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Eco Friendly Synthesis and Characterization of Iron Oxide Nano-Particles by Using *Amaranthusspinosus* Leave Extract and Apply It for Domestic Wastewater Treatment

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Abstract
Owing to the limitations of conventional synthesis methods for iron nanoparticles and its wide range of environmental applications, there is a need to develop green synthesis protocols by exploring newer biological resources. In this study, for the first time, Turmeric (AmaranthusspinosusL.) leaves were used to synthesize iron oxide nanoparticles by calcination (FeNPs). As prepared nanoparticles were compared for their efficiency to treat domestic wastewater in terms of orthophosphate (PO4), Chemical Oxygen Demand (COD) and Escherichia coli (E. coli) removal. FeNPs(90%, 92% and 95% respectively) in 24 hours. FeNPs exhibited superior antimicrobial activity inhibited E. coli.

Introduction
Water is one of the most important and basic natural resources. Owing to increasing industrialization and exploding population, there is continuous increase in demand of water supply. On the other hand, the problem of water pollution is getting very severe, especially in developing countries with disposal of untreated sewage. This disposal has resulted in additional load of inorganic, organic and microbial pollutants which further deteriorates the water quality[1,2]. Conventionally, the pollutants from sewage are removed to a certain extent in wastewater treatment facilities (WWTFs). But, the major limitations of existing WWTFs include time required for the complete process (around 15-20 hours), installation, maintenance, labor and energy cost along with the sludge handling. So, the researchers need to develop advanced technologies which are cost effective, durable and more efficient as compared to the existing treatment options. In this context, “Nanotechnology” could help in solving the problems concerning water purification and quality[3].

Iron nanoparticles, namely nano zero-valent iron (nZVI), magnetite (Fe3O4) and maghemite (_-Fe2O3) are widely used 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[4]. These nanoparticles, when synthesized by conventional physical and chemical methods, lose their reactivity due to aggregate formation[5]and magnetism and dispersibility on air exposure[6]. In addition to these limitations, the concern arising due to use of non polar solvents and toxic reducing agents such as sodium borohydride during synthesishave 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 [7]. In the present study, for the first time, (*Amaranthusspinosus*) leaves were used as biotemplate for iron nanoparticle synthesis. India is the largest producer, consumer and exporter of (*Amaranthusspinosus*)[8]. *Amaranthusspinosus* rhizomes are widely used but the leaves, except for its use in Indian and Malaysian cooking, are mostly treated as an agro-waste[9]. The magnetic nanoparticles were synthesized by two methods – calcination and microwave assisted synthesis. The iron nanoparticles synthesized by both these methods were characterized and tested for their efficiency to treat municipal wastewater. This is the first of its kind study that evaluates the efficiency of biologically synthesized magnetic nanoparticles for the treatment of municipal wastewater in terms of COD, *E. coli* and PO4 removal.

Material and Methods

Amaranthusspinosus leaves were obtained from farm in Satara district in Maharashtra. The leaves were repeatedly washed with double distilled water and sun dried. These were further dried in an oven (Metalab) at 500 C for 48 hours, fine powdered using domestic blender and stored in air tight container and used as biotemplate. From now on, it is denoted as *Amaranthusspinosus* leaf powder (TLP).purified anhydrous iron (III) chloride (FeCl3), pure sodium chloride (NaCl), concentrated sulfuric acid (H2SO4), silver sulfate (Ag2SO4), ammonium iron (II) sulfatehexahydrate[(NH4)2Fe(SO4)2.6H2O], ferroin indicator were purchased from erck India. Potassium dichromate (K2Cr2O7), Mercuric sulphate (HgSO4), Ammonium molybdate, stannous chloride and liquor ammonia solution 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.

Synthesis of Iron Oxide Nanoparticles by plant extract:

grams of FeCl3 was dissolved in 150 ml saturated NaCl solution and 18 grams of *Amaranthusspinosus* powder was added to it[9]. 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 FeCl3. It was then dried overnight in an oven at 800C. The dried material was calcined in a muffle furnace at 4500C for 6 hours. After cooling to room temperature in a desiccator, the calcinedmaterial was homogenized using mortar and pestle [10].

