Research Article

Utilization of induction furnace steel slag based iron oxide nanocomposites for antibacterial studies

J. Baalamurugan^{1,2} · V. Ganesh Kumar¹ · T. Stalin Dhas¹ · S. Taran^{1,3} · S. Nalini¹ · V. Karthick¹ · M. Ravi¹ · K. Govindaraju¹

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Abstract

Metals and metal oxide-based nanocomposites play a significant role over the control of microbes. In this study, antibacterial activity of iron oxide (Fe_2O_3) nanocomposites based on induction furnace (IF) steel slag has been carried out. IF steel slag is an industrial by-product generated from secondary steel manufacturing process and has various metal oxides which includes Al_2O_3 (7.89%), MnO (5.06), CaO (1.49%) and specifically Fe_2O_3 (14.30%) in higher content along with metalloid SiO_2 (66.42). Antibacterial activity of iron oxide nanocomposites has been revealed on bacterial species such as *Micrococcus luteus*, *Bacillus subtilis* and *Staphylococcus aureus*. *Micrococcus luteus* has undergone maximum zone of inhibition (ZOI) of 12 mm for 10 mg/mL concentration of steel slag iron oxide nanocomposite. Growth inhibitory kinetics of bacterial species has been studied using ELISA microplate reader at 660 nm by varying the concentration of steel slag iron oxide nanocomposites. The results illustrate that IF steel slag is a potential material and can be utilized in building materials to increase the resistance against biodeterioration.

✉ V. Ganesh Kumar, ganeshkumar@sathyabama.ac.in | ¹Centre for Ocean Research (DST-FIST Sponsored Centre), Earth Science and Technology Cell (ESTC), Sathyabama Institute of Science and Technology, Chennai 600119, India. ²Department of Chemistry, Sathyabama Institute of Science and Technology, Chennai 600119, India. ³Department of Biotechnology, School of Bio and Chemical, Sathyabama Institute of Science and Technology, Chennai 600119, India.

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Graphic abstract

Keywords Induction furnace steel slag · Nanocomposite · Iron oxide · Antibacterial activity · Antimicrobial agent · Biodeterioration

1 Introduction

Industrial by-products utilization and management is a major challenge among researchers globally, and many solutions have been initiated. In steel industry, steel manufacturing process generates enormous quantity of industrial by-product called 'slag' [1]. Land filling of steel slag in industrial sites has not been encouraged due to progressive saturation [2]. The energy and emission associated with natural aggregate extraction, crushing and cleaning can be reduced by utilizing of steel slag as an alternate aggregate [3]. Several research studies have been carried out to utilize steel slag in various fields. Steel slag has been utilized in concrete/mortar [4, 5], cementing material [6], bituminous paving mixtures [7], road construction [8], fire resistance [9], thermal energy storage [10], heavy metal removal [11, 12], organic pollutant and dye removal [13], synthesis of mesoporous nanosilica [14], improvement in soil quality [15], water filter [16], radiation shielding [17], CO₂ sequestration [18], crop production [19], green artificial reef [20] and micro-fouling [21].

Historically, it is evident that, iron slag was used in wound healing by Greek physician Aristotle in 350 BC [22]. Antibacterial agents seek considerable attention in health sector for its prevention against bacterial pathogens, and they are also used in various industries including food, textile and as water disinfectant etc., [23]. There is

an ever-growing need for prevention of biofilm formation, and different strategies are conferred towards antimicrobial studies [24]. The dimensions of metals/metal oxides nanomaterial are between bulk materials and molecules/atoms/ions which interact with cell to make stable entity with less energy [25]. Iron oxide nanoparticles have been studied for various antimicrobial activities [26, 27].

