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Camellia sinensis mediated synthesis of Iron nanoparticles and its encapsulation for decolorization of dyes

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ABSTRACT

Textile industry has been condemned as one of the world's worst offenders in terms of pollution because 10-15% of all the dyes used in the industry are lost within wastewater during processing which is degrading the environment. Owing to the complex nature of synthetic dyes, conventional biological treatment methods are ineffective. Hence there exist an urgent need to develop novel treatment techniques that lead to the degradation of dye molecules from waste stream. The current study focused to phytosynthesize the Iron nanoparticles using *Camellia sinensis* leaf extract, its encapsulation in alginate beads for repeated use and to use it to degrade Methyl red and Methyl orange dye in an effective way. UV-visible spectra showed the maximum absorbance of 270 nm due to the excitation of surface plasmon vibrations in the FeNPs. FTIR spectrum exhibited the characteristic band at 543.63 cm^{-1} which indicated the Fe-O stretching of Fe_2O_3 nanoparticles. The XRD spectrum showed three different diffraction peaks (°2θ values) corresponding to the crystal planes of crystalline Fe_2O_3. The average particle size of the synthesized FeNPs was 44 nm using NTA analysis. TEM study revealed that the particles were predominantly spherical in shape. The results of this study show the successful degradation of Methyl red and Methyl orange dyes using synthesized FeNPs encapsulated in calcium alginate beads. The removal of methyl red after 5 hours is greater than that of methyl orange, with more than 50% of the dye being removed. Calcium alginate was used as a green support for immobilizing the nanoparticles.

KEYWORDS

Fe nanoparticles;
Encapsulation;
Camellia sinensis;
Dye degradation.

INTRODUCTION

Nanoscale iron particles are recently gaining great interest in environmental remediation circles. One of the prominent applications in this regard is the removal of organic and inorganic pollutants from aqueous solutions[3, 14]. Iron nanoparticles containing iron oxides and zero valent iron (ZVI) can be used as a Fenton-like catalyst for the degradation of aqueous organic solutes[13, 17]. The nano-scale size offers high surface area and high surface reactivity.

Green synthesis of iron nanoparticles is evolv-

ing as a method that would impart steric stabilization of iron nanoparticles against aggregation, and help to overcome the concerns related with the use of sodium borohydride as a reducing agent in routine synthesis reported so far. This chemical is known for its corrosiveness and flammability[4]. Recently, successful synthesis of iron nanoparticles utilizing green tea leaf and sorghum bran extracts have been reported[4, 8, 9].

In this work, iron nanoparticles were readily synthesized using green tea leaf extracts. Green tea is known to contain polyphenols that act both as a reducing agent and a capping agent. Iron nanoparticles which were synthesized using green tea extract were less susceptible to oxidation and were stable. Iron nanoparticles formed were encapsulated with alginate beads to provide resistance to changes in pH or temperature. The synthesized Iron nanoparticles encapsulated with alginate beads are used for degradation of dyes in this work.

The main objective of the current study is to test the applicability of green tea synthesized FeNPs as a catalyst in the removal of anionic (methyl orange and methyl red) model dyes. The synthesized FeNPs were characterized using UV-vis spectroscopy, NTA, XRD, FTIR and TEM. The decolorization was studied over a wide range of periods of contact of aqueous solutions of methyl red (MR) and methyl orange (MO). Methyl orange with the formula $C_{14}H_{14}N_3NaO_3S$ and methyl red with the formula $C_{15}H_{15}N_3O_2$ are anionic dyes which are usually chosen as one of the model azo dyes in literature. Azo dyes are a major class of synthetic, colored organic compounds that account for about half of the textile dye stuffs used today[7].

MATERIALS AND METHODS

Preparation of green tea extract

For preparing the extract, 1 gm dried leaves of green tea were added in 100 ml of distilled water and the beaker was kept on a heating mantle at 80°C for 20 minutes. Then it was filtered to obtain green tea extract.

Preparation of iron nanoparticles (FeNPs)

Figure 1 : Color change from yellow to black on synthesis of iron nanoparticles

5 ml of the leaf extract was added to 1mM $FeSO_4.7H_2O$ solution at pH5. During the synthesis of iron nanoparticles, both the aqueous leaf extract and the precursor salt solution were mixed in 1:5 proportions. After the addition of leaf extract to the salt solution, the color change from colorless to black (Figure 1) was noted. The reaction mixture was kept in shaking condition overnight to enhance the synthesis of nanoparticles. Next day the reaction mixture was centrifuged for 20 minutes at 8000 rpm. The supernatant was discarded and the pellets were repeatedly washed with distilled water.

Characterization of FeNPs

The synthesized FeNPs were characterized using UV-vis spectrophotometer, NTA, XRD, FTIR and TEM techniques. The bioreduction of iron nanoparticles was monitored by UV-visible absorption measurements at room temperature as function of time using Shimadzu UV 1700 spectrometer. 1mM of $FeSO_4.7H_2O$ solution was used for base line correction.

Nanoparticle tracking analysis (NTA) was done using NTA Model LM 20 from Nanosight UK for the visualization and analyses of nanoparticles for their particle size. NTA works on the principle of Brownian motion. NTA allows the determination of nanoparticle size distribution profile with diameter in the range of 10-1000nm in liquid suspension.

