Immobilization of Bacteria in Natural Fiber to Remediate the Crude Oil Contamination – A Laboratory Scale

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Abstract

A chronic environmental threat, crude oil contamination calls for creative and long-lasting remedial techniques. In order to improve bioremediation capacities on a laboratory scale, the present research investigates the effectiveness of immobilizing a group of hydrocarbon-degrading bacteria inside natural fibers. The bacteria isolated that may capable of breaking down crude oil are characterized and analyzed for degradation purposes. In-depth, the growth kinetics study was conducted for 30 hours shows better stability and growth of the isolated bacteria. To immobilize the strains the natural fiber were used as a bio-carrier to carry out the experiment. The adherence of consortium was confirmed through High-resolution Scanning Electron Microscopy (HR-SEM) analysis. In order to understand the efficiency and pathways of hydrocarbon degradation, Gas Chromatography Mass Spectroscopy (GC-MS) is used to examine the components which showed 31.70% degradation by the consortium. For extended periods, natural fibers maintain the sustainability of bacteria and metabolic activity by acting as a supporting matrix. The study emphasizes how this immobilization technique can increase bioremediation efficiency and is a viable way to reduce crude oil pollution in a variety of environmental settings. This study highlights the potential of natural fiber-immobilized consortia as an economical and environmentally friendly alternative that advances sustainable bioremediation technology.

Keywords: Biodegradation, Crude oil, Consortium, Immobilization, Natural fiber, GC-MS

1. Introduction

Crude oil leaks and spills are major global environmental hazards that have long-lasting consequences for both aquatic and terrestrial ecosystems. Conventional cleanup techniques, such as mechanical confinement and chemical dispersants, might increase environmental dangers while having a low efficacy rate. On the other hand, bioremediation provides a sustainable method by utilizing microorganism innate ability to break down and change dangerous contaminants into less dangerous forms (Neethu et al. 2019). Microbial consortiums have demonstrated potential as a bioremediation strategy to improve hydrocarbon breakdown efficiency. Comprising various bacterial species with compatible metabolic pathways, these consortia have the ability to breakdown complex hydrocarbon compounds found in crude oil in a synergistic manner. Researchers have investigated the immobility of these ensembles within natural fibers such as jute and coconut fiber in order to maximize their effectiveness (Partovinia et al. 2023). These fibers shield bacteria from external stresses and maintain metabolic activity for extended periods of time, creating an environment that is favorable for bacterial growth. The goal of this research is to examine, in a laboratory environment, the potential of bacterial consortia immobilized in natural fiber for the remediation of settings contaminated by crude oil. The first step in the study is to isolate and describe bacterial strains that have a reputation for successfully breaking down crude oil. In the studies that follow, the kinetics of growth of these bacteria will be examined, and their capacity for biodegradation within natural fibers will be assessed. The efficiency and mechanisms of hydrocarbon degradation are revealed by analyzing degradation products and routes using sophisticated analytical techniques like gas chromatography-mass spectrometry (Atakpa et al. 2023). This research is aimed at contributing to the creation of sustainable methods for environmental cleaning by expanding our knowledge of bacterial immobility inside natural fibers and improving bioremediation conditions. The results have implications for stepping up bioremediation initiatives, providing a possible way to lessen the negative effects of crude oil spills on the environment and successfully recover impacted ecosystems (Wang et al. 2023).

2. Objective

To isolate the microbes from oil contaminated soil sample and identified through PCR reaction and Sanger's sequencing techniques. The identified bacterial strains were utilized for crude oil degradation process. For the metabolic and degrading activity confirmation, growth kinetics of two chosen bacterial strains was analysed. In this micro scale study immobilization of bacteria in natural sisal fiber which acts as a Bio-carrier for microbes were performed to decrease the crude oil contamination. For this experimentation, immobilized sisal fiber was placed onto a 5-liter container with sea water and crude oil and maintained for 14 days of incubation and degradation process was performed in an open laboratory uncontrolled condition at room temperature.

