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The Impact of Iron Oxide Nanoparticles on Crude Oil Biodegradation with Bacterial Consortium

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ABSTRACT

This study was performed to determine the effect of synthesized iron oxide nanoparticles on the consortium of isolated bacterial strains from the crude oil-contaminated site. The iron oxide nanoparticle (FeNPs) was synthesized by chemical co-precipitation method and confirmed with its characterization results such as UV-spectroscopy, X-ray Diffraction (XRD), High-Resolution Scanning Electron Microscopy (HR-SEM), Zeta potential and Particle Size Analyser studies. The isolates were cultured in LBBH (Luria-Bertani and Bushnell Haas) medium containing crude oil as a carbon source with incubation for 7 days. This study was performed using FeNPs with four different concentrations (10, 50, 100 and 150mg) incorporated with the isolated microbes clubbed as a consortium. The rate of biodegradation was investigated by gas chromatography-mass spectrometry (GC-MS) analysis. By comparing the control sample (crude oil) there was a better degradation in FeNPs added bacterial culture than consortium degradation. The obtained results conclude that studying different concentrations of FeNPs with the consortium of isolated microbes showed degradation differences, whereas 150mg concentration has a better degradation effect compared to other variations. It should be carried out to avoid agglomeration of nanoparticles by improving their biocompatibility and quality to influence the biodegradation of crude oil.

INTRODUCTION

The pollution of hydrocarbons due to oil and gas exploration and exploitation operations is one of the main environmental issues of the day. The discharge of this vital energy source into the environment is unavoidable and it has disastrous effects on marine/coastal waterways, shorelines, and land as well. Due to the danger that large oil spills pose to the environment, it is crucial to find clean-up methods that are affordable, quick, environmentally friendly, and long-lasting. These methods should also optimize and make it easier for highly petroleum hydrocarbon-inundated terrestrial ecosystems to be bio-restored for agricultural use or ecological balance (Bertha et al. 2021). Following a discharge or spill, a variety of concurrent physicochemical processes control the distribution and destiny of oil. Short-chain volatile chemicals rapidly evaporate after first spreading out on the surface. The amount that the mass of oil is exposed to air and light depends on the physical form of the oil, which affects the rate of oxidation (Yusaf 1985). Accidents involving oil spills have a history of declining in recent decades. The need to continue studying the function of microorganisms in the natural and induced attenuation of pollutants is driven by the greatest industrial catastrophe in history and the continual input of several additional pollutants into the coastal environment. By understanding the role of microorganisms in the restoration of an ecosystem, greater efforts are needed to better understand how these microbial communities function. In situ, investigations at the contaminated sites and simulation experiments in microcosms and mesocosm under controlled laboratory settings have been the two primary approaches utilized to investigate the microbiological response to oil pollution (Acosta-González et al. 2016). The primary use of environmental biotechnology is bioremediation, which is presently thought of as an emerging technique. This can completely degrade the pollutant into carbon dioxide aerobically or water and methane under anaerobiosis, or it can mineralize the pollutant, partially transforming it into a less dangerous form. Each microbe has a unique tolerance profile, and there are instances where large amounts of organic contaminants might limit microbial activity (San Martín 2011). Although bioremediation has not yet been proven to be efficient for the treatment of open ocean oil after a spill it is far more challenging to assess hydrocarbon biodegradation in situ than it is to do so in lab experiments (Atlas 1995). The restricted accessibility of oil pollutants to microorganisms is the limiting factor in the biodegradation of oil pollution in the environment. Since many complex hydrocarbon molecules are carcinogenic, they also pose a real risk to human health. Here, to solve these issues, nanotechnology has drawn a lot of attention due to its large specific surface areas and has been widely exploited as reductants and/or catalysts to enhance a variety of processes. On the other hand, the impact of nanoparticles on microbes has also attracted a lot of interest. Although nanoparticles can help with microbe activity, there has only been very little research on how they affect the rates of microbiological response. Typically, nanoparticle catalysts increase the speed of microbiological reactions by attaching to cells and promoting microbial activity (El-Sheshtawy et al. 2017). Even if biological methods are effective, the efficiency and efficacy of nanoparticles have been widely employed to remove a range of organic and inorganic toxins from the environment. Nanoparticles make excellent adsorbents and may significantly increase the adsorption capabilities of sorbent materials. Among them, Iron oxide nanoparticles have gained a lot of interest for a variety of applications due to their special characteristics and relatively simple manufacture. Iron oxide nanoparticles with appropriate surface chemistry are prepared by various methods such as wet chemical, dry processes, or microbiological techniques. The most effective and simplest technique to obtain iron oxide nanoparticles is through the Chemical co-precipitation method. The potential of iron oxide nanoparticles for oil clean-up was emphasized by several investigations (Ali et al. 2016 & Alabresm et al. 2018). The method for synthesizing iron oxide nanoparticles is environmentally friendly and requires a short reaction time compared to the physical and chemical methodologies (Oyewole et al. 2019). This research work exhibits the enhancement in crude oil biodegradation of isolated strains as a consortium and with the addition of synthesized iron oxide nanoparticles (FeNPs) was performed and analyzed using GC-MS study.