The completely bioreduced sample was concen-

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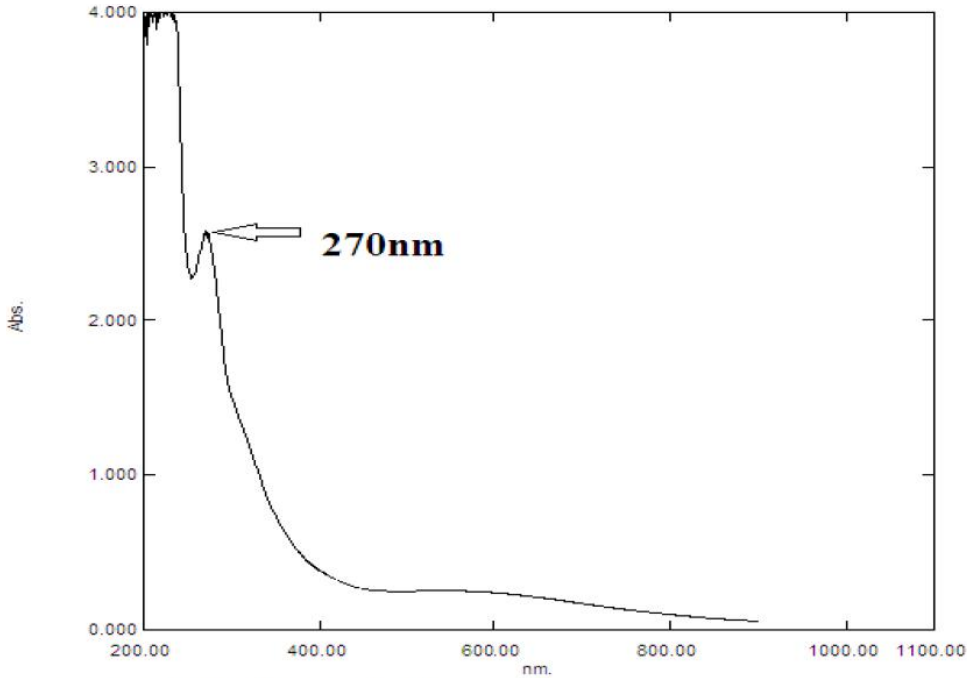

Figure 2 : UV-vis spectra of synthesized FeNPs with the peak at 270 nm

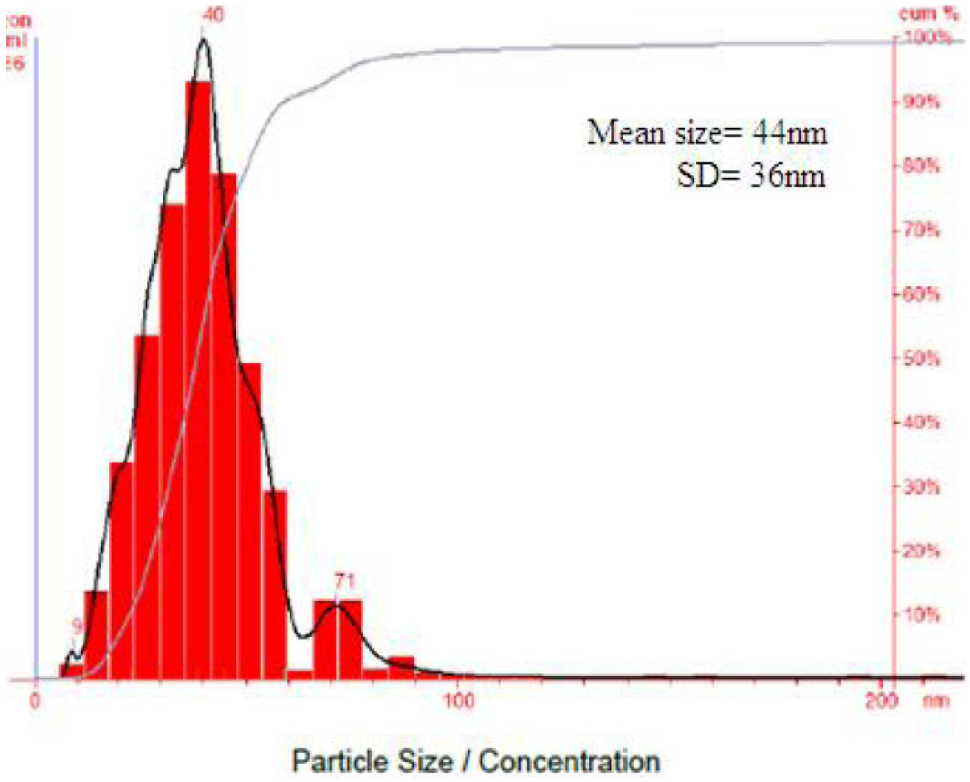

Figure 3 : Frequency of occurrence of varying sizes of synthesized FeNPs calculated using nanoparticle tracking analyzer

trated and then centrifuged at 8000 rpm for 20 minutes. The pellet obtained, was washed and re-dispersed in deionized water. The repeated centrifugation and re-dispersion in deionized water was carried out to remove the water soluble biomolecules such as proteins and secondary metabolites. XRD measurements of purified iron nanoparticle solution casted onto the glass substrate was carried out using

Rigaku Mini Flex Bench Top X-ray spectrophotometer instrument operating at a voltage of 40 kV and current of 20 Ma.

