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Bacteria-Mediated Synthesis of Iron Oxide Nanoparticles and Their Efficiency in Ammonia Removal from Fish Culture Tanks

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ABSTRACT

Background: Aquaculture is one of the most rapidly expanding food production sectors. Due to intensification, declining water quality parameters negatively impact fish production and the surrounding environment. Thus, it is essential to maintain optimum water quality parameters and aquatic ecosystems for sustainable fish production. This study aims to synthesize nanoparticles (NPs) and evaluate their efficiency against ammonia in cultured fish tanks under diverse conditions.

Methods: Bacillus megaterium from the soil sample was isolated and identified. Iron oxide NPs were synthesized using *Bacillus megaterium* collected from soil samples and characterized by DLS-zeta potential, UV-Vis, XRD, FTIR and TEM analysis. Next, synthesized NPs were evaluated under ex-situ and in-situ conditions to determine their efficacy in removing ammonia from Common carp, *Cyprinus carpio* culture tanks.

Result: *B. megaterium* was isolated, identified and 16S rRNA gene PCR-amplified sequences confirm the identification of *B megaterium*. DLS-zeta potential, UV-Vis, XRD, FTIR and TEM analysis revealed the quality of synthesized NPs. *B. megaterium* from the soil sample was isolated and identified. The mean zeta potential of the biosynthesized NPs was between -4.65 to -6.45 mV and had an average size of 5 nm. The concentration of ammonia in aerated tanks treated with iron NPs reduced to less than 0.03 mg/L from 0.3 mg/L. Also, fish mortality in these tanks was significantly controlled with the application of NPs. The application of NPs considerably reduced fish mortality in these fish tanks. Iron oxide NPs from *B. megaterium* can produce high-quality NPs that are efficient in removing ammonia from fish culture tanks. Further, exploratory studies are necessary to determine the efficacy of iron oxide NPs on aquatic organisms cultured in different environments.

Key words: Ammonia, *B. megaterium,* Fish culture, Iron oxide, Nanoparticles.

INTRODUCTION

Aquaculture is one of the most rapidly expanding food production industries and has enormous potential for global food security and employment. It is an age-old practice and can be traced back as far as 4000 years in Egypt (Chimits 1957) and about 2000 years in Europe (Buschmann *et al*., 2006) and China (Edwards, 2004; Lu and Li 2006). Aquaculture was developed as traditional aquatic ecosystems could not sustain human population growth. The rapid and robust expansion of the aquaculture sector began in Asia in the 1960s and then spread to the rest of the world. The rise in the aquaculture sector is popularly known as the blue revolution (Costa-Pierce, 2002). Since then, the aquaculture industry has grown significantly towards diversification and intensification. Aquaculture tends to release nutrients and chemicals into the water. These nutrients and chemicals interact with local biodiversity and lead to long-term adverse effects on its surroundings and the environment. Over time, environmental issues such as eutrophication, climate change, the degradation of local biodiversity and resource depletion emerge (Ottinger *et al*., 2016, Bohnes *et al*., 2019). Moreover, deterioration in water quality parameters, especially ammonia, significantly affects the productivity of the pond. Increased ammonia concentrations affect other water quality parameters, such as nitrite, dissolved oxygen and phosphate levels. These water quality parameters can be controlled with physical, chemical and biological remedial methods. However, these conventional approaches have their own limits. For instance, chemical remediation may exacerbate the problem in the long term. Thus, for a sustainable solution, bioremediation is the most reliable and cost-effective method for addressing environmental issues. With the advancement of

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nanotechnology, bioremediation utilizing nanoparticles (NPs) is the most reliable method for controlling aquaculture-related waste management.

Advances in nanotechnology push bioremediation beyond its current boundaries. This specific approach offers a diverse range of ways to manage water quality parameters in wastewater (Jain *et al*., 2021), heavy metal- and hydrocarbon-contaminated sediments (Sawan *et al*., 2020, Iravani and Varma 2022) and organic or inorganic compounds in soil (Cai *et al*., 2020; Pareek *et al*., 2020) by reducing process intermediates and mitigating their negative environmental effects. Thus, aquaculture effluents are increasingly treated with NPs to remove inorganic chemicals, microbes, pesticides and heavy metals. These NPs can be synthesized using bacteria since they are adept at multiple processes, such as biomineralization, biotransformation, bioleaching and bioaccumulation. Bacteria can solubilize metal ions by modifying their oxidation state via reduction, oxidation, or both (Raĭkher *et al*., 2010). Among these processes, biotransformation is especially helpful for nanoparticle synthesis. Bacterial cells can synthesize metallic NPs by using their defense against soluble metal ions (Ramanathan *et al*., 2013). NPs catalyzes the degradation of pollutants, thereby accelerating their breakdown improving water quality. Improvement in water quality increases the growth of cultured species as well as reduces the chance of pollutants entering neighboring water bodies. Thus, this study aims to synthesize NPs and evaluate their efficiency against ammonia under diverse culture conditions.