Characterization of Iron Oxide Nanoparticles:

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 were sonicated for 5 minutes in double distilled water and then centrifuged at 1000 rpm for 5 minutes so that *Amaranthusspinosus*powder gets separated. The procedure was repeated thrice to ensure maximum recovery. The morphological features and elemental composition of assyn the size dnanocomposites was analyzed using SEM-EDX (FEI ESEM Quanta 200). To identify the phase of iron oxide formed, X-Ray Diffraction (XRD) analysis was done using Shimadzu 6000 with Cu-K_ radiation source with wavelength of 0.154 nm and was operated at 40kV/30mA over 2_ range of 2 to 800. The scanning speed was maintained at 50 min-1. Fourier Transform Infrared Spectroscopy (FTIR) analysis of TLP and nanocomposite was done over the range of wave number 4000- 400 cm-1. The measurements were carried out on Perkin Elmer Spectrum BX FTIR spectrophotometer.

Domestic Wastewater Treatment Efficiency Comparison:

The nanoparticles were evaluated for their efficiency to treat domestic sewage collected from a municipal wastewatertreatment facility located inHyderabad, India. pH was measured using pH meter. Initial and final concentration of COD, E.coliand PO4 was measured by Open Reflux Method, Plate Count Method and Stannous Chloride Method respectively as per the Standard Methods for Water and

Wastewater Analysis [11]. A dose of 1 gramL-1 of nanoparticles was added to wastewater. The solution was stirred at 160 rpm at room temperature without any pH adjustment for 24 hours. After treatment, the nanocomposites were magnetically separated and the supernatant was filtered through Whatman Grade GF/C filter paper and the filtrate was analyzed for PO4, COD and E. coli. All the experiments were done in triplicates and average values are reported.

Results and Discussion

Characteristics of raw sewage:

The average initial concentration of PO4 was 2.10, COD was 353 mg L-1 and E. colicount in raw sewage was 4.9 x 107 /100 ml at pH 7.3.

Characterization of nanoparticles:

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 scanned over the 300-700 nm range. The formation of iron nanoparticles was confirmed by the characteristic peak at 423 nm in case of FeNPs depicted in figure-1. Similar observation was noted in the study in which green synthesis of iron oxide nanoparticles was achieved by leaf extract of Rumexacetosa plant and the characteristic peak was observed at 420 nm [12].

SEM images of FeNPs are seen in figure-2. As-synthesized nanoparticles were in cuboid and pyramid shaped. Similar observation was noted by when tea waste was used as template for magnetite synthesis [13]. 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. The elemental composition of FeNPsstudied using EDS is exhibited in figure-3a. As can be seen from the figures, the predominant peaks were of iron (Fe), Oxygen (O) and Carbon (C). This confirmed the presence of Fe in the synthesized nanoparticles. The signals for C and O were mainly due to the different phytochemicals present in turmeric leaf powder used during the synthesis. The signal for oxygen also confirms the fact that iron oxide nanoparticles have been synthesized. The weight percent (wt %) of elements in FeNPs was measured to be 41.72% for Fe, 35.03 % for O and 20.03 % for C. Some minor loading from sodium (Na) and (Cl) was also observed. It would be arising from the use of NaCl as spacer. 1.98 wt % of Na and 1.23 % of Cl was detected. Other elements observed were 36.98% of Carbon and 35.99% of Oxygen in addition to traces of Silica (Si) arising from plant material. The high Fe loading enables easy recovery of as-prepared nanoparticles.

XRD patterns obtained for FeNPs shown in figure-4. The diffraction peaks were observed at 2_ values of 31.730, 45.450 and 66.250. The peak at 31.730 can be indexed to the formation of magnetite. The peak at 45.450 corresponds to Fe with associated peak at 66.250 matching with the XRD standard for the magnetite nanoparticles [13]. The diffraction peaks were observed at 2_ values of 29.67, 35.47 and 44.52. The peaks at 29.67 and 35.47 can be indexed to the formation of magnetite [14]. The peak at 44.52 corresponds to zero valent iron nanoparticles [11,15].