3. Materials and Methods

3.1. Isolation and Identification of Bacteria

Bacterial colonies were identified as a consequence of the serial dilutions used in this study to isolate strains from the crude oil polluted soil sample collected from an oil refinery site, in Chennai, Tamil Nadu (13°10'36" N 80°16'25" E). Two strains were chosen at random from the collected colonies to correspond with their capacity to break down crude oil. Then, using a 100-based pair DNA marker, a 16S rRNA gene of each bacterium was amplified through PCR and sequenced using Sanger sequencing technique (Neethu et al. 2018). The biological identification of each strain was validated by phylogenetic tree construction and sequencing data analysis. A remarkable degree of similarity (99% - 100%) between the sequencing data and known genetic sequences was observed. This methodology guaranteed accurate identification of the bacterial isolates necessary for further bioremediation research in areas contaminated with crude oil.

3.2. Growth Kinetics of Isolated Bacteria

The examination of isolated bacterial strains were analyzed with its growth kinetics at 37°C and the growth curve was monitored for 2hrs until the time period up to 30 hours. The strains were separated and compared it as control with experimental sample (Lin et al. 2014). An experimental sample includes 2ml of crude oil, two isolated microbes cultured in LBBH medium, and control sample consist same set of microbes without crude oil. For thirty hours, the two samples are incubated at 37°C in shaking incubator at 120rpm. Every two hours, growth was observed in UV visible spectroscopy at absorbance of 600nm (OD600) is measured to create growth curves that show the dynamics of growth. Approximately 26 hours after the start of incubation, the experimental sample is anticipated to reach the stationary phase. Providing vital information for the isolated strains possible use in bioremediation procedures, this experiment attempts to evaluate the metabolic activity and growth properties of the strains in the absence of crude oil.

3.3. Biodegradation of Crude Oil Using Consortium

In this section of the research, we evaluated the crude oil degradation capabilities of a combination of two bacteria: *Bacillus glycinifermentans*, *Bacillus subtilis subsp. Stercoris*. The bacterial mix had been prepared, so we combined it with a mixture of seawater, crude oil, and LBBH, a nutritional medium (Mohammadpour et al. 2020). Then, in order to encourage the bacteria's growth and improve the oil's degradation, we used sisal fiber as a bio-carrier to withstand a supportive material for the micro scale experimentation. Two groups were established: the control group, which contained only sea water, crude oil, and LBBH, and the experimental group, which also included the bacterial consortium. For seven days, the two groups were kept at 37 °C in an incubator that shook at a speed of 150rpm. To track the degradation process, we examined the samples on a regular basis. We used gas chromatography-mass spectrometry, or GC, to examine the oil residues from both groups after 14 days to determine the amount of oil that had been broken down. This investigation gave specific details on the breakdown of products and the degree of crude oil decomposition that the bacterial consortium was able to accomplish (Yang et al. 2023). The efficiency of the bacterial mixture in degrading the crude oil was ascertained by contrasting the outcomes of both the control and group experiments. Understanding the effectiveness of using these bacteria to clean up spills of oil in the natural world depends on completing this step.

3.4. Immobilization of Isolated Bacteria in Natural Fiber for Biodegrading Activity

For micro scale set up experimentation, sisal fiber immobilized with the isolated consortium was placed onto a 5-liter container with sea water and crude oil. The arrangement was then maintained for 14 days for incubation and degradation process in an open lab at 37 °C. We monitored the amount of crude oil being broken down in the container on a regular basis during this time. By keeping those bacteria in one location for the duration of the two-week timeframe, using the fiber increases their efficiency in breaking down the oil. Better and more durable biodegradation result from this technique, which keeps the bacteria alive according to the growth curve studies performed, and prevents their removal. At the end of incubation time period, the biodegraded crude oil residues were analyzed using GC-MS studies (Lin et al. 2014; Yang et al. 2023).

