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Anti-Microbial Efficacy of Fe-TiO_2 Nano-Particles Application In Conventional Water Disinfection And Purification Strategy

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Abstract: *Waterborne diseases pose a significant global health challenge due to antimicrobial resistance, climate change, inadequate water sanitation, and emerging pathogens, further exacerbated by pollution from industrial and agricultural sources.Photocatalysis presents an advanced and promising strategy for water disinfection by using simultaneous oxidation and reduction reactions. Iron-doped titanium dioxide (Fe-TiO_2) nanoparticles enhance photo-catalytic efficiency, facilitating the degradation of both Gram-negative and Gram-positive bacteria. The Fe-TiO_2 nano-particles were synthesized via the co-precipitation method. The synthesized nanoparticles were characterized using X-ray diffraction (XRD),Scanning Electron Microscopy (SEM) techniques, and Fourier Transform Infrared Spectroscopy (FTIR). The experimental procedure involved coating Fe-TiO_2 nanoparticles onto the inner surface of the mud pot, which was subsequently filled with water. The water samples were tested using the Most Probable Number (MPN) method in the first experiment. In the second experiment, the aforementioned microbial strains were inoculated to evaluate the antimicrobial efficacy of the Fe-TiO_2 in disintegrating Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), and Salmonella typhi (S. typhi). The results consistently demonstrated that Fe-TiO_2 nanoparticles exhibit significant antimicrobial activity, effectively inactivating the tested microorganisms within a specific time frame. These findings suggest the potential application of Fe-TiO_2 nanoparticles in conventional water disinfection systems. This work contributes to the cost-effective development of mud pots that have anti-microbial properties and can be effectively used in conventionalcommunity-oriented practices.*

Keywords: *Fe-TiO_2; Nano-particles;Water quality; Photo catalysis;Water disinfection; E. coli*

INTRODUCTION

Clean drinking water is essential for sustaining a healthy life, as it serves as an essential nutrient, a structural component, and a medium for biochemical reactions[1]. It also plays a vital role in preventing nutrition-related non-communicable diseases[2]. This in turn, results in the development of micro-organisms,organic pollutants, heavy metals, and inorganic compounds. This water, when consumed by humans and animals, can cause fever, vomiting, burning sensations, limb swelling, and even disorientation. Proper disinfection of water is essential to prevent such health hazards.According to Ayurveda, environmental conditions such as disturbance in air (*vayu*), water (*jala*), land (*desha*), and time (*kala*) are the main causes for *Janapadodhwansa or* epidemics[3]. As an increase in population, various forms of pollution and improper practices (*adharma*)[4]led to water contamination. Ayurveda reports common methods to get clean water, which include boiling, keeping it under sunlight, and dipping a red-hot iron in water[5]. Several other methods include processing of water with *Kataka*(Strychnos potatorum), *Gomedak*(Hessonite), *Bisa-granthi* (Lotus/waterlily), *Shaivalmoola* (Algae), *Mukta*(Pearl), *Mani*(Moon stone), and Vastra (filtration with thick cloth)[6] and also with mixing ash of certain medicines[7].In contemporary science, contaminated water spreadsinfections such as cholera, typhoid, amoebiasis, hepatitis A, and jaundice. The rainy season and natural disasters such as floods facilitate the growth of microbes. The most commonly found microbes responsible for illness are *E. coli, S. typhi,* and *S. aureus*.Several other disinfection methods include chlorination, UV treatment, and ozone treatment. A widely used disinfectant to eliminate microorganisms in water is chlorine. However, it may cause toxicity and disrupt beneficial gut microbiota[8]. Additionally, approximately 10 case-control studies conducted before 2001 indicate a potential association between chlorinated water disinfection by-products such as chloramines and an increased risk of bladder cancer[9]. Water treatment by ozonation also leads to the formation of bromate (BrO_3) through a reaction involving ozone (O_3) and hydroxyl radicals (OH)[10], which is a genotoxic human carcinogen[11] primarily affecting the kidney, peritoneum, testes, and thyroid, with its carcinogenicity due to oxidative stress-induced Deoxyribonucleic acid damage in kidney cells[12,13].Traditional water treatment methods, including sedimentation, filtration, chemical processes, and membrane technologies, often have high operational costs and may produce toxic secondary pollutants that harm the environment[14,15].To minimize such health risks, photocatalysis offers an effective and safe approach to water disinfection. The photocatalytic process begins when a semiconducting material absorbs light energy equal to or greater than its band gap energy giving rise to excitation of electron-hole pairs. This leads to movement of electrons (e^-) from the valence band to the conduction band, leaving holes (h^+) behind. The smaller the band gap, the more photons it captures under visible light.The next step is the separation of the electron pair occurs along with the recombination of carriers. These holes oxidize hydroxyl ions to produce hydroxyl radicals, which are the main agents for the degradation of organic pollution. In this step, the electrons that are photogenerated act as reducing agents and react with dissolved oxygen, leading to the formation of superoxide radicals ($\bullet O_2^-$), which generate (•OH). Degradation of contaminants through active species occurred by adsorption on the catalytic surface, enhancing charge mobility and redox ability[16].Titanium dioxide (TiO_2) has recently gained prominence as an efficient photocatalytic material in environmental science. This is due to its chemical and biological stability, insolubility in water, and resistance to acidic and basic environments. In addition to this, its nontoxicity, corrosion resistance, low cost, and availability make it more effective compared to other oxides, sulphides, and other materials[17.18].A common method for titanium synthesis includes sol-gel, co-precipitation, and hydrothermal process. Currently,semiconductorphoto catalysis has originated as a cost-effective, eco-friendly, and sustainable treatment technology supporting zero waste in waste wastewater industry. These materials trigger a redox reaction that degrades thepollutant. This paper represents the efficacy of Fe-TiO_2 photocatalysis in destroying harmful microorganisms from water, showcasing its effectiveness as an innovative approach to water purification.Iron(Fe^{3+}) improves titanium's photocatalytic properties by directing photogeneration and separation of electron-hole pairs to prevent recombination, and enabling oxidation and reduction with adsorbed species[19].Nanoparticle size has a crucial role in photocatalysis by affecting the surface area of the catalyst. Generally, water purification using nanomaterials involves adsorption, oxidation, and reduction. Nanomaterials are more common among these procedures as catalysts since they not only eliminate or break down organic contaminants during the purification process but also convert hazardous contaminants into nontoxic or useful pharmaceutical products.

