Research article

Biosynthesis of iron oxide nanoparticles for the degradation of methylene blue dye, sulfisoxazole antibiotic and removal of bacteria from real water

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HIGHLIGHTS

- Biosynthesis of Fe₃O₄ NPs using M. burkeana for the first time.
- 99% and 60% degradation of MB dye and antibiotic SSX, respectively.
- Superoxides were the major species responsible for MB degradation.
- Materials could be reused several times.
- High potency against gram positive strains using real water.

ARTICLE INFO

Keywords:
Fe₃O₄ NPs
Green synthesis
Photocatalytic degradation
Bacteria

ABSTRACT

Water pollution that is caused by dyes, bacteria and antibiotics, has resulted in a threat to living organisms, animals and humans, hence there is a need to synthesize multifunctional materials that can be used for the degradation of various pollutants. The aim of this study was to synthesize Iron oxide (Fe₃O₄) NPs and test this material for photocatalytic degradation and antibacterial activity. The synthesis of Iron oxide (Fe₃O₄) NPs was conducted using M. burkeana extract and characterised using UV-vis, XRD, BET, SEM, EDS and TGA. The material was then tested for its photocatalytic and antibacterial efficiency against methylene blue dye, antibiotic sulfisoxazole and E. coli and S. aureus bacterial strains. XRD confirmed the formation of Fe₃O₄ NPs. UV-vis gave optical information whereby an excitation at 320 nm and a bandgap of 3.74 eV was noted. The deposition of the phytochemicals onto the Fe₃O₄ NPs was demonstrated using FTIR. From the surface analysis, the morphology of the synthesized NPs was found to be rod like and mesoporous. Upon testing for methylene blue degradation, the Fe₃O₄ NPs were more potent under basic conditions (pH 12) and the O₂ radicals were found to be the species responsible for the degradation. Against sulfisoxazole, a 60% degradation was observed. Lastly, when testing these materials against bacterial strains found in tap, pond, river and sewage water, they were potent in particular against gram positive strains. These results show that at optimum conditions, these materials are able to degrade various pollutants in wastewater.

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https://doi.org/10.1016/j.heliyon.2022.e10536

Received 7 April 2022; Received in revised form 2 August 2022; Accepted 31 August 2022

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1. Introduction

The increase in human population worldwide, has necessitated the need and expansion of industries such as the textile and pharmaceutical industry, where dyes are required to change the colour of the cloths and the antibiotics are for human treatment [1]. Consequently, the demand has increased thus leading to a large production and large quantity usage. The improper disposal and direct discharge of dyes and pharmaceutical pollutants generated from the textile industries and the hospital effluents has led to water pollution within the ecosystem [2]. Toxins from these effluents enter the water bodies and worsen the quality and condition of the eco-system globally, thus making drinking water unstable for consumption [3, 4]. Water is the source of life and exposure to these toxins badly affects the ecosystem placing animals and human health at risk [5]. Organic dyes are notorious for causing damage to the central nervous system, gastrointestinal and respiratory diseases [6]. Also, the increased usage of antibiotics may cause irreversible and long term effects to microbes by leading to genetic exchange, consequently accelerating the resistance of (bacteria species) pathogens against antibiotics [6]. Thus, promoting human health threat when disorders, syndromes and diseases are therefore developed [7, 8].

Several methods such as floatation, ion exchange, chlorination, adsorption, electrochemical and photocatalytic degradation have been used for the removal of dyes, bacteria and pharmaceutical drugs in water in particular pollutants such as methylene blue (MB), malachite green (MG), E. coli, Amoxicillin, Diclofenac, S. aureus, ciprofloxin, sulfamethoxazole (SMX) and sulfoxazole (SSX) respectively [4, 6]. A majority of these methods are expensive, require sensitive equipment and the products cannot be reused [7, 8]. For ion exchange, the pre-treatment of all the samples is always a requirement and for biological methods, standardization is required and in most cases, these methods have a poor efficiency. Of these methods, photocatalysis is the most preferred method by researchers as it has been shown to degrade complex pollutants, is easy to use and can produce reproducible results [9, 10, 11].