3.5. Biodegradation analysis using GC-MS studies

We employed a technique known as gas chromatography-mass spectrometry (GC-MS) to investigate the changes in the chemical composition of the leftover oil following the breakdown of the crude oil by the bacterial consortium using natural fiber as a bio-carrier. We utilized the GC-MS machine's DB5, a unique column. In order for us to investigate the various chemicals in the oil, this column assisted in their separation. The gas of nitrogen was used to force the samples of oil through the GC-MS apparatus at a constant flow rate of one milliliter per minute throughout the study. In order to aid in the chemical separation process, the machine was gradually heated from 50°C to 280°C. To guarantee precise measurements, the machine's injector and detector were both maintained at 280°C. To ensure that the leftover crude oil was equally mixed, we mixed it with dichloromethane (DCM) before we could test the samples. Our GC-MS study produced trustworthy results thanks to this procedure (Kegere et al. 2023). The degree to which the bacteria degraded the crude oil was determined by comparing the diameters of the increases on the GC-MS graphs of the samples addressed by the bacterial community to those that weren't. We were able to comprehend the efficacy of the bacteria in cleaning up the oil spill thanks to this analysis, which provided comprehensive details about the various compounds present in the crude oil both before and after treatment.

4. Results

This study investigated a unique method of cleaning up crude oil contamination by immobilizing bacterial strains that are known to break down hydrocarbons using natural fiber. The goal of the experiment was to isolate and identify species of bacteria that have been shown to be capable of decomposing crude oil. Then, the effectiveness of these bacteria was tested when they were placed within sisal fiber. Two significant species, *Bacillus glycinifermentans* and *Bacillus subtilis subsp. stercoris*, were identified by the isolation and identification of bacterial strains from soil polluted with crude oil. Through the use of Sanger sequencing and PCR, these strains were successfully identified, indicating that they have the capacity to degrade the heavy crude oil. With growth kinetics **(Figure 1)** indicating that they entered a stationary phase after roughly 26 hours, the strains showed that they were able to grow on crude oil and sustained until the mentioned time period, demonstrating their successful adaptation to utilize it as a food. This growth kinetics was performed to analyze and determine the ability in growth of isolated microbes helps to adhere on the natural sisal fiber to undergo the micro scale experimentation purpose.

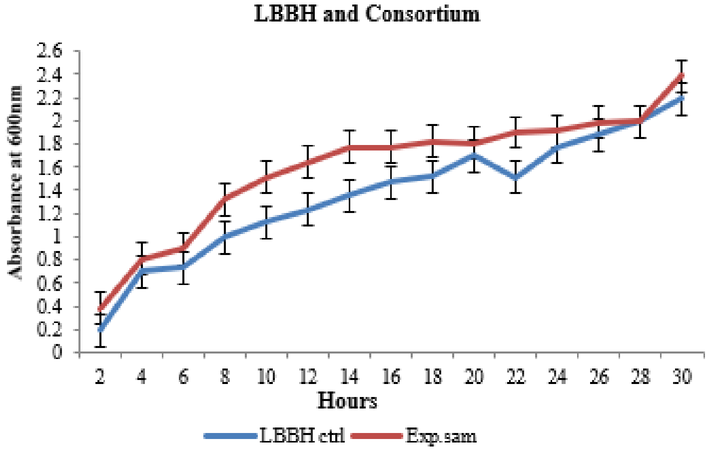
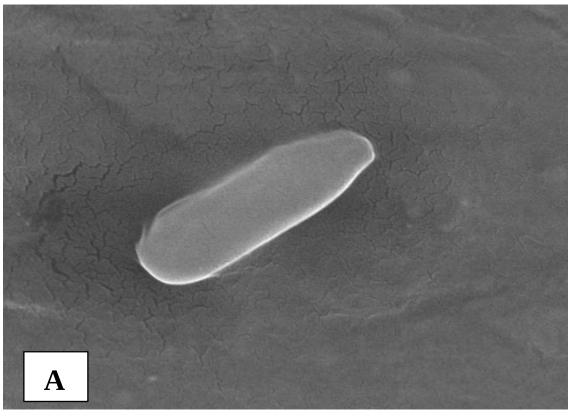


Figure: 1. Growth kinetics of isolated consortium (30 hours incubation)

The immobilization of *Bacillus glycinifermentans* and *Bacillus subtilis subsp. stercoris*, in sisal fibers **(Figure 2 A, B)** was confirmed by HR-SEM analysis showed the rod shaped bacterium was adhered onto the surface of the fiber resulted to process the crude oil degradation procedure.