MATERIALS AND METHODS

Sample collection

500 g of soil samples were collected from four locations in Versova and Gorai mangrove area, Mumbai, India. These selected sampling sites represent the soil quality of mangrove areas on the west coast of India. All samples are collected in accordance with the guidelines established by the Department of Aquatic Environmental and Fish Health Management of the Central Institute of Education Fisheries in Versova, Mumbai, India. All the soil samples were collected and placed in sterile bags for transportation and storage purposes.

Isolation and identification of bacteria

The soil microorganisms were isolated using the serial dilution method on a nutrient agar medium (Bharathi *et al*., 2019; Mogana *et al*., 2020). Briefly, 1 g of soil was diluted with 10 mL of sterile water and serially diluted from 10-1 to 10-6. 100 µL of diluted sample was mixed with nutrient agar medium (HiMedia, India) and incubated. After incubation, the most prominent colonies were streaked on Zobel Marine Agar (Marine agar 2216). Next, prominent colonies were selected to obtain a pure culture. All unique strains in the culture were preserved in glycerol at -80°C. The isolated bacterial strains were identified by gram staining. All isolated colonies were Gram-stained in accordance with standard procedure. Isolated and gram-stained bacteria were identified using the VITEK-2 system (Biomerieux, India) according to the manufacturer's instructions. Fig 1 depicts all aspects of bacterium isolation and identification from collected samples and Schematic diagram showing the experimental design of the study.

DNA extraction and sequencing

All the identified strains were cultured in nutrient agar for 24-48 h. The DNA was extracted using DNA extraction buffer (Sigma-Aldrich, India). 5-10 colonies were inoculated to 1 mL distilled water and vortexed to make a suspension. Bacterial suspension was centrifuged to obtain a pellet. 100 µL of DNA extraction solution was added and mixed thoroughly and the mixture was boiled at 100°C for 15 min. The bacterial 16S rRNA gene was PCR-amplified using the 27F (5$^{\circ}_{\circ}$-AGAGTTTGATCMTGGCTCAG-3$^{\circ}_{\circ}$) and 1492R (5$^{\circ}_{\circ}$ TACGGYTACCTTGTTACGACTT-3$^{\circ}_{\circ}$) primers. For sequencing, PCR products were sent to Eurofins, Bangalore, India, for analysis. The 16s r RNA sequences were evaluated for similarity using the BLAST similarity search tool and a phylogenetic tree was constructed as described earlier (Biasini *et al*., 2014, Lisak *et al*., 2015).

Synthesis of iron oxide nanoparticles (NPs)

Identified and sequence-verified bacterial species Bacillus megaterium was selected for synthesis of NPs. For NP synthesis, 50 mL of bacterial suspension was resuspended in 50 mL of 0.01 mmol $FeCl_3$ and incubated for 72 h in a shaker at 37°C. The resultant bacterial suspension was centrifuged and the pellet containing NPs was washed 3-4 times with deionized water to remove Fe ions and seed extract residue. The visible colour change of the solution indicated the formation of NPs. A secondary reaction mixture devoid of ions was prepared and used as a control for each and ion.

Characterization of biosynthesized NPs

The formation of NPs confirmed from UV-visible analysis of the reaction mixture obtained in the range of 100-200 nm at different time intervals using Schimazdu UV-1800 scanning spectrometer (Schimazdu, Kyoto, Japan). Further characterization involved use of Fourier-transform infrared spectroscopy (FTIR). The emission spectra were recorded with IRAffinity-1 (Shimadzu, Japan) in the wavelength range 4000-400 cm^{-1}. Next, TEM was employed to understand the morphology, size and distribution of NPs (Majeed *et al*., 2021). Briefly, synthesized NPs sonicated, coated onto a carbon-coated TEM grid and evaporated to remove excess solution. All the operations were done with a maximum resolution of 0.2 nm and operated at an accelerating voltage of 20KV. The size and distribution of the synthesized NPs were observed using Philips Technai 10 transmission electron microscope (Amsterdam, Netherlands).

Antimicrobial activity

Antimicrobial activity was evaluated with agar-well-diffusion method *in vitro* (Burygin, Khlebtsov *et al*., 2009). Briefly,

Overnight cultures of bacteria were treated with 1 µg/ml- 25 mg/ml of NPs and incubated at standard growth conditions for 24 h. After incubation, culture plates were observed for the formation of growth inhibitions zone.

