Effects of iron nanoparticles on iron-corroding bacteria

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Abstract The toxicological effects of Fe₃O₄ nanoparticles were evaluated with an iron-corroding bacterium (ICB) for preventing the biocorrosion of iron. Fe₃O₄ nanoparticles of 18 nm were successfully prepared and characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD) patterns and scanning electron microscopy–energy dispersive X-ray spectroscopy (SEM–EDS). A halophilic ICB strain L4 was isolated from Ribandar saltpan Goa, India and identified biochemically and by 16S rRNA gene sequence analysis as *Halanaerobium* sp. The Fe₃O₄ nanoparticles in increasing doses (0.1–100 mg/L) caused transformation in growth and sulfide production of ICB strain L4. SEM–EDS analysis revealed a deformed cell structure with adsorption of nanoparticle on the cell surface and increased cell size. Comet assay revealed genotoxic effect of Fe₃O₄ nanoparticles on strain L4 which resulted in dose-dependent DNA damage by increasing percentage tail DNA from 5 to 88% with increasing Fe₃O₄ nanoparticles concentration. Furthermore, sulfide production rate was reduced to 11.8% in presence of 100 mg/L Fe₃O₄ nanoparticles which reduced the corroding property of ICB strain L4; thus, it was unable to corrode the iron nail in presence of Fe₃O₄ nanoparticle. This work suggests the

possible application of Fe₃O₄ nanoparticle in addressing biocorrosion problems faced by different industries.

Keywords Fe₃O₄ nanoparticles · Bacteria · Comet assay · Sulfide production rate · DNA damage

Introduction

Nano-iron is gaining high commercial value due to its multipurpose application in catalysis, magnetism, electronics, biomedical, environmental remediation and various industrial applications, however, its potential toxicological risks and environmental impact has not been studied in depth. With the increasing demand for engineered nanostructures, release of such materials into the environment is unavoidable and the associated environmental risks have attracted increasing concern. The increased production and use of iron oxide nanoparticles will inevitably result in a greater exposure risk in the environment. Nanoscale zero-valent iron (nZVI) has been used increasingly over the last decade to clean up polluted waters, soils and sediments (Kirschling et al. 2010; Muller et al. 2012). Nano-form of iron has been used extensively for ground water remediation and disinfection of waste water (Diao and Yao 2009). Release of nanomaterials to soil systems might be beneficial to certain microbes (Nemcek et al. 2014) but it can also have a negative impact on the beneficial bacteria and thus increases the possibility of affecting biogeochemical cycles like nitrogen or sulfur cycles (Shahrokh et al. 2014). It has been reported that the Fe₃O₄ nanoparticles showed antimicrobial activity by inducing oxidative stress, morphological variation and cytotoxic effect on *Escherichia coli* (Auffan et al. 2008)

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and also have a bactericidal effect on various pathogenic bacteria (Prema and Selvarani 2012).