Iron oxide nanostructures in the phase of hematite (α -Fe₂O₃) have been synthesized and experimented for its antibacterial activity using *Escherichia coli* and *Staphylococcus aureus*. The experimental result shows that 20 µg/µl concentration of α -Fe₂O₃ delivered the ZOI 45 ± 2 39 ± 2 against the bacterial species *E. coli* and *S. aureus*, respectively [28]. Fe₂O₃ nanoparticles have been functionalized using 3-aminopropyltriethoxysilane (APTES) and studied for its antibacterial activity. The binding of inorganic nanoparticles and organic functional groups (i.e. APTES–Fe₂O₃) has been studied using spectral analysis (absorption and emission). *Pseudomonas aeruginosa* and *S. aureus* are the bacterial species used in this study. The measured zone of inhibition was 21 mm and 11 mm for *S. aureus* and *P. aeruginosa*, respectively. The results illustrate that the APTES–Fe₂O₃ nanocomposites can be used as a potential antibacterial agent [29]. Iron oxide (α -Fe₂O₃) nanoparticles were synthesized using the extract of *S. cordifolia* plant and studied for its antibacterial activities using bacterial species such as *B. subtilis*, *S. aureus*, *E. coli* and

K. pneumonia. Agar well diffusion procedure has been adopted for antibacterial study, and the obtained zones of inhibition values are 16.00 ± 1.00 , 13.67 ± 0.58 , 11.33 ± 0.58 and 12.00 ± 1.00 mm for the bacterial species *B. subtilis*, *S. aureus*, *E. coli* and *K. pneumonia*, respectively. From the results, it can be inferred that *B. subtilis* shows maximum inhibition (ZOI 16.00 ± 1.00) towards $\alpha\text{-Fe}_2\text{O}_3$ [30].

Iron/iron oxides are also utilized with other metal ion nanoparticles for antibacterial/antimicrobial activities as Cu-Fe [31], Fe/Zn oxide [32], fibreglass- $\text{Fe}_2\text{O}_3/\text{Ag}$ [33] and $\gamma\text{-Fe}_2\text{O}_3/\text{SiO}_2/\text{TiO}_2\text{-Ag}$ nanocomposites [34]. In concrete, corrosion occurs by microbes (producing inorganic/organic acids) causing deterioration which degrades the components of concrete and it is a serious issue to be addressed [35]. Induction furnace steel slag has been utilized in concrete by our research team earlier [36] which reveals that it can be used as a partial replacement of natural coarse aggregate. This study aims to provide antibacterial property of induction furnace steel slag on *M. luteus*, *B. subtilis* and *S. aureus* which can be a significant research data in the building environment of concrete constructions under microbial environment, thereby contributing to the sustainable constructions.

2 Materials and methods

2.1 IF steel slag sample collection and characterization

Iron oxide-based slag materials used in the present study have been obtained from Jeppiaar furnace and steels, Pvt. Ltd., Kanchipuram, Tamil Nadu, India. Slag material is subjected to size reduction using ball milling technique. X-ray diffraction (XRD) is operated at 9 kW with CuK-alpha radiations using Bruker D8 to analyse mineral phases of slag material. Surface morphology of IF steel slag has been analysed using field emission scanning electron microscope (FE-SEM) (CARL ZEISS, SUPRA-55, Germany) equipped with energy-dispersive X-ray analysis (EDAX). The presence of various metal oxides in steel slag material has been analysed using X-ray fluorescence spectroscopy (Bruker S8 Tiger).

2.2 Antibacterial experiments

Antibacterial activity of iron oxide nanocomposite has been studied against *M. luteus*, *B. subtilis* and *S. aureus* bacterial species. Microbes were maintained at Marine microbiology laboratory, Centre for Ocean Research, Sathyabama Institute of Science and Technology, Chennai. Agar well diffusion assay has been adopted to determine the zone of inhibition (ZOI) of microbes against iron oxide

nanocomposite. Luria Bertani agar (LBA) media plates were made and wells of 5 mm diameter were created using cork borer. Iron oxide nanocomposite of 2, 5 and 10 mg/mL concentrations was added to the wells and incubated for 24 h at 37 °C.

The growth kinetics of bacterial species were studied using ELISA microplate reader (BioTek—ELX 800) to examine the effect of iron oxide nanocomposite in various concentrations (2, 5 and 10 mg/mL) with 10^6 cells of each individual bacterial species (20 μL) grown in Luria Bertani broth medium (200 μL). A control is maintained in identical conditions. Growth rate of bacterial species was examined by measuring OD values at 660 nm up to 27 h at an interval of 3 h. In this research work, all the experiments were carried out thrice and the mean value has been taken.