Jar test to determine the efficacy of synthesized NPs for ammonia removal

The experiment was performed using a micro-controlled jar-test apparatus. The experimental unit contained 500 mL Mason jars containing 150 g of air-dried soil and the jars were filled with wastewater containing 10 ppm ammonia and 1 ppm of Iron Oxide NPs. All the physical parameters of the prepared wastewater are described in Table 1. Each jar was filled with a mixture of samples and 1 ppm iron NPs. Each treatment was replicated three times. All of the jars were stirred at 100 rpm for 5 h. The observations were recorded at 1h, 2h, 3h, 4h and 5h. The ammonia was immediately analysed and the proportion of ammonia removed was calculated.

Effect of synthesized NPs in indoor aquaculture ponds

A completely randomized design was selected to investigate the effect of synthesized NPs on ammonia in different conditions, such as with sediment, without sediment, with aeration and without aeration. In this experiment, common carp were cultured in a 500 ltr tank with a stocking density of 14 kg/m^2. The fish were cultured for 30 days with a standard feeding rate of 3%. After 30 days of culture, tanks were treated with NPs at 1 ppm. Later, the concentration of ammonia in the pond was measured daily for ten days.

Statistical analysis

Multiple comparisons were made by one-way analysis of variance (ANOVA), followed by tukey's post hoc test using

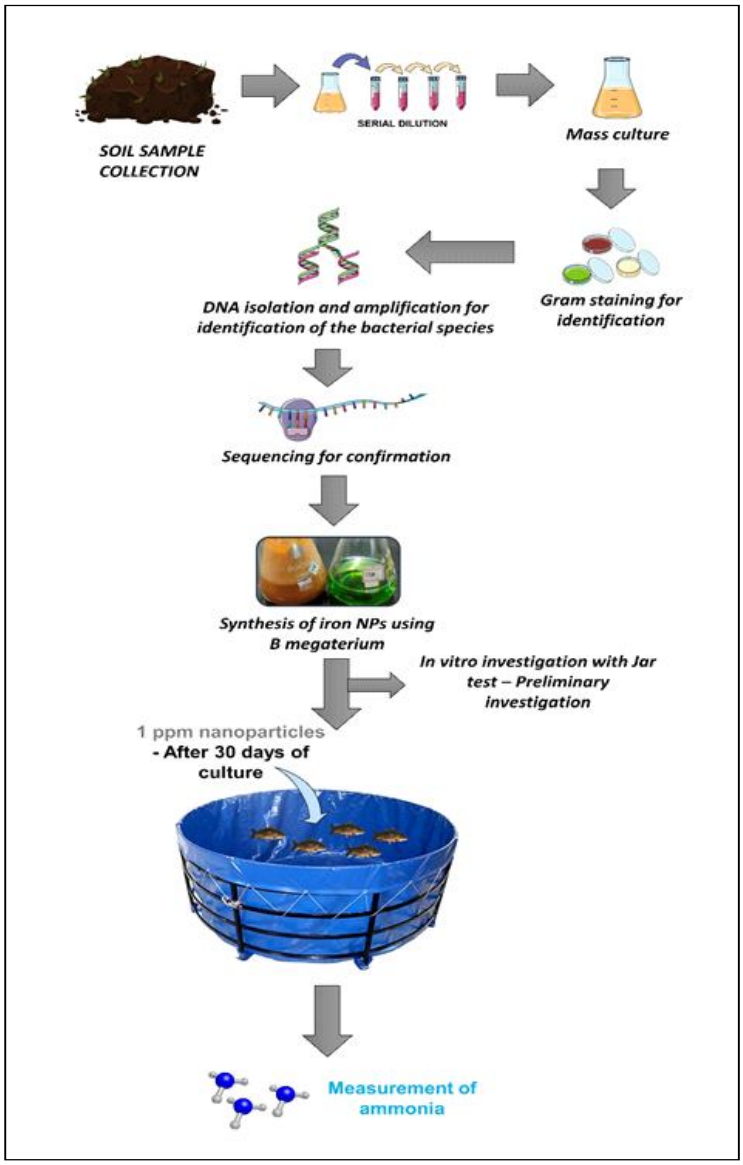


Fig 1: Schematic diagram showing the experimental design of the study.

the statistical software OriginPro8, USA (Version 8.0951). All parameters were tested in triplicate. A p-value of <0.05 was considered statistically significant. All data are expressed as mean±SD.