The crystallite size of iron nanoparticles was estimated using the Debye-Scherrer equation, which gives a relationship between peak broadening in XRD and particle size that is demonstrated by following equation:

$$D = \frac{k\lambda}{\beta \cos\theta} \quad (1)$$

Where, d is the particle size of the crystal, k is Scherrer constant, _ is the X-ray wavelength (0.154 nm), _ is the width of the XRD peak at half-height, and _ is the Bragg diffraction angle. Using the Scherrer equation the average crystallite sizes of the magnetic nanoparticles synthesized by calcination method was estimated to be in the range of 66 to 148 nm.

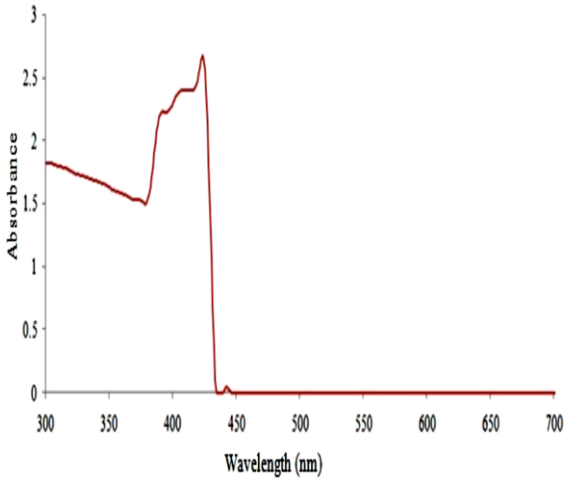

Figure-1UV-visible Absorption Spectra of FeNPs

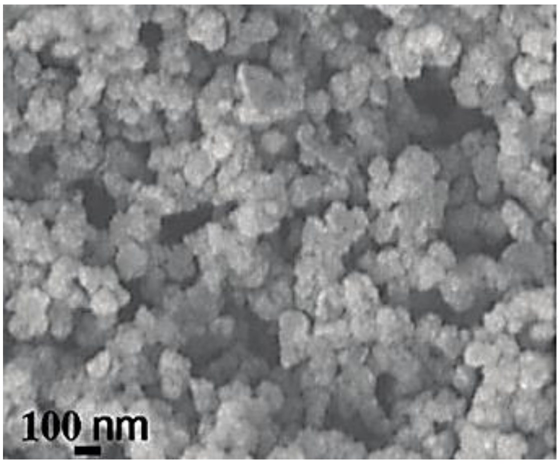

Figure-2SEM Image of FeNPs

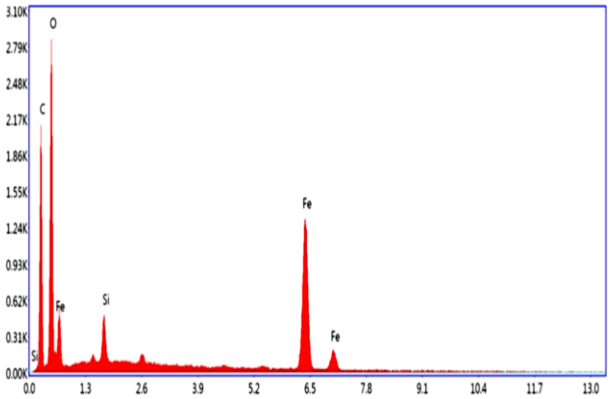

Figure-3 Elemental Composition of FeNPs

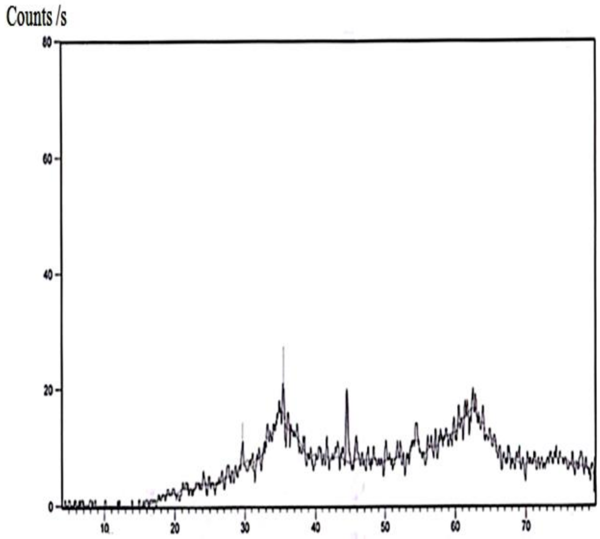

Figure-4 XRD Pattern of FeNPs

Mechanism of Magnetite synthesis:

FTIR measurements of TLP and nanoparticles (figure-5) were carried out to understand the involvement of biomolecules in nanoparticle synthesis. Turmeric leaves have high polyphenol content [9,16]and authors have already highlighted its role in forming complex with metal ions and reduce the metals [17,20]. The shift in the FTIR peaks of *Amaranthusspinosus* powder from 3260.43 (attributed to O-H stretch) to 3289.30 in FTIR spectra of oven dried material indicated involvement of polyphenols from *Amaranthusspinosus* powder in reduction of iron. Also, involvement of aldehydes group can be seen in peak shift from 1670.10 in *Amaranthusspinosus* powder to 1643.37 in oven dried material. Oxidation of reduced Fe in oven dried material to iron oxide during calcination resulted in the formation of FeNPs 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 21. Calcined material also shows strong and broad absorption band at 3367.44 cm-1 due to stretching vibration of -OH which can beassigned to OH- absorbed by iron oxide nanoparticles. The peakat 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 FeNPs even after calcination. The peak at 1634.11 cm-1indicates C=O stretch of aldehydes in turmeric leaves [18]. In iron oxide nanoparticles synthesized by microwave method, involvement of polyphenols and aldehydes in turmeric leaves is highlighted by the shift of peak to 3420.37 cm-1 and 1628.13 cm-1 respectively in microwaved material. Alkali treatment andfurther microwaving has resulted the formation of reduced iron nanoparticles [11]. Iron oxide peaks are characterized by strong bands in low wavelength region of 1000-400 cm-1. The peak at 827.61 cm-1 is attributed to Fe-OH vibration [19].

Sewage Treatability Studies:

The comparative percent removal of pollutants under study is depicted in figure-6. Plant material ash without iron nanoparticles resulted in slight increase in PO4 concentration; this is commonly reported forbiochars prepared from biomass [21]. Low PO4 removal by FeNPs can be attributed to ash content in the chosen plant materials (around 15 wt % of ash was generated when only turmeric leaves were calcined). The silicates being negatively charged results in the repulsion of negatively charged phosphate ions from the sewage sample [23]. Other reasons attributed to low PO4 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 [24]. Eucalyptus leaves extract synthesized iron nanoparticles have been reported to remove 30.4% of total phosphorus from swine wastewater [25].

Only plant material treated with alkali in 32% of PO4 removal. In case of FeNPs, significantly higher PO4 removal was observed. The reason for high efficiency of this nanocomposite in PO4 removal is due to its ability to form monodentate inner sphere complex with phosphorus at around neutral pH which holds true for sewage [26]. Highly efficient phosphorus adsorbing magnetic nanocomposites have been previously synthesized for phosphate removal from synthetic samples [11].

