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Degradation of crude oil using the indigenous isolate *Bacillus* sp SEA18

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Hydrocarbon contamination in the environment today is gaining more importance as they are carcinogenic and neurotoxic. Methods to degrade these hydrocarbons are rightfully demanding and researchers are on a lookout for new and indigenous species as they are sustained in that niche by utilizing the resources available. Because of the capabilities exhibited by bacteria in environmental remediation, this study, focuses on isolating an indigenous bacterium from oil-contaminated site and evaluate its potential in degrading oils. The isolate obtained was identified as *Bacillus sp* and was found to show the degradation of crude oil to an extent of 80% after 60 days of incubation. The analysis was confirmed by GC-MS analysis that showed a significant reduction in the number of hydrocarbons. This capability of this bacterium to produce biosurfactants promises this species to play a role in degradation as biosurfactants would enhance the degradation process. This study, therefore, reinforces the fact that indigenous species are potential hydrocarbon degraders due to their adaptability and endurance.

Keywords: *Bacillus* sp, GC-MS, Gravimetric analysis, Hydrocarbons

Oil contamination of water has been identified as one of the most upsetting pollution sources. This kind of wastewater is widely produced from a variety of sources such as crude oil production, automotive garage, oil refinery, petrochemical industry, metal processing, lubricant, and car washing. They are the major contributors to environmental problems, especially in soil and water. Both the waste and unused compounds from the prescribed sources are grouped as oily waste which is difficult to treat or recycle. Petroleum contaminated soil contains various hazardous materials such as aromatic hydrocarbons and polycyclic aromatic hydrocarbons and they are potentially toxic, mutagenic, and carcinogenic.

Crude oil an important pollutant contains a mixture of low to high molecular weight hydrocarbons including alkanes, aromatics, asphaltenes and resins in composites[1]. Remediation is possible through physical and chemical methods but still bioremediation remains a valuable option as it is eco-friendly, more efficient and cost effective[2,3].

Microorganisms as biodegraders of crude oil were accounted over a century ago. Exploiting the biological agents, *i.e.*, fungi, bacteria, or algae, to remove the hydrocarbons is bioremediation. The most crucial factor influencing the process is the hydrophobic nature of hydrocarbons and it is well documented that native species are more effective than the non-native microbes as the former produces metabolites that can solubilize and readily convert the hydrocarbons since they are adapted[4]. Consortium of native microbes proved to be effective in removing contaminants in a short span of time[5].

Bacteria is shown to display adaptability to the environment they dwell in and the ability to utilize carbon compounds such as oils as a source of energy with their characteristic enzyme system compared to that of other groups of microbes[6]. Of late many species of bacteria are reported that showed potential to degrade petroleum hydrocarbons which include *Marinobacter*, *Achromobacter*, *Acinetobacter*, *Mycobacterium*, *Pseudomonas*, *Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Rhodococcus Dietzia*, *Enterobacter*, *Kocuria*, *Pandoraea*, *Staphylococcus*, *Streptobacillus*, and *Streptococcus*[7-14]. It is reported that around 79 bacterial genera are adept in hydrocarbon degradation[15].

At this juncture, taking a cue from the potential displayed by bacteria, an attempt has been made to isolate bacteria from oil-contaminated soil and appraise its ability to degrade hydrocarbons.

Materials and methods

Isolation of bacteria

Isolation of bacteria from oil-contaminated soil was performed by serial dilution followed by culturing in

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solid nutrient agar[16]. One gram of soil was dissolved in 9 mL of distilled water and agitated. Different aqueous dilutions 10^{-1}, 10^{-2}…10^{-10} of the suspension were prepared and 10^{-10} dilution was applied onto nutrient agar plates and incubated at 37°C for 24 h. Single colonies were isolated and subcultured and the pure isolate obtained was preserved on nutrient agar slants.

Screening for the oil-degrading capacity

The ability of the bacteria to degrade oil was determined by overlaying Bushnell Haas (BH) agar with 0.1 mL oil and inoculating bacteria onto the agar. The plates were incubated at 37°C for 1 week to 15 days in an incubator. The ability of the isolated bacteria in degrading the oil was displayed by the zone of clearance obtained.

