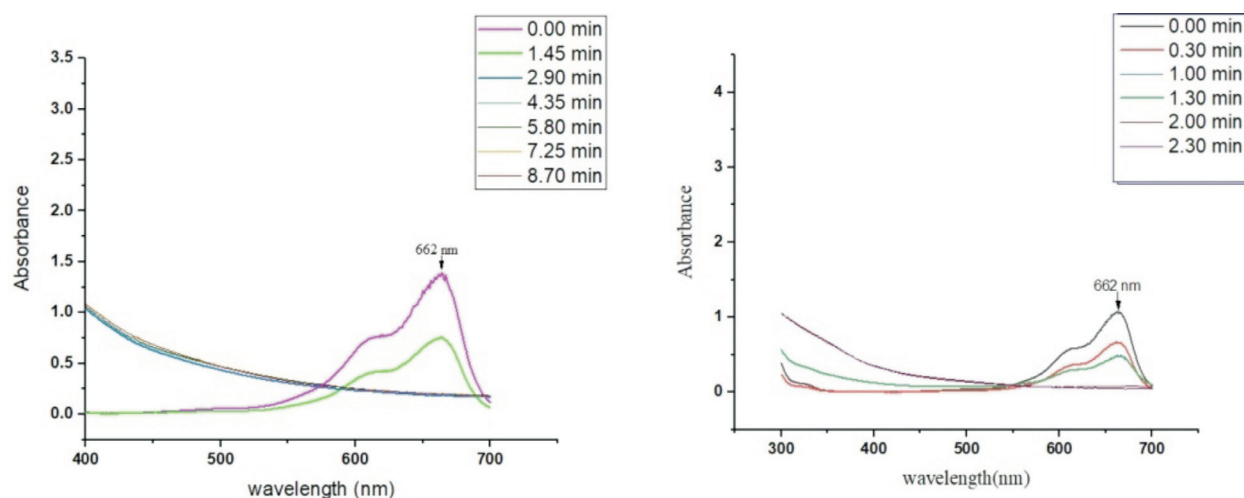


Fig. 8. Catalytic degradation of AgNPs with Methylene blue: a) 2Mm b) 1Mm

methylene, carbonyl, carboxyl, phenol, alcohol and amide groups in the humic substances obtained from the mangrove sediment.

The catalytic studies of the AgNPs showed that these are excellent catalysts for the rapid, economic and effective degradation of a wide spectrum of hazardous organic pollutants like Congo red and Methylene blue using  $\text{NaBH}_4$  as the reductant, which was indicated by the absolute disappearance of deep blue colour of their action mixture. The degradation of Congo red occurred in 4 minutes with a concentration of 1 mM AgNPS, whereas it took 3 minutes (1 mM concentration) for the complete degradation of Methylene blue. This was visually observed with the absolute disappearance of deep colour and was confirmed using UV Visible Spectroscopy.

These biodegradation reactions depended upon the concentration of the catalyst. It was noticed that the degradation occurs more rapidly with a concentration of 1 mM concentration of the catalyst (AgNPs) for both Congo red and Methylene blue. This strongly supports the characteristics property of nanoparticles. In both the reactions silver nanoparticles act as electron transfer mediator between the dye and  $\text{NaBH}_4$  (reductant). Thus the humic acid medicated biosynthesized silver nanoparticles act as effective ecofriendly catalyst in the degradation of toxic chemicals, hence act as promising materials in the prevention of environmental pollution.

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