Metal oxides such as Zinc Oxide (ZnO), Iron Oxide (Fe₂O₃), Titanium dioxide (TiO₂), Nickel Oxide (NiO) etc have been used as photocatalysts for the degradation of various pollutants from wastewater [12, 13, 14]. Of these, TiO₂ and ZnO are the most commonly used semiconductors due to their photoelectric properties, thus extensive studies have focused on these materials. Akinola et al synthesized multifunctional TiO₂ using the extracts of Cola nitida [3]. At optimum conditions, a maximum degradation of 86% was achieved for malachite green dye. Ngoepe et al also synthesized green TiO₂ using Monsonia Burkeana, a 85% degradation of MB was achieved [15]. From these studies, it can be noted that the traditional green derived semiconductors are also highly effective even though they still suffer from known limitations such fast recombination rate, broad bandgap, reusability etc. Moreover, because they are green derived, they are less toxic and due to that these materials can also be used in a variety of other applications in particular the biomedical field as antioxidant, antioxidative and antibacterial agents. Of interest lately has been Iron oxide nanoparticles. These materials are preferred since they generally have a low bandgap but more importantly because they are magnetic, which means that they can also be easily recovered during the water treatment process.

Of interest lately has been Iron oxide nanoparticles. These materials are preferred since they generally have a low bandgap but more importantly because they are magnetic thus making them recyclable. These materials can be classified into three categories, namely; Hematite (α Fe₂O₃), Maghemite (γ Fe₂O₃) and the magnetic (Fe₃O₄) [2,9]. Hematite is the most preferred form as a photocatalyst, where-as maghemite and magnetite are primarily used for drug delivery, energy storage and data storage [10]. The degradation of pollutants using iron-based materials depends on the stability and the surface area of the material. Thus, this necessitates a synthetic method that can achieve that. Synthesis strategies such as sol gel, chemical vapour deposition and pyrolysis have been found to promote the usage of unsafe, hazardous solvents and with synthesis conditions requiring extremely high pressures and temperatures. Furthermore, they generate a large surface ratio and higher energy levels on the surface of the material [11, 12]. Moreover, the chemical synthesis methods tend to affect the iron-based nanoparticles by reducing their magnetic properties. This is because, in chemical synthesis, the iron-based nanomaterials are easily oxidized by high chemical activity resulting in loss of the magnetic property of the material [13]. Thus, researchers have moved towards the use of safer materials through green chemistry.

Green chemistry is an area in science, which aims at synthesizing materials that are environmentally friendly through the use of plant extracts, fungus and bacteria to name a few [14, 16]. The use of plants extracts for the synthesis is more preferred as this method has the ability to reduce the energy levels, increase neutrality and also display the ability to oxidize [17]. Moreover, green synthesis is not time-consuming, does not require several steps and the plants are mostly readily available. The plant extracts have been shown to consists of phytochemicals such as terpenoids, tannin, polyphenols which are rich in hydroxyl and carboxylic groups which normally act as reducing agents for the synthesis of nanoparticles [15, 18, 19]. Several studies have shown that biomedical synthesis of iron oxide nanoparticles have been effectively used in the degradation of pollutants. Bibi et al [6], synthesized iron oxide using the promegranate seeds for the removal of reactive blue dye. The study showed a degradation of 95% of the dyes using a spherical nanomaterial with the sizes of 25 and 55 nm. Another study was performed for the removal of Remazol yellow (77%) dye by the green (Carica papaya leaf extract) derived iron oxide nanoparticles [7]. Canthium coromandelicum leaf extract was used to synthesize iron oxide nanomaterial and used for the degradation of Janus green dye, a 97% degradation was obtained [20]. From these studies, it could be noted that iron oxide nanoparticles were highly efficient in the degradation of dyes.

In this study, a low cost, green and recyclable material, Iron oxide (Fe₃O₄) nanoparticles was synthesized using M. Burkeana plant extract for the degradation of methylene blue dyes and sulfosaxazole antibiotics in wastewater. The effect of pH against the MB dye, surface analysis, spectroscopic, thermal stability studies, reactive oxygen species and reusability studies were conducted. Furthermore, the activity of this material against the sulfosaxazole antibiotic was also evaluated. Lastly, the potency of this material against Total Coliforms, E. coli and S. aureus against real water pollutants was also investigated.