FTIR analysis was carried out for FeNPs using Shimadzu IR Affinity-1 (S/N ratio 30,000:1, 1-minute accumulation, neighborhood of 2,100cm^{-1}, peak-to-peak). The spectra were acquired by direct analysis of FeNPs. The maximum resolution of 0.5cm^{-1} was used for phytosynthesized iron nanoparticles. The scans recorded were the average of 150 scans, and the contribution of the background was accounted for. Each sample was measured in a transmission mode at a resolution of 4cm^{-1}.

The sample purified as stated in XRD measurement section was sonicated for 10 minutes. A drop of the sonicated solution was placed on carbon coated copper grid and later exposed to heating mantle (30 minutes) for solvent evaporation. TEM measurements were performed on FEI Tecnai T20 TEM instrument operated at an accelerating voltage of 200 kV with resolution of 0.22 nm at Tata Institute of Fundamental Research, Mumbai.

Preparation of alginate beads

3% of calcium chloride solution was prepared and refrigerated. 2% solution of sodium alginate was prepared and 50mg of iron nanoparticles were added to it. The sodium alginate and iron nanoparticle mixture was added drop by drop to the chilled calcium chloride solution. Due to the cross-linking between sodium alginate and calcium chloride, iron nanoparticles encapsulated within alginate beads were formed.

Preparation of dye

The aqueous solution of 1mM Methyl orange and Methyl red dyes (each) was prepared as a test sample in present experiment.

Decolorization experiments

All the decolorization experiments were carried out under atmospheric pressure, by shaking Erlenmeyer flasks containing 1mM dye solution and alginate encapsulated iron nanoparticles. The UV–vis absorbance readings were taken using a Shimadzu UV 1800 spectrophotometer at λmax = 520 nm for Methyl red and λmax = 465 nm for Methyl orange. In addition, parallel blank experiments were carried out under the same conditions, with just alginate beads without FeNPs.

The solutions after treatment were separated from the FeNPs by simple decantation. Subsequently, the UV–vis absorbance readings were obtained after different times of contact (30.0 min, 60.0 min, 120.0 min, 180.0 min, 240.0 min and 300.0 min). The absorbance of the prepared dye solutions and the effluents collected were measured before and after the degradation at different degradation times. Measurements were carried out using Shimadzu UV 1800 spectrophotometer in the photon energy range of wavelength from 200 to 600 nm. The color removal of the dye solution was measured at the λ maximum of the absorption spectrum of each dye. Decolorization efficiency was calculated from a mathematical equation adapted from measurements of decolorization. From the respective absorbances obtained, percentage color disappearance was calculated using the following:

$$\text{Decolorization \%} = \frac{(\text{Absorbance})_0 - (\text{Absorbance})_t}{(\text{Absorbance})_0} \times 100$$

Where (Absorbance) 0 is the absorbance before treatment and (Absorbance) t is the absorbance at time t.

Degradation of the dye solutions was confirmed with IR spectroscopy which involves collecting absorption information and analyzing it in the form of a spectrum. FTIR spectrum was used as a measure to confirm the degradation of dye solutions. Measurements were carried out using Shimadzu IR Affinity-1; FTIR reading were taken before treatment and after 240 minutes of treatment.

RESULTS AND DISCUSSION

Preparation and characterization of Iron nanoparticles

Phytosynthesis method was adopted to synthesize Fe nanoparticles. In this method, the tea leaf extract was mixed with 1mM $FeSO_4$ in 1:5 proportions at pH 5 and color change was observed from pale yellow to black indicating the formation of FeNPs (Figure 1). More or less similar type of change in color was noticed during the synthesis

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FeNPs in previous work using Neem plant leaves extract[11]. Change in color was due to excitation of surface plasmon resonance (SPR) which is characterized by UV-vis spectroscopy indicating formation FeNPs[16]. The formation of nanoscale FeNP at pH 5 was confirmed by UV, NTA and XRD analysis.

UV-vis spectroscopy

The bioreduction of Fe^{+3} in aqueous solutions was monitored by periodic sampling of aliquots of the mixture and subsequently measuring UV–Vis spectra. UV-Vis spectral analysis was done using Shimadzu UV 1700 spectrometer at the range of 200-900 nm and observed the absorption peaks at 270 nm region due to the excitation of surface plasmon vibrations in the iron nanoparticles, which are identical to the characteristics UV visible spectrum of metallic iron and it was recorded. The lambda maxima of synthesized iron oxide nanoparticles were quite similar to those reported for Fe_2O_3[6]. The room temperature absorption spectrum of the FeNP nanoparticles is shown in Figure-2.