Corrosion is an electrochemical process consisting of an anodic reaction involving the ionization (oxidation) of the metal and a cathodic reaction on the reduction of a chemical species. These corrosion reactions when governed by microorganism or the product of their metabolic activity such as organic acids or ammonia or hydrogen sulfides on the metal surfaces termed as biocorrosion. Microbially influenced corrosion (MIC) is a common problem for oil (Neria-González et al. 2006), gas (Zhu et al. 2003) and shipping industries (Beech and Gaylarde 1999) where microbes initiate or accelerates a corrosion reaction on metallic surface. It causes economical losses to various industries by affecting operational and maintenance cost (Rajasekar et al. 2010). Microbially influenced corrosion (MIC) causes serious economical problem to various industries in particular the anaerobic corrosion of iron by sulfide producing microbes. Sulfidogenic bacteria (reducing sulfate, thiosulfate or sulfur to sulfide), iron oxidizing microbes, metal reducing bacteria and acid producing fermentative microbes are known to induce MIC through various processes (Vigneron et al. 2016). Anaerobic corrosion of metallic material is linked to activity of thiosulfate reducing bacteria (TRB) and sulfate reducing bacteria (SRB), as they produce hydrogen sulfide (Boudaud et al. 2010) as a corrosive agent acting primarily up on iron metals forming their metal sulfide (Hang 2003). Under anaerobic conditions, *Halanaerobium* sp. utilizes the available thiosulfate or sulfur to oxidize organic compounds and generate sulfide (S^{2-}). It reacts with dissolved metals to form metal-sulfide precipitates, since the solubilities of most toxic metal sulfides are generally very low (Al-Zuhair et al. 2008). Members of genus *Halanaerobium* are well known for carbohydrate fermentation and sulfide production (Liang et al. 2016) thus associated with biocorrosion. Better understanding of their metabolic activity will help in controlling these microbes in mitigating biocorrosion. The effects of nanoparticles on such sulfide producing microbes are very important because they can sequester heavy metals from the environment and play significant role in various biogeochemical cycles. Fe_3O_4 nanoparticles tend to agglomerate, which is determined by the nanoparticle's surface properties depending on temperature, ionic strength, pH, particle size and concentration variations in the surrounding environment (Nowack and Bucheli 2007). The effect of nanoparticles against these microorganisms should be evaluated to provide guidance on their field application (Liang et al. 2016).

Thus, the objective of this study was to examine the effects of iron nanoparticles on an iron-corroding bacterium to develop control measures against biocorrosion. We isolated an ICB (*Halanaerobium* sp. strain L4) from saltpan ecosystem. Subsequently, capabilities in corrosion

were tested with iron nail corrosion study. Further, the efficacy of Fe_3O_4 nanoparticle against strain L4 was evaluated and its impact on biocorrosion was assessed.

Materials and methods

Synthesis of iron oxide nanoparticles

The iron nanoparticles were synthesized by co-precipitation technique by reducing 1 M iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) and 2 M iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) solution with 1 M liquid ammonia (NH_3) under constant stirring (Berger et al. 1999; Lopez et al. 2010). Black precipitates of magnetite formed was centrifuged for 5 min at 4000 rpm and repeatedly washed with ultrapure water. A stable redispersable powder was obtained after lyophilisation. Subsequently nanoparticles were characterized by X-ray diffraction (XRD) of the powder samples using a Rigaku X-ray diffractometer equipped with a Cu K α monochromatic radiation source. Morphology of Fe_3O_4 nanoparticles were measured with transmission electron microscopy (TEM) using an FEI, TECNAI G2 F30, S-TWIN microscope operating at 300 kV equipped with a GATAN Orius SC1000B CCD camera. Elemental composition was determined by SEM-EDS, JEOL-JSM-5800LV.

Cultivation of culture and characterization

The iron-corroding bacterial (ICB) strain L4 was isolated from the overlying water of the Ribandar saltpan of Goa, India (15°30.166 N and 73°51.245 E) on modified Hatchikian's medium prepared in sterile sea water (Harithsa et al. 2002). Gram staining was performed with Hi-media Gram staining Kit by following manufacturer's instruction. Cell structure was acquired with ZEISS EVO 18 Scanning Electron Microscope (SEM). Motility, catalase and oxidase tests were carried out for physiological characterization. Substrate utilization was tested in 25 ml glass vials containing growth medium supplemented with a sterile stock solution of substrates (5 mM final concentration) and growth was detected with an increase in sulfide concentration on the 14th day of incubation.

DNA extraction and PCR amplification

For molecular characterization genomic DNA was extracted from strain L4, using Axygen genomic DNA extraction kit following manufacturer's instructions. 16S rRNA gene was PCR amplified using universal primer 27F and 1492R (Lane 1991) from the genomic DNA. The PCR conditions includes an initial denaturation at 94 °C for 5 min followed by 30 cycles with denaturation at 94 °C for 1 min,

annealing at 56 °C for 1 min and extension at 72 °C for 1 min followed by a final extension at 72 °C for 7 min. The Purified PCR product was sequenced by Sanger's dideoxynucleotide sequencing method.