2. Materials and methods

2.1. Materials

All the chemicals used for the preparation of the photocatalysts and photodegradation experiment studies were of analytical grade. Methylene blue dye (MB) (C₁₆H₁₄ClN₃S, Sigma-Aldrich), Iron nitrate nonahydrate Fe(NO₃)₂·9H₂O, 98%; Sulfoxazole (C₁₅H₂₄N₄O₅S, >98%) were purchased from Sigma Aldrich and used in their original state. A membrane filter with a pore size 0.45 μm and 47 nm diameter were purchased from Sigma Aldrich, Germany. M. burkeana plant was harvested from the Limpopo Experimental Farm, Mankweng.

2.2. Extraction of M. burkeana plant

The method used for the extraction of M. Burkeana (MB) was reported by Motene et al. [18]. Briefly, 5 g of M. burkeana plant powder was added to 250 ml of hot water and allowed to boil at 80 °C for 15 min. The extract was cooled down at room temperature and was filtered using the 0.45 filters through gravity filtration. Upon collection, the extract was stored in the refrigerator at 4 °C until further use.

2.3. Synthesis of Fe₃O₄ using M. burkeana plant extract

The synthesis Fe₃O₄ NPs was conducted using a wet chemistry technique. Briefly, 0.1 L of the prepared M. Burkeana extract was poured in to
The use of X-Ray Diffraction (XRD) using a Philips PW 1830 at a scan range of 0–80°. To confirm the formed nanoparticles, FTIR (Bruker, Alpha) using a scan range of 500–4500 cm⁻¹ was conducted. Furthermore, the physico-chemical analysis from the plant deposition was also determined. To understand their thermal stability, a Pyris PerkinElmer system was used, where the Fe₃O₄ samples were heated to 900 °C at a flow rate of 10 °C/min under air gas. Lastly, the surface area analysis and pore size measurements were conducted using a BET Micrometrics Tri Star.

2.5. Photocatalytic degradation of MB dye using iron oxide nanoparticles

The photocatalytic degradation of Methylene blue (MB) dye using Fe₃O₄ nanoparticles was studied using a 150 W UV lamp following a method reported previously by several authors [15, 18]. Briefly, the effect of pH was investigated whereby pH 3, pH 7 and pH 12 were the conditions of choice. In these degradation experiments, in a 250 ml beaker, 25 mg of the Iron oxide photocatalyst was added to 100 ml of the 5 ppm MB solution. Prior to the light being emitted, an adsorption-desorption experiment was conducted in the darkness for about 30 min to achieve equilibrium. Thereafter, the solution was exposed to the UV light for 60 min. At 15 min intervals, 5 ml of the solutions were aliquoted and taken for analysis using a UV-Vis spectrophotometer at a wavelength of 668 nm. The percentage degradation was calculated as follows, using Eq. (1):

\[
\text{% Degradation} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

where “\(A_0\)” indicates the initial absorption of the MB dye solution and “\(A_t\)” indicates the final absorption of the MB dye at specified time intervals.

2.6. Reusability and stability studies

The reusability and recovery of the Fe₃O₄ NPs towards the degradation of MB dye was studied using a method reported previously. More than four trials were conducted and after each trial, the photocatalyst was removed from the solution through centrifugation, washed several times thoroughly with deionized water, and then dried in an oven for 60 min at 80 °C.

2.7. Influence of reactive species on the photocatalytic degradation of MB dye

The effect of reactive species on the degradation of MB dye was investigated through the addition of B- benzoquinone, EDTA, isopropyl alcohol and AgNO₃ as the main scavengers for \(O_2^\cdot\), \(h^+\), \(\text{OH}^-\), and \(e^-\) radicals, respectively, in the experiments. Upon testing the effect of these species, the experiments were conducted the same way as the normal degradation experiments except that 2.0 mmol/L of the different scavenger solutions were added.

2.8. Photodegradation of the antibiotic sulfisoxazole

The photocatalytic degradation of antibiotic sulfisoxazole (SSX) was conducted using a 150 W UV lamp. Briefly, 100 mg of Fe₃O₄ NPs was added into a 100 ml 10 ppm SSX solution. To ensure an adsorption-desorption equilibrium, the solution was stirred for 30 min in the dark prior to irradiation. Thereafter, the mixture was exposed to UV-light irradiation for 2 h. At 30 min intervals, about 3 ml aliquots of antibiotic solution were drawn of the solution and the rate of decomposition was determined using a UV-vis spectrophotometer at 285 nm in the process.