MATERIALS AND METHODS

Synthesis of Iron Oxide Nanoparticles (FeNPs)

Iron oxide nanoparticles were synthesized using the chemical co-precipitation method (Ganapathe et al. 2022, Kalantari et al. 2014). The iron source was first prepared by dissolving 0.2 M $FeSO_4 \cdot 2H_2O$ and 0.1 M $FeCl_3 \cdot 6H_2O$ in 50 mL of distilled water. The base solution was prepared by dissolving 2M NaOH into 200 mL of distilled water. The prepared iron source was added dropwise into the flask containing the base solution under vigorous magnetic stirring of 1000 rpm at 37°C for 30 minutes. At the end of the reaction, the observed black precipitate was centrifuged at 5000 rpm for 10min, and washed thrice with distilled water and ethanol. The precipitate was then dried in a hot air oven at 100°C overnight. The dried black granules were finely ground into powdered form. The sample has been labeled as FeNPs for further reference.

Characterization Studies of Synthesized FeNPs

The UV spectrophotometric analysis of the synthesized iron oxide nanoparticles was carried out at 200 to 800 nm wavelength range (Oyewole et al. 2018). The surface charge and the size distribution were analyzed using a Zeta-potential and Particle Size analyzer. The crystalline nature of synthesized nanoparticles was analyzed using X-ray diffraction (XRD) at an angle range of 2θ (10–80 degrees). The surface morphology of the iron oxide nanoparticles was examined using High-Resolution Scanning Electron Microscopy (HR-SEM) equipped with (EDS) Energy Dispersive Spectroscopy.

Isolation and Identification of Bacteria

The crude oil-polluted soil sample was collected from an oil refinery site, in Chennai, Tamil Nadu (13°10’36” N 80°16’25” E). The sample was serial diluted and inoculated in LBBH medium and kept in a shaking incubator overnight at 37°C at 100 rpm. After initial serial dilution and growth observation, four strains (SH 1- 4) were isolated and maintained as pure cultures (Chikere & Ekwuabu 2014). To assess biodegradation, these isolates were used as a consortium, inoculated in LBBH medium with 2% crude oil, and incubated in a rotary shaker at 37°C in 150 rpm (Li et al. 2019). Flask with LBBH medium and crude oil served as a control for the comparative study.

Degradation of Crude Oil Using Synthesized Iron Oxide Nanoparticles (FeNPs)

For biodegradation activity, LBBH medium was prepared with different concentrations of synthesized nanoparticles

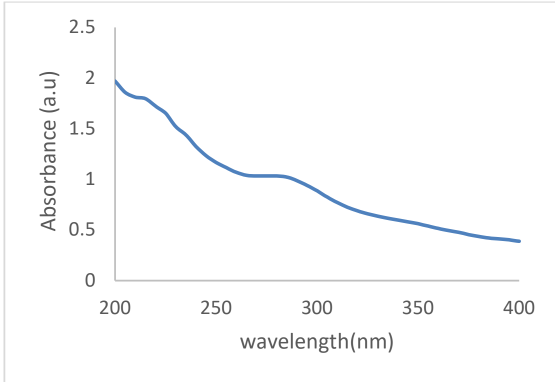


Fig. 1: The UV-visible analysis of FeNPs.

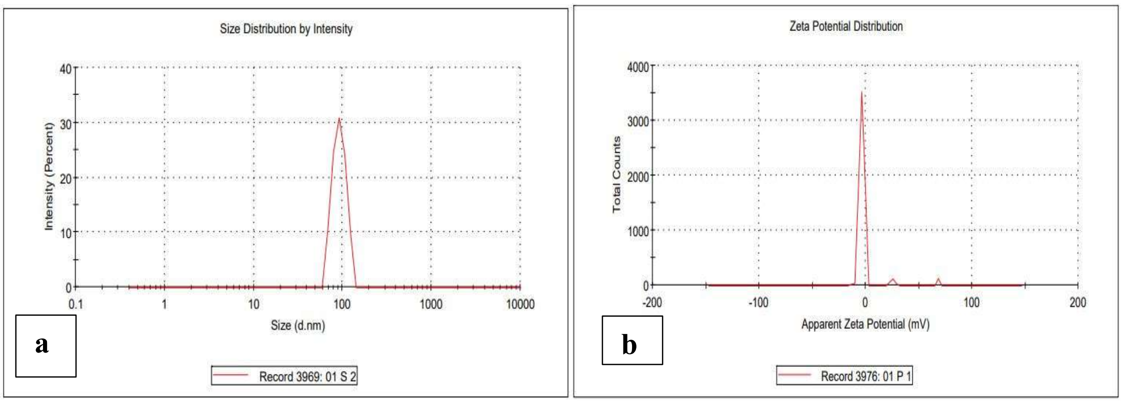


Fig. 2: (a) Particle size and (b) zeta potential distribution of FeNPs.