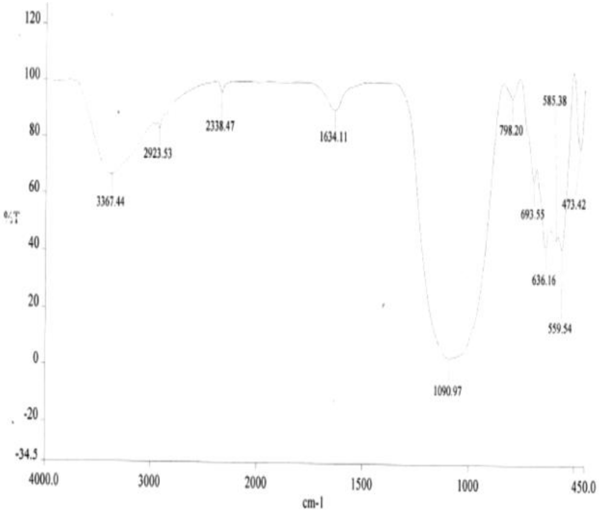

Figure-5FTIR Measurements of TLP

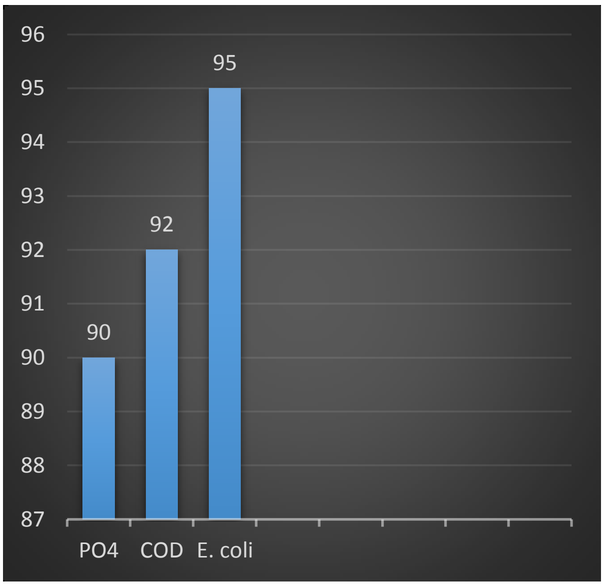

Figure-6 Comparison of Percent Pollutant Removal

Calcined plant material resulted in 92% COD reductionfrom the domestic wastewater within 24 hours. The calcination at low temperature has been reported to result in the formation of porous structure due to the elimination ofbiotemplate which resulted in adsorption of organic matter [27]. Higher ash content also might have led to better COD removal29. Only 88% reduction was achieved using plant material treated with alkali and subsequent microwaving.

The amount of iron/iron oxide incorporated into in the composite played an important role in organic matter removal. Not much literature could be cited with regards to COD removal from actual wastewater. However, one of the studies reported the efficacy of 91% of COD removal in 21 days from swine wastewater [28]. Similarly, chemically synthesized 5% modified neodymium-doped TiO_2 nanoparticles could remove 95% of COD from municipal wastewater in 3 hours in presence of sunlight [29]. The growth of *E. coli* was completely inhibited using plant material ash as well as synthesized FeNPs. Reactive Oxygen Species (ROS) are known to cause damage to proteins and DNA in bacteria [30,31]. The same mechanism seems to exhibit antimicrobial activity in the present study. The other reason could be trapping of microbial cells by amine groups in the nanocomposite as indicated by peaks at 1304.09 cm^{-1} in FTIR of the microwaved nanocomposite. *E.coli*cells were successfully trapped by amino acid functionalized magnetic nanoparticles [32]. The efficiency of percent removal of *E. coli* by microwaved plant material and synthesized FeNPs to be comparable with that nanocomposite obtained by calcination method. But, the count is too high than the discharge standard of 10,000 CFU /100 ml for the discharge of treated sewage as per India's Central Pollution Control Board norms [33]. Effective inhibition of *E. coli* has been reported for nZVI synthesized by *Dodonaeaviscosa*leaf extract [34].Further studies are being carried out to understand the reactionkinetics and adsorption mechanism for removal of pollutants.

Conclusion:

In this study, for the first time, magnetite nanoparticles were successfully synthesized using low-cost, renewable, eco-friendly biotemplate like *Amaranthusspinosus* leaves. The nanoparticles synthesized by calcination and microwave method were characterized using different techniques. Another innovative feature of this study is the comparative efficiency estimation of the as prepared nanocomposites for the municipal wastewater treatment at room temperature and without any pH adjustment. FeNPs exhibited significant COD removal capacity and excellent antimicrobial activity whereas, significantly higherPO_4 removal and almost similar COD removal was achieved using nanocomposites synthesized by microwave method. This underlines future potential of both these nanoadsorbents for the treatment of municipal wastewater and other industrial effluents.

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