2.9. Antibacterial activity of Fe₃O₄ NPs against various bacterial pollutants

The antibacterial activity of M. burkeana Fe₃O₄ NPs against the Total Coliform, E. coli and S. Aureus was investigated following the method described previously without any modification. From the analysis, 4 water samples from the river, tap, pond and sewage were investigated. In this study, 2 concentrations (5 and 50 mg/L) of the catalyst were added onto the various water samples, allowed to be incubated for overnight at 37 °C and after the treatment, analysis was taken using the tempo reader.

3. Results and discussion

3.1. Phase identification and structural analysis (XRD)

X-ray diffraction analysis was conducted for the plant synthesised Fe₃O₄ NPs in order to gain knowledge regarding the structural features of the synthesized nanoparticle. In Figure 1, the characteristic diffraction peaks for Fe₃O₄ were observed and assigned as follows (220), (311), (400), (422), (511), (440), (620) and (622) corresponding to the following 2θ values 14, 30, 35, 41, 45, 54 and 62, 66, 74° respectively [21, 22]. The diffraction pattern for the plant synthesised Fe₃O₄ NPs (JCP 22-33-0664) matches those of previously published chemical synthesized materials except the green materials have additional peaks and a slight peak shifting may have occurred, whereby they were also a bit broader, which then would also have an effect on the particle size of these materials [23]. These results corroborate with work that was done by Yogamoorthi et al. [24] and Latha et al [17] from the XRD pattern obtained when Fe₃O₄ NPs were synthesized using papaya leaves.

Generally, the mechanical and physical properties of nanomaterials mostly depend on how crystalline the material is and their size. When a material is highly crystalline and has a small crystalline size, it is mostly expected that these properties could enhance their photocatalytic capacity. From the Debye-Scherer equation, the green synthesized nanoparticles were calculated to be 60.2 nm. To obtain more information about the materials, Optical analysis was conducted to obtain more information about the functional groups and the formation of the material.
3.2. Optical analysis via UV-vis and FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups found in the plant and the formed nanoparticles. The *M. Burkenana* plant extract and Fe3O4 nanoparticles both showed a stretch at 3250 cm⁻¹, which was assigned to O–H and N–H, indicating the presence of phenolic compounds. These are the functional groups of the biomolecules which are responsible for the reduction, capping and efficient stabilization of the metal ion precursor to its corresponding nanoparticles. Other groups from the plant extract that have been integrated onto the Fe3O4 NPs as shown by dotted lines in Figure 2 are C–O, C–N(bent) and C–O(amid) at 1100, 1300 and 1600 cm⁻¹, respectively. The peak in the fingerprint region at 750 cm⁻¹ due to Fe–O confirmed the formation of Fe3O4 NPs [15, 23]. Senhil et al. (2022) synthesized Fe3O4 NPs at room temperature using *Tridax procumbens* plant extract and reported that the carbohydrates from the plant extract acted as the reducing agents [20]. Hydrogen bonded (OH) groups were also identified at 3446 cm⁻¹ and 1611 cm⁻¹. The presence of carboxylic groups (–COOH–) at 1300 cm⁻¹ were also identified, these are known to be responsible for the formation of Fe3O4 nanoparticles and C–O–C stretching frequency.

How iron oxide nanoparticles normally form from the use of plants as a reducing agent is not well understood but several authors have suggested that the formation could be a two-step reaction mechanism. In this process, the phytochemicals identified which contain the hydroxyl and aldehyde groups generally play a role in the partial reduction of Fe³⁺ to Fe₃O₄ nanoparticles. The hydrolysis of FeCl₃ to Fe(OH)₃ takes place and it is followed by the release of H⁺. Juarez-Rojop et al. 2014, further explained that phytochemicals such as tannis, flavonoids and phenols, which are some the phytochemicals found in the *M. burkeana* plant extract in this study act as both reducing and stabilizing agents during synthesis. Anchan et al. 2019, stated that the formation of the brown colour, the bubbles and precipitation as noted in this study, were caused by the reaction between the phytochemicals and the metal ions and served as an indication that the iron oxide NPs had formed. Devi et al., 2019 also elaborated that though many reactions take place when the iron salt is reacted with the extracts, it does not reduce Fe³⁺ to Fe⁰ as oxidation takes place, instead the phytochemicals react with the ions of the iron to form the NPs [9]. To further understand the potential characteristics of these materials, UV-vis DRS was conducted as it is known that optical properties of semiconducting materials are strongly linked to their optical absorption capability which might later affect their photocatalytic activity.

