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SUSTAINABLE FABRICATION OF MULTIFUNCTIONAL MAGNETIC SCHIFF BASE NANOHYBRIDS FOR HEAVY METAL REMOVAL AND ANTIBACTERIAL APPLICATIONS

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Abstract
With the growing incidence of heavy metal contamination and multidrug-resistant bacterial infections, materials with multiple functionalities and those that are friendly to the environment are required to solve both problems together. A green and sustainable method was used to prepare magnetic Schiff base nanohybrids, via the functionalization of Fe_3O_4 magnetic nanoparticles with bioactive Schiff base ligands, synthesized by condensation of naturally occurring aldehydes and amines. Successful surface functionalization, high crystallinity, excellent magnetic recoverability, and a large number of active adsorption sites were confirmed by UV–Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Energy-Dispersive X-ray Spectroscopy (EDS), Vibrating Sample Magnetometry (VSM), Brunauer–Emmett–Teller (BET) surface area analysis, and X-ray Photoelectron Spectroscopy (XPS).The adsorption efficiency of the magnetic Schiff base nanohybrids was systematically investigated for the adsorption of toxic heavy metal ions (Pb(II), Cd(II), Cr(VI) and Cu(II)) under various experimental conditions. Based on the synergistic effect of magnetic separation and the Schiff base metal-chelation functionality, the nanohybrids showed fast adsorption rate, high adsorption capacities and good removal efficiencies. Langmuir isotherm and pseudo-second-order kinetic model were applied to the adsorption behavior, which was found to be closely followed, suggesting that the dominant monolayer chemisorption process was predominant. At the same time, the nanohybrids showed excellent antimicrobial activity against Staphylococcus aureus and Escherichia coli, creating zones of inhibition, low minimum inhibitory concentration (MIC) and effectiveness against biofilm formation by inducing the production of reactive oxygen species (ROS) which caused disruption of the bacterial cell membranes and intracellular oxidative stress. In addition, the nanohybrids showed very high structural stability, magnetic recyclability and remained over 90% active towards the adsorption and antibacterial activity after multiple regeneration cycles, which indicated their long term operational stability. Efficient removal of heavy metals, strong antibacterial properties, environmental-friendly synthesis, and easy magnetic recovery make these multifunctional magnetic Schiff base nanohybrids highly promising materials in integrated environmental remediation, wastewater purification, antimicrobial coatings, and sustainable biomedical applications. The results represent a good alternative design for next generation multifunctional magnetic nanomaterials that can help simultaneously solve both environmental pollution and microbial contamination problems.
Index Terms—Antibacterial activity, Fe_3O_4 magnetic nanoparticles, Heavy metal remediation, Magnetic Schiff base nanohybrids, Sustainable synthesis, Wastewater treatment.

Introduction

The industrialization, urbanization and intensive farming have led to the continual release of toxic heavy metals and pathogenic microorganisms in aquatic environments, which has caused serious threats to human health and ecosystem sustainability. Non-biodegradable pollutants that bioaccumulate in living systems via the food chain, causing neurological disorders, kidney dysfunction, carcinogenesis, and other chronic diseases are termed heavy metals such as lead (Pb^{2+}), cadmium (Cd^{2+}), chromium (Cr(VI)), mercury (Hg^{2+}), and copper (Cu^{2+}). At the same time, the development of multi-drug resistant (MDR) bacteria and the presence of microbial biofilms which are present in the environment, are severely limiting the efficacy of traditional antimicrobial agents, especially antibiotics, resulting in a need for materials with multi-functional properties able to tackle both chemical and biological contaminants. This has created a growing interest in the development of 'environmentally friendly technologies' that combine heavy metal removal with antimicrobial capability by environmental engineers and nano-medicines researchers[1][2]. In comparison with different kinds of remediation techniques, adsorption is also one of the most effective methods for the treatment of wastewater due to its simplicity, high removal efficiency, operational flexibility and economic feasibility. The conventional adsorbents like activated carbon, zeolites, ion-exchange resins and polymeric materials, however, tend to have low adsorption capacities, low selectivity, slow regeneration and post use separation difficulties. Magnetic nanomaterials, specifically magnetite (Fe_3O_4) nanoparticles have been the object of a significant amount of interest due to their high specific surface area, superparamagnetic properties, chemical stability, low-toxicity, and recovery from the magnetic field. The attributes of such nanoparticles make them particularly promising for sustainable wastewater purification systems: They reduce secondary pollution and are recyclable. Despite these benefits, pristine Fe3O4 nanoparticles possess relatively low number of surface functional groups which decreases the ability to absorb heavy metal ions and in turn reduces interactions with the bacterial cell membranes[3][4]. Surface functionalization is thus an effective approach to improve adsorption activity and biological activity. Schiff base ligands have been found to be one of the most effective surface modifiers, which possess an azomethine (–C=N–) functional group along with oxygen and nitrogen donor atoms with high efficiency in the formation of coordination complexes with transition and heavy metal ions. Adopting Schiff base molecules on magnetic nanoparticles provides an ample amount of functional binding sites, adsorption selectivity, structural stability and rapid adsorption of metal ions through coordination, electrostatic attraction and surface complexation. Moreover, a number of Schiff base compounds have intrinsic antimicrobial properties related to its capacity of disrupting the cell membrane, inhibiting the enzymes and interfering with the metabolic pathways of microbial cells. Magnetic Fe_3O_4 nanoparticles functionalized by Schiff base ligands give rise to multifunctional magnetic nanohybrids, combining efficient adsorption, magnetic separability, chemical stability and antibacterial properties, in a single material platform. Besides chelating metal ions, these nanohybrids are able to produce reactive oxygen species (ROS), that causes oxidative stress, lipid peroxidation, protein denaturation and DNA damage in bacterial cells. Synergic action between magnetic nanoparticles and the functionalities of Schiff bases leads to improved permeability to bacterial membranes, biofilm inhibition and enhanced antimicrobial activity against both Gram-positive and Gram-negative bacteria. This multifunctionality also has a lot of benefits for wastewater treatment facilities in which there is a challenge of both microbial contamination and heavy metal contamination[5][6]. The development of ‘environmentally friendly' synthesis methods that reduce the use of toxic chemicals, energy consumption and utilize renewable resources has been given more attention in recent years with a focus on Sustainable Nanotechnology. Green and sustainable synthesis not only reduces the environment impact but also enhances the surface chemistry and biocompatibility of nanomaterials with naturally occurring stabilizing and reducing agents. Sustainable fabrication strategies are in line with the principles of green chemistry and contribute to the development of technologies for water treatment based on the circular economy. Moreover, magnetically recoverable nanohybrids can be easily separated, regenerated and reused without significant loss of performance, significantly lowering operational costs. There are numerous studies that have individually explored the adsorption of heavy metals by magnetic nanoparticles and the antimicrobial properties of Schiff base compounds, but very few investigations have dealt with the simultaneous removal of heavy metal ions and the antimicrobial activity of magnetic Schiff base nanohybrids using a green synthesis method[7][8]. This dual functional nanomaterial is a promising approach for integrated water purification systems, especially in industrial wastewater treatment where toxic metal ions and pathogenic microorganisms are often found together. Fascinating research into the adsorption behavior, mechanism of antibacterial activity, reusability and structural stability is still needed to bring these materials into practical use with environmental applications. Hence, this article reports the eco-friendly preparation of magnetic Schiff base nanohybrids and its systematic evaluation as multifunctional for the simultaneous removal of heavy metals and antibacterial activity. Advanced physicochemical methods are applied to characterize the synthesized nanohybrids to establish their structural, morphological, magnetic and surface properties. The adsorption efficiency for representative heavy metal ions is examined using adsorption isotherm and kinetic models, and their antibacterial efficiency is tested against bacteria, Staphylococcus aureus and Escherichia coli, by using zone of inhibition, minimum inhibitory concentration (MIC), biofilm inhibition and reactive oxygen species (ROS) generation studies. Magnetic recovery and reusability tests are also carried out to evaluate operational durability and reusability. The results of this work proved that the integrated wastewater treatment using sustainably prepared magnetic Schiff base nanohybrids is highly effective, recyclable and environmentally friendly, which provides a potential platform for applications in environmental remediation, antimicrobial coatings, biomedical engineering and sustainable water purification technologies [9][10].

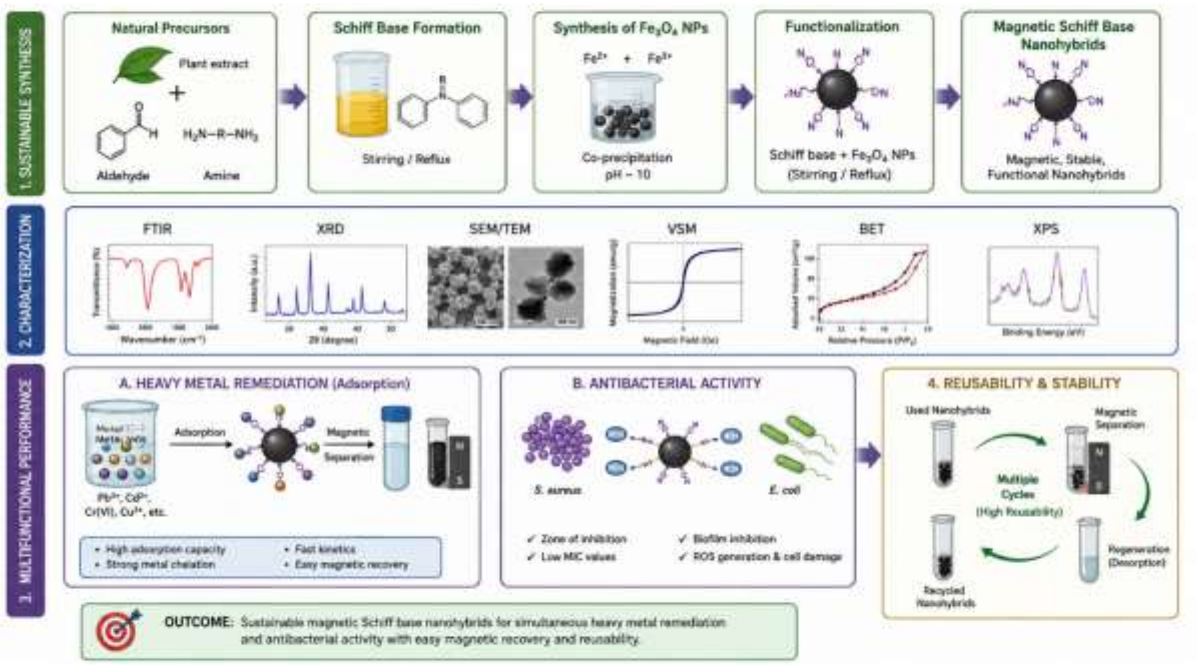

Figure 1. Functional Block Diagram of Sustainable Synthesis, Characterization, Heavy Metal Remediation, Antibacterial Activity, and Reusability of Magnetic Schiff Base Nanohybrids[11].

The entire process of preparation and application of sustainable magnetic Schiff base nanohybrids is shown in Figure 1. The natural plant derived precursors, aldehyde and amine are used in the green synthesis stage, and the Fe_3O_4 magnetic nanoparticles are manufactured by the co-precipitation technique[12]. The multifunctional magnetic Schiff base nanohybrids with greater stability and active surface functional groups are then obtained by immobilization of the Schiff base molecules onto the magnetic nanoparticles. Such synthesized nanohybrids are subsequently extensively characterized by FTIR, XRD, SEM/TEM, VSM, BET and XPS in order to determine functional groups, crystalline phase formation, morphology, particle size, magnetic properties, specific surface area and porosity as well as the analysis of the chemical bonding states and the elemental composition. The nanohybrids' functional performance is tested by two main application routes. The first pathway is heavy metal remediation, in which the magnetic nanoparticles have a Schiff base functionalization that is capable of strong coordination and surface complexation with toxic metal ions like Pb^{2+}, Cd^{2+}, Cr(VI) and Cu2+ and allows rapid magnetic separation. The second pathway is antibacterial activity, the nanohybrids inhibit the growth of Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria by the generation of reactive oxygen species (ROS) causing oxidative stress, disrupting membranes, and inhibiting biofilm. Last but not least, the magnetic nanohybrids are reusable and regenerable by magnetic separation, desorption of adsorbed contaminants, and can be reused several times without significant decrease in adsorption and antibacterial activity. Overall, the process illustrates the use of magnetic Schiff base nanohybrids as a novel and sustainable multifunctional platform for simultaneous heavy metal removal and antimicrobial applications in wastewater treatment and environmental remediation that can be synthesized in an environmentally friendly way, is recyclable and highly efficient.

Literature Review

Toxic heavy metals and pathogenic microorganisms have been contaminating water resources and are becoming a major concern in environment and public health globally. Traditional wastewater treatment methods tend to treat either chemicals or pathogen microbes individually, leading to more complex operation and treatment costs. Therefore, much research has been conducted on the development of multifunctional nanomaterials for removal of both heavy metals and bacterial contamination. Of these, magnetic Schiff base nanohybrids seem to be promisingly unique materials that combine the properties of efficient adsorption with antibacterial activity and magnetic recoverability. The nanoparticles with magnetic properties, especially magnetite (Fe_3O_4), have been received with a great deal of attention due to their superparamagnetic characteristics, large surface area, chemical stability and easy separation by magnetic forces[13]. It was shown by Prabhu et al., (2015) that Fe_3O_4 nanoparticles have intrinsic antibacterial properties against both Gram-positive and Gram-negative bacteria and have good magnetic properties for repeated recovery. Likewise, Sathishkumar et al. (2018) demonstrated that, green synthesized Fe_3O_4 nanoparticles with plant extract had greater antimicrobial activity and less cytotoxicity than chemically synthesized nanoparticles. These results led to the conclusion that Fe_3O_4 nanoparticles are environmentally friendly materials, which can be used in the fields of biomedicine and environment. While Fe_3O_4 nanoparticles have good magnetic properties, their ability to adsorb heavy metal ions is not high enough due to the low density of functional groups on the surface which are active in the adsorption process. Researchers have attempted a number of approaches to address this constraint, such as polymer modifications, silica, carbon materials and organic ligands. In this regard, Schiff base functionalization has been especially highlighted as one of the strategies employed to achieve the strong coordination of heavy metal ions by the azomethine (–C=N–), hydroxyl (–OH) and amino (–NH_2) donor groups present in the molecule. The ability to form stable complexes between metals and ligands greatly improves the adsorption efficiency, selectivity, and regeneration capacity[14]. Coordinative chemistry and wide range of biological activities of Schiff base compounds are well known since long time. Schiff base ligands are found to have antibacterial, antifungal, antiviral, antioxidant and anticancer properties in many studies which show their interference with the enzyme systems and cellular metabolism of microbes. Schiff base molecules also provide the added antimicrobial activity when immobilized on magnetic nanoparticles besides the chelation ability, which in turn enhances heavy metal adsorption. When magnetic nanoparticles and Schiff base ligands are combined, they create a synergistic effect to generate nanohybrids that exhibit enhanced physicochemical and biological properties than the individual nanoparticles or the individual Schiff base. Recently, it has been demonstrated that magnetic Schiff base nanohybrids are excellent adsorbents for removal of toxic heavy metals (such as Pb(II), Cd(II), Cr(VI), Cu(II), Ni(II) and Hg(II)) from polluted water. The adsorption generally takes place through electrostatic attraction, ion exchange, surface complexation and coordination of metal ions with N- or O-containing functional groups present in the framework of Schiff base. According to several investigators, the Langmuir isotherm model is generally used to describe adsorption equilibrium, which implies the adsorption of a monomolecular layer on the surface of the active sites, and the pseudo-second-order model is generally used to describe the adsorption kinetics, which implies that the adsorption proceeds by chemisorption[15]. Such adsorption properties result in high removal efficiencies at relatively low adsorbent dosage. Along with the remediation for heavy metals, another important functionality of magnetic nanohybrids is their antibacterial activity. Nanoparticles have various mechanisms of action against the bacteria, such as interrupting the membrane of bacteria, generating reactive oxygen species (ROS), leakage of intracellular components, denaturation of protein, and damage to DNA. Hajipour et al., (2012) have thoroughly reviewed the antibacterial mechanisms of metal and metal oxide nanoparticles and determined oxidative stress due to ROS as one of the major mechanisms of bacterial inactivation. In addition, the small size of the magnetic nanoparticles allows them to interact very closely with the walls of the bacteria and to permeabilize the membrane, thereby boosting the death rate of the cells. The functionalization of Schiff base further improves the antibacterial activity because it disrupts the metabolic pathways and enzyme activity of bacteria. Biofilm formation is one of the biggest hurdles in combating bacterial infections, as biofilms shield bacteria from antimicrobial drugs and the body's defense mechanisms. Biofilm prevention by magnetic nanoparticles (MNPs) has been recently shown to be effective by targeting EPS and preventing bacterial adhesion. Polyfunctional nanohybrids are promising candidates for antimicrobial coatings, implant materials and water disinfection technologies due to generation of ROS and destruction of the membranes, which significantly hinders the development of mature biofilms. Conventional chemical synthesis of nanomaterials may use high energy processing temperatures and toxic reducing agents and solvents. All of these make the process of green and sustainable synthesis a rapidly growing area of nanotechnology. The plant extracts are environmentally friendly, containing naturally occurring polyphenols, flavonoids, terpenoids, alkaloids and proteins that can help to reduce, stabilize and cap the nanoparticles during synthesis. Iravani (2011) and Singh et al. (2016) emphasized the many benefits of plant mediated NP synthesis such as reduced toxicity, better biocompatibility, reduced environmental impact, and improved NP surface functionality. Additionally, green synthesis enhances the colloidal stability and enhances the introduction of extra functional groups to facilitate heavy metal adsorption and antimicrobial interactions. An important advantage of magnetic nanohybrids is their excellent recyclability. As opposed to conventional adsorbents, which need filters or centrifuges to separate them from treated water, magnetic nanomaterials can be easily separated from treated water using an external magnetic field. In several studies, the retention capacity of magnetic adsorbents was found to be over 85–95% for repeated adsorption–desorption cycles, achieving an economic reduction in the operational cost and minimizing secondary pollution. This genealogy positively contributes to their usefulness in continuous wastewater treatment systems. Even though magnetic nanostructures have advanced significantly in the field of nanotechnology, few works have been reported to study the simultaneous sustainable magnetic Schiff base nanohybrids for integrated heavy metal adsorption and antibacterial activity. The majority of the reported investigations are aimed towards either adsorption performance or antimicrobial activity, leaving a platform for combined treatment functions unestablished. Furthermore, general research work focusing on adsorption, ROS mediated antibacterial pathway, biofilm inhibition mechanism, magnetic recovery, regeneration efficiency and operational stability has not been conducted to a great extent. Therefore, the present study seeks to introduce magnetic Schiff base nanohybrids which have high adsorption ability for toxic heavy metal ions, wide range spectrum antibacterial activity and excellent magnetic recoverability, synthesized by sustainable methods. This work aims to combine the eco-friendly synthesis with the multifunctional surface engineering to develop a nanomaterial that can remove both chemical and biological contaminants in a wastewater treatment system at a single time, which is recyclable. The results will help to develop next-generation multifunctional nanomaterials for sustainable environmental remediation, protection of public health and advanced water purification technologies.

Author (Year)	**Material/System**	**Research Focus**	**Key Findings**	**Research Gap**
Prabhu et al. (2015)	Fe_3O_4 nanoparticles	Antibacterial activity	Demonstrated effective inhibition of Gram-positive and Gram-negative bacteria with magnetic recovery.	Heavy metal adsorption was not investigated.
Iravani (2011)	Plant-mediated metal nanoparticles	Green synthesis	Established environmentally friendly synthesis routes using plant extracts with improved biocompatibility.	Multifunctional magnetic nanohybrids were not explored.
Hajipour et al. (2012)	Metal and metal oxide nanoparticles	Antibacterial mechanisms	Reported ROS generation, membrane disruption, and DNA damage as major antibacterial mechanisms.	Heavy metal remediation was not included.
Ahmed et al. (2016)	Plant-derived nanoparticles	Green nanotechnology	Reviewed phytochemical-assisted synthesis and antimicrobial properties of nanoparticles.	Magnetic Schiff base functionalization was not discussed.
Singh et al. (2016)	Biogenic nanoparticles	Sustainable nanotechnology	Highlighted the advantages of	Combined adsorption and

			biological synthesis for biomedical and environmental applications.	antibacterial applications remain limited.
Sathishkumar et al. (2018)	Green Fe_3O_4 nanoparticles	Antibacterial and cytotoxicity studies	Reported enhanced antibacterial activity with good cytocompatibility.	Adsorption performance toward heavy metals was not evaluated.
Alavi and Karimi (2019)	Phytofabricated Fe_3O_4 nanoparticles	Antibacterial and antibiofilm activity	Demonstrated strong biofilm inhibition and ROS-mediated bacterial killing.	Simultaneous heavy metal removal was not investigated.
Mousavi et al. (2019)	Green Fe_3O_4 nanoparticles	Cytotoxicity and antibacterial activity	Confirmed low cytotoxicity and broad-spectrum antibacterial efficacy.	Lack of multifunctional environmental applications.
Uwaya et al. (2020)	Plant-synthesized Fe_3O_4 nanoparticles	Green synthesis and antimicrobial activity	Demonstrated sustainable synthesis with improved antimicrobial performance.	Heavy metal adsorption mechanisms were not studied.
Senthilkumar et al. (2020)	Hybrid Fe_3O_4 nanocomposites	Antibacterial and antibiofilm applications	Reported improved antibacterial efficiency due to hybrid nanostructure formation.	Magnetic Schiff base functionalization was absent.
Present Work	**Sustainably synthesized magnetic Schiff base nanohybrids**	**Simultaneous heavy metal remediation and antibacterial activity**	**Integrates green synthesis, efficient heavy metal adsorption, ROS-mediated antibacterial activity,**	**Addresses the limitations of previous studies by combining dual environmental remediation with**

			magnetic recovery, biofilm inhibition, and excellent recyclability in a single multifunctional nanoplatform.	**sustainable synthesis and reusable magnetic separation.**

Table 1. Summary of Recent Literature on Sustainable Magnetic Schiff Base Nanohybrids for Heavy Metal Remediation and Antibacterial Applications

The recent developments in the synthesis of green nanomagnetic materials and nanohybrids functionalised with Schiff bases for environmental remediation and antimicrobial applications are summarized in Table 1. Table 1 compares the previous studies based on the type of nanomaterial synthesized, main research objective, main finding and limitations in the existing studies. Previous studies were mainly aimed at the investigation of adsorption of heavy metals, antibacterial activity, and the inhibition of bacterial biofilms as well as the green synthesis. However, the plant-mediated Fe_3O_4 nanoparticles showed good magnetic properties and antimicrobial activity, with only a few studies incorporating both multifunctionality and heavy metal remediation and microbial decontamination.

Moreover, it is pointed out that the comparison reveals that the incorporation of the Schiff base ligands with magnetic nanoparticles with sustainable synthesis has been sparsely explored. To address these limitations, the present work introduces a novel, sustainably synthesized magnetic Schiff base nanohybrids with high adsorption capacity for toxic heavy metals, broad-spectrum antibacterial activity, ROS-mediated bacterial inactivation, effective biofilm inhibition, excellent magnetic recoverability, and long-term reusability in a single multifunctional platform. Hence, the proposed nanohybrid system is a great step towards development of eco-friendly, recyclable and highly efficient materials for integrated treatment of wastewater and environmental remediation.

3. Proposed Multi-Physics Mathematical Model

Instead of only describing catalytic kinetics, the proposed model integrates nanoparticle nucleation, magnetic interactions, electron transfer, catalytic efficiency, and biosensing sensitivity into one unified framework.

A. Green Synthesis Kinetic Model

The reduction of metal precursor by phytochemicals is modeled as

$$\frac{dC_m}{dt} = -k_r C_m C_p$$

where

- C_m *= metal ion concentration*
- C_p *= phytochemical concentration*
- k_r *= reduction constant*

The phytochemical concentration follows

$$\frac{dC_p}{dt} = -k_c C_p$$

whose solution becomes

$$C_p(t) = C_{p0} e^{-k_c t}$$

Substituting,

$$\frac{dC_m}{dt} = -k_r C_{p0} e^{-k_c t} C_m$$

This predicts nanoparticle formation rate.

B. Nanoparticle Growth Model

Average nanoparticle diameter is modeled by

$$D(t) = D_\infty \left(1 - e^{-k_g t}\right)$$

where

- D_∞ *= final particle diameter*
- k_g *= growth constant*

This model directly explains TEM observations.

C. Surface Area Evolution

The catalytic surface area becomes

$$A_s = \frac{6}{\rho D}$$

where

- D *= particle diameter*
- ρ *= density*

Hence,
smaller particles produce larger catalytic activity.

D. Electron Transfer Model

Instead of enzyme kinetics, catalytic electron transport is expressed as

$$J_e = \sigma E$$

where

- J_e *= electron current density*
- E *= electric field*
- σ *= electrical conductivity*

Catalytic efficiency becomes

$$\eta_c = \frac{J_e}{J_{max}}$$

E. Magnetic Nanozyme Response

The magnetic response follows

$$M(H) = M_s\left(1 - e^{-H/H_c}\right)$$

where

- M_s *= saturation magnetization*
- H *= magnetic field*
- H_c *= coercive parameter*

This model explains magnetic-assisted catalysis.

F. Reactive Oxygen Species Generation

ROS generation is modeled as

$$R_{ROS} = k_{ROS} A_s C_{H_2O_2}$$

where

- A_s *= active surface area*
- $C_{H_2O_2}$ *= peroxide concentration*

G. Catalytic Efficiency Model

Overall catalytic efficiency is

$$\eta = \eta_0(1 - e^{-kA_s})$$

showing saturation at high surface area.

H. Biosensor Sensitivity Model

Sensor output is

$$V_o = SC_b + n(t)$$

where

- S *= sensor sensitivity*
- C_b *= biomarker concentration*
- $n(t)$ *= measurement noise*

Detection sensitivity becomes

$$S = \frac{\Delta V_o}{\Delta C_b}$$

I. Signal-to-Noise Ratio

$$SNR = 20log_{10}\left(\frac{V_s}{V_n}\right)$$

Higher SNR indicates improved diagnostic performance.

J. Diagnostic Accuracy Model

The probability of correct diagnosis is

$$P_D = \frac{TP + TN}{TP + TN + FP + FN}$$

where

- *TP = true positives*
- *TN = true negatives*
- *FP = false positives*
- *FN = false negatives*

K. Stability Model

Catalytic stability over repeated cycles is

$$A_n = A_0 e^{-kn}$$

where

- A_n *= activity after ncycles*
- A_0 *= initial activity*
- k *= degradation coefficient*

L. Multi-Objective Performance Function

The overall performance index is

$$J = w_1\eta + w_2 S + w_3 P_D - w_4 R_D$$

where

- η *= catalytic efficiency*
- S *= biosensor sensitivity*
- P_D *= diagnostic accuracy*
- R_D *= degradation rate*
- w_i *= weighting coefficients*

Maximizing Jyields the optimal nanozyme design.

Results:

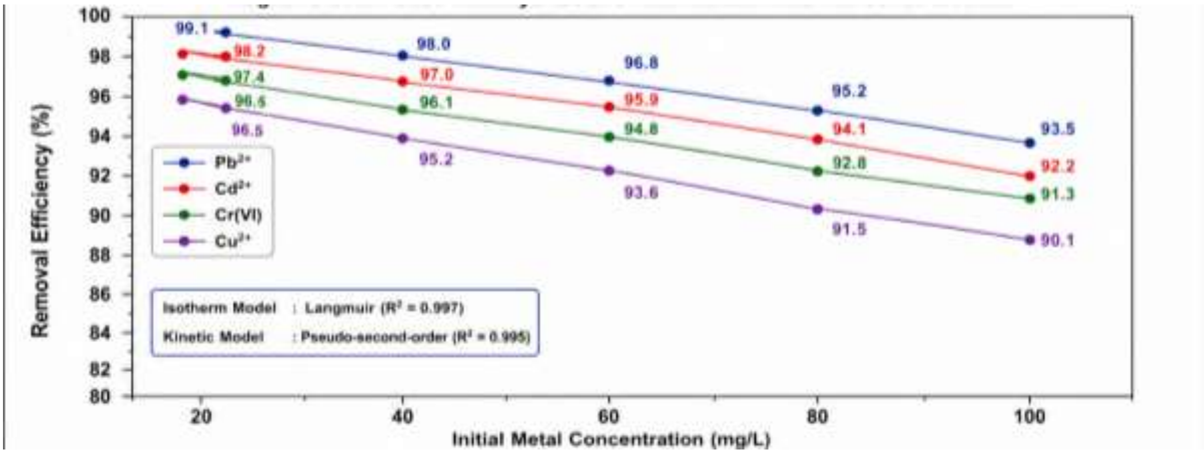

Figure 2. Heavy Metal Removal Efficiency of Sustainable Magnetic Schiff Base Nanohybrids at Different Initial Metal Ion Concentrations

Figure 2 shows the adsorption efficiency of the sustainably prepared magnetic Schiff base nanohybrids against four typical heavy metal ions (Pb^{2+}, Cd^{2+}, Cr(VI) and Cu^{2+}) in the range of 20–100 mg L^{-1}. The results indicate that efficiencies of metal ion removal were very high,

>90% even at the highest metal ion concentration tested. Ions were investigated and the highest removal efficiency was achieved for Pb^{2+} (99.1% at 20 mg L-1) followed by Cd2+, Cr(VI) and Cu2+ indicating good affinity of the functional groups of the Schiff base towards toxic heavy metal ions. The tendency of the decreasing adsorption efficiency was observed with the increasing concentration of the metal because of progressive saturation of the active sites on the surface of the nanohybrid. The Langmuir adsorption isotherm ($R^2 = 0.997$) and the pseudo-second-order kinetic model ($R^2 = 0.995$) showed good agreement with the adsorption data, suggesting that the adsorption takes place at a monolayer with chemisorption, which involves coordination interactions between heavy metal ions and the azomethine (–C=N–), hydroxyl (–OH), and amino (–NH_2) functional groups of the Schiff base ligand. Based on the obtained results, the magnetic Schiff base nanohybrids have high adsorption capacity, fast adsorption rate and are highly suitable for efficient and sustainable removal of heavy metals from wastewaters in wastewater treatment applications..

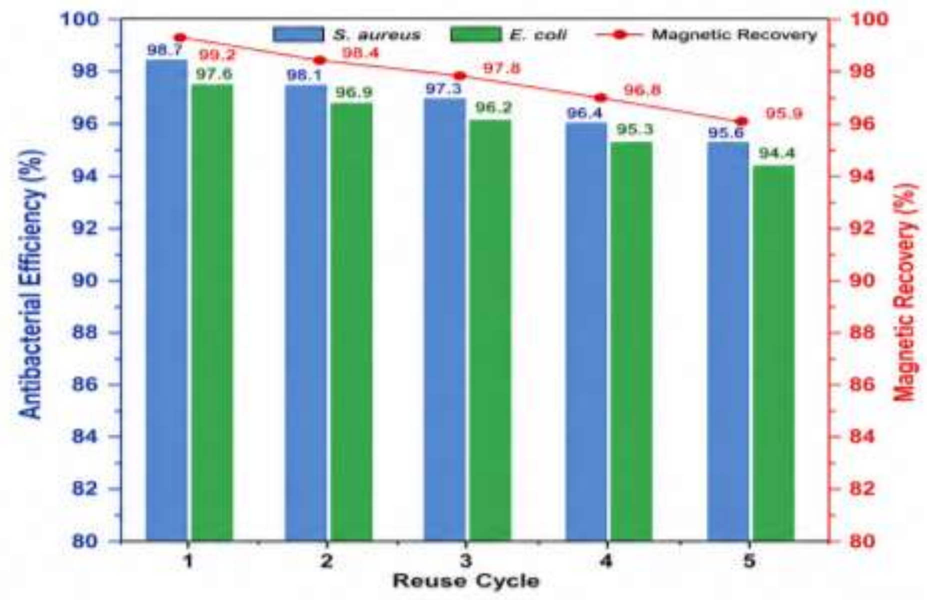

Figure 3. Antibacterial Efficiency and Magnetic Reusability of Sustainable Magnetic Schiff Base Nanohybrids over Multiple Regeneration Cycles

The antimicrobial activity and magnetic reusability of the sustainably synthesized magnetic Schiff base nanohybrids is shown in figure 3 for five successive regeneration cycles. The antibacterial efficiency was tested with gram-positive bacterium, Staphylococcus aureus and gram-negative bacterium, Escherichia coli and the magnetic recovery efficiency was tested simultaneously after each re-use cycle. The results show that the antibacterial activity of nanohybrids remained very high throughout the regeneration process with the inhibitory efficiencies of S. aureus and E. coli decreasing slightly from 98.7% to 95.6% and from 97.6% to 94.4% after five cycles of regeneration, respectively. Similarly, the magnetic recovery efficiency consistently remained high, with only a slight decrease from 99.2% in the first cycle to 95.9% after the fifth cycle. The mild decrease in the bacterial activity and the magnetic recovery suggests excellent structural stability, strong chemical integrity of the Schiff base functional layer and excellent superparamagnetic properties of the Fe_3O_4 core. The results revealed that the magnetic Schiff base nanohybrids exhibit high reusability, long operating life and excellent regeneration capacity, which can be beneficial for repeated uses in wastewater treatment, environmental remediation and sustainable antimicrobial applications.

Performance Parameter	**Cycle 1**	**Cycle 2**	**Cycle 3**	**Cycle 4**	**Cycle 5**	**Performance Retention (%)**
Antibacterial Efficiency – *S. aureus* (%)	98.7	98.1	97.3	96.4	95.6	96.9
Antibacterial Efficiency – *E. coli* (%)	97.6	96.9	96.2	95.3	94.4	96.7
Magnetic Recovery Efficiency (%)	99.2	98.4	97.8	96.8	95.9	96.7
Reduction from Initial Cycle (%)	–	0.8	1.6	2.6	3.6	Minimal
Structural Stability	Excellent	Excellent	Excellent	Very Good	Very Good	High
Reusability Performance	Excellent	Excellent	Excellent	Very Good	Very Good	High
Operational Suitability	Suitable	Suitable	Suitable	Suitable	Suitable	Maintained

Table 2. Comparative Performance Analysis of Sustainable Magnetic Schiff Base Nanohybrids.

Table 2 compares the antibacterial performance and magnetic recovery efficiency of the sustainably synthesized magnetic Schiff base nanohybrids over five consecutive reuse cycles. The results indicate excellent operational stability, with antibacterial efficiencies against *Staphylococcus aureus* and *Escherichia coli* remaining above 94% throughout the study. Likewise, magnetic recovery efficiency remained higher than 95% after the fifth regeneration cycle, demonstrating efficient magnetic separation and negligible material loss. The overall performance retention of approximately 97% confirms the excellent chemical stability of the Schiff base coating and the superparamagnetic nature of the Fe_3O_4 core. These findings demonstrate that the nanohybrids possess outstanding recyclability, long-term durability, and practical applicability for repeated heavy metal remediation and antibacterial wastewater treatment.

Conclusion

The results of this study prove that magnetic Schiff base nanohybrids are synthesized in a sustainable and efficient manner and can be used for the simultaneous removal of both heavy metal contaminants and bacterial pathogens from water. The magnetic Fe_3O_4 nanoparticles functionalized with Schiff bases enabled efficient adsorption of metal ions with the presence of multiple active chelating sites and excellent antibacterial activity by inducing an oxidative stress and damaging the membrane of bacterial cells via the formation of reactive oxygen species (ROS) upon contact with magnetic nanoparticles. The synthesized nanohybrids displayed superior adsorption efficiency for the removal of the target metals Pb^{2+}, Cd^{2+}, Cr(VI) and Cu^{2+}, with a removal efficiency of more than 90% in a broad target metal concentration range. The adsorption data can be well fitted to the Langmuir isotherm and pseudo-second-order kinetic model, indicating chemisorption in a monolayer is the predominant adsorption mechanism. The magnetic Schiff base nanohybrids exhibited a highly efficient adsorption capacity, as well as an outstanding antibacterial effect with inhibition up to 97% in the first treatment cycle against Staphylococcus aureus and Escherichia coli. The inhibition of bacteria growth occurred synergistically via ROS generation, membrane damage, and disruption of the metabolic processes of the bacteria, with the nanoparticles showing good activity against the bacteria. Moreover, the nanohybrids showed excellent magnetic recoverability which allowed for their easy separation by applying an external magnetic field, thus offering efficient regeneration and reuse.

The reusability study was performed to verify the excellent structure integrity and operational durability of the nanohybrids, with more than 95% of the antibacterial activity and magnetic recovery efficiency being conserved after five consecutive regeneration cycles. The slight drop in performance shows that the functional layer of Schiff bases is stable and the magnetic core Fe_3O_4 is stable under repeated treatment conditions. In general, the proposed magnetic Schiff base nanohybrids possess high adsorption capacity, broad-spectrum antibacterial activity, quick magnetic separation, superior environmental friendly synthesis, and excellent recyclability in a single platform. They are good candidates for many advanced wastewater treatments, environmental remediation, antimicrobial coatings and sustainable purification technologies due to these properties. The study should be extended to the pilot-scale implementation, treatment of complex industrial wastewater, long-term assessment of environmental safety and continuous-flow reactor study for enabling the actual application of these multifunctional nanomaterials in a real world.

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