RESULTS AND DISCUSSION

Isolation of bacteria and morphological characteristics

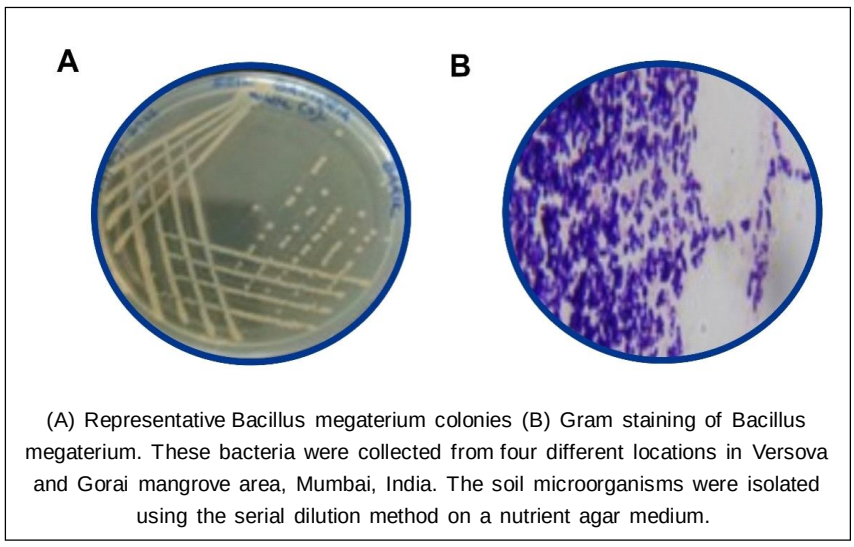
VITEK2 observations indicated a 92% probability for *Bacillus megaterium*. Morphologically, colonies were of medium size and round shape, had mucoid characteristics and appeared non-pigmented (Fig 2). Microorganisms show structured physical and biosynthetic activity, which can be used to obtain NPs with controlled shape and size (Sunkar and Nachiyar 2012; Koul *et al*., 2021). Previously, *Bacillus spp* was effectively utilized for NP synthesis (Fouad *et al*., 2017; Alsamhary 2020; Kumar *et al*., 2020, Kabeerdass *et al*., 2021; Ullah *et al*., 2021; Halder *et al*., 2022). These investigations strongly suggest the stability of NPs synthesized using *Bacillus spp*. Thus, *B megaterium* is a potential candidate for the synthesis of iron NPs. Furthermore, there are few studies in which NPs produced by *B. megaterium* were utilized to reduce ammonia in fish culture systems. Hence, this study will be one of the first to examine the application of NPs produced from *B megaterium.*

Synthesis of iron oxide NPs

In NP synthesis, absorption and wavelength play a vital role in determining the properties of NPs. Thus, the quality of synthesized NPs was meticulously evaluated. Understanding the characteristic properties of synthesized NPs facilitates the determination of their applications and efficacy. Bioreduction of metal ions into NPs can be basically characterized using UV-Vis spectroscopy. The transition from green to thick orange indicated the formation of NPs by $FeCl_3$ (Fig 3A). Excitation of surface plasmon resonance (SPR) in metal NPs is responsible for the resulting color change. (Thangaraju, Venkatalakshmi *et al*., 2012). Within 48 hours, the color of the metal ion solution in the flask containing bacterial culture filtrate changed. At a concentration of 1 mM, a significant amount of iron NPs were obtained. To confirm the NP synthesis, UV-spectra is used to provide convincing proof of NP synthesis. In this study, the absorption peaks of synthesized NPs formed by chemical reduction using $FeCl_3$ were concentrated within 300-1000 nm and the maximum absorption was detected at 425 nm. Fig 3B shows the representative absorbance spectrum at ëmax= 425 nm, whereas absorption intensity was about 1.1-1.4 for NPs, suggesting the formation of NPs in the solution. Previous reports indicate that the usual iron NP SPR pattern is present at the wavelength of ~350-1000 nm (Tang *et al*., 2013; Saranya *et al*., 2017). Interestingly, produced NPs showed a similar wavelength spectrum. These observations on absorption and wavelength reflect the quality of synthesized NPs.

Table 1: Physicochemical parameters of wastewater used in the Jar test.