In Figure 3a, the UV-vis showed that the material got excited at 320 nm, which confirmed the formation of Fe₃O₄ as this was a characteristic feature of Iron oxide nanoparticles. This result was further corroborated by Lakshminaranayan et al. [13] and other researchers whereby they found that Iron oxide peaks are normally found between 300–350 nm. This also depending on the phytochemicals used as reducing agents for the synthesis of green derived Iron oxide nanoparticles. It is known that iron nanoparticles generally absorb a certain amount of UV radiation which is caused by the movement of the electrons from the valence band to the conduction band. The bandgap was also measured (Figure 3b) through the Tauc plot where it was found to be 3.75 eV. Morphological analysis of this material through SEM/EDS for the plant derived Fe₃O₄ was conducted to obtain more information.

3.3. Morphological analysis

SEM and EDS analysis was conducted to determine the morphology and elemental composition of the synthesized Fe₃O₄ NPs. Figure 4(a, b, c) shows the morphology of the Fe₃O₄ NPs to be rodlike. This was different from what was observed by Pattanayak et al. (2013) who successfully...
synthesized Fe$_3$O$_4$ NPs using various plant extracts. The SEM image of the synthesized NPs revealed they were mostly spherical in shape \[25\]. These differences could be due to the varying phytochemicals identified in the plant which could have played a role in the formation of spherical and rodlike Fe$_3$O$_4$ nanoparticles. Figure 4(d) shows the elemental composition of the Fe$_3$O$_4$ NPs which demonstrated that the elements Fe and O are in abundance (Figure 4e), confirming the formation of the NPs in addition to the C peak, identified to be from the plant extract \[21\]. To understand the thermal stability of the synthesized Fe$_3$O$_4$ NPs, as it is an important factor in catalytic activities, TGA was conducted.

### 3.4. Thermal stability of Fe$_3$O$_4$ NPs

The percentage weight loss was studied by TGA for green synthesized Fe$_3$O$_4$ NPs. The purpose of this was to investigate the decomposition and to gain structural information about the NPs. From Figure 5(a), the TGA curve shows a weight loss over the range. Four derivative peaks in the DTG curve (Figure 5b), (more than three for Fe$_3$O$_4$ NPS implying the presence of impurities) were identified which correlated to the mass losses found in the TGA curve. The peak just under 100 °C, had a mass loss of 22%, was due to decomposition of water, caused by the OH groups present in the Fe$_3$O$_4$ NPs. A peak at 280 °C, with a percentage mass loss of 5% was caused by the decomposition of the protein chains from the plant extract. At 444 °C, a percentage weight loss of 15% due to the capping agents from the plant extract in the Fe$_3$O$_4$ NPs was noted. The last peak at

| Element | Wt% | Wt% Sigma |
|---------|-----|-----------|
| C       | 6.52| 0.08      |
| O       | 28.57| 0.12     |
| Fe      | 64.91| 0.23     |

Figure 4. (a, b, c) SEM at various magnifications, (d) EDS of Fe$_3$O$_4$ NPs and (e) Weight.

Figure 5. (a) TGA and (b) DTG of Fe$_3$O$_4$ NPs.
780 °C, with a mass loss of 15% could have been due to phase transition from Fe3O4 to FeO since it has been shown that FeO is highly stable beyond 570 °C.