(10, 50, 100, 150mg), and 2mL of crude oil was added and kept in a shaking incubator at 100rpm for 30 minutes. Then 100µL of overnight incubated consortium culture was added in each medium containing various concentrations of nanoparticles again kept for incubation in a shaker incubator for 7 days at 37°C at 150rpm. A sample containing crude oil without inoculum was taken as a control. The cultures were kept in a shaking incubator at 150 rpm for 7 days (El-Sheshtawy & Ahmed 2017). Degraded crude oil residues were performed by Gas Chromatography-Mass Spectroscopy (GC-MS) analysis equipped with a fused silica capillary column (DB5, 30m long 0.25mm inner diameter). The oven temperature was programmed from 50°C (3 min) to 280°C at 10°C/min. The carrier gas was Helium at a flow rate of 1 mL/min. The injector temperature was 230°C, and the samples were injected for 2mL. The mass spectrum of the components present in the crude oil was confirmed in the National Institute of Standard and Technology (NIST) mass spectra library database to determine the amount of residual crude oil in the analyzed degraded samples (Kazemzadeh et al. 2020, Iwabuchi et al. 2002).

RESULTS

This work evaluates the possibility of using isolated bacterial strains in combination with iron oxide nanoparticles (FeNPs) to improve crude oil biodegradation. FeNPs characterization verifies their applicability, and enhanced degradation is revealed by GC-MS analysis. With ramifications for environmental cleanup initiatives, our findings highlight FeNPs' potential as a potent instrument for enhancing crude oil remediation procedures. The effective production of FeNPs was validated by characterization investigations; UV-Vis examination revealed an absorption peak at 278nm (Fig. 1), which is suggestive of iron oxide nanoparticles.

The Zeta potential and size of particles analysis in Fig. 2 (a) & (b) showed that the FeNPs were stable, with a zeta potential of ±3.53 mV, a particle size of about 148.3 nm, and a PDI of 0.373. Zeta potential was also determined and obtained to be ±3.53 mV signifies the stability of synthesized nanoparticles.

FeNPs' crystalline structure was validated by X-ray diffraction (XRD) research. In X-ray diffraction analysis

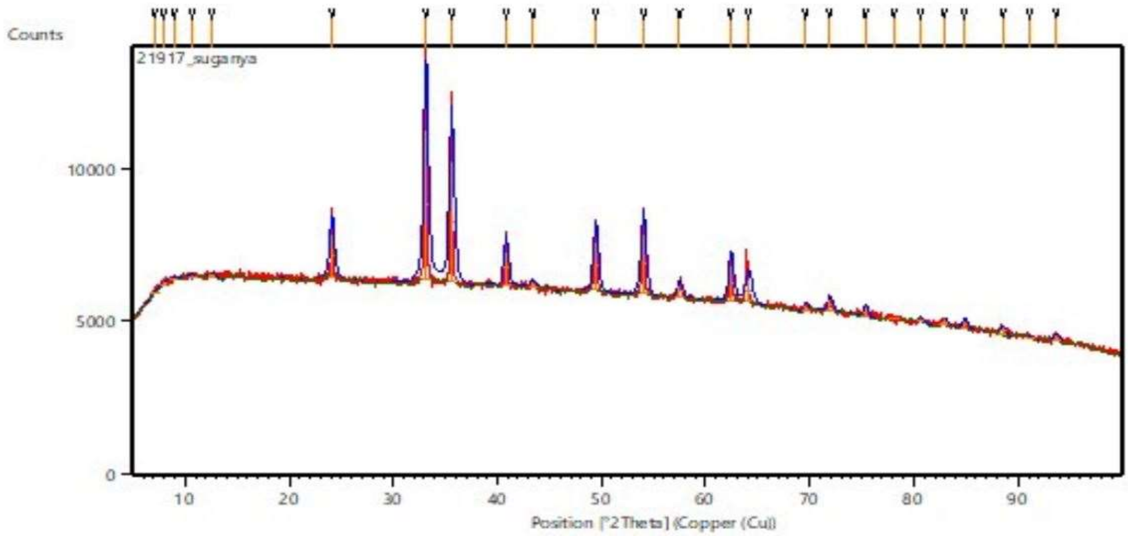


Fig. 3: XRD spectrum of FeNPs.

(Fig. 3) of synthesized FeNPs, a cathode film coating was used to determine the type of crystals of nanoparticles (2 θ = 10–80). The spectrum obtained by the XRD method showed the peak range at 2θ = 33.50, 38, 41.35, 50.01, and 63 positions confirming the crystalline structure of nanoparticles.

Morphological Characterization of FeNPs by HR-SEM Analysis

The surface morphology of the iron nanoparticles was examined using HR-SEM and EDX. In Fig. 4 (A & B) and Fig. 5, the results of the synthesized nanoparticles were visible in HR-SEM images as cylindrical and rod-shaped spheres at a magnification of 2 µm, and 500 nm images were confirmed with clear morphology of synthesized FeNPs. Agglomeration in the SEM images of the synthesized nanoparticles might be due to electrostatic interaction between layers of nanoparticles' surface. In terms of EDAX analysis, the main components found in the sample were Fe (81.79 by weight) proved the expected iron oxide nanoparticles.