NTA analysis for size distribution of FeNPs

The NTA analysis showed good sizing accuracy and relatively narrow distributions for all monodispersed samples. The measurement condition used for the analysis includes temperature: 22°C, viscosity: 0.95 cP, frames per second: 30, measurement time: 178 of 0 seconds and drift velocity: 0nm/s. NTA analysis of phytosynthesized FeNPs using green tea leaves extract revealed the synthesis of FeNPs. The size of synthesized FeNPs was recorded in the range of 9 to 80 nm with the mean size of 44 nm and standard deviation (SD) of 36 nm (Figure 3). The concentration of phytosynthesized FeNPs was found to be 4.9 x 10^7 particles/ ml.

X-ray diffraction (XRD)

The XRD spectrum showed different diffraction peaks with 2θ values of 31.5°, 47.3°, 56° and 67.5° corresponding to the crystal planes of (221), (410), (510) and (622) of crystalline Fe_2O_3. The obtained data matched with the Joint Committee on Powder Diffraction Standards (JCPDS) File No. (39-1346)[1]. The X-ray diffraction patterns obtained for the FeNPs synthesized using green tea leaves extract is shown in Figure-4.

FTIR spectroscopy

FTIR spectroscopy was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. Figure-5 shows FTIR spectra of FeNPs synthesized in the leaf extract of *Camellia sinensis*. After complete bioreduction of iron ions, the extract was centrifuged for 20 min to isolate the FeNPs from the compounds present in the solution.

The band at 1,121 cm^{-1} is assigned to the symmetric C–O vibration associated with a C–O–SO_3

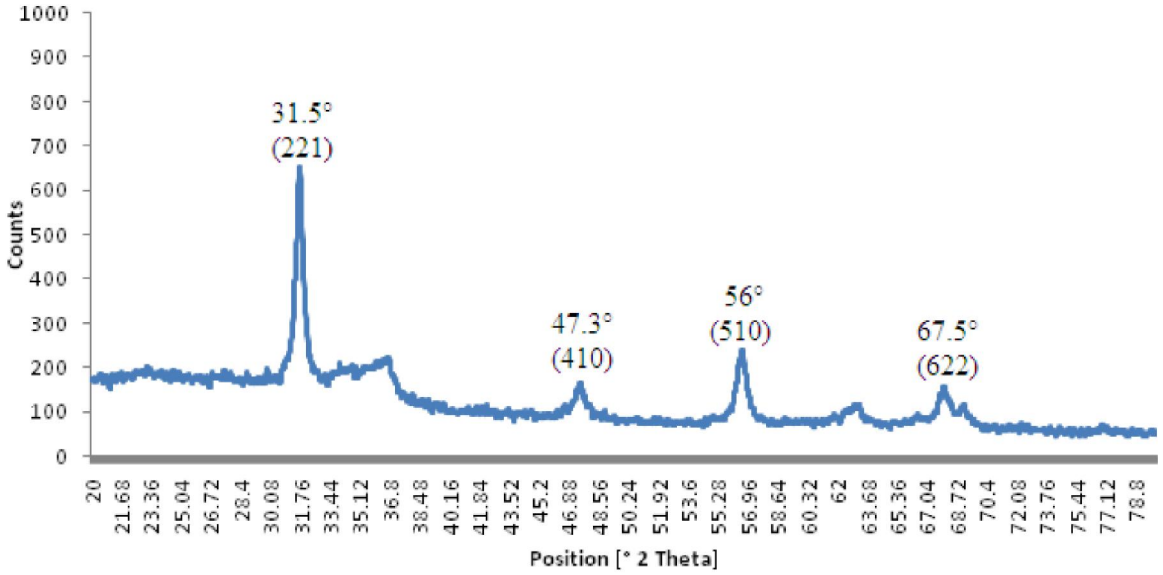

Figure 4 : XRD pattern of FeNPs synthesized using *Camellia sinensis* leaf extract