Physicochemical parameter	Concentration
Ammonia	10 ppm
Nitrite	3.1 ppm
Nitrate	8 ppm
Dissolved oxygen	1.01 ppm
pH	8
Chemical oxygen demand (COD)	200 ppm
Biological oxygen demand (BOD)	24 ppm
Alkalinity	220 ppm
Hardness	140 N/mm^2
H_2S	0.5 ppm
Dissolved organic matter (DOM)	1 ppm
Salinity	3.3%
Microbial load	10^6 CFU/mL



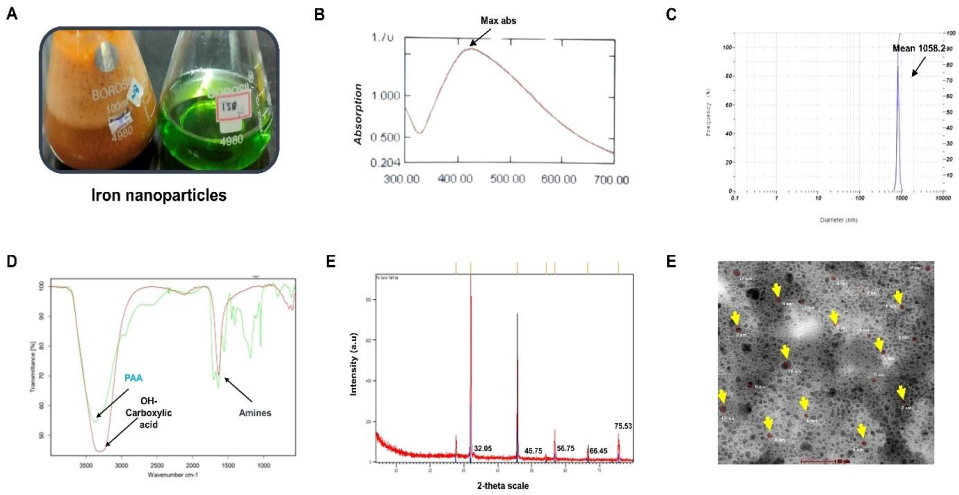
(A) Representative Bacillus megaterium colonies (B) Gram staining of Bacillus megaterium. These bacteria were collected from four different locations in Versova and Gorai mangrove area, Mumbai, India. The soil microorganisms were isolated using the serial dilution method on a nutrient agar medium.

Fig 2: Isolation of bacteria and morphological characteristics.

Particle size, zeta potential and X-ray diffraction analysis

Particle size, zeta potential and X-ray diffraction (XRD) analysis are essential for NP synthesis and characterization techniques. Particle size measurement provides crucial information about NP size distribution and homogeneity, directly influencing their properties and applications. Zeta potential analysis helps determine the surface charge of NPs, which affects their stability and interaction with surrounding media. XRD analysis is used to identify the crystal structure and phase composition of NPs, providing insights into their crystallinity and potential applications. In this study, the DLS observations indicate that the mean particle size of iron NPs was 8.2 nm (Fig 3C). Also, the mean zeta potential of the biosynthesized iron NPs ranges from -4.65 to -6.45 mV indicating negatively charged NPs. These findings on particle size and zeta potential demonstrate the repulsion and stability of NPs (Anandalakshmi, Venugobal *et al.*, 2016). Additionally, X-ray diffraction analysis (XRD) is adopted to assess the crystallographic structure of synthesized NPs. The diffraction peaks above 32° and XRD reveal five prominent reflections at 2θ = 32.05, 45.72, 56.75, 66.45 and 75.53, suggesting the face-centered cubic (FCC) structure of synthesized NPs (Fig 3E).

Further, iron NPs were evaluated under TEM to understand their form, size and distribution. It is a powerful method to understand morphology and size (Mahdieh, Zolanvari *et al.*, 2012), while FTIR and X-ray diffraction provides crucial information on crystallographic structure and interaction between bacteria and metal ions. Observation clearly indicates that the NPs were spherical-shaped and smooth. TEM images suggest that NP sizes range from 3 to 13 nm with an average size of 5 nm (Fig 3F). FTIR reveals how simple metal ions transform into elemental metals under the influence of various phytochemicals. Simultaneously, these photochemical act as reducing, stabilizing and capping agents (Elkomy 2020). In this study, FTIR findings (Fig 3D) indicate that the analyzed bacteria belong to bioactive groups, which could be responsible for the reduction of metal ions to metal NPs. These data show that synthesized NPs may have a variety of applications. The biosynthesis of NPs is economical and can produce high-quality NPs at room temperature. Here, synthesized NPs were characterized by DLS-zeta potential, UV-Vis, XRD, FTIR and TEM analysis. All these methods demonstrated the quality and distribution of synthesized NPs. Together, these observations strongly indicate the quality of NPs and also allow for the optimization