Phylogenetic analysis

The partial sequences generated were assembled by Bioedit software. Further the sequence was subjected to similarity search using BLAST algorithm. The closest taxa sequences were retrieved from NCBI GenBank repository. The phylogenetic tree was constructed from the nearest taxa sequences by using Molecular Evolution Genetics Analysis software (MEGA version 6.0). The sequences were aligned by Clustal W parameter and maximum likelihood tree was constructed based on Tamura–Nei model with 1000 bootstrap replications were carried out to validate internal branches (Tamura and Kumar 2002; Nei and Kumar 2000).

Nanoparticle exposure

The ICB strain L4 was allowed to grow in different concentrations (0.1, 0.5, 1, 5, 10, 50 and 100 mg/L) of Fe₃O₄ nanoparticles. The growth was measured by optical density measurement with spectrophotometry at 480 nm and expressed as cell density per mL of the culture. The growth was monitored after 7th, 14th, 21st and 28th days of incubation.

Sulfide production and Morphology

Sulfide production by strain L4 was estimated by Parchmayer's method (Harithsa et al. 2002) on 7th, 14th and 21st days of incubation. Rate of sulfide production was calculated based on the amount of peak sulfide produced and expressed as $\mu\text{M ml}^{-1} \text{ day}^{-1}$. All the experiments were carried out in triplicates. Morphological changes were observed under SEM using a ZEISS Evo18 and JEOL-JSM-5800LV SEM and also the bacterial surface elemental composition was detected with the EDS analysis.

DNA damage study

DNA damage was investigated by neutral microgel electrophoresis technique (comet assay) as explained by Singh et al. (1999) with a slight modification, by staining the slide with ethidium bromide and observed under a LM-52-3000 epifluorescence microscope at 100× magnification. Comet assay was performed on 14-day-old cultures of *Halanaerobium* sp. Hundred random nuclear images from each bacterial slide were taken and number of comet pattern migration was counted per sample (normal cells and nanoparticle-exposed cells). The images of these comets

were captured using AMCap 9.2. The captured images were analyzed by CASP comet assay image analysis software. The percentage of tail DNA content (% tail DNA), the most reliable parameter of comet image analysis (Kumaravel and Jha 2006), was measured which reflects the extent of DNA damage (Praveen Kumar et al. 2014).

Iron corrosion study

For the evaluation of iron corrosion, nails of size 15 × 1 mm were sterilized by exposing to 99.9% ethanol for 24 h (Bhola et al. 2014). Two conditions were used in this experiment, iron nail in the presence of 100 mg/L Fe₃O₄ nanoparticle and in the absence of nanoparticle in growth medium inoculated with 2 ml of ICB strain L4 at 10⁶ cells/mL to check its iron-corroding property. The changes occurring in iron nail was compared to the control (iron nail in growth medium only) after 30 days of incubation and nail's surface was observed under SEM.

Results

Nanoparticle synthesis and characterization

The synthesized iron nanoparticles were characterized as crystalline Fe₃O₄ nanoparticle from the XRD pattern (Fig. 1a), with an average diameter of 18 nm measured by TEM analysis (Fig. 1b) and made up of Fe and oxygen as detected in EDS analysis (Fig. 1c). TEM micrograph demonstrated the spherical shape of the nanoparticles ranging over 10–35 nm in diameter and their aggregation property observed due to dipolar interactions arising from magnetic interaction (Lopez et al. 2010). This result confirmed the uniformity in particle size and the average size was measured to be 18 nm. Iron nanoparticles prepared by this method have been previously described as amorphous or cubic (Lopez et al. 2010) and its diffraction pattern was found similar to ICDD file no: 75-0449, Fe₃O₄, Magnetite particle. From the EDS results, the elemental composition was found to be iron and oxygen with an atomic percentage of 47.53 and 48.24, respectively, confirming the element to be an iron oxide nanoparticle.

