# Biological Synthesis of Iron Oxide Nanoparticles Using Agro-Wastes and Feasibility for Municipal Wastewater Treatment

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# Biological Synthesis of Iron Oxide Nanoparticles Using Agro-Wastes and Feasibility for Municipal Wastewater Treatment


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***Abstract-*** In this work, iron oxide (magnetite) nanoparticles were successfully synthesized using two abundantly found agro wastes in India, Turmeric (*Curcuma longa* L.) leaves and Sweet lime / Mosambi (*Citrus limetta*) peel. UV-Visible Spectroscopy (UV-Vis Spectroscopy) and Scanning Electron Microscopy (SEM) coupled with X-ray energy-dispersive spectrometer (SEM-EDX) was used to confirm the formation of iron oxide nanoparticles. The synthesized nanoparticles using turmeric leaves (TL -Fe NPs) and Mosambi peel (MP – Fe NPs) were tested for removal of ortho phosphate ($PO_4$), Chemical Oxygen Demand (COD) and *Escherichia coli* (*E. coli*) from domestic sewage. TL - Fe NPs showed better removal of organic matter (82%) and complete inhibition of *E. coli* whereas MP - Fe NPs showed higher removal of $PO_4$ (65%) within 24 hours. The preliminary feasibility studies proved that agro wastes can be potentially used as biotemplates for iron nanoparticle synthesis which can provide a novel option for municipal wastewater treatment.



***Index Terms -*** Iron oxide Nanoparticles; Agro Wastes; *Curcuma longa* L.; *Citrus limetta*; Municipal Wastewater


## I. Introduction

Iron nanoparticles, namely nano zero-valent iron (nZVI), magnetite ($Fe_3O_4$) and maghemite ($\gamma$-$Fe_2O_3$) have emerged as a new class of important nanoparticles to be used widely in the field of environmental remediation. This is mainly due to their very efficient pollutant removal capacity, fast reaction kinetics and most importantly due to magnetism which enables its easy recovery [1]. These nanoparticles, when synthesized by conventional physical and chemical methods, lose their reactivity due to aggregate formation [2; 3] and magnetism and dispersibility on air exposure [4]. In addition to these limitations, the concern arising due to use of non polar solvents and toxic reducing agents such as sodium borohydride during synthesis [5] have not only limited their environmental application but also have highlighted the need to develop clean, non toxic and environment friendly procedures for iron nanoparticle synthesis.

Plant-mediated synthesis of magnetic nanoparticles has remained a relatively unexplored research area with the majority of papers being published only in the last two years. Several authors have successfully explored the use of different agro-wastes for iron nanoparticle synthesis [6]. The use of agro waste for nanoparticle synthesis serves dual purpose of using this inexpensive, easily available source of active biochemical constituents and also helps in the prevention of pollution which might result due to its improper disposal.

In the present study, for the first time, Turmeric (*Curcuma longa* L.) leaves and Mosambi (*Citrus limetta*) peel were used as biotemplates for iron nanoparticle synthesis. India is the largest producer, consumer and exporter of Turmeric (*Curcuma longa* L.) [7] whereas Maharashtra ranks 1$^{st}$ amongst the Indian states in Mosambi production [8]. Both these plant materials have high polyphenol content [9, 10] which is known to form complex with metal ions and reduce it [11]. The preliminary characterization of as-synthesized nanoparticles was done using UV-Vis spectroscopy and SEM-EDX. The magnetic nanoparticles were checked for efficacy to treat municipal wastewater in terms of $PO_4$, COD and *E. coli* removal. TL – Fe NPs showed better treatment efficiency (except for $PO_4$ removal) and hence its detailed characterization was done. To the best of our knowledge, this is a novel study that evaluates the efficiency of biologically synthesized magnetic nanoparticles for the treatment of municipal wastewater.

## II. Materials and methods

### *A. Reagents*

Purified Anhydrous Iron (III) Chloride ($FeCl_3$), pure Sodium Chloride (NaCl), Concentrated Sulfuric Acid ($H_2SO_4$), Silver Sulfate ($Ag_2SO_4$), Ammonium Iron (II) Sulfate hexahydrate [$(NH_4)_2Fe(SO_4)_2.6H_2O$], Ferroin indicator were purchased from Merck India. Potassium Dichromate ($K_2Cr_2O_7$), Mercuric Sulfate ($HgSO_4$), Ammonium Molybdate, Stannous Chloride was obtained from Qualigens Fine Chemicals Pvt. Ltd. India. All the chemicals were of analytical grade and were used without further purification. M-EC Test Agar was purchased from Hi-Media, India.