(A) The transition from green to thick orange indicated the formation of nanoparticles by $FeCl_3$. (B) Representative UV-visible absorption spectra of iron NPs after 48 h incubation. The absorption peaks were concentrated within 300-700 nm and the maximum absorption was detected at 425 nm. (C) The distribution of iron nanopaticles with a mean size of 8.2 nm. (D) Representative FTIR spectra of synthesized nanoparticle suspension. The nanoparticles showed peaks at 3300 cm^{-1} and 1600 cm^{-1}, indicating the presence of polyacrylic acid (PAA), OH stretch of carboxylic acid and N-H bending of primary amines. (E) Representative XRD pattern for synthesized nanoparticles. The XRD reveals five notable reflections at 2θ = 32.05, 45.72, 56.75, 66.45 and 75.53, indicating that synthesized nanoparticles have a face-centered cubic (FCC) structure. (F) Representative TEM images from iron NPs. Size ranges from 3 to 13 nm.NP, Nanoparticles; XRD, X-ray diffraction analysis; FTIR, Fourier-transform infrared spectroscopy.

Fig 3: Synthesis of iron nanoparticles and their characterization with UV-Vis, XRD and FTIR.

of synthesis parameters, which facilitates the customization of NPs' properties for specific applications.

Antimicrobial activity of iron NPs

The antimicrobial activity was determined as the diameters of the inhibition zones developed. The antimicrobial test revealed that NPs synthesized by *B. megaterium* could inhibit the growth of microorganisms. The optimal inhibitory zone of various NPs varies between 24-30 mm. These observations suggest that synthesized iron NPs appear equally bactericidal against gram-positive and gram-negative bacteria. This indicates that iron NPs can interact with the proteins of both gram-positive and gram-negative bacteria, subsequently affecting respiratory link and cell division, inevitably resulting in cell death (Feng *et al*., 2000). The antimicrobial activity of iron nanoparticles (NPs) has gained significant attention in recent years, mainly due to their potential applications in various fields, including aquaculture. Iron NPs exhibit unique properties, such as a high surface-to-volume ratio and improved reactivity, which enhance the antimicrobial efficacy of iron NPs. Specifically, in aquaculture, controlling pathogens and diseases that can adversely impact fish health and production presents significant challenges during the culture period. The antimicrobial effect of iron NPs represents a promising alternative to conventional antimicrobial agents, which are frequently associated with the development of antibiotic resistance.

Impact of iron NPs on ammonia in ex-situ condition

In jar test apparatus, coagulant substances precipitate, trap and form flocs to remove the pollutants. However, the jar test is modified in this study to accommodate NP investigation. This specific test was adopted to check the efficacy of synthesized NPs in regulating ammonia concentration in controlled conditions. The unique optical and electrical properties like SPR of iron NPs can be examined thoroughly in a closed jar apparatus instrument under regular observation. Additionally, iron NPs synthesized by the green method efficiently removed the ammonia and phosphates in the water through chemisorption (Xu *et al*., 2020). Despite scientific advances, the ammonia-removal effectiveness of NPs still needs to be determined. Thus, the jar test was used to assess synthesized NPs' effectiveness in removing ammonia.

Generally, ammonia, nitrite, dissolved oxygen (DO), pH and alkalinity all play a crucial role in the productivity of any culture pond. Reports suggest that iron oxide NPs from eucalyptus plant extract efficiently reduced the ammonia from the wastewater (Xu *et al*., 2020; Eljamal *et al*., 2022). Here, the presence of 10 ppm ammonia in ex-situ water is predicted to have a negative effect on water quality. However, within 5 hours, the addition of 1 ppm of iron NPs lowered the ammonia concentration from 10 ppm to ppm Additionally, NPs significantly increased the DO levels within 5 h of treatment. Wastewater containing jar had 1 mg/L of DO, but treatment with iron NPs improved the DO to 4 mg/L. However, there are no major reports indicating the effect of iron NPs on DO. Data showing influence of iron NPs on water quality parameter in jar test are represented in Table 2 and Fig 4. This specific observation suggests the necessity of further investigation.