Culture characterization

ICB strain L4 cells were observed as gram negative, motile, rod shaped bacteria with 0.8–1.2 μm in length and 0.4–0.7 μm in diameter. The phenotypic characteristics of the strain L4 are listed in Table 1 and Table S1. The strain L4 shared similar physiological and biochemical characteristics with genus *Halanaerobium*. Being a saltpan isolate the strain 14 require 1.3 M NaCl and alkaline pH for its

Fig. 1 Characterization of synthesized iron nanoparticle **a** X-ray diffraction pattern of synthesized Fe_3O_4 nanopowder, **b** TEM image of Fe_3O_4 nanoparticle used in this study. Bar denotes 20 nm. **c** EDS result of synthesized Fe_3O_4 nanoparticle showing elemental composition

Table 1 Partial biochemical characterization of ICB strain L4 from Ribandar saltpan compared with its closest relative *Halanaerobium acetethylicum* EIGI

Characteristics	<i>Halanaerobium</i> sp. strain L4	<i>Halanaerobium</i> <i>acetethylicum</i> EIGI
Isolation source	Saltpan sediment	Offshore oil rig Brines
Morphology	Rod	Rod
Cell size	0.4–0.7 × 0.8–1.2	0.4–0.7 × 1.0–1.6
Gram stain reaction	–ve	–ve
Motility	Motile	Motile
Optimum salinity (NaCl)	1.3 M	1.7 M
Temperature range	25–45	15–45
Optimum temperature	30	34
pH range	6.5–8.2	6.3–7.4
Sulfite	+	+
Sulfate	+	–
Thiosulfate	+	+

optimum growth. It was found to utilize sulfite, sulfate and thiosulfate as electron acceptor. The strain L4 was found to corrode iron nails present in their growth media thus further molecular identification was carried out by 16S rRNA gene sequence analysis.

Molecular identification

Phylogeny of 16S rRNA gene sequence (1466 base pair) of ICB strain L4 showed a distinct clade in the genus *Halanaerobium*. It had highest sequence similarity of 99% in BLAST search with *Halanaerobium acetethylicum* strain EIGI and also shares a bootstrap value of 99 (Fig. 2). The 16S rRNA gene sequence of the strain L4 was deposited in GenBank under accession number: KX784553. Based on the morphological, physiological characteristic similarity to its closest relative *Halanaerobium acetethylicum* strain EIGI (Table 1; Table S1) and 16S rRNA gene sequence analysis of ICB strain L4, was affiliated to *Halanaerobium* sp. It corrodes iron as evident from the iron nail corrosion occurred in the growth media only in the presence of strain L4.

Effects of Fe_3O_4 nanoparticle on ICB

Effect on growth

The growth of ICB, *Halanaerobium* sp. strain L4, at different concentrations of Fe_3O_4 nanoparticle (0.1, 0.5, 1, 5, 10, 50 and 100 mg/L) presented as growth percentage (Fig. 3a) derived from comparing with control (optimum

Fig. 2 Molecular Phylogenetic analysis of *Halanaerobium* sp. strain L4. The evolutionary history was inferred by the Maximum Likelihood method based on the Tamura–Nei model