### B. Biotemplate Preparation

Turmeric (*Curcuma longa* L.) leaves were obtained from farm in Satara district in Maharashtra. Mosambi peels were obtained from a local vendor in Mumbai. These plant materials were thoroughly washed with double distilled water and sun dried. These were further dried in an oven (*Metalab*) at $50^0$ C for 48 hours, fine powdered (TLP and MPP) using domestic blender and stored in air tight container.

### C. Synthesis of Iron Oxide Nanoparticles

The synthesis of iron oxide nanoparticles was carried out by dissolving 9 grams of $FeCl_3$ in 150 ml saturated NaCl solution and 18 grams of biotemplate was added to it. This mixture was kept on a rotary shaker at 100 rpm overnight. The plant material was then separated by vacuum filtration and filter residue was washed with double distilled water to remove any unbound $FeCl_3$. It was then dried overnight in an oven at $80^0$ C. The dried material was calcined in a muffle furnace at $450^0$ C for 6 hours. After cooling to room temperature in a desiccator, the calcined material was homogenized using mortar and pestle [12] and used for the treatment of municipal wastewater.

### D. Efficacy Testing for Municipal Wastewater Treatment

The nanoparticles were evaluated for their efficiency to treat domestic sewage collected from a municipal wastewater treatment facility located in Mumbai, India. pH was measured using pH meter. pH of raw sewage was 7.3. Initial and final concentration of COD, *E. coli* and $PO_4$ was measured by Open Reflux Method, Plate Count Method and Stannous Chloride Method respectively as per the Standard Methods for Water and Wastewater Analysis [13]. A dose of 1 gram of nanoparticles was added to 1 liter wastewater. The solution was mixed at 160 rpm at a temperature of $30 \pm 2^0$C without any pH adjustment for 24 hours. After treatment, the nanoparticles were separated from the sample by filtering it through Whatman® Grade GF/C filter paper. All the experiments were done in triplicates and average values are reported.

### E. Characterization of Fe NPs

The preliminary characterization of nanoparticles was done using Chemito UV-Visible spectrophotometer (Model UV 2100) after recovering the embedded nanoparticles from the plant matrix. For this purpose, the nanocomposite was sonicated for 5 minutes in double distilled water and then centrifuged at 1000 rpm for 5 minutes so that TLP and MPP gets separated. The procedure was repeated thrice to ensure maximum recovery. The morphological features and elemental composition of as-synthesized nanocomposite was analyzed using SEM-EDX (FEI ESEM Quanta 200). To identify the phase of iron oxide formed in case of TL - Fe NPs, X-Ray Diffraction (XRD) analysis was performed using Shimadzu 6000 with Cu-Kα radiation source with wavelength of 0.154 nm and was operated at 40kV/30mA over 2θ range of 2 to $80^0$. The scanning speed was maintained at $5^0$ $min^{-1}$. Fourier Transform Infrared Spectroscopy (FTIR) analysis of TLP and nanocomposite was done over the range of wavenumber 4000 - 400 $cm^{-1}$. The measurements were carried out on Perkin Elmer Spectrum BX FTIR spectrophotometer.

## III. Results And Discussion

### A. Characterization

The preliminary characterization of synthesized nanoparticles by UV-Visible Spectroscopy has proven to be very useful technique for the analysis of nanoparticles [14]. The UV- Vis spectra of supernatant solution containing nanoparticles was taken against the spectra of double distilled water as blank. The UV-visible spectra were recorded over the 300 -700 nm range. The formation of iron nanoparticles was confirmed by the characteristic peak at 423 nm for TL- Fe NPs and MP- Fe NPs as depicted in Fig. 1. Similar observation was noted during green synthesis of iron oxide nanoparticles was achieved by leaf extract of *Rumex acetosa* plant and the characteristic peak was observed at 420 nm [15]. UV spectra of $Fe_3O_4$ nanoparticles synthesized using Brown seaweed (*Sargassum muticum*) showed peaks at around same wavelength i.e. at 402 and 415 nm [16].
SEM images of iron oxide nanoparticles are shown in Fig. 2. The nanoparticles synthesized using TLP were in cuboid and pyramid shaped whereas MP – Fe NPs were irregular and rod shaped. The size of nanoparticles ranged from 338.2 nm to 488.1 nm and 176.8 nm to 685.6 nm for TL- Fe NPs and MP- Fe NPs respectively. The nanoparticles were found to be evenly dispersed in the plant matrix. Sodium chloride acted as spacer and thus prevented the formation of aggregated nanoparticles.