3.5. Surface area studies (BET)

BET analysis (Fig. 6a, b, c), was conducted in order to gain information regarding surface properties such as surface area, pore size and volume of the green synthesised Fe3O4 NPs. The results for this experiment are given in Table 1. The surface area was shown to be 12.83, with a pore size of 35.67 nm, which falls within the range 2–50 nm implying that the sorbent is mesoporous. Venkateswarlu et al. (2013) synthesized green iron oxide NPs using plantain leaf extract. The surface area of the synthesized NPs was comparable to the one reported in our study and found to be 11.31 mg2/g. The structure of the NPs was assigned to be mesoporous based on the BET surface area and pore volume results. This data clearly shows that Fe3O4 NPs can be used as a photocatalyst for the degradation of various pollutants from wastewater.

| Plant                | dosage (mg) | Time (min) | Dye     | pH | % Removal | Refs.               |
|---------------------|-------------|------------|---------|----|-----------|---------------------|
| Carica papaya       | 800         | 250        | RRRY    | 2  | 76        | Bhuiyane et al. [6]  |
| Withania coagulans  | 5           | 180        | Saffarin| NR | 68        | Qasim et al. [26]   |
| Ruellia tuberosa    | -           | 150        | CV      | NR | 79        | Vasanthiwar et al.  |
| Crotalaria arborescens | 10         | 180        | Janus Green | NR | 97%       | Sudhakar et al. [9] |
| Chlorelastrum comosum | 360       | 60         | MO      | NR | 77        | Aradani et al. [3]  |
| Monsonia burkeana   | 25          | 30         | MB      | 12 | 99.8%     | Present study       |

*RRRY-Remazol RR Yellow *CV-Crystal Violet *NR-Not recorded. *MB-Methylene Blue * JG-Janus Green.

3.6. Effect of pH on the degradation of MB using Fe3O4 NPs

The pH is one of the most important factors to be considered when testing a material’s ability to degrade. In this study, the effect of pH (Figure 7a, b, c, d) was investigated, whereby acidic, neutral and highly basic conditions were created. From the analysis, pH 12 (basic conditions), produced the highest degradation of 99% in less than 30 min. This is because MB dye as a thiazine dye has a cationic nature that tends to dissociate into negatively charged ions, especially at pH 12 [2]. This then increases the OH\(^+\) ions on the surface of the material. Thus increasing the OH\(^-\) radicals as active sites on the surface [23]. This leads to a higher and complete degradation percentage of MB. Moreover, since Iron oxide materials are magnetic, it therefore, would have undergone the electric
dipole moments where the positively and negatively charged ions are easily separated, thus allowing the surface material to adsorb and degrade the dye [26, 25, 28]. These results showed an improved and complete degradation of MB dye within 60 min. Various studies also reported a degradation of more than 95% for MB at basic conditions [6, 26]. At pH 3, the lowest degradation of 18% was noted. This is because at acidic conditions more positively charged ions are formed and when they interact with a cationic dye they tend to form repulsion, thus exhibiting minimal dye degradation. At neutral conditions though, not much degradation was noted, with only 25% of MB degraded.

The rate of the reaction was also investigated as exhibited in Figure 7(e). The photocatalytic results were calculated using the Langmuir Hinshelwood equation:

$$\ln(C_0/C_t) = kt$$

Where the rate of the reaction is represented by the ln, $C_0$ is the initial concentration, $C_t$ is the final concentration, $k$ is the rate of reaction constant and $t$ is reaction time [19]. According to the plotted results indicated in Figure 7(e), the rate of the reaction is following the Pseudo first-order kinetic with the kinetics rate equal to $k = 0.096 \text{ min}^{-1}$, thus leading to the fastest rate of reaction conducted at pH 12 than the rest of the pH measurements. When comparing our results with previously published data, it can be noted that iron oxide nanoparticles are effective in dye removal in general [29, 30]. Bhuyan et al. got a 99% removal at basic conditions of pH 12 as well with the minimal rate of 0.008 min$^{-1}$ following first order ($R^2 = 0.99$) kinetic. Another study, followed first order $R^2 = 0.9943$ kinetic with the rate of 0.00016 min$^{-1}$ [31]. Also importantly to note, our green derived Fe$_3$O$_4$ was highly efficient for dye degradation as a 99% efficiency was noted for MB at 30 min, whereas for other studies (Table 1) a longer period of time (300 min, 250 min, 180 min etc) was required to have a high degradation. Moreover, in some instances a higher catalyst amount (800 mg) with prolonged periods was required but still not a complete degradation was achieved. It can also be noted also that in all the studies, different dye pollutants were used, as that maybe could also play a role in the differing degradation efficiencies. The complex aromatic nature of the various dyes is one of the reasons that has made researchers to move towards the synthesis of multifunctional materials, that can be used for the removal and degradation of various pollutants. The reusability studies were performed for 4 trials to assess the stability and the reusability property of our material.