growth of strain L4 in medium with our nanoparticle). Irrespective of the nanoparticle concentration, the higher growth was measured for 14-day incubation, which was used for evaluating growth percentage with respect to the control growth. The growth was found to be decreasing with increasing concentration of Fe_3O_4 nanoparticle. As illustrated in Fig. 3a, Fe_3O_4 nanoparticle readily reduced the growth to 50% at 1 mg/L. The growth curve of *Halanaerobium* sp. showed a clear inhibition of log phase with increasing concentration of nanoparticle with respect to control. Gradual shortening of log phase indicates bacteriostatic effect of the Fe_3O_4 nanoparticle on *Halanaerobium* sp. strain L4 in a concentration-dependent manner. On a long-term exposure of 28 days to Fe_3O_4 nanoparticle, the cell numbers decreased followed by cell inactivation and cell death suggestive of a bactericidal property of the particle probably resulting from a disturbance of the electron and ionic transport chains (Auffan et al. 2008; Boudaud et al. 2010) between the intra- and extra-cellular media. The nano-form of Fe does not support bacterial growth even though iron is an essential element for strain L4 growth.

Effect on sulfide production

The concentration-dependent growth inhibition of *Halanaerobium* sp. strain L4 was supported with the

decreased sulfide production rate (Fig. 3a), indicative of a simultaneous reduction in respiration of the bacterium. Hydrogen sulfide is the main metabolite produced by *Halanaerobium* sp. which corrodes metals by forming its metal sulfide (Boudaud et al. 2010; Bhola et al. 2014); thus, the Fe_3O_4 nanoparticle impact on sulfide production has a significant value as it could interfere with biogeochemical cycle of sulfur. It was observed that the bacterium could respire with an optimal concentration up to 0.5 mg/L Fe_3O_4 nanoparticle resulting in $0.023 \text{ nM ml}^{-1} \text{ day}^{-1}$ sulfide production. At higher nanoparticle concentration ($> 1 \text{ mg/L}$), an inhibition in sulfide production was observed and the value reached to $0.0046 \text{ nM ml}^{-1} \text{ day}^{-1}$ (11.8%) at 100 mg/L resulting in a gradual inactivation of the cells due to the excess of nanoparticle in the medium.

Phenotypic effect

From SEM images of the bacterium (Fig. 3b) normal cells of *Halanaerobium* sp. cells are observed to be rod shaped with $0.8\text{--}1.2 \mu\text{m} \times 0.4\text{--}0.7 \mu\text{m}$ in size; however, in the presence of the nanoparticle, an uneven change in the morphology of few cells of *Halanaerobium* sp. (Fig. 3c) with a curved, irregular shape and increase in size was observed. EDS spectrum taken on its cell surface detected the presence of the Fe_3O_4 nanoparticle as it detected iron (39%) and oxygen (42%) concentration to be predominant.

Fig. 3 Effect of Fe_3O_4 nanoparticle on *Halanaerobium* sp., **a** showing percentage survival and sulfide production rate with increase in nanoparticle concentration, **b** SEM images of normal cells of *Halanaerobium* sp. with average length of 0.7 μm , **c** cells of *Halanaerobium* sp. incubated with Fe_3O_4 nanoparticle showing morphological deformations and Fe_3O_4 nanoparticle adsorption on cell surface detected with EDS analysis

These adsorbed Fe_3O_4 nanoparticle on the bacteria could probably be attributed to the cellular activities. Thus, Fe_3O_4 nanoparticle could interfere with the corrosion property of strain L4.

Genotoxic effect

DNA breakage was visually detected as the difference in DNA migration (comet type migration) pattern in the slides. Genotoxic effect on bacteria assessed by comet assay showing the normal cell with intact DNA of *Halanaerobium* sp. (Figure S1a) and comet pattern migration (Figure S1b) was indicating the DNA damage in the cells of ICB. DNA damage (% tail DNA) induced by different nanoparticle concentration was presented in Fig. 4. It was found to be increasing in a concentration-dependent manner. Lower DNA damage was observed at 0.1 mg/L suggesting it as the tolerant nanoparticle concentration for the ICB while beyond 0.1 mg/L, the %tail DNA increased from 5 to 88%. Due to the nano-dimension, these particles could easily gain entry into the cellular environment and attack the genetic material of these test organisms, indicating the genotoxic effect of the nanoparticles.