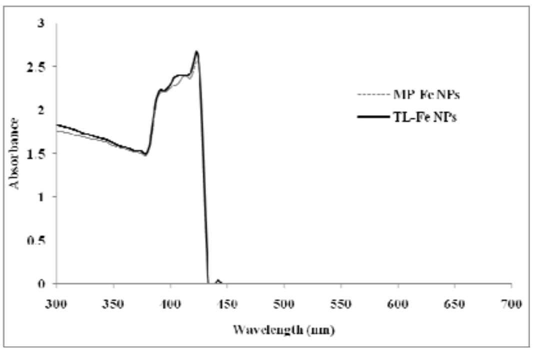


Figure 1. UV-Vis spectra for Iron Oxide Nanoparticles

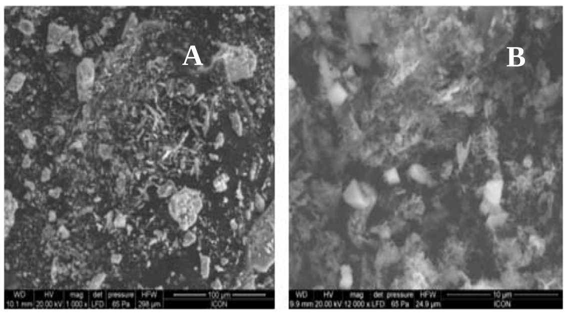


Figure 2. SEM images A) MP Fe NPs, B) TL Fe NPs

The elemental composition of Fe NPs was studied using EDS and is exhibited in Fig. 3. As can be seen from the figure, the predominant peaks were of iron (Fe), Oxygen (O) and Carbon (C). The signals for C and O were mainly due to the different phytochemicals present in plant powder. The signal for oxygen also confirms the fact that iron oxide nanoparticles have been synthesized. The weight percent (wt %) of nanoparticles was measured to be 41.72% for Fe, 35.03 % for O and 20.03% for C for TL – Fe NPs and 29.45% for Fe, 28.40% for O and 38.24% for C for MP – Fe NPs respectively. The high Fe loading enables easy magnetic recovery of as-prepared nanoparticles. Some minor loading from sodium (Na) and (Cl) was also observed. It would be arising from the use of NaCl as spacer. As cited from relevant literature, the iron content of Fe NPs synthesized during the study is found to be higher than iron nanoparticles obtained from plant mediated synthesis.

XRD patterns obtained for TL - Fe NPs is shown in Fig. 4. The diffraction peaks were observed at 2θ values of $31.73^0$, $45.45^0$ and $66.25^0$. The peak at $31.73^0$ can be indexed to the formation of magnetite. The peak at $45.45^0$ corresponds to Fe with associated peak at $66.25^0$ and the results are in agreement with the XRD standard for the magnetite nanoparticles [17].

FTIR measurements of TLP and TL – Fe NPs (Fig. 5) were carried out to understand the involvement of biomolecules in nanoparticle synthesis. The shift in the FTIR peaks of TLP from 3260.43 (attributed to O-H stretch) to 3289.30 in FTIR spectra of oven dried material indicated involvement of polyphenols from TLP in reduction of iron. Also, involvement of aldehydes group can be seen in peak shift from 1670.10 in TLP to 1643.37 in oven dried material. Oxidation of reduced Fe to iron oxide during calcination resulted in the formation of TL – Fe NPs and was confirmed by the peaks at 636.16 $cm^{-1}$ and 585.38 $cm^{-1}$ which are attributed to Fe-O bond vibration of $Fe_3O_4$ [18, 19].