3 Results and discussion

3.1 Slag-based iron oxide nanocomposite

Mineralogical phases of components present in steel slag have been identified using XRD analysis (Bruker D8 advance) (Fig. 1). The presence of major mineralogical phases in slag material is quartz (SiO_2 , 2θ value 26.6), iscorite ($\text{Fe}_7\text{SiO}_{10}$, 2θ values 50.32 and 60.30), almandine ($\text{Fe}_3\text{Al}_2\text{Si}_3\text{O}_{12}$, 2θ values 22.20 and 68.48) and hematite ($\epsilon\text{-Fe}_2\text{O}_3$, 2θ values 36.7 and 39.94). Also minor peaks with less intensity have been identified and is similar to the results reported [37, 38]. The presence of minor phases in steel slag materials is manganocalcite [(Ca, Mn) CO_3 , 2θ value 29.73], merwinite [$\text{Ca}_3\text{Mg}(\text{SiO}_4)_2$, 2θ values 31.69 and 34.92], periclase (MgO , 2θ value 46.12)

Fig. 1 XRD analysis of IF steel slag

and mayenite ($\text{Ca}_{12}\text{Al}_{14}\text{O}_{33}$, 2 θ value 55.07) [37]. Iron silicates such as FeSiO_4 (2 θ value 35.10) and FeSiO_{10} (2 θ value 51.48) are along with MnO_2 (2 θ value 42.74) and are also present in slag materials, and the similar results have been reported earlier [38].

Surface morphology of slag material is recorded using FE-SEM (SUPRA 55 CARL ZEISS, Germany) and is shown in Fig. 2.a–c. SEM analysis shows that the particles are heterogeneous in nature. Also, the metal oxides of slag material are in dissimilar in size and shape. The slag particles were sub-rounded to angular shape with heterogeneous in nature. The roughness and edges were visible in bulk and angular particles. EDAX analysis was carried out to identify the elemental composition of slag material (Fig. 2d). Among various metals present in slag elemental composition (Fig. 3 and Table 1), iron (Fe—17.25%) and silicon (Si—18.54%) contribute majorly along with aluminium (Al—5.80%), manganese (Mn—3.48%), calcium (Ca—1.58%) and magnesium (Mg—0.65%) with some trace elements. The presence of various metal oxides composition in steel slag has been analysed using XRF technique (Bruker S8 Tiger) and is shown in Table 2. The major metal oxides present in slag are Fe_2O_3 (14.30%), Al_2O_3 (7.89%), MnO (5.06) and CaO (1.49%) along with metalloid SiO_2 (66.42).

Fig. 3 EDAX pattern—Elemental composition of IF steel slag

3.2 Antibacterial studies of iron oxide slag nanocomposites

3.2.1 Zone of inhibition

Antibacterial efficacy of slag-based iron oxide nanocomposite against *M. luteus*, *B. subtilis* and *S. aureus* was evaluated by agar well diffusion assay. Iron oxide nanocomposite-introduced bacterial species have undergone bactericidal effect with respect to the amount of IF steel slag taken. The antibacterial effect of bacterial species

Fig. 2 SEM analysis of steel slag: **a**, **b** and **c** surface morphology; **d** EDAX analysis

Table 1 Elements present in IF steel slag

Elements	O	Mg	Al	Si	K	Ca	Ti	Cr	Mn	Fe	Zn
Weight %	50.72	0.65	5.80	18.54	0.54	1.58	0.50	0.59	3.48	17.25	0.35
Atomic %	70.06	0.59	4.75	14.59	0.30	0.87	0.23	0.25	1.40	6.83	0.12