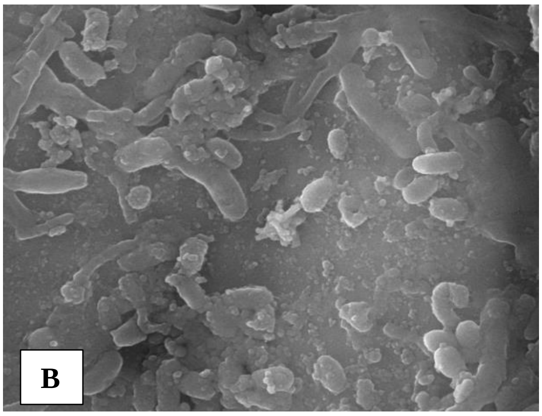


Figure: 2 (A) and (B) HRSEM images of consortium adhered on natural (sisal) fiber

The two isolated strains were chosen to investigate the biodegradation of crude oil immobilized in sisal fiber. After 14 days of incubation, when compared with the control sample containing crude oil, the degraded residues were analysed using GC-MS determination for the components absence and partial degradation performed in the micro scale experimentation. To ensure that the leftover crude oil was equally mixed, we mixed it with dichloromethane (DCM) before we could test the samples. The isolated bacteria degraded the crude oil was determined by comparing the control and experimental sample diameters on the GC-MS graph **(Figure 3 and 4)** of the samples addressed by the bacterial community. We were able to comprehend the efficacy of the bacteria which provided comprehensive details about the various compounds present in the crude oil both before and after treatment.

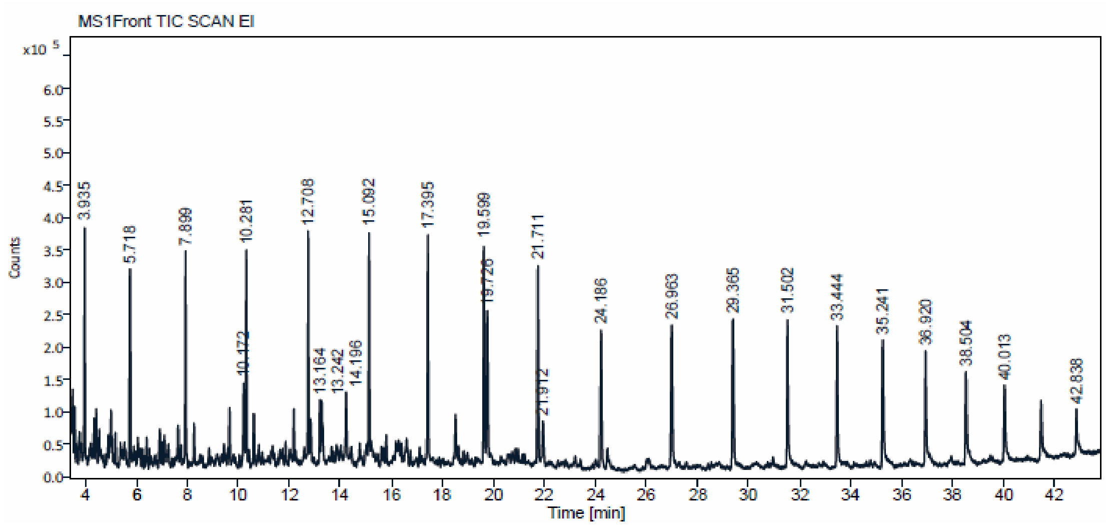


Figure: 3. GC-MS data (control)