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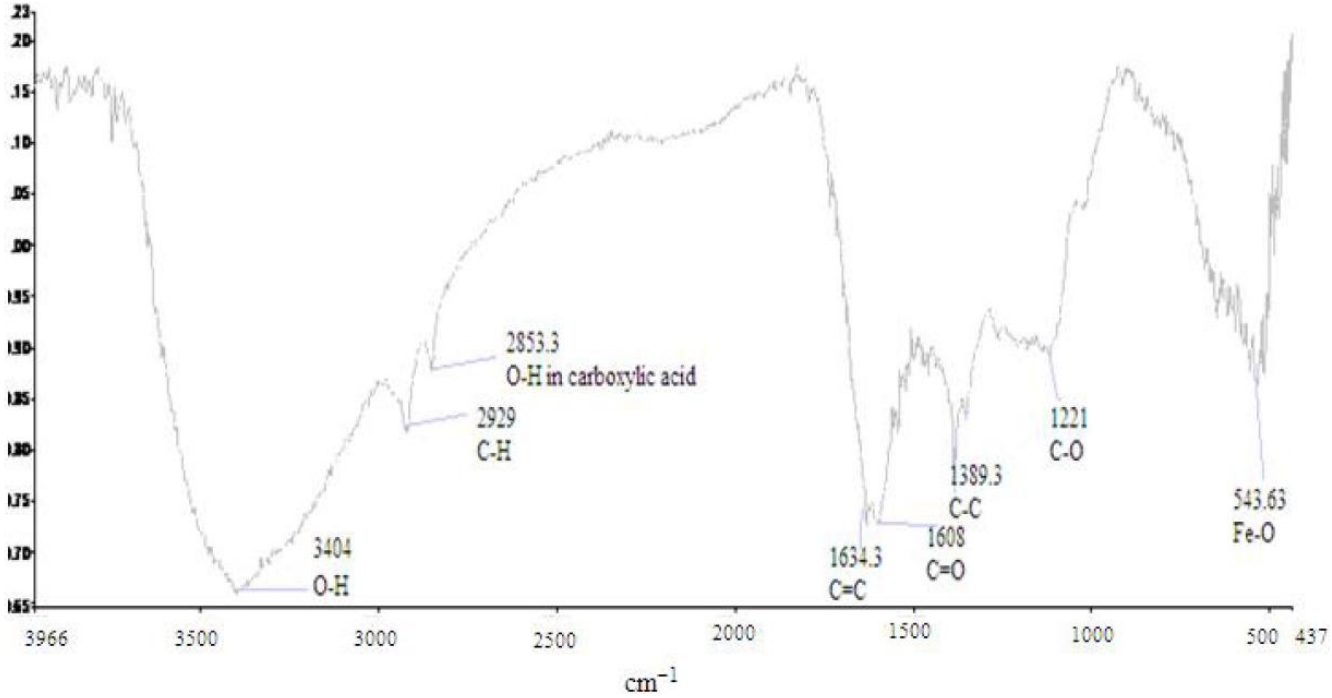

Figure 5 : FTIR spectra of phytosynthesized FeNPs

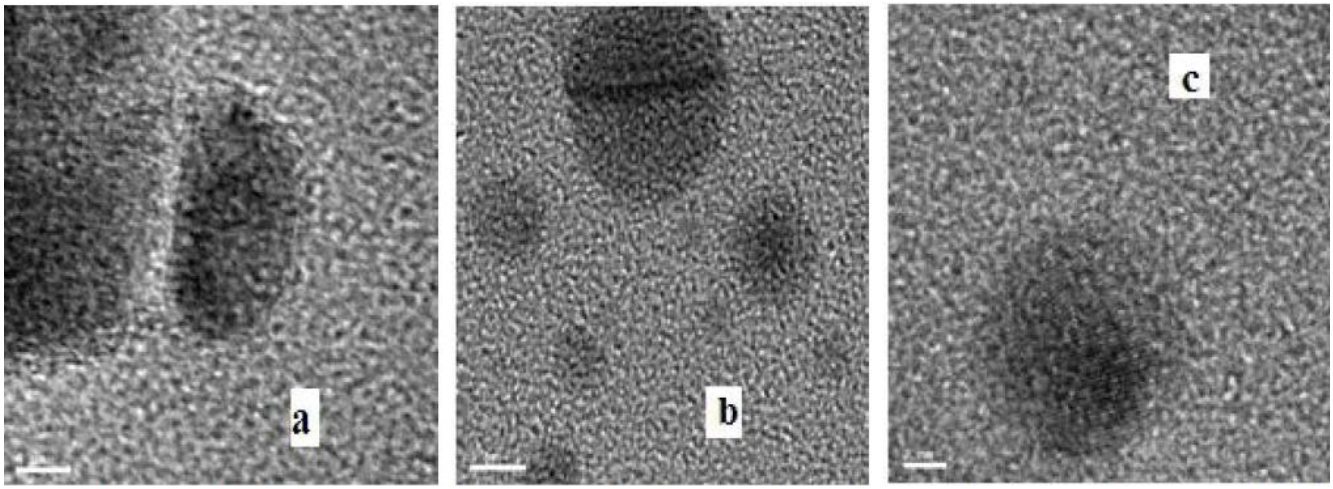

Figure 6 : TEM images of *Camellia sinensis* leaf extract mediated synthesized FeNPs

group[2]. In addition, signals at 3,404 cm^{-1} (O-H stretching) and 2,929 cm^{-1} (C-H stretching in alkanes) were also recorded. The band at 3,404 cm^{-1} is due to stretching vibrations of O-H groups in water, alcohol and phenols. The peak at 1,389.3 cm^{-1} indicates the C-C groups derived from aromatic rings that are present in the green tea leaf extract and also the peak at 1,608 cm^{-1} is attributed to the conjugated carbonyl (–C=O) group stretching vibration in polyphenols, and it was attributed to the binding of a C=O group with the nanoparticles[5, 12]. The O-H stretch in carboxylic acid appears at 2853.3 cm^{-1}. The band at 1634.3 cm^{-1} is attributed to the C=C stretch in aromatic ring. The formation of FeNP is characterized by one absorption band at 543.63 cm^{-1} which correspond to the Fe–O bond in magnetite[10]. From the FTIR result it appears that the soluble elements present in green tea extract having the presence of higher percentage of molecules of phenolic group, thus, phenolic group acts as a capping agent and contributes in preventing the aggregation of nanoparticles in solution, thereby playing a relevant role in their extracellular synthesis and shaping as suggested by Singh *et al*., (2013).