Table 2 Metal oxides present in IF steel slag

Formula	Concentration (%)
SiO ₂	66.42
Fe ₂ O ₃	14.30
Al ₂ O ₃	7.89
MnO	5.06
CaO	1.49
Cr ₂ O ₃	0.93
SO ₃	0.92
TiO ₂	0.84
K ₂ O	0.53
Na ₂ O	0.42
MgO	0.40
BaO	0.20
ZnO	0.17
PbO	0.12
Cl	0.08
V ₂ O ₅	0.06
P ₂ O ₅	0.03
MoO ₃	0.03
Nb ₂ O ₅	0.02
ZrO ₂	0.02
SrO	0.02
CuO	77 PPM

increases with increasing concentrations of iron oxide nanocomposite (Fig. 4). It was observed that *M. luteus* has high receptive of antibacterial activity of iron oxide nanocomposite with ZOI of 12.1 mm (10 mg/mL), 9.1 mm (5 mg/mL) and 6.5 mm (2 mg/mL) followed by *B. subtilis* of ZOI of 10 mm (10 mg/mL), 8.2 mm (5 mg/mL) and 6.2 mm (2 mg/mL). *S. aureus* exhibits least ZOI among the microbes studied with 8, 6.7 and 5.9 mm for 10 mg/mL, 5 mg/mL and 2 mg/mL of iron oxide nanocomposite concentrations, respectively. Iron oxide of slag material inhibits microbial growth and can be utilized in concrete related to microbial attack [39].

3.2.2 Growth kinetics and cell death

Growth inhibitory kinetics of bacterial species has been studied using ELISA microplate reader. Accuracy in determination, usage of less chemicals/time saving is the reason behind this method [40]. Optical density (OD) values were measured with respect to time at 660 nm, for different species with various concentration of iron oxide

Fig. 4 Antibacterial activity of iron oxide slag nanocomposite (2, 5 and 10 mg/mL)

nanocomposite (2, 5 and 10 mg/mL), and are plotted in a graph (Fig. 5a–c). Antibacterial activity increases with increasing concentration of iron oxide with respect to time in the order of $10 > 5 > 2$ mg/mL.

Iron oxide nanoparticles are capable of extending reactive oxygen species (ROS) in the media culture possessing antimicrobial activity [41]. IF steel slag iron oxide nanocomposite involved in the growth inhibition of *M. luteus*, *B. subtilis* and *S. aureus* bacterial species. Oxido-reduction reactions involve in ROS generation via Fe^{3+} and Fe^{2+} [42] present in slag-based iron oxide. The free radicals formed via oxido-reduction process are adequate to deposit stress on the bacterial cell causing death [41]. The rupturing of cell wall is due to the production of ROS such as hydroxyl radicals ($\text{OH}\cdot$), singlet oxygen ($\text{HO}_2\cdot$) [41, 43]. Microbial-influenced biocorrosion occurs when suitable conditions are present in the environment [44]. In concrete, corrosion occurs by microbes (producing inorganic/organic acids) causing deterioration which degrades the components of concrete. Metabolic process of microorganisms generates ammonia and hydrogen sulphide causes which can also cause corrosion of concretes; these metabolites of microorganisms are aggressive in nature and reacts with the components of concrete leading to the formation of

Fig. 5 Inhibitory growth kinetics of bacterial species: **a** *M. luteus*; **b** *B. subtilis*; **c** *S. aureus*

non-binding calcareous salts, and degradation of sulphate causes expansion of concrete results destruction of structural elements in concrete [45] 002E Blast furnace slag has

been utilized in concrete, and the results show positive impact on deterioration of concrete [46, 47]. Iron oxide nanocomposite of IF steel slag exhibits inhibitory effect against microbial growth and can be utilized in building materials to increase the resistance against biodeterioration [39].

4 Conclusion

Antibacterial potential of IF steel slag-based iron oxide nanocomposite on *M. luteus*, *B. subtilis* and *S. aureus* bacterial species is effective. IF steel slag is available widely as an industrial by-product at very low cost and can be used as an active antimicrobial agent in building materials. Induction furnace slag-based iron oxide nanocomposites can improve the safety measures of concretes to increase the resistance against biodeterioration. This initiative will lead to utilization of IF steel slag in concrete on large scale under microbial environment.

5 Future work

Biodeterioration and biodegradation of concrete causes serious impacts on construction structures. The results of this research study will make an impact to work with IF steel slag as an alternative. Our research team has already incorporated IF steel slag in concrete, and the mechanical properties of concrete were admissible. Hence, antibacterial efficacy of IF steel slag paves a way to study the concrete specimens incorporated with IF steel slag under microbial environment. This initiative will lead to lesser microbial attack in concrete structures.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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