Effect of iron NPs on ammonia and nitrite levels in common carp tank

Indoor aquaculture tanks are known to produce a substantial amount of ammonia and nitrite, which has an adverse effect on production. In addition, dissolved oxygen will deteriorate if no action is undertaken to maintain the optimum DO. Collectively, ammonia, DO and nitrite influences fish production as well as the aquatic ecosystem. In this in vitro experiment, NPs are employed to reduce the ammonia and nitrite and to maintain optimum DO throughout the culture period under different conditions. Here, common carp were grown intensively for 30 days. In these 30 days of culture, fish mortality ranged from 30 to 57% due to various factors (Fig 5A). Among these tanks, tanks with aeration had a lower mortality rate than the other tanks. Similarly, ammonia concentrations range from 0.47 to 0.79 mg/L, while nitrite levels were 2.25 to 2.75 mg/L. Moreover, DO levels were observed to be around 3.1-5.4 mg/L. These observations are higher than the optimum ranges required for the best results. The high ammonia content can be attributed to the high organic matter in the tanks, such as feed residues and animal excretions, due to high fish density (Liu *et al*., 2016). High ammonia and nitrite levels might have exerted pressure on fish growth as well as survival. Also, ammonia poisoning results in slowed growth, oxidative stress, diminished immunity and even death (Cheng *et al*., 2015). Consequently, they had shown its impact on fish mortality (0-day values are the values observed following 30 days of culture) in the investigation. Still, these adverse parameters created a suitable environment investigation.

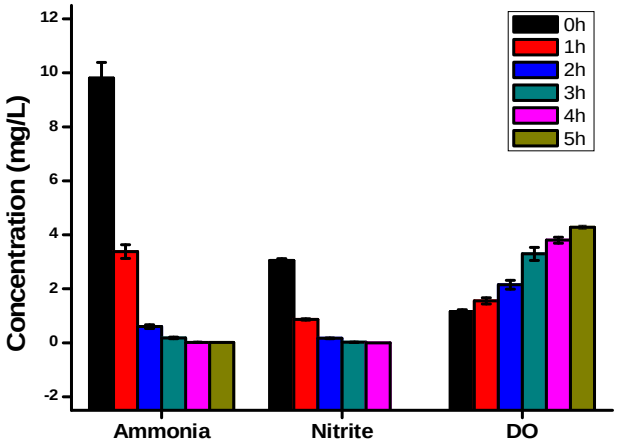
During the 10-day monitoring period, ammonia levels in fish culture tanks treated with nitrifying bacteria appear to remain stable or slightly increased. Interestingly, aeration in culture tanks regulates the ammonia levels modestly.

Table 2: Influence of iron NPs on water quality parameter in jar test.

Parameters	0 hour	1 hour	2 hours	3 hours	4 hours	5 hours
Ammonia	9.81±0.57	3.37±0.25	0.6±0.07	0.18±0.03	0.015±0.003	0.01±0.001
DO	1.16±0.06	1.55±0.11	2.15±0.16	3.29±0.23	3.80±0.10	4.28±.0.07
Nitrite	3.05±0.06	0.86±0.02	0.16±0.01	0.018±0.001	NA	NA

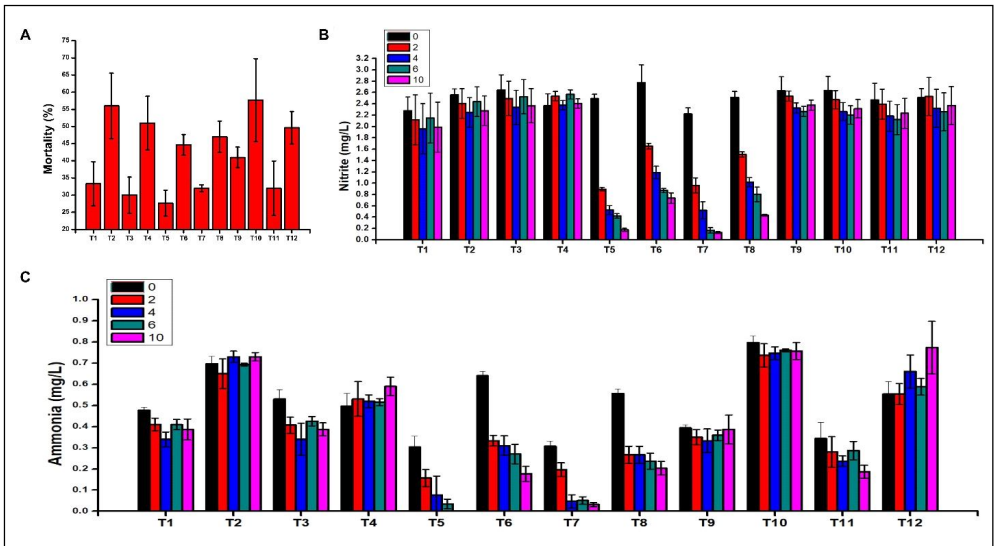
Note: All the measurements are expressed as mg/L. DO, Dissolved oxygen. Data are expressed as mean±SD.