Calcined material also shows strong and broad absorption band at 3367.44 cm-1 due to stretching vibration of –OH which can be assigned to OH- absorbed by iron oxide nanoparticles. The peak at 2923.53 cm-1 and 2338.47 cm-1 can be attributed to C - H stretching of aliphatic carbon. These peaks along with peaks at 1090 cm-1 (C-O stretch), 798.20 cm-1 (C-H) stretch) indicate that minute quantity of residual carbon of turmeric leaves is present in TL – Fe NPs even after calcination. The peak at 1634.11 cm-1 indicate C=O stretch of aldehydes in turmeric leaves [20].

## *B. Efficacy Testing for Municipal Wastewater Treatment*

The treatment efficiency of as – synthesized nanoparticles was tested using municipal wastewater collected from local wastewater treatment facility. The initial COD was 353 mg/L and $PO_4$ concentration was 2.10 mg/L. E. coli count was 4.9 X $10^7$ Colony Forming Units (CFU) /100 ml.

In present investigation, TL- Fe NPs could remove 17% $PO_4$. In comparison, 65% $PO_4$ removal was achieved using MP-Fe NPs. Low $PO_4$ removal can be attributed to ash content in the chosen plant materials (around 15 wt % of ash was generated when only turmeric leaves were calcined as compared to 4% in Mosambi peel). The silicates being negatively charged results in the repulsion of negatively charged phosphate ions from the sewage sample [21]. Other reasons attributed to low $PO_4$ removal can be trapping of iron oxide in the residue of calcined biomass making it less available for adsorption which highlights the importance of adsorbent preparation temperature on the location of iron oxide in the composite [22]. Only 9.3 + 1.6 % of phosphate removal was achieved when magnetic biochar was prepared at lower temperature ($400^0$ C) [23]. Eucalyptus leaves extract synthesized iron nanoparticles have been reported to remove 30.4% of total phosphorus from swine wastewater [24]. Highly efficient phosphorus adsorbing magnetic nanocomposites have been synthesized by various workers [25, 26, 17] for synthetic applications.