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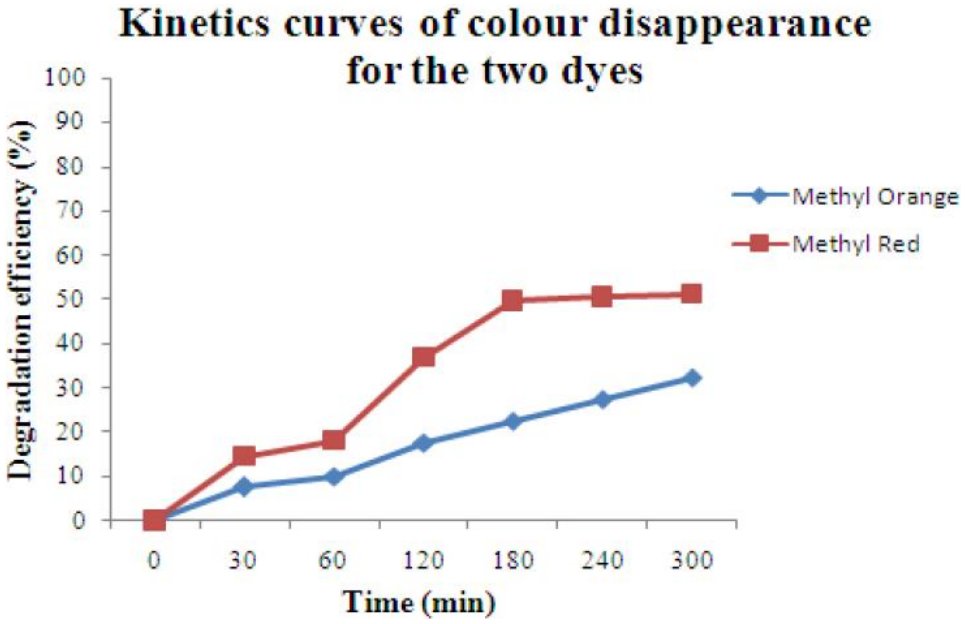

Figure 7 : Percentage degradation efficiency of the dyes after treatment with encapsulated FeNPs

Morphology and size distribution of nanoparticles

Transmission electron microscopy (TEM) has been employed to characterize the size, shape and morphology of synthesized iron nanoparticles. The TEM image of iron nanoparticles is shown in Figure 6a, b and c. From the images, it is evident that the morphology of iron nanoparticles is predominantly spherical. The overall morphology of the iron nanoparticles produced by reduction of Fe^{+} ions with 1mM $FeSO_4.7H_2O$ salt. In the TEM imaging of the synthesized FeNPs, the field of focus showed particles in the range of 10 nm to 20 nm. The phytochemical constituents in the tea leaves such as tannins, polyphenols, and flavonoids may act as reducing agents during the synthesis of FeNPs.

Decolorization experiments

Kinetic experiments

The degradation efficiency of Methyl Red and Methyl Orange concentration with time is shown in Figure 7. It is clear from Figure 7 that the removal of Methyl red precedes Methyl orange almost instantaneously, with more than 50% of the dye being removed after 5 hours of operation. Comparatively, the removal of MO is slower, with 32% of the dye being removed after about 5 hours of operation. Almost complete removal of the dyes requires more than 5 hours of operation and probably with high concentration of iron nanoparticles.

Infrared spectrometric evaluation for decolorization of dyes

The FTIR spectra of control Methyl orange (Figure 8A), displays a peak at 3326.01 cm^{-1} for asymmetric O-H stretching vibrations; peaks at 1,544.80 and 1,411.78 cm^{-1} for the C = C–H in plane C–H bend and $–CH_3$ vibrations, respectively; peak at 1,118.00 for –S=O bond; and a peak at 832.87 cm^{-1} for the1, 4 di-substituted (Para) benzene ring. The peak at 1141.98 cm^{-1} is due to methyl group vibrations ($-CH_3$). All these peaks confirm the aromatic nature of the dye. But in Figure-8B the peaks between 1600-1400 cm^{-1} indicating aromatic C=C bond and Phenyl ring substitution band, and C-H at 832.87 cm^{-1} was entirely absent. The FTIR spectrum of the degradation products formed by degradation due to FeNPs had displayed entirely new peaks (Figure 8B) compared to the initial control dye (Figure 8A), which confirms the degradation of Methyl orange. The new peaks at 1585.6 cm^{-1} and 1389.3 cm^{-1} correspond to the N-H stretch and asymmetrical stretch of nitro compounds (C=N). This indicates that Iron nanoparticle immobilized in Calcium Alginate used in the present experiment had resulted in the degradation of the dye to form new products.