Molecular Identification of Selected Bacterial Strains

The study involved the isolation and selection of bacterial

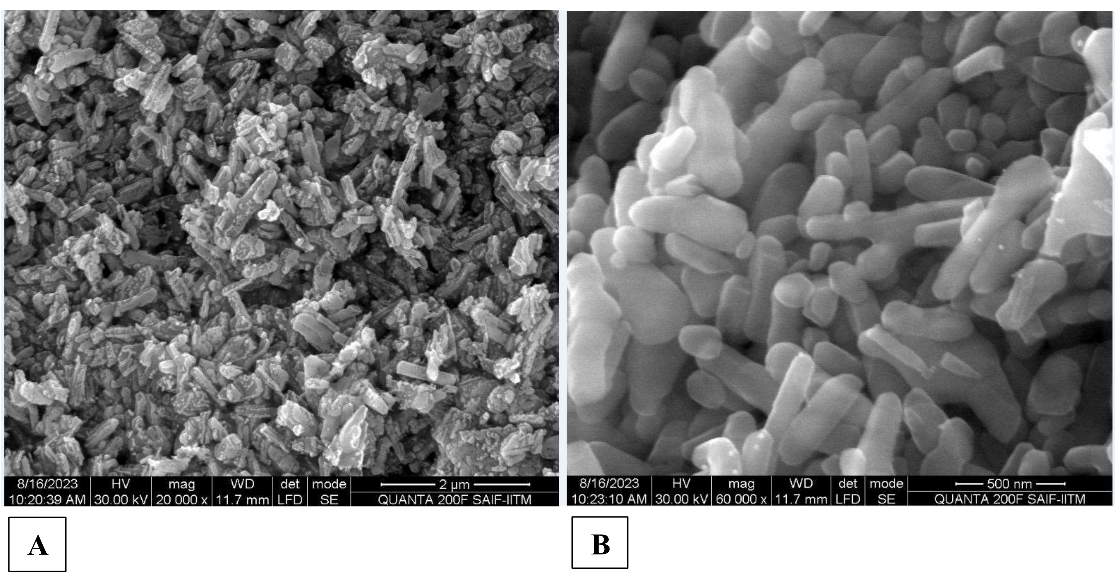
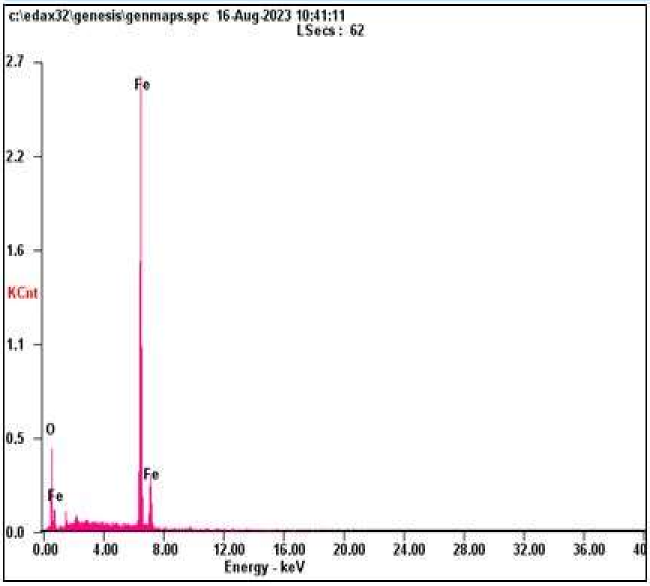


Fig. 4: HR-SEM images (2µm - A and 500nm - B magnification) of FeNPs.



Element	Wt%	At%
OK	18.21	43.73
FeK	81.79	56.27
Matrix	Correction	ZAF

Fig. 5: EDS data of FeNPs.

strains from a soil sample contaminated with crude oil. The collected soil sample was incubated in LBBH medium overnight at 37°C and 100 rpm. After serial dilutions and growth observations, four bacterial strains (SH 1-4) were isolated and maintained as pure cultures. These strains were identified as *Bacillus glycinifermentans* (MN399919.1), *Bacillus subtilis* subsp. *Stercoris* (MN704442.1), *Enterobacter cloacae* (MT367840.1), and *Enterobacter ludwigii* (CP018785.1). To confirm the identities of these strains, molecular-based techniques, specifically PCR amplification, were employed. The obtained 16S rRNA sequences were compared with those available in the database. Subsequently, the selected strains were used in degradation studies, focusing on their ability to utilize crude oil as the sole carbon source (Tian et al. 2018). This research describes improving the biodegradation process by using the consortium of isolated bacteria which confirms the degradation activity and the synthesized nanoparticles tend to break down the crude oil components into simpler forms to provide one of the nutrient sources for microbes. FeNPs are produced by the chemical co-precipitation technique, and their appropriateness and efficacy in promoting biodegradation processes are studied. The separated bacterial strains that are grown from soil samples polluted with crude oil are then mixed with these nanoparticles. Grown in a medium containing FeNPs, the consortium of bacterial strains identified by molecular techniques shows that they can break down crude oil.