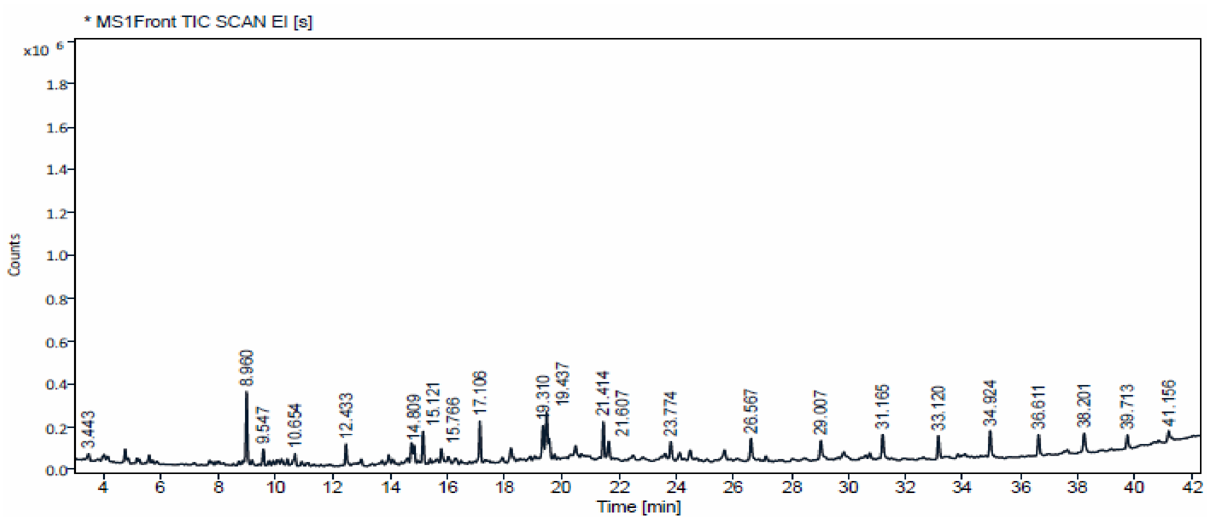


Figure: 4. GC-MS data (Experimental sample)

The control sample included untreated crude oil, and the GC-MS analysis revealed a variety of hydrocarbon molecules typical of the composition of crude oil. Important chemicals were Bicyclo (4.4.1), Undeca-1,3,5,7,9-pentaene, Undecane, Dodecane, and Decane, Tetradecane, Pentadecane, and 2,7-dimethyl-naphthalene. The complexity of the crude oil sample prior to treatment was confirmed by its abundance of high-molecular-weight hydrocarbons such as Tricosane and Eicosane. In comparison, there were noticeable alterations in the hydrocarbon profile in the experimental sample that was treated with the bacterial consortia immobilized in sisal fiber. Numerous substances found in the control had either undergone reduction or transformation. Decane, for example, was substituted with Octadecane, 6-methyl, and Undecane with 1,3-bis(1,1-dimethylethyl) - benzene. The appearance of new chemicals, including hexadecane thiol and hexadecane-9-hexyl, showed that biodegradation processes were in motion. For tetradecane 2,6,10-trimethyl, the experimental material likewise showed several peaks, indicating intermediate breakdown. The bacterial consortium ability to break down crude oil effectively and produce simpler molecules highlights the oil potential for environmental remediation. This is evidenced by the decreased amount of high-molecular-weight hydrocarbons. The description **(Table 1)** of degraded crude oil compounds were discussed in table of components present in both control and experimental sample of micro scale procedure.

Table 1: GC-MS result: The crude oil compounds present in control were degraded by the consortium immobilized in natural fiber.

S.No	Retention time (Control)	Compound name (Control)	Retention time (experimental sample)	Compound name (experimental name)
1	3.935	Decane	3.443	Octadecane,6-methyl
2	5.718	Undecane	8.960	Benzene, 1,3-bis(1,1-dimethylethyl)-
3	7.899	Dodecane	9.547	Pentadecane
4	10.172	Bicyclo(4.4.1)undeca – 1,3,5,7,9 pentaene	10.654	Tetradecane 2,6,10- trimethyl
5	10.281	Pentadecane	12.433	Tetradecane
6	12.708	Tetradecane	14.809	Pentadecane
7	13.164	Naphthalene, 2,7 – dimethyl-	15.121	2,4-di-tert-butyl phenol
8	13.242	Naphthalene, 2,7 – dimethyl-	15.766	Tetradecane 2,6,10- trimethyl
9	14.196	Tetradecane 2,6,10- trimethyl	17.106	Hexadecane
10	15.092	Pentadecane	19.310	Heptadecane
11	17.395	Hexadecane	19.437	Tetradecane 2,6,10- trimethyl
12	19.599	Heptadecane	21.414	Tetradecane 2,6,10- trimethyl
13	19.726	Hexadecane 2,6,10- trimethyl	21.607	Tetradecane 2,6,10- trimethyl
14	21.711	Octadecane	23.774	Tetradecane 2,6,10- trimethyl
15	21.912	Tetradecane 2,6,10- trimethyl	26.567	Tert-hexadecane thiol
16	24.186	Nonadecane	29.007	Tetradecane 2,6,10- trimethyl
17	26.963	Eicosane	31.165	Heptadecane-9-hexyl
18	29.365	Heneicosane	33.120	Heptadecane-9-hexyl
19	31.502	Docosane	34.924	Octadecane 3,ethyl-5-(2-ethylbutyl)-
20	33.444	Tricosane	36.611	Octadecane 3,ethyl-5-(2-ethylbutyl)-
21	35.241	Tetracosane	38.201	Octadecane 3,ethyl-5-(2-ethylbutyl)-
22	36.920	Pentacosane	39.713	Octadecane 3,ethyl-5-(2-ethylbutyl)-
23	38.504	Pentacosane	41.156	Octadecane 3,ethyl-5-(2-ethylbutyl)-
24	40.013	Octadecane 3,ethyl-5-(2-ethylbutyl)-		