The FTIR spectra of Methyl red (Figure 8C), displays the peak at 1621.70 cm^{-1} and 1547.73 cm^{-1}

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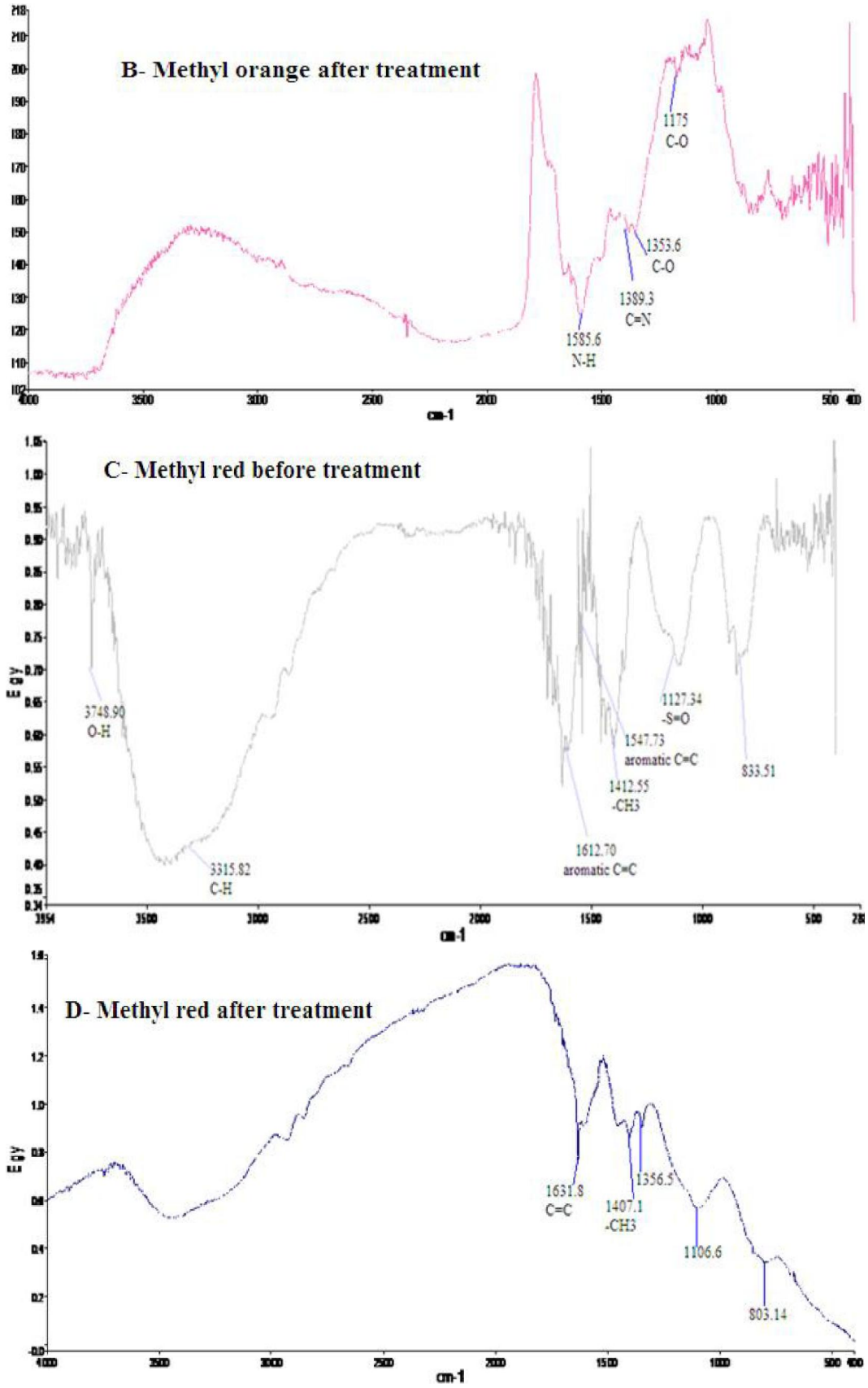

Figure 8 : Infrared spectra of methyl orange and methyl red dyes before and after decolorization studies with encapsulated FeNPs

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for aromatic C=C bond; peak at 3748.90 cm^{-1} for the O-H stretch; peak at 3315.82 cm^{-1} for the C-H stretch. The peak at 1412.55 cm^{-1} is due to the methyl group vibration (-CH_3). All these peaks confirm the aromatic nature of the dye. After the degradation reaction between the dye and encapsulated FeNPs, the peaks between 3748 cm^{-1} to 3315 cm^{-1} which indicates aromatic C=C bond and C=O at 1850 cm^{-1} to 1650 cm^{-1} was entirely absent (Figure 8D). Instead there was a new peak observed at 1356.5 cm^{-1}. This indicates that the encapsulated Iron nanoparticles are effective in the degradation of the dye in to new products, thus acting as the catalyst in the degradation of the dye.

CONCLUSION

The rapid biological synthesis of iron oxide nanoparticles using leaf broth of *Camellia sinensis* provides an environment friendly, simple and efficient route. FTIR spectroscopy indicated that the phenolic compounds and soluble elements present in green tea leaf extract acts as surface capping agent and responsible for the reduction of ferrous ions. The XRD spectrum matched with the JCPDS File No. (39-1346) for ferric ions; the average size of FeNPs was estimated to be 44 nm by NTA analysis. TEM micrographs showed that the synthesized iron nanoparticles are in spherical form. Degradation studies of the two dyes were successfully carried out via encapsulated FeNPs. FTIR spectra revealed the degradation of the dyes after 4 hours treatment with encapsulated FeNP. This elimination of peaks of spectra for characterized component was clearly evident in spectrometric analysis. Calcium alginate can be used as a green support for immobilizing nanoparticles and can be used for developing a new environment friendly immobilization system for large scale water treatment. Also the commercial potential of this technique as an economic means of treating water with dye effluent is very high.