GC-MS Analysis of Crude Oil Degradation Studies

Gas chromatography-mass spectrometry (GC-MS) analysis is used to assess the biodegradation process and determine how much crude oil has degraded. The utilization of bacterial consortia and FeNPs in this system presents a promising approach to crude oil pollution remediation, with the potential to yield more environmentally sustainable and effective remediation methods. When using chemically synthesized FeNPs in the consortium of isolated bacterial strains for the biodegradation of crude oil, the results are suitable based on characterization investigations, which demonstrate well-dispersed nanoparticles with the right size and stability. As a consortium, these strains were used to study the biodegradation of crude oil. In GC-MS analysis the group of four distinct bacterial strains were cultivated on LBBH medium with 2% crude oil to evaluate the biodegradation of crude oil. After that, the cultures were kept in an incubator that was shaking and running at 150 rpm for 7 days. To assess their effects and variation in crude oil biodegradation activity, FeNPs were introduced to the consortium in four different concentrations (10, 50, 100, 150 mg). By contrasting the group of organisms and FeNP-treated cultures with a control sample, the biological degradation effectiveness of both was validated using the gas chromatography-mass spectrometry used to assess the amount of biodegradation and detect any alterations in the hydrocarbon component composition of the degraded oil residues during incubation. This all-inclusive method made it possible to evaluate the bacterial consortium's capacity for biodegradation and the impact of FeNPs on crude oil clean-up in detail.

The complicated structure of hydrocarbon chemicals within the crude oil substrate was revealed by the gas chromatography-mass spectrometry (GC-MS) analysis of

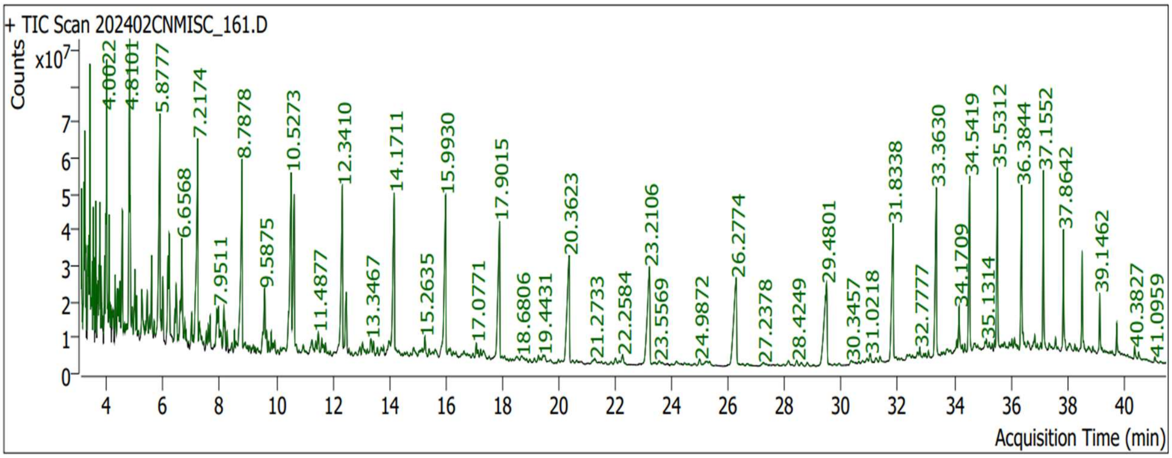


Fig. 6: GC-MS analysis - Control (crude oil).

the control sample (Fig. 6). The complicated character of the crude oil sample was reflected in the wide spectrum of hydrocarbon compounds that were found. This thorough evaluation offered a fundamental comprehension of the hydrocarbon profile found in the raw crude oil and was an essential point of reference for further investigations. The existence of several hydrocarbon compounds emphasized the difficulty of crude oil pollution and the need for efficient clean-up techniques. The control sample's outcomes provided the framework for assessing how well biodegradation processes reduce the pollution caused by crude oil.

When exposed to crude oil, the bacterial consortium showed moderate biodegradation capabilities without the presence of iron oxide nanoparticles (FeNPs). After research, it became clear that the consortium had started a degrading process that resulted in a noticeable drop in the amount of different hydrocarbon compounds (Fig. 7). The consortium's natural capacity to metabolize and degrade complex hydrocarbons was demonstrated by the mild biodegradation. The consortium's capacity as a natural agent for reducing pollution from crude oil is highlighted by this decrease in hydrocarbon compounds. To maximize the consortium's biodegradation efficiency, more studies may be needed to improve its efficacy in environmental clean-up initiatives. The consortium only includes a restricted amount of bacterial strains (SH 1-4), and this could not be diverse enough to break down every component of crude oil effectively. A microbial community with greater diversity may be able to target a broader variety of hydrocarbon molecules for breakdown.