25	42.838	Ethanol, 2-(octadecyloxy)		

The efficiency of crude oil hydrocarbon degradation by the immobilized isolated bacterial cells adhered in sisal fiber attained 31.70% degradation maintained for 14 days of incubation time period. The degradation may increase to high percentage relies and proportional to incubation of bacteria leads to well dissipation and degradation of crude oil components. GC-MS analysis demonstrated that the bacterial consortium was successful in breaking down crude oil in the bioremediation studies. High-molecular-weight hydrocarbons were significantly reduced after 14 days of treatment, and simpler, less hazardous chemicals were produced in their place.

5. Discussion

The outcomes showed that during the course of the experiment, the immobilized bacterial consortia showed strong biological degradation of crude oil components. A significant drop in hydrocarbon concentrations was verified by GC-MS analysis, demonstrating the immobilized bacteria's efficacious activity (Ferreira et al. 2023). Further research could focus on enhancing the immobilization procedure to further improve bacterial performance and examining the long-term ecological impacts of natural fiber based bioremediation techniques. The study concludes that immobilizing bacteria for crude oil biodegradation using natural fiber is both feasible and effective. This approach is a potential development in environmental research that will open up new possibilities for improving environmentally friendly methods of environmental clean-up and reducing the negative ecological effects of oil spills (Sozina et al. 2023). Bacterial immobilization and adherence in sisal fiber provides a better connect in between given nutrient supplements and the strains. The capacity of adsorption and strength of binding are the two considerations for the supporting material (Hsu and Lo. 2003). In this study, the utilization of sisal fiber provided the isolated bacteria a steady support, which improved their capacity to break down crude oil. By keeping the bacteria in constant contact with the oil and preventing them from being rinsed away, this method increased the bioremediation process life expectancy and efficiency (Hossein et al. 2020). The potential of *Bacillus glycinifermentans* and *Bacillus subtilis subsp. stercoris* as efficient agents for crude oil bioremediation has been validated by their successful isolation and identification. These strains showed significant growth when crude oil was present, suggesting that they may use it as a source of carbon (Neethu et al. 2019). By giving the bacterial consortia on sisal fiber a robust support structure, immobilization increased the effectiveness of their degradation and allowed for greater oil breakdown and prolonged microbial activity. The combined effects of natural fiber immobilization and bacterial consortia demonstrate the efficacy of this approach in repairing crude oil spills, and it presents a viable method for large-scale environmental restoration initiatives. The possibility of employing such techniques to treat oil pollution in contaminated locations is highlighted by this study (Lin et al. 2014).

Conclusion

The degrading abilities of the bacterial strains *Bacillus glycinifermentans* and *Bacillus subtilis subsp. stercoris* in crude oil were successfully demonstrated by the investigation. By creating a steady setting for ongoing microbial activity, immobilizing these bacteria on the sisal fiber greatly increased the effectiveness of their destruction. It was verified by GC-MS analysis that complex hydrocarbons may be efficiently broken down into simpler molecules. The findings demonstrate the potential for effective oil spill bioremediation through the use of bacterial consortia and natural fiber supports. This strategy presents a viable way to deal with crude oil pollution in the environment.

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Data Availability Statement The authors confirm that the data supporting the findings of this study are available within the article.

Disclosure Statement The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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