Iron corrosion study

Our test organism belongs to the sulfide producing group of bacteria that corrodes iron evidenced from the experiment showing *Halanaerobium* sp. strain L4 at normal growth conditions corroding iron pins. Figure 5 illustrates visual inspection of the corrosion induced by the *Halanaerobium* sp., as they grow they produce sulfide in the growth media which reacted with the iron pin and black colouration of the pin was observed due to production of iron sulfide on the pin surface. In the presence of Fe_3O_4 nanoparticle (100 mg/L) *Halanaerobium* sp. failed to produce sulfide and struggled to survive as evidenced from prior experiment; thus, the iron pin remained unaffected as compared

Fig. 4 Comet assay for bacterial DNA damage showing percentage of DNA in tail with increasing nanoparticle concentration. The error bars represent the standard deviation of three replicates

Fig. 5 Iron nail corrosion experiment. (1) control: Iron nail in Growth media. (2) Iron nail in growth media inoculated with *Halanaerobium* sp. showing corrosion. (3) Iron nail in growth media inoculated with *Halanaerobium* sp. and Fe_3O_4 nanoparticle shows preventing corrosion

to the control. Further a significant difference in appearance, morphology and structure was observed from SEM images of iron nail surface in absence of nanoparticles (Figure S2a, c) and in the presence of nanoparticles (Figure S2b). SEM micrograph revealed the extent of corrosive behavior of the strain L4 (Figure S2b) compared to the control (Figure S2a). The control and nanoparticle-exposed iron nail surfaces were observed to have a smooth surface whereas the ICB strain L4 exposed nail was observed to be corroded with rough surface.

Discussions

Magnetite (Fe_3O_4) nanoparticles are naturally present in the environment (Guo and Barnard 2013). They have various useful applications in small concentrations; however, above a certain threshold, they possess the potential to produce ecotoxicity, challenging the eco-friendly nature of the particles. Iron plays a vital role in the growth and metabolic activities of bacteria including *Halanaerobium* sp. but different forms of iron could have different impacts on these species. The molecular characterization of the ICB revealed that the strain L4 belongs to genus *Halanaerobium*, which are abundantly found in the bacterial community of biocorrosion sites (Rajasekar et al. 2010; Gales et al. 2016; Liang et al. 2016). Exposure to increasing concentrations of Fe_3O_4 nanoparticle resulted in a dose-dependent growth inhibition in *Halanaerobium* sp. strain L4. The sulfides produced by the bacteria declined in presence of Fe_3O_4 nanoparticle in the growth media, resulting from the poor growth of the bacteria. Since the Fe_3O_4 nanoparticle employed, were toxic to ICB by inhibiting its growth and sulfide production, they eventually had an anti-corrosion effect. Our results suggest that the Fe_3O_4 nanoparticle toxicity is concentration dependent

and primarily they induce genomic damage and morphological variation. Fe_3O_4 nanoparticle was found to be toxic to bacteria even at lower concentration of 0.5 mg/L and toxicity increases in a dose-dependent manner with reduction in sulfide production. Small particle size improves the permeability through cell membrane and influence the bacterial inhibition (Azzam et al. 2012). As the particle size was 18 nm, it could easily penetrate through the cell membrane and may possibly react with various cellular organelles in the cytoplasm or caused damage to the DNA of cell and may interrupt different cellular activities. The effect of Fe_3O_4 nanoparticles on the cell size of strain L4 may be a result of its toxic impact on the bacteria during the cell division resulting in an uneven increase in cell size (Chatterjee et al. 2011). Previous studies on Fe_3O_4 nanoparticles also reported that it has a potential to penetrate the biofilm, may cause loss of membrane integrity in bacteria and change cell structure (Darwish et al. 2015). These could lead to increase in membrane permeability, leakage of intracellular constituents and generation of reactive oxygen species (Darwish et al. 2015). Bacterial response to the nanoparticle toxicity determined by the nature of the particle. Fe_3O_4 nanoparticle toxicity in bacteria can be explained by various mechanisms viz. oxygenated stress generated by Reactive Oxygen Species, including the radicals (O_2^-), hydroxyl radicals ($-\text{OH}$), hydrogen Peroxide (H_2O_2), and singlet oxygen ($^1\text{O}_2$), can cause damage to proteins and DNA in bacteria (Barnes et al. 2010; Youssef et al. 2009; Chatterjee et al. 2011; Singh et al. 2010). He et al. (2011) has reported the cell wall and outer membrane damage in *E.coli* cells by iron oxide nanoparticle. Other mechanisms may involve the disruption of cell membrane integrity or disturbance of electron transport chain and ions after the adsorption of nanoparticle on the cell wall. The probable reason is that the particles get adsorbed on the bacterial