A significant improvement in crude oil biological degradation was seen at the lowest concentration of 10mg of FeNPs, which outperformed the effectiveness of a control sample (Fig. 8). This result emphasizes how FeNPs, even at comparatively modest dosages, can help bioremediation processes. The effect showed that FeNPs had a beneficial

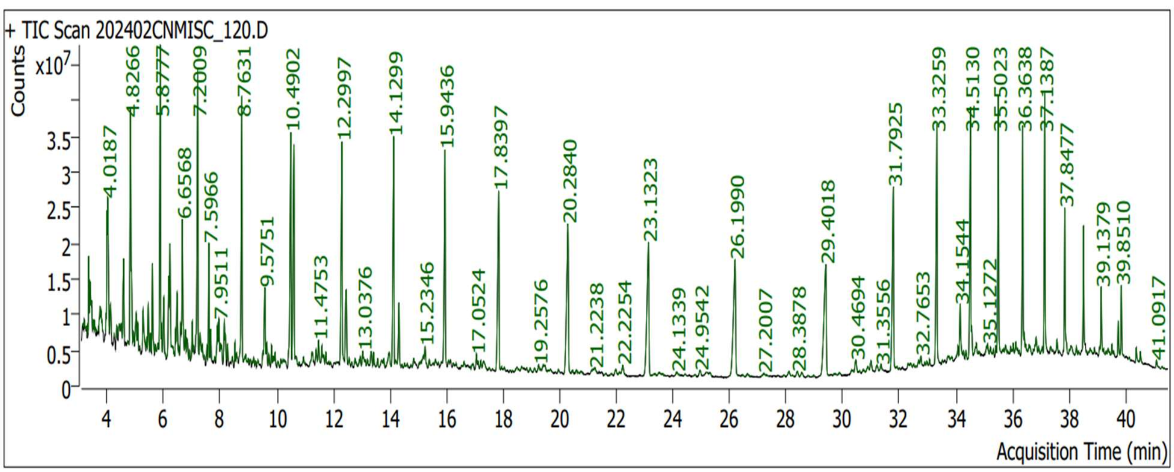


Fig. 7: GC-MS analysis– Consortium (SH 1-4).

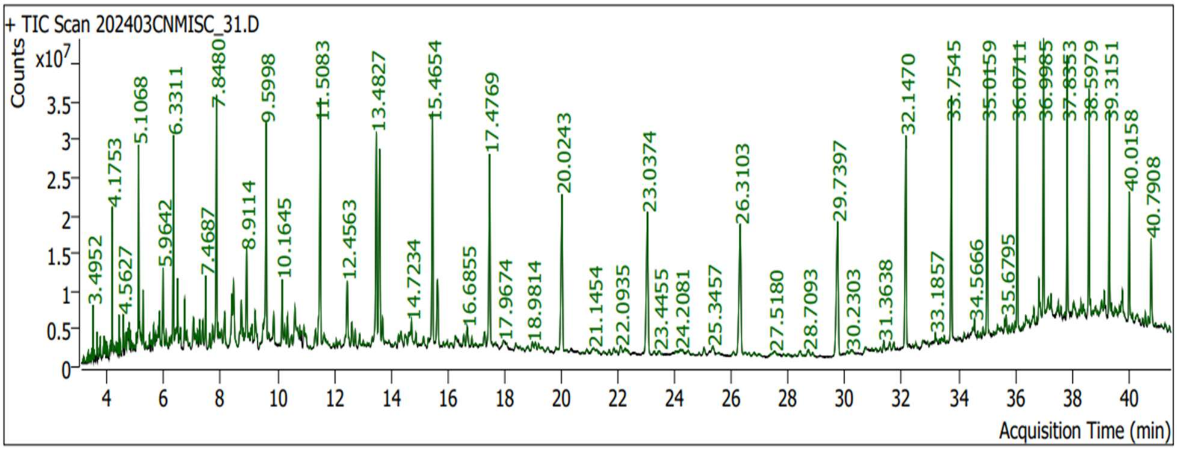


Fig. 8: GC-MS result-10mg concentration.

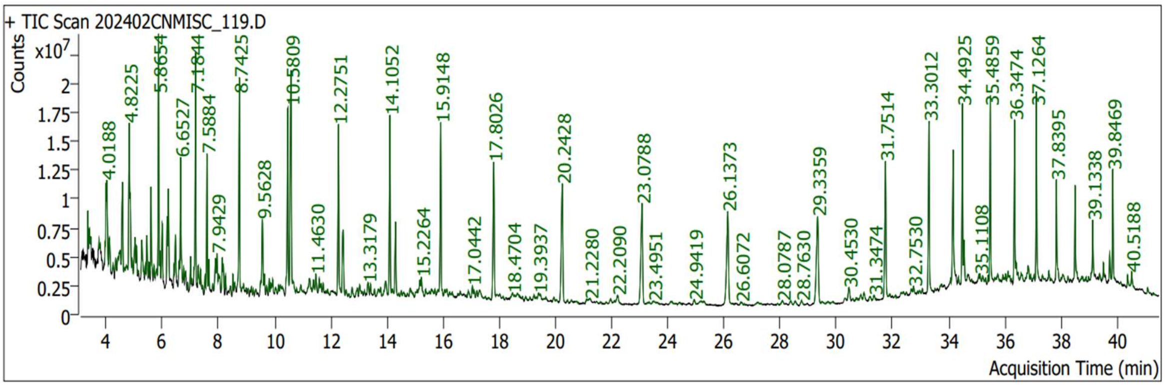


Fig. 9: GC-MS result – 50mg concentration.

effect on hastening the hydrocarbon breakdown of crude oil, even if it was less pronounced than at higher FeNPs concentrations. This study is noteworthy because it implies that FeNPs, even at low concentrations, can aid in environmental clean-up operations. It also emphasizes the versatility and possible application of FeNPs in remediation tactics targeted at treating crude oil contamination.

The biodegradation efficacy significantly improved as the concentration of FeNPs was raised to 50 mg, which is a significant improvement over lesser concentration (Fig. 9). The quantity of hydrocarbon compounds decreased noticeably as the concentration of FeNPs increased, demonstrating the critical role FeNPs play in quickening the degradation process. FeNPs may be able to improve the bacterial consortium's interaction with the crude oil substrate, leading to more effective hydrocarbon compound breakdown, as indicated by the increased efficacy at this concentration.

A much-improved capacity for crude oil clean-up was demonstrated by raising the FeNPs content to 100mg, which produced an even more noticeable biodegradation effect (Fig. 10). A more thorough clean-up of hydrocarbon compounds from the surroundings and increased rates of degradation were probably caused by higher levels of FeNPs, which also encouraged increased interaction and mutually beneficial outcomes that occurred between FeNPs and the bacterial consortia.

The biodegradation effect peaked at a remarkably high concentration of 150 mg of FeNPs, suggesting that FeNPs have the greatest potential for facilitating crude oil bioremediation (Fig. 11). The hydrocarbon compound abundance was most significantly reduced at this concentration, highlighting the important role that FeNPs play in enhancing the effectiveness and efficiency of the processes involved in the degradation of crude oil. These results imply that a higher number of FeNPs can result in more comprehensive and effective crude oil bioremediation procedures, and they also highlight the significance of optimizing the FeNPs amount for maximum elimination performance. Finally, the study's conclusions highlight the favorable relationship between rising iron

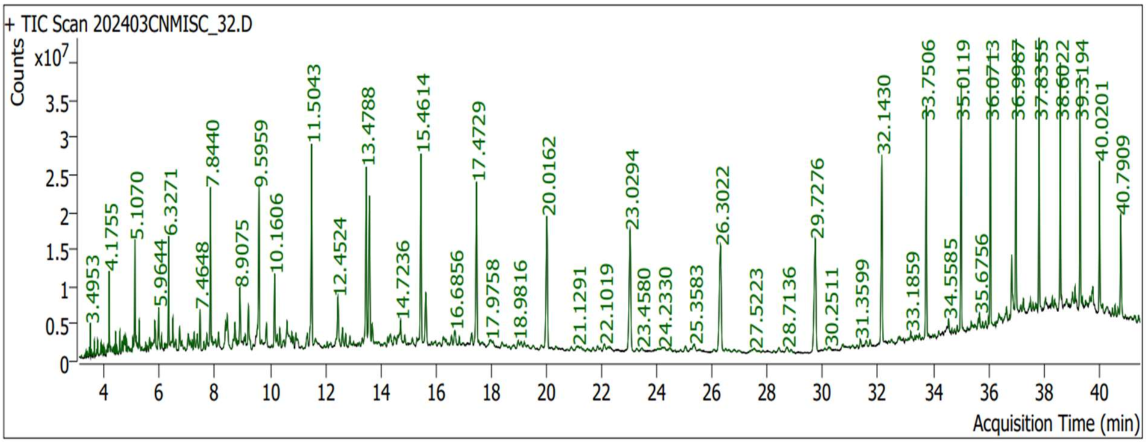


Fig. 10: GC-MS result – 100mg concentration.

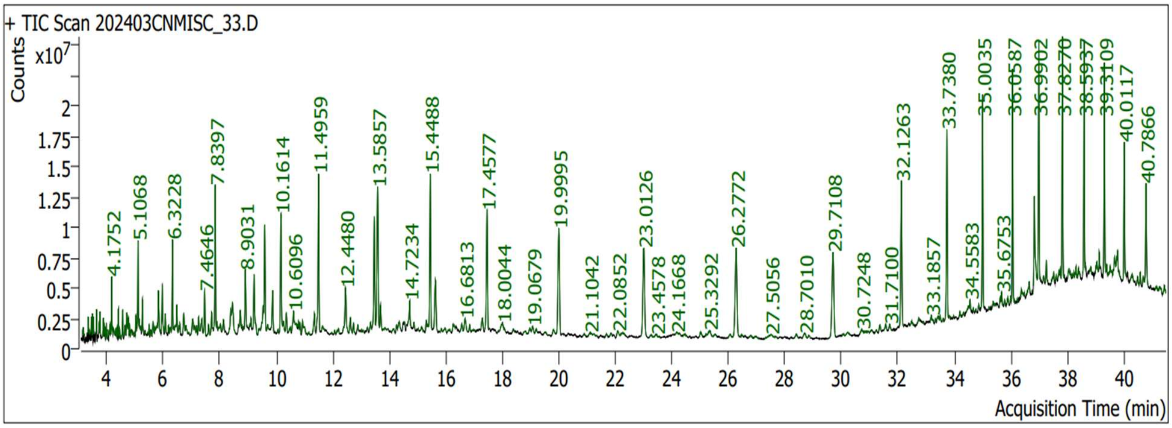


Fig. 11: GC-MS result – 150mg concentration.

oxide nanoparticle (FeNPs) concentrations and improved crude oil biodegradation efficiency. The results indicate that FeNPs play a crucial role in expediting bioremediation processes, as evidenced by the substantial decrease in hydrocarbon compounds observed at the highest dosage of 150 mg. FeNPs ability to support cleanup efforts was further validated by real evidence of crude oil chemical degradation obtained through gas chromatography-mass spectrometry research. These findings suggest that FeNPs may be a viable and long-term instrument for improving the effectiveness of crude oil cleaning operations. These findings have important ramifications for projects aimed at protecting the environment and restoring ecosystems.