3.7. Reusability studies of Fe$_3$O$_4$ NPS against MB dye

The reusability studies of Fe$_3$O$_4$ NPs was evaluated at optimal conditions of 25 mg dosage, 5 ppm concentration and pH 12 for 4 cycles (Figure 8a, b, c, d). The stability of a material is an important factor as it also contributes to the cost factor of the synthesized materials. The results illustrate that the material is stable and maintained its excellent photocatalytic efficiency. This is noticed when the degradation activity of all these cycles obtained was above the 98% degradation even with the last cycle (Figure 8e). Since the stability and the recyclability efficiency was perceived, the reactive species responsible for the degradation of MB and the mechanism is further explored in the following section.
3.8. Effect of reactive species on the degradation of MB and mechanism

P-benzoquinone, EDTA, isopropyl alcohol and AgNO₃ were used as the four quenchers, to evaluate the contribution of scavengers and which one was responsible on the degradation against MB dye. The reactive species (main scavengers) of superoxide (O₂⁻), holes (h⁺), hydroxyl radicals (•OH), and electrons (e⁻) radicals, respectively were added to the trapping experiments [15]. Upon testing the effect of these species, the experiments were conducted the same way as the normal degradation experiments except that 2.0 mmol/L of the different scavengers were added. The results from the trapping studies are displayed in Figure 9(a, b, c, d).

From the results (Figure 9e), it can be noted that the degradation of MB was not affected by the addition of OH and h⁺ quenchers. However, trappers such as the electrons (e⁻) and O₂⁻ radicals played a major role as the degradation of MB decreased to 45.4 and 16.8 respectively. The O₂⁻ radicals were the major species responsible for the degradation. The study done by Makofane et al. [15], showed that electrons were the main contributors to the degradation process. This could have been because their material was a metal iron-based material for the removal of dye. Other authors also had the most effective species as a superoxide which correlates with our study, followed by the hydroxyl and the electrons. Whereas in our study, the most effective reactive species are the superoxide, followed by the electrons for the photocatalytic degradation. This shows that when there was enough light absorbed on the Iron oxide surface (Scheme 1) and they had reached the valence band (VB), the excited e⁻ moved to the conductive band (CB) whilst leaving the holes behind. In the process, the O₂ and OH radicals were also forming and as noted in radicals. The O₂ radicals which were absorbed on the surface of the material, contributed to the removal of MB dye during photocatalytic degradation. Our material was further evaluated for the photo-degradation of the SSX antibiotic pollutant.

3.9. Photodegradation of antibiotic sulfisoxazole (SSX) using Fe₃O₄ NPs

Sulfonamides are pharmaceutical pollutants that have gained a lot of attention in the last decade due to the increased over reliance on medications such as antibiotics. Through excretion and urine feaces, these antibiotics have found their way into the water streams and the concentrations are increasing uncontrollably, hence the need for viable methods and newer materials to be used. In this study, the effect of time on the degradation of Sulfisoxazole (SSX) was investigated. The UV-Vis spectra of the results are exhibited in Figure 10(a). It can be noted that the degradation effect was monitored over 120 min at 30 min intervals. A maximum degradation of 60% was achieved after 120 min. These results also indicate that the degradation of SSX using Fe₃O₄ followed second-order kinetic as displayed in Figure 10(c). When comparing with the previously published work, a 67% degradation was noted against SSX using a nickel ferrite nanoparticle [15]. When iron oxide was degraded against another antibiotic, tetracycline, a low degradation of 40% was achieved [21]. They noted that when there is a high level of adsorption taking place between the catalyst surface and the pollutant before degradation occurs, photodegradation of the antibiotic might be impared. Lack of penetration of light might take place due to the screening, which would then mean that the formation of the excited electrons and holes might be limited. To further test potency of this material against other pollutants, the antibacterial efficiency of this material against various bacterial strains from real water samples was investigated.
3.10. Antibacterial efficiency of M. Burkeana Fe$_3$O$_4$ NPs against various strains

The antibacterial potency of *M. Burkeana* Fe$_3$O$_4$ NPs was investigated against Total Coliforms, *E. Coli* and *S. Aureus*. From Table 2, it can be noted that water from the pond, river, tap and sewage was investigated. In all the water types, a high number of total coliforms in particular for sewage water was detected. This could be due to the fact that total coliforms generally consist of all the different types of bacteria as there is no classification or differentiation from the other strains. In pond, river and tap water there was no gram negative *E. coli* detected and in tap water even the gram positive *S. aureus* was not identified. Only in sewage water

![Figure 9](image)

(Figure 9. (a, b, c, d) the degradation of the scavengers and (e) the percentage degradation of active species.)