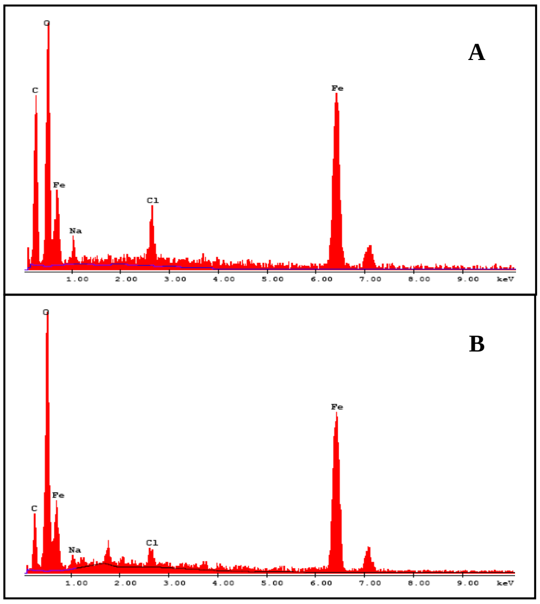


Figure 3. EDX A) MP Fe NPs, B) TL Fe NPs

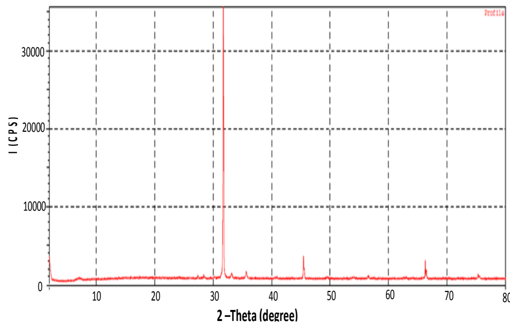


Figure 4. XRD Pattern of $Fe_3O_4$

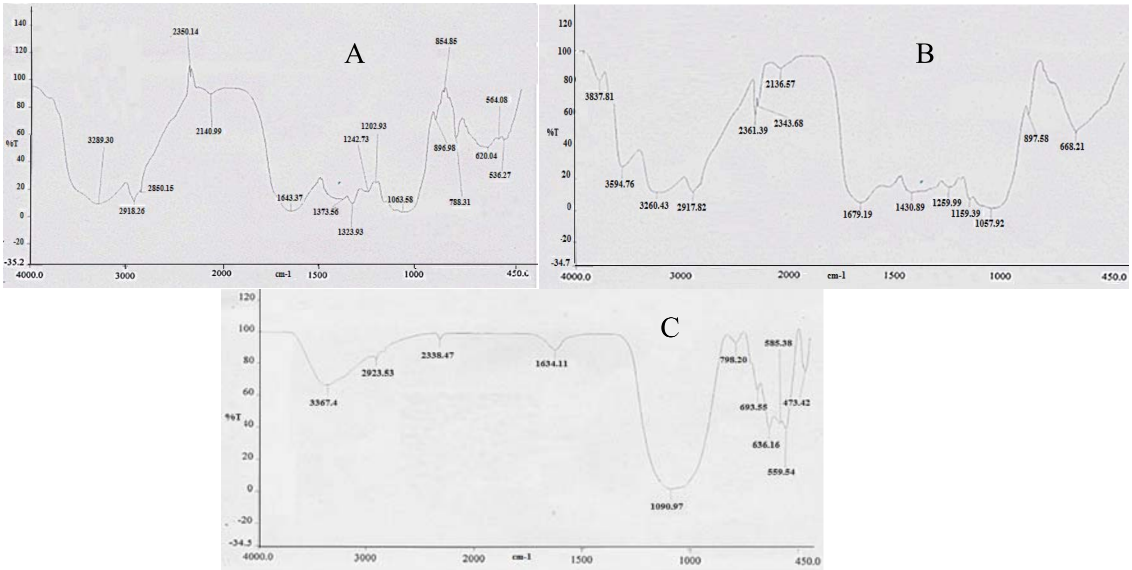


Figure 5. FTIR Spectra of A) TLP, B) Oven dried material and C) Calcined material

The iron nanoparticles synthesized using turmeric leaves could remove 82% of organic content (COD) from the wastewater within 24 hours. The calcination at low temperature has been reported to result in the formation of porous structure due to the elimination of biotemplate [27, 28] which along with the presence of residual carbon as seen in FTIR spectra and high iron content (confirmed by EDS) resulted in adsorption of organic matter [25]. Higher ash content also might have led to better COD removal [29]. Low iron and ash content in MP- Fe NPs might have resulted in their decreased efficiency for COD removal (65%). Not much literature could be cited in this regard. However, one of the studies reported the efficacy of 84.5% COD removal in 21 days from swine wastewater [24]. Similarly, chemically synthesized 5 % modified neodymium-doped $TiO_2$ nanoparticles could remove 95% of COD reduction of municipal wastewater was achieved in 3 hours in presence of sunlight [30].

Complete inhibition of *E. coli*'s growth was achieved using as - synthesized TL - Fe NPs. In case of MP - Fe NPs, *E. coli* number was significantly reduced (5.3 X $10^5$) but complete inhibition was not observed. It has been reported that Reactive oxygen species (ROS) can cause damage to proteins and DNA in bacteria [31, 32]. The same mechanism seems to exhibit antimicrobial activity in the present study. Effective inhibition of *E. coli* has been reported for nZVI synthesized by *Dodonaea viscosa* leaf extract [33]. The microbial load from municipal wastewater was reduced by 90 % by poly (ethylenimine) (PEI) functionalized magnetic nanoparticles [34].

The field of nanoparticle application for waste treatment has immense potential, attempts are needed to synthesize iron nanoparticle with more homogenous size distribution using newer substrates especially biological ones. The synthesis protocol needs to be refined further for higher efficiency. Further studies are also required to get insights into the reaction kinetics and adsorption mechanism. Nonetheless, present study, for the first time has demonstrated the successful synthesis and application of iron nanoparticles agro wastes for municipal wastewater treatment.

## IV. Conclusion

Magnetite nanoparticles were successfully synthesized using low-cost, renewable, eco-friendly biotemplates for the first time. The nanoparticles were characterized using different techniques. Another innovative feature of this study is the application of the as - prepared nanoparticles for the municipal wastewater treatment at room temperature and without any pH adjustment. TL- Fe NPs exhibited significant COD removal capacity and excellent antimicrobial activity. MP - Fe NPs showed significant $PO_4$ removal even at a low dose. This underlines future potential of nanoadsorbent for remediation of municipal wastewater and similar such systems.

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