DISCUSSION

This work analyses the use of isolated microorganisms in combination with synthetic iron oxide nanoparticles (FeNPs) as a possible remediation approach for the biodegradation of crude oil. The effectiveness of nanoparticles in accelerating the breakdown of crude oil has been found that the various types of nanoparticles have distinct properties that affect how well they work (Bertha et al. 2021). Enhancing reaction speeds also greatly depends on choosing the right microorganisms and nanoparticles. The ideal concentration of nanoparticles must be carefully determined, though, since too much can negatively impact microbial activity and perhaps slow down the breakdown process (Iwabuchi et al. 2002). The purpose of this study's iron oxide nanoparticle creation was to use bacterial isolates to accelerate the breakdown of crude oil. The propensity of iron oxide with bare surfaces to aggregate because of van der Waals forces and extremely energetic surfaces, however, presents a significant obstacle. The ability of the nanoparticles to promote biodegradation may be hampered by this aggregation. Although iron nanoparticles' size distribution, shape, and surface characteristics have been altered in an attempt to improve their biocompatibility, stability is still a major challenge. Iron nanoparticles' strong reactivity with oxidizing substances, especially when air is present, is a drawback to employing them. This reactivity may cause unintentional oxidation, which could reduce the nanoparticles' usefulness in bioremediation applications. Preparation procedures frequently need to be carried out in anaerobic environments to minimize oxidation and help address this problem. Creating efficient defense mechanisms, including appropriate polymer or biomolecule surface coatings, is also crucial for preserving the stability of nanoparticles and guaranteeing their long-term effectiveness in clean-up operations. Therefore, even though iron oxide nanoparticles have the potential for biodegrading crude oil, resolving issues with stability and reactivity is essential to maximizing their use in environmental remediation. In the work by Rizi et al. (2017), isolated bacterial colonies were cultivated in media containing varied concentrations of TiO_2, Fe_2O_3, and nanoparticles of Fe_3O_4 to examine the effect of nanoparticles on sludge from oil refinery biodegradation. Using GC-MS analysis, the maximum microbial growth was detected at a value of 1mg/ml of Fe_2O_3 and Fe_3O_4 nanoparticles. In contrast, the inclusion of Fe_3O_4 nanoparticles, particularly under light circumstances, resulted in a significant decrease in the amount of cyclosiloxane compound, suggesting improved biodegradation. Furthermore, even in dark settings, bacterial activity in conjunction with Fe_3O_4 nanoparticles led to the elimination of some compounds, whereas the presence of Fe_2O_3 nanoparticles demonstrated a decrease in some chemicals. Overall, the study shows that Fe_2O_3 and Fe_3O_4 nanoparticles have a beneficial effect on the biodegradation of oil sludge. In a further study, El-Sheshtawy & Ahmed (2017) used GC-MS analysis to find variations in total paraffins as samples of leftover crude oil from different microcosms biodegraded. The most efficient breakdown of total paraffins, especially those with long chains (C31–C46), was achieved by adding the biosurfactant containing 0.2g of Fe_2O_3. This finding indicates a new and significant trend in biodegradation, which is enhanced bacterial strain performance and total paraffin consumption when biosurfactant with Fe_2O_3 nanoparticles is present. Cao et al. (2021) produced magnetic nanoparticles adorned with bacteria to mitigate the effects of crude oil. The research focused on the biodegradation of low-molecular-weight chemicals, including aromatics and alkanes. According to GC-MS analysis, when combined with pure bacteria, magnetic nanoparticles improved the breakdown of lower molecular-weight aromatic compounds compared to when pure bacteria were used alone. Moreover, the recovery procedure made it easier to get rid of oil-bacteria clumps, which eliminated high-molecular-weight aromatic compounds. The study's conclusions highlight how magnetic nanoparticles can enhance the biodegradation of crude oil, especially for low-molecular-weight chemicals, potentially leading to improvements in remediation techniques.

CONCLUSIONS

According to the results of this study, the four bacteria species were isolated from the oil-contaminated site to check its crude oil biodegradation activity. Thus, these strains can deteriorate the long complex chains to short chain compounds and there was partial degradation of the complex compounds present in crude oil that was provided as the sole carbon source. The synthesized iron oxide nanoparticles showed heavy components reduction at 150 mg concentration than other used concentrations, which was better than the consortium sample when compared to the control. So, the synthesized iron oxide nanoparticles confirm its efficacy in degrading crude oil. Further, different concentrations of nanoparticles should be performed to analyze the degradation levels and to achieve the complete efficiency of crude oil in a consortium for bioremediation purposes.

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