![Scheme 1](image)

(Scheme 1. Schematic depicting photocatalytic degradation of MB dye.)
were all the strains detected. Upon treating these various water bodies using *M. burkeana* Fe₃O₄ NPs with 2 differing concentrations, for tap water which had the least number of microbes and only total coliforms, a 100% removal was noted at the highest concentration of the catalyst. In pond water, only a 98% removal could be achieved for total coliform and *S. aureus*. In both river and sewage water, a 100% total removal of *S. aureus* was achieved even at the lowest concentration. In sewage water the total coliform even after treatment with the highest concentration, was still very high. From this analysis it can be concluded that our synthesized material was more potent towards gram positive strains. Generally it is expected that the highest potency will be achieved against gram negative strains since they have a single peptidoglycan layer, they should be more prone to the breakdown of the bacterial cell but surprisingly as noted in the sewage water, the *E. coli* was highly resistant.

Several factors other than the cell wall of the strains can play a role in the potency of the material, factors such as the formation of the radical species, particle size, morphology and stability also can be factored in. Also, other studies have noted that because of the positive charge of the metal cation and negative charge of the bacteria, due to the attraction, these materials become equally enticed, which then results in a denaturation of the proteins, resulting in the destruction of the pathogen. The efficiency of nanoparticles against gram positive strains more than gram negative strains though it is not expected it is also not unusual as several studies have reported such a pattern before [19]. Furthermore, it is known that gram negative strains tend to be defiant against several antibodies since their cell wall though thin can be impenetrable. This study has therefore shown that our green derived iron oxide material can be used for the degradation and removal of several pollutants in water.

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**Figure 10.** (a) Uv degradation spectra of Fe₃O₄ against the antibiotic SSX, (b) the degradation % of SSX versus the time and (c) kinetic plot versus time.
Table 2. Effect of Fe3O4 NPs against various bacterial strains in real water samples.

| Water sample | Total coliform | E. coli | S. aureus |
|--------------|---------------|---------|-----------|
| Pond         | 1.7 x 10^3   | 0       | 66        |
| B            | 2.0 x 10^2   | 0       | 53        |
| C            | 10           | 0       | 10        |
| River        | 5.8 x 10^3   | 1.0 x 10^7 | 0       |
| B            | 67           | 0       | 0         |
| C            | 10           | 0       | 0         |
| Tap          | 1.0 x 10^2   | 0       | 0         |
| B            | 30           | 0       | 0         |
| C            | 0            | 0       | 0         |
| Sewage       | 4.9 x 10^4   | 3.0 x 10^4 | 50       |
| B            | 2.0 x 10^3   | 67      | 0         |
| C            | 1.3 x 10^3   | 10      | 0         |

4. Conclusion

This study has successfully reported on the green synthesis Fe3O4 NPs as confirmed by FTIR, XRD, EDS, SEM, BET, TGA and DTG. FTIR confirmed the presence of carbohydrate groups from the plant extract on the synthesized Fe3O4 NPs. The characteristic peaks of Fe3O4 NPs were observed on the XRD plots of the green synthesized NPs. With this information, the crystallites sizes were calculated and found to be around 60 nm for the green synthesized NPs. DTA and TGA revealed the presence of organic moieties on the synthesized NPs, with their decomposition identified between 450–600 °C. BET analysis showed that Fe3O4 NPs was mesoporous in structure, meaning that their structure can adsorb and degrade organic and antibiotic pollutants. Fe3O4 was effective in degrading the organic and the antibiotic pollutants, obtaining the highest degradation of 99 and 60% removal for MB and SSX, respectively. The kinetic followed first order and seconder kinetics for the MB and SSX, respectively. The superoxide was the most effective radical followed by the electrons radical on the trapping studies. The reusability study for our samples.

Additional information

No additional information is available for this paper.

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