Screening for the production of biosurfactant

Biosurfactant production by the isolate was checked as this helps in the emulsification of oils thereby facilitating the degradation process. A drop collapse test was performed initially followed by determining the emulsification index. The emulsification index (E24) of the biosurfactant was determined by adding 2 mL of petroleum hydrocarbon (used engine oil) individually to the same amount of cell- free culture broth followed by vortexing for 2 min. This mixture was allowed to stand for 24 h at room temperature. The E24 index was determined by the formula[17].

$$\text{E24 index (\%)} = \frac{\text{Height of emulsified layer (mm)}}{\text{Total height of the liquid column (mm)}} \times 100$$

The isolate was inoculated in MSM medium supplemented with 2% (*v/v*) of the most preferred hydrocarbon supplement *i.e.*, engine oil and incubated. After incubation, the culture was centrifuged at 10000 g for 15 min at 4°C to separate the biomass. Crude biosurfactant was precipitated by adding three volumes of chilled acetone to the cell- free supernatant, maintained at 4°C with vigorous stirring for 10 h on a magnetic stirrer[17]. The crude biosurfactant was recovered by separating the precipitate under 10000 g for 10 min followed by air-drying and the amount of biosurfactant was quantified.

Identification of the bacteria

Identification of bacteria was initially done by carrying out biochemical tests, followed by 16S rDNA sequencing. The former represents the preliminary identification like the bacterial genus and the latter gives complete information including its phylogenetic relationship.

Oil degradation assay

The gravimetric analysis method was used to study oil degradation. Mineral salts medium (MSM)[18] was prepared by dissolving 1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.01 g $FeSO_4.7H_2O$ in 1 L of distilled water to which 1.0% (v/v) crude oil was added which acts as sole carbon source. After the incubation period, the flasks were taken out and the reaction was terminated by adding 1% 1N-HCl. The oil was extracted using 20 mL petroleum ether: acetone (1:1) and 50 mL culture broth in a separating funnel and was shaken vigorously to get a single emulsified layer. Acetone was then added to it and shaken gently to break the emulsification, which resulted in three layers. The layer on the top was a mixture of petroleum ether, oil, and acetone while the middle layer is made of clumped cells. The bottom aqueous layer contains acetone, water, and biosurfactant insoluble form. The top layer was taken in a preweighed clean beaker. The moisture content was removed by using anhydrous sodium sulphate and the solvents were evaporated on a water bath. The residual oil left after biodegradation was found by weighing the quantity of oil in the beaker. The percentage of oil degradation was calculated as follows:

$$\text{Weight of Residual oil} = \frac{\text{Weight of beaker containing extracted oil}}{\text{Weight of empty beaker}}$$

$$\text{Amount of crude oil degraded} = \text{Weight of oil added} - \text{Weight of residual oil}$$

$$\text{\% degradation} = \frac{\text{Amount of oil degraded in the media}}{\text{Oil added in media}} \times 100$$

Optimization of pH and temperature

Isolated bacteria was aseptically transferred into 100 freshly prepared MSM supplemented with 1% hydrocarbon supplement (used engine oil) taken in four different Erlenmeyer flasks. Appropriate blanks were maintained under similar conditions. Different pH (3, 5, 7, 9 and 11) and temperatures (20, 30, 40, 50, and 60°C) were maintained and periodic evaluation of growth and degradation efficiency was calculated[19].

Analysis of degraded compounds using GC-MS analysis

The products of oil degradation were analyzed after 24 days, and 60 days along with the 0th day using Agilent GC-MSD (6890N-5973) with pole temperature kept at 80°C for 4 min, then increased at a rate of 5°C·min^{-1} to 250°C and maintained at 250°C for 20 min.

Results and Discussion

Microorganisms are one class of assorted species that are capable of consuming contaminants as energy and carbon source for their survival. This process simultaneously enables the riddance of a varied range of chemical compounds that are pollutants from the environment thereby maintaining a healthy ecosystem. The inherent catabolic activity of the microorganisms facilitates this process of converting harmful chemicals into less toxic and harmless substances through redox reactions[20]. The recent past has witnessed so much pollution in terms of oil contamination in water and soil disturbing the natural ecosystem balance by being a threat to the very survival of different species. In this context, the present investigation is carried out to isolate such bacteria that can degrade oils from oil- contaminated soil and evaluate its efficiency in oil degradation.

Isolation of bacteria from oil-contaminated soil

One bacterium was isolated from the crude oil contaminated soil at 10^{-10} dilution using nutrient agar medium. Based on the staining technique, it was identified to be rod-shaped and gram-positive bacteria. This isolate was identified to be *Bacillus* and named as Isolate SEA18 (Fig 2A).

Screening for the ability of bacteria to degrade oil

The bacterial isolate SEA 18 was grown in Bushnell Haas agar containing engine oil as a sole carbon source and was incubated. After 48 h, growth was observed along with a clear zone in the particular area where it grew indicating the ability of the bacteria to utilize crude oil as a carbon source. Many species apart from *Bacillus* are known to be degraders of hydrocarbons like *