Acharya Sushruta mentioned different vesselsused for drinking water, such as *Swarna*(gold), *Rajata*(silver), *Tamra*(copper), *Kansya*(bronze), and *Mrittikapaatra* (earthen vessel). Among these,mud pot is considered the most compatible because of its low cost, easy availability, and eco-friendly nature [20].Several studies have been conducted using Fe-doped TiO_2 materials under visible light irradiation for the removal of organic pollutants by photocatalytic treatment[21,22,23,24]. In a study, it was concluded that the bacterial activity of Fe^{3+} dopped TiO_2 thin film towards *E.coli* on grass substrate under UV light[25].Disinfection of *E. coli was* investigated using Fe-doped TiO_2 electrodes fabricated through spin coating on titanium mesh,which required UV light for effective bacterial inactivation[26].The present study aims to synthesize Fe-TiO_2 nanoparticles using the co-precipitation method and characterize them through X-ray diffraction (XRD), Scanning Electron Microscopy (SEM), and Fourier Transform Infrared Spectroscopy (FTIR). The synthesized nanoparticles were evaluated for their antimicrobial efficacy by coating them onto the surface of a mud pot. Their effectiveness in disinfecting waterborne pathogens, including *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive), and *Salmonella typhi*, were assessed. The antimicrobial activity of the Fe-TiO_2-coated vessel wasanalysed using the Most Probable Number (MPN) method and two experimental procedures involving microbial inoculation in the coated pots, followed by quantitative assessment of bacterial reduction. This study aims to explore the possibilities of Fe-TiO_2 nanoparticles as an effective and sustainable water purification strategywith objectives includingthe synthesis of Fe-TiO_2 nanoparticles, characterisation of Fe-$TiO_2$and itsmode of action in purifying water.

MATERIALS AND METHODS:

Chemicals:

Ferrous sulphate heptahydrate ($FeSO_4 \cdot 7H_2O$,Molychem LR grade, 98.5-104.5%assay) Titanium dioxide (TiO_2,Labogens LR grade, 99% assay) Ammonia Solution (NH_4OH, Ranchem, LR grade, 25% assay), Demineralized water (demineralization unit, conductivity < 10µS/cm), Ethanol (C_2 H_6O_4, Changshu Hongsheng Fine Chemicals Co.Ltd,)Whatman filter paper (no 41 GE Healthcare UK Ltd).