surface and interfere in its metabolic processes; as a consequence, the growth and activity are inhibited. *Halanaerobium* sp. was found to corrode the downstream production facilities such as gathering pipelines and storage tanks (Liang et al. 2016). Various companies worldwide suffer economic losses due to MIC mostly affecting the pipe lines in almost every related industry like oil, gas, nuclear power (Zhu et al. 2003) primarily by the anaerobic corrosion caused by SRB and TRB. We isolated and identified a halophilic ICB from saltpan ecosystem. Anaerobic biocorrosion of iron is mainly driven by cathodic depolarization mechanism which is driven by removal of hydrogen by hydrogenase systems of most anaerobic microorganisms (Parthipan et al. 2017). In presence of H_2S an increase in proton discharge occurs. The hydrogenase enzyme may be either directly involved in depolarizing cathodic hydrogen from metal surface or may involve in synthesis of metabolic end product (H_2S) and play a major role in anaerobic biocorrosion (Parthipan et al. 2017). MIC process starts with a biofilm development on the metal surface (Parthipan et al. 2017). The sulfidogenic activity of the strain L4 contributed to the observed corrosion in the iron nail. The release of significant H_2S content during the growth of strain L4 explains the formation of black ferrous sulfide layer on the iron nail. Biogenic sulfide is the main cause for MIC primarily involving sulfidogenic bacteria. Fe_3O_4 nanoparticle could be used as a remedy in preventing corrosion since it inhibited the sulfide production and prevented iron pin corrosion. This suggests a protective covering of Fe_3O_4 nanoparticle on the surface prone to corrosion may inhibit microbial activity around it and thus render protection from MIC. These Fe_3O_4 nanoparticles were found to be an efficient biocide by inhibiting growth and sulfide production of ICB strain L4. To diminish the corrosive effect of *Halanaerobium* sp., the efficacy of Fe_3O_4 nanoparticle was tested against strain L4. At a lower dose of nanoparticle (0.5 mg/L), the growth inhibition was observed. Therefore, Fe_3O_4 nanoparticle may be considered for using as an anti-biocorrosion agent to reduce the sulfidogenic activity of ICB.

Conclusion

The research presented here showed biocorrosion induced by sulfidogenic microorganisms and its prevention with iron nanoparticles. Fe_3O_4 nanoparticle was found to be toxic to the iron-corroding *Halanaerobium* sp. strain L4 and its toxicity acts in a dose-dependent manner, inducing morphological variations with interruption at the genetic level. This suggests that Fe_3O_4 nanoparticle could provide an alternative option for industries to use it as a nano-

anticorrosion compound for the surfaces prone to corrosion by sulfidogenic bacteria. The adverse impact it may cause on the microbial community in the environment restricts its broad spectrum environmental applications. Further work is needed to develop Fe_3O_4 nanoparticle-based application strategy for mitigating the biocorrosion problems faced by various industries.

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Compliance with ethical standards

Conflict of interest All the authors have approved the submission of this manuscript to 3 Biotech and declare that there is no conflict of interest.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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