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REFERENCES

[1] M.G.Balamurughan, S.Mohanraj, S.Kodhaiyolii, V.Pugalenthi; Ocimum sanctum leaf extract mediated green synthesis of iron oxide nanoparticles: Spectroscopic and microscopic studies, Journal of Chemical and Pharmaceutical Sciences, **4**, 201-204 **(2014)**.

[2] R.B.G.Camara, S.L.Costa, G.P.Fidelis; Heterofucans from the brown seaweed Canistrocarpus cervicornis with anticoagulant and antioxidant activities, Marine Drugs, **9**, 124–138 **(2011)**.

[3] N.Efecan, T.Shahwan, A.E.Ero˘glu, I.Lieberwirth; Characterization of the uptake of aqueous Ni^{2+} ions on nanoparticles of zero-valent iron (nZVI), Desalination, **249**, 1048–1054 **(2009)**.

[4] G.Hoag, J.Collins, J.Holcomb, J.Hoag, M.Nadagouda, R.Varma; Degradation of bromothylmol blue by 'greener' nano-scale zero-valent iron synthesized using tea polyphenols, Journal of Materials Chemistry, **19**, 8671–8677 **(2009)**.

[5] R.R.R.Kannan, W.A.Stirk, van J.Staden; Synthesis of silver nanoparticles using the seaweed Codium capitatum P.C. Silva (Chlorophyceae), South African Journal of Botany, **86**, 1–4 **(2013)**.

[6] K.Klacanova, P.Fodran, P.Simon, P.Rapta, R.Boca, V.Jorik, M.Miglierini, E.Kolek, L.Caplovik; Formation of Fe (0) – Nanoparticles via reduction of Fe (II) compounds by amino acids and their subsequent oxidation to iron oxides, Journal of Chemistry, 961629 doi.org/10.1155/2013/961629, **(2013)**.

[7] B.H.Moon, Y.B.Park, K.H.Park; Fenton oxidation of Orange II by prereduction using nanoscale zero-valent iron, Desalination, **268**, 249–252 **(2011)**.

[8] M.N.Nadagouda, A.B.Castle, R.C.Murdock, S.M.Hussain, R.S.Varma; In vitro biocompatibility of nanoscale zerovalent iron particles (NZVI) synthesized using tea polyphenols, Green Chemistry, **12**, 114–122 **(2010)**.

[9] E.C.Njagi, H.Huang, L.Stafford, H.Genuino, H.M.Galindo, J.B.Collins, G.E.Hoag, S.L.Suib; Biosynthesis of iron and silver nanoparticles at room temperature using aqueous sorghum bran extracts, Langmuir, **27**, 264–271 **(2011)**.

[10] J.S.Nunes, De C.L.Vasconcelos, F.A.O.Cabral, de J.H.Araújo, M.R.Pereira, J.L.C.Fonseca; Synthesis and characterization of poly (ethyl methacrylate-co-methacrylic acid) magnetic particles via miniemulsion polymerization, Polymer, **47**, 7646–7652 **(2006)**.

[11] M.Pattanayak, P.L.Nayak; Green synthesis and characterization of zero valent iron nanoparticles from the leaf extract of azadirachta indica (Neem), World Journal of Nano Science & Technology, **2(1)**, 06-09 **(2013)**.

[12] R.W.Raut, N.S.Kolekar, J.R.Lakkakula, V.D.Mendhulkar, S.B.Kashid; Extracellular synthesis of silver nanoparticles using dried leaves of Pongamia pinnata (L) pierre, Nano-Micro Letters, **2**, 106-113 **(2010)**.

[13] S.Shin, H.Yoon, J.Jang; Polymer-encapsulated iron oxide nanoparticles as highly efficient Fenton catalysts, Catalysis Communications, **10**, 178–182 **(2008)**.

[14] H.Y.Shu, M.C.Chang, H.H.Yu, W.H.Chen; Reduction of an azo dye acid black 24 solution using synthesized nanoscale zerovalent iron particles, Journal of Colloid and Interface Science, **314**, 89–97 **(2007)**.

[15] M.Singh, R.Kalaivani, S.Manikandan, N.Sangeetha, A.K.Kumaraguru; Facile green synthesis of variable metallic gold nanoparticle using Padina gymnospora, A brown marine macroalga, Applied Nanoscience, **3**, 145–151 **(2013)**.

[16] J.Y.Song, B.S.Kim; Rapid biological synthesis of silver nanoparticles using plant leaf extracts, Bioprocess and Biosystems Engineering, **32**, 79-84 **(2009)**.

[17] L.Xu, J.Wang; A heterogeneous Fenton-like system with nanoparticulate zerovalent iron for removal of 4-chloro-3-methyl phenol, Journal of Hazardous Materials, **186**, 256–264 **(2011)**.