Among all the groups, the concentration of ammonia in aerated tanks treated with nitrifying bacteria and iron NPs reduced to less than 0.03 mg/L from (Fig 5c). However, this positive impact was less in non-aerated tanks treated with nitrifying bacteria and iron NPs, suggesting the significance of aeration in the culture ponds. Next, nitrite levels reached 2.22 to 2.6 mg/L in 30 days of culture and the levels remained in a similar range during the 10-day monitoring period in all



This experiment was performed using a micro-controlled jar-test apparatus. Each jar contains 500 mL of wastewater with 10 ppm ammonia. Each jar was filled with samples and 1 ppm of iron oxide NPs. Each treatment was replicated three times. All of the jars were stirred at 100 rpm for 5 h. The observations were recorded at 1h, 2h, 3h, 4h and 5h.

Fig 4: Impact of iron NPs on ammonia, nitrite and DO concentrations.



Common carp was intensively cultured in a 500 ltr tank for 30 days. After 30 days, cultures were maintained under different culture conditions and ammonia concentrations were evaluated for the following 10 days. 0-day values are the values observed following 30 days of culture. I1= With microbial inoculum ($1.8x10^7$ CFU/mL); I2= Nanoparticle + inoculum ($1.8x10^7$ CFU/mL); I3= Uninoculated; S1= With soil base 15 cm; S2=Without soil base; A1= With aeration; A2= Without aeration. T_1= I_1+S_1+A_1; T_2= I_1+S_1+A_2; T_3= I_1+S_2+A_1; T_4= I_1+S_2+A_2; T_5= I_2+S_1+A_1; T_6= I_2+S_1+A_2; T_7= I_2+S_2+A_1; T_8= I_2+S_2+A_2; T_{9} = I_3+S_1+A_1; T_{10}= I_3+S_1+A_2; T_{11}= I_3+S_2+A_1; T_{12} = I_3+S_2+A_2.

Fig 5: Influence of iron NPs on ammonia and nitritelevels that determines the fish growth and production.

the groups except tanks treated with NPs (Fig 5B). The level of nitrites in tanks treated with nitrifying bacteria and iron NPs significantly reduced these levels in 10 days, irrespective of aeration. These elevated levels of ammonia, nitrite and low DO concentrations contributed to fish mortality. In culture tanks, fish mortality after a 30-day culture period ranged from 33-57%, which is considered high for any aquaculture system. During the 10-day experimental period, fish mortality increased in all tanks except those treated with nitrifying bacteria and iron NPs. The mortality rate in non-aerated tanks increased by up to 76%. Collectively, the observations demonstrate the influence of iron NPs and aeration on the key indices of water quality.

By aerating a pond, toxic ammonia dissolved in the water will diffuse into the air (Environmental 2010; Ip and Chew 2010). This specific process occurs in small tanks but may not yield effective outcomes in larger ponds. Since the small size of our experimental tanks, aeration may have positively influenced the maintenance of ammonia and nitrite levels. Additionally, treatment NPs and nitrifying bacteria are known to control ammonia and nitrite levels. Previously, iron or nickel NPs at 0.2 g/L regulated the nitrite levels (Valiyeva *et al*., 2019). In tanks containing NPs, the rate of mortality decreased dramatically, especially in aerated tanks. These key observations strongly indicate that aeration and nanoparticle treatment played a vital role in improving the water quality and sustaining fish mortality in cultured tanks. Further, these unambiguous observations on the concentrations of ammonia and nitrite following aeration and nanoparticle treatment diminish the significance of other parameters like DO. However, DO observe to be > 3 mg/L in all the groups indicating near-optimal DO levels (Fig 4-5). Together, these findings strongly suggest the use of iron NPs to eliminate or reduce ammonia concentrations in stressed fish culture ponds. The study has few limitations. Firstly, multiple factors are involved in the synthesis of NPs, including microbes used, pH, temperature, pore size and pressure (Patra and Baek 2014, Yaqoob *et al*., 2020, Ahmad *et al*., 2021). Thus, there could be lag or compromise in quality when preparing NPs in large quantities. Secondly, the study has considered only intensive freshwater farming while testing the efficacy of NPs. However, aquatic organisms are megacultured in different environments where the presence of salinity may alter the behavior of NPs (Khosravi-Katuli *et al*., 2017) and may demonstrate different outcomes than this study.

CONCLUSION

Bacillus sp is best suited for synthesis of iron NPs. DLS-zeta potential, UV-Vis, XRD, FTIR and TEM analysis confirms the quality and size of the iron NPs, which significantly reduced ammonia and nitrite levels in culture tanks with high ammonia and nitrite levels. However, aquatic organisms are cultured in different environments where the presence of salinity may alter the behavior of NPs. Thus, it is essential to perform extensive exploratory investigations to study the efficacy of NPs.

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Conflicts of Interest

The authors declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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