Microbial media and culture:

MacConkey Agar with crystal violet, NaCl, and 0.15% bile salt (MAAT Biotech, dehydrated powder, microbiological grade 50.83 g/L), Soybean Casein Digest Agar (HiMedia, microbiological grade, REF MH290) Bacteria -*Escherichia coli*(ATCC-25922), *Staphylococcus aureus*(ATCC-25923), *Salmonella typhi*(ATCC-6539), Six mud pots of 250 mL capacity were used,of which three were coatedwith cement andtaken as the control group,while the remaining three were coated with cement along withFe-$TiO_2$and taken as the trial group.

Experiment for Synthesis of Nanoparticles:

The Fe-TiO_2 nano-particles were synthesized via the co-precipitation method [27] by dissolving in distilled water under continuous magnetic stirring to create a homogenous solution with a total molar concentration of 0.3 M. NH_4OH was titrated to the mixed solution while stirring continuously until the pH reached ~6.9. After being undisturbedthe slurry for 48 hours, the slurry was filtered, followed by thorough washing with ethanol. This washing will result in keeping the slurry free from sulphate ions. This filtered precipitate was dried in an oven at 80°C for 24 hours.The dried product was ground manually to a fine powder and calcined in a muffle furnace at 200°C for 2 hours to enhance crystallinity. This optimized process ensures the efficient synthesis of Fe-TiO_2.

Characterization of Fe-TiO_2:

Powder XRD, SEM, and FTIR characterized the produced sample's crystal structure.

Pot Preparation:

Mud pots were taken andexposed to washing with water, followed by air drying for 8 hours. Water was added and left to stand for 6 hours. After discarding water, those were subjected to UV disinfection for 2 hours and autoclaving for 30 minutes. In each experiment, two pots were taken, one asa control (coated with only cement) and one trial (coated with a combination of Cement and Fe-TiO_2)

Experiment to check the antimicrobial effect:

Twosets of experiments were conducted to assess the antimicrobial efficiency of Fe-TiO_2.

Water testing by MPN:

To assess bacterial contamination, water samples were analysed using the MPN method. The idea behind choosingthe MPN method is that it is a standard and cost-effective technique for estimating coliform bacteria in water bodies. It also requires minimal instrumentation and provides statistically reliable estimates of bacteria[28,29,30,31]. Water sample was collected aseptically using sterile bottles from the Rakaskopa water reservoir in Belagavi District of Karnataka, India, during September 2024. Bottles were filled to eliminate air space, sealed, and labelled to prevent contamination. After that, the samples were put through MPN testing, which involved adding water to mud pots to assess the bacterial load. At 0, 1, 2, 4, 6, 7, and 24 hours, aliquots of 100 µL, 1 mL, and 10 mL from both pots were inoculated into test tubes containing single-strength and double-strength MacConkey broth for bacterial analysis. The inoculated tubes were incubated for 24 hours to allow bacterial growth. After incubation, streaking was performed on pre-prepared Petri dishes to isolate and identify bacterial colonies.This approach makes it possible for a comparative assessment of the antimicrobial efficacy of Fe-TiO_2 in reducing bacterial contamination in drinking water.

Microbial Inoculation Procedure:

Two pots were taken with 250 mL of water and were inoculated with *Salmonella Typhi* and *Escherichia coli*[32]. Another two pots were inoculated with *Escherichia coli* and *Staphylococcus aureus*. Water samples were collected from each pot at 0, 1, 2, 4, 6, 7, and 24 hours. These samples were streaked onto pre-prepared Petri dishes for bacterial enumeration and analysis.

RESULTS

Fe-Ti bimetal oxide nanoparticles characterization

The synthesized nanoparticles were characterised by XRD and SEM.XRD analysis was performed using an X-ray diffractometer (Rigaku Smart Lab) equipped with Cu K_a radiation (k = 1.541 Å) ata scan speed of 0.020°/s in the 2θ range from 5◦ to 90◦. The Joint Committee on Powder Diffraction Standards (JCPDS) database was used for phase SEM-EDS analysis was carried out using a JEOL JSM-IT500 after coating with gold powder by sputtering.

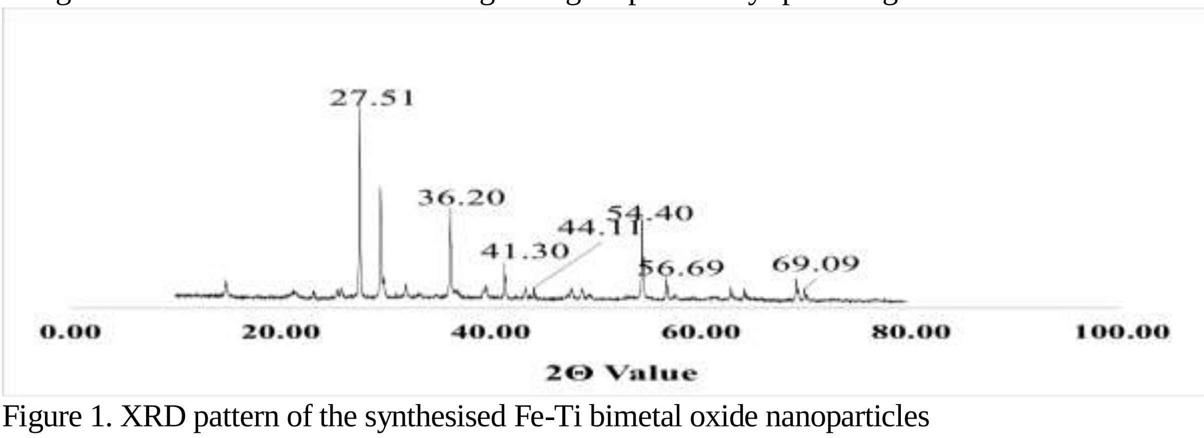

Figure 1. XRD pattern of the synthesised Fe-Ti bimetal oxide nanoparticles

XRD analysis was carried out to study the composition of the synthesised Fe-Ti oxide nanoparticles. The XRD analysis is shown in Fig.1. The nanoparticles clearly show peaks

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corresponding to Fe-TiO_2 and Fe-TiO at 2θ values of 41.30, 54.40, and 36.20 according to the JCPDS (Joint Committee for Powder Diffraction Standards) card number 47-1777.The peaks at 2q values of 27.51, 44.11, 54.4, 56.69, and 69.09 match the JCPDS card number 84-1284, thereby confirming the existence of TiO_2. These XRD results confirm the presence of Fe-Ti bimetal oxide nanoparticles.

SEM analysis:

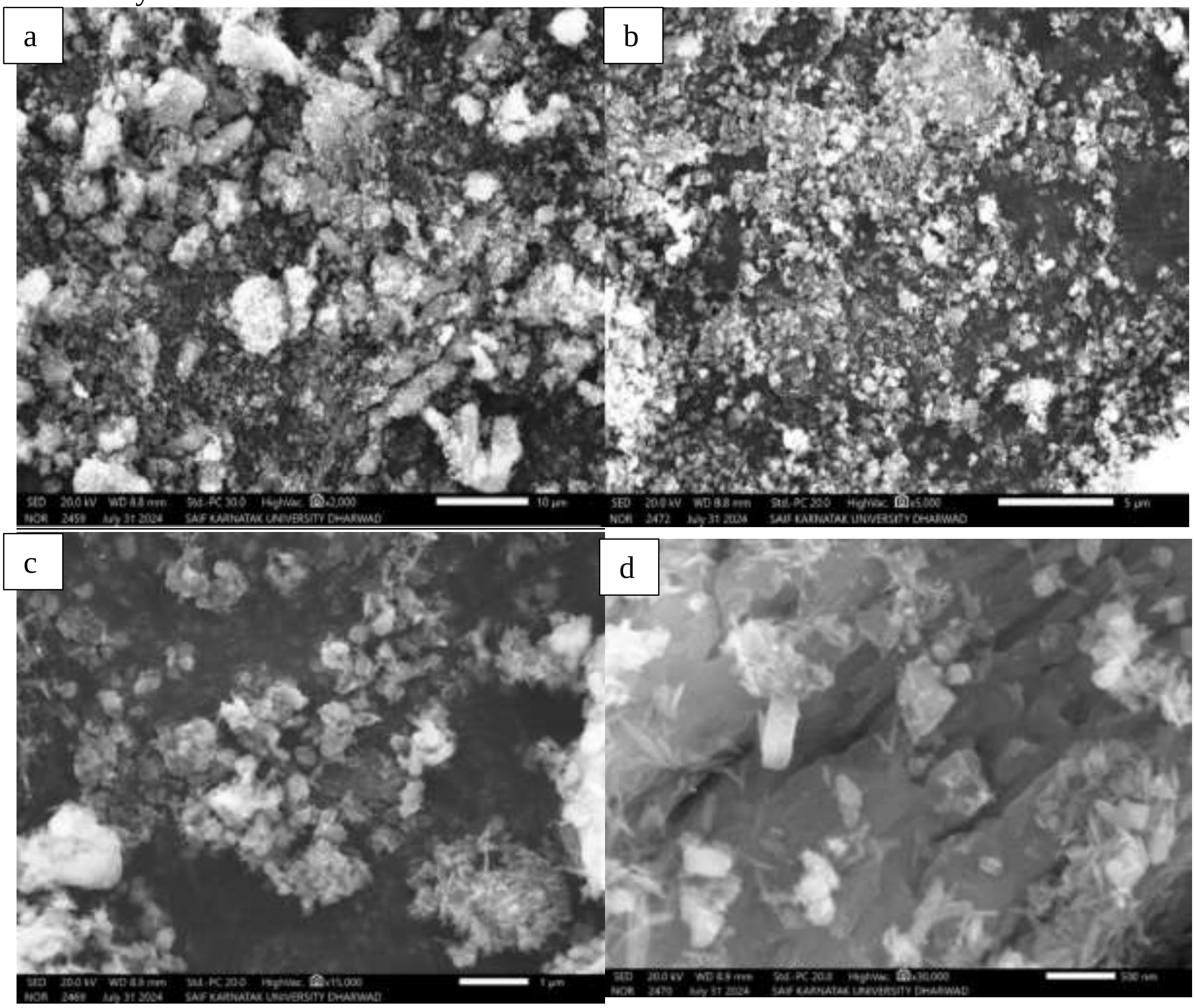

Figure 2. SEM analysis of Fe-Ti bimetal oxide nanoparticles:(a) Magnification 2000x (10µm), (b) Magnification 5000x (5µm), (c) Magnification 15,000x (1µm),(d) Magnification 30,000x (500nm)

SEM images of Fe-TiO_2show that theshape of the synthesised nanoparticles is a fine nanosphere.A Similar spherical morphology of Fe-TiO_2 nanoparticles was reported by earlier researchers,indicating that Fe was incorporated into Ti and was not just a mixture[27].

FTIR Interpretation:

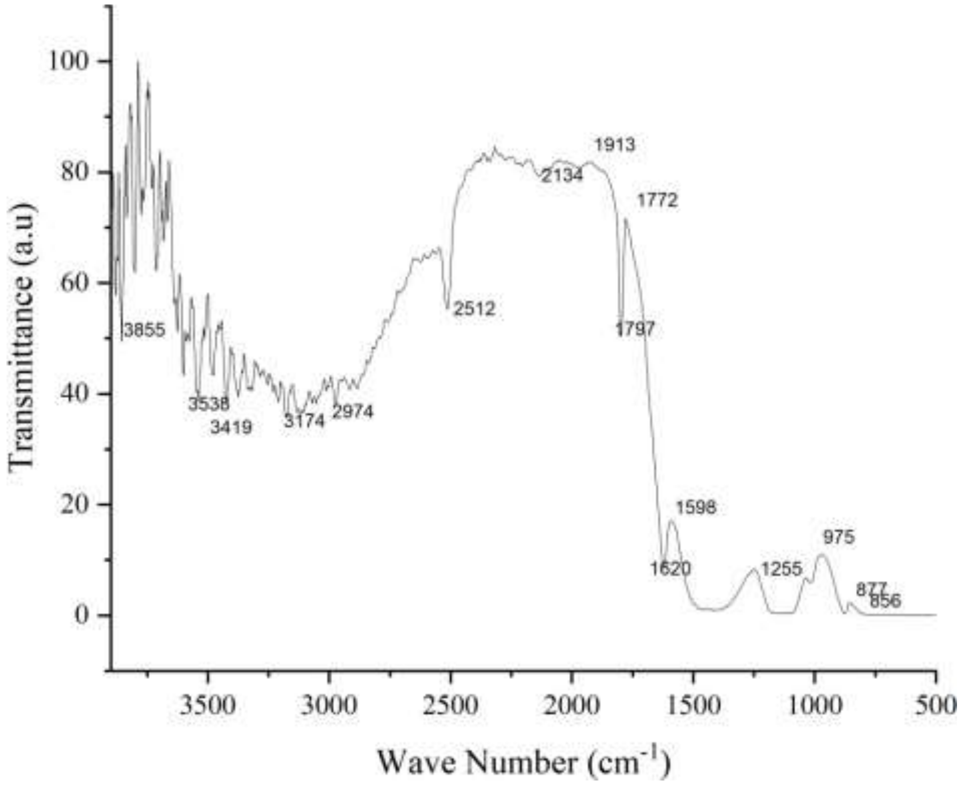

Figure 3. FTIRanalysis of the synthesised Fe-Ti bimetal oxide nanoparticles

Fourier Transform Infrared Spectroscopy (FTIR) analysis was done to determine the functional groups present in the sample.The presence of strong broad peaks at 3855 cm^{-1} indicates O–H stretching vibrations characteristic of alcohols, which is because of ethanol washing during the synthesis. A further O–H peak at 3538 cm^{-1} supports this interpretation. Strong peaks in the region of 3174 cm^{-1} and 2974 cm^{-1} suggest aromatic C–H stretching. A weak absorption at 2512 cm^{-1} indicates possible S–H stretching from thiol groups, though the intensity is low and may represent trace components. The strong absorption at 1797 $cm^{-1}$is attributed to C=O stretching, suggesting the presence of anhydrides, esters, or acid chlorides. The 1620 $cm^{-1}$and 1598 cm^{-1} bands correspond to C=C stretching or N–H bending, indicating the presence of aromatic systems or amine functionalities. Lastly, the medium intensity peak at 1255 $cm^{-1}$is indicative of C–O stretching, typical of alcohols, esters, and related oxygen-containing groups.

Comparison of Microbial Growth and Most Probable Number Index (MPN)

Table 1. Showing water tested at different time intervals using the MPN in both the control and experimental groups

Time in hours	100µL (SS) Control	1 mL (SS) Control	10 mL (DS) Control	MPN Index Control	100 µL (SS) $Fe\text{-}TiO_2$	1mL (SS) $Fe\text{-}TiO_2$	10mL(DS) $Fe\text{-}TiO_2$	MPN Index $Fe\text{-}TiO_2$
0	Growth	Growth	Growth	10	Growth	Growth	Growth	10
1	Growth	Growth	Growth	10	Growth	Growth	Growth	10

2	Growth	Growth	Growth	10	No Growth	No Growth	No Growth	0
4	Growth	Growth	Growth	10	No Growth	No Growth	No Growth	0
6	Growth	Growth	Growth	10	No Growth	No Growth	No Growth	0
7	No Growth	No Growth	Growth	4	No Growth	No Growth	No Growth	0
24	No Growth	No Growth	No Growth	0	No Growth	No Growth	No Growth	0

One MPN is equal to one CFU. Both units measure the estimated number of bacteria in a water sample.The MPN or multi-tube method (McCrady) is a statistical method used to estimate the number of viable cells of a particular microorganism in a sample, most commonly food or water. It is used when samples contain too few bacteria to provide reliable viable cell numbers by classicalplatecount.

From Table1,itwas observed that the MPN index remains high up to 7 hours in pots that are not coated with Fe-TiO_2,whereas a sudden reduction in MPN number occurs after 1 hour, indicatingthe antimicrobial effect of Fe-TiO_2

After 7 hours, no microbial growth was observed in the uncoated pot, likely due to the natural decline in viable bacteria caused by nutrition depletion and unfavourable environmental conditions.

Figure 4:Experimental setup and microbial analysis: (a) Prepared mud pots with and without Fe-TiO_2 coating; (b) Autoclaving of pots before experimentation; (c) Preparation for MPN method and streaking onto petri dishes (d) Showing bacterial growth without coating of Fe-TiO_2 on one side and no growth in Fe-TiO_2 on the other side at 7 hours, done by the MPN method.
Microbial Inoculation Procedure:

Table 2. Bacterial growth analysis on MacConkey Agar for water samples stored in Fe-TiO_2coated and uncoated pots at specified time intervals.

Mac-Conkey Agar				
Timein hours	*Escherichia coli and Salmonella typhi* (Pot Coated with $Fe\text{-}TiO_2$)	*Escherichia coli and Salmonella typhi* (Pot Without $Fe\text{-}TiO_2$ Coating)	*Escherichia coli + Staphylococcus aureus* (Pot Coated with $Fe\text{-}TiO_2$)	*Escherichia coli + Staphylococcus aureus* (Pot Without $Fe\text{-}TiO_2$ Coating)
0	No growth	Growth	No growth	Growth
1	No growth	Growth	No growth	Growth
2	No growth	No growth	No growth	Growth
4	No growth	No growth	No growth	No growth
6	No growths	No growth	No growth	No growth
7	No growth	No growth	No growth	No growth
24	No growth	No growth	No growth	No growth

It is observed from the above table that there is no bacterial growth at 0 hour in water stored in a Pot coated with $Fe\text{-}TiO_2$,whereas no microbial growth was observed in the uncoated pot after 2 hours, likely due to the natural decline in viable bacteria caused by nutrition depletion and unfavourable environmental conditions.

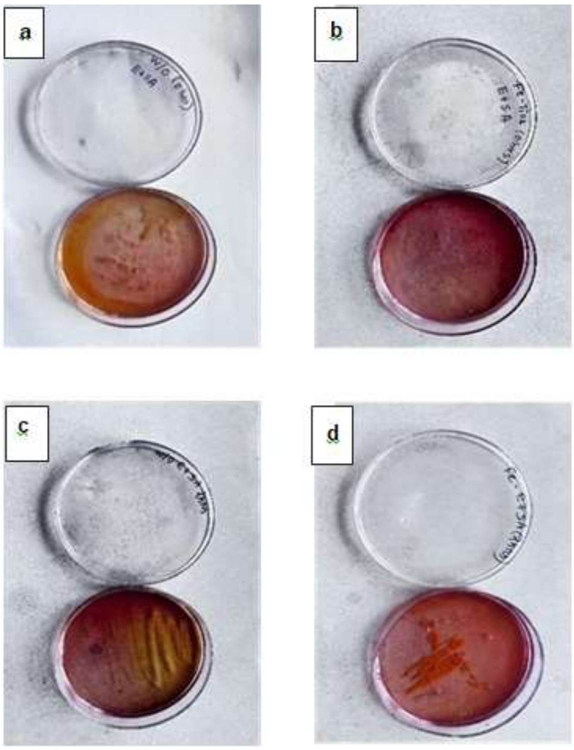

Figure 5: Bacterial growth observed on Mac-Conkey agar (a) [*E. coli* + *S. aureus*] without $Fe\text{-}TiO_2$ coating at 0 hour; (b) with coating of $Fe\text{-}TiO_2$ at 0 hour;(c) Bacterial growth [*E. coli*+ *S. typhi*] without $Fe\text{-}TiO_2$coatingat 2 hours; (d) with $Fe\text{-}TiO_2$ coating at 2 hours.The reduction in bacterial growth in (b) and (d) demonstrates the antimicrobial efficacy of Fe-Ti bimetallic oxide nanoparticles.

Table 3: Showing bacterial growth analysis on Soybean Casein Digest Agar for water sample stored in $Fe\text{-}TiO_2$ coated and uncoated pots at specified time intervals

Soybean Casein Digest Agar				
Time in hours	*Escherichia coli and Salmonellatyphi*(Pot Coated with Fe-TiO_2)	*Escherichia coli and Salmonella typhi* (Pot Without Fe-TiO_2 Coating)	*Escherichia coli and Staphylococcus aureus* (Pot Coated with Fe-TiO_2)	*Escherichia coli and Staphylococcus aureus* (Pot Without Fe-TiO_2 Coating)
0	Growth	Growth	Growth	Growth
1	Growth	Growth	Growth	Growth
2	Growth	Growth	No growth	No growth
4	No growth	No growth	No growth	No growth
6	No growth	No growth	No growth	No growth
7	No growth	No growth	No growth	No growth
24	No growth	No growth	No growth	No growth

A similar trend of bacterial growth reduction was observed in water samples stored in both Fe-TiO_2 coated and uncoated pots on soybean casein digest agar, indicating disintegration of microbes at 2 hours.

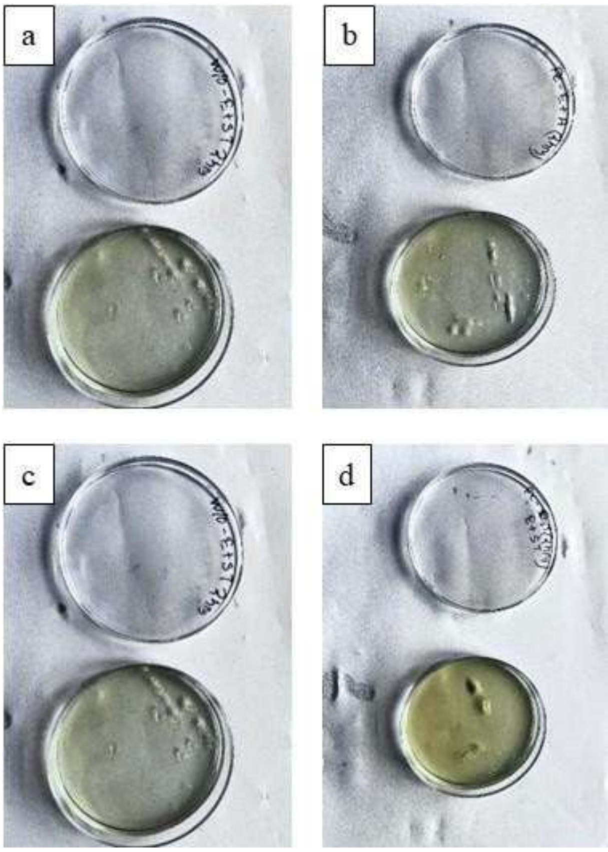

Figure 6: Bacterial growth observed on Soybean Casein Digest agar at 2 hours: (a) [*E. coli* + *S. aureus*] without Fe-TiO_2 coating; (b) with Fe-TiO_2 coating(c)Bacterial growth [*E. coli*+ *S. typhi*] without Fe-TiO_2 coating; (d) with Fe-TiO_2 coating, indicating antimicrobial efficiency of Fe-Ti bimetallic oxide nanoparticles.

Discussion

A series of experiments were carried out to check to efficacy of Fe-TiO_2 in disinfecting water using different microbes.The findings of the Present study elaborate that Fe-$TiO_2$is efficient enough against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*,three common waterborne pathogens[32]. A comparison of treated and untreated water samples showed that Fe-TiO_2nanoparticles have strong antibacterial properties.

In pots without Fe-TiO_2coating, the MPN index remained high,i.e.,10 up to 4 hours, indicating persistent bacterial contamination. Only after 6-7 hours was a slight decline detected, followed by total microbial suppression, i.e., MPN index 0 at 24 hours. Pots treated with Fe-TiO_2demonstrated faster and stronger disinfection effects. While the initial microbial load was identical MPN index 10, bacterial growth was completely inhibited as early as 2 hours after treatment. This considerable reduction, validated by the MPN technique, confirms the improved photocatalytic activity of Fe-TiO_2nanoparticles.The observed microbial inactivation is attributed to the production of Reactive Oxygen Species (ROS) under light exposure, which disrupts cell membranes and intracellular components.

Results of microbial inoculation procedure show no growth of *Escherichia coli, Salmonella typhi*atany point on Mac-Conkey agar, but the control pots show growth of microbes up to 1 hour. A slight delay in Soybean Casein Digest Agar media was observed, where both the trial and control groups revealed bacterial persistence for up to 2 hours. A similar trend was noted in the group inoculated with *Escherichia coli* and *Staphylococcus aureus*,where no bacterial growth was detected after 2 hours,which implies the coated surface was ultimately effective against Gram-negative and Gram-positive organisms.

Adding iron to titanium dioxide,Fe-$TiO_{2,}$improves its photocatalytic capabilities by increasing visible light absorption and lowering charge carrier recombination. Because iron traps electrons, it decreases electron-hole recombination and increases the generation of reactive oxygen species (ROS), including superoxide anions and hydroxyl radicals. Because they damage intracellular components and rupture cell membranes, these ROS play a significant part in microbial inactivation. Fe-TiO_2has higher antibacterial efficacy compared to only TiO_2[33,34,35].

The above experiments highlight the potential of Fe-TiO_2 nano-particles as a photocatalytic disinfectant when exposed to conventional water storage materials like earthen vessels.The poroussurface of the mud pot eases the contact between water and the coated surface,boosting the disinfection process. Earthen vesselsare a traditional and widely used method of storing water. This study also shows an economical and culturally acceptable way to incorporate nanotechnology into public health.

Additionally, Fe-TiO_2photocatalysis is a clean process with no toxic residues, in contrast to chlorine-based disinfection, which can result in the development of hazardous byproducts such as Trihalomethanes (THMs) when chlorine reacts with NaturalOrganic Matter (NOM) present in the water. It is therefore a viable, environmentally acceptable substitute for safe water treatment[36].

CONCLUSION

To limit the growth of microbes in water is one of the most dangerous challenges to global healthcare. This study demonstrates the effectiveness of Fe-TiO_2nanoparticles as a promising and innovative method for water disinfection. The nano-particles exhibit significant microbial reduction in certain hours, making them a viable alternative to conventional disinfection techniques.The photo-catalytic properties of Fe-TiO_2pivotal role in microbial inactivation.Fe-TiO_2 nanoparticles are an effective, eco-friendly, and promising solution for improving water quality. They could be useful for large-scale water treatment, especially in areas with limited access to advanced technologies. From a public health perspective, the adoption of Fe-TiO_2could minimise the burden of waterborne infections, especially in under-resourced locations.The technology shows promise for usage in household water filters, community tanks, and decentralized water treatment systems.However, more research is needed to understand their long-term effects on the environment, reduce costs, and test their use in real-life situations to ensure they are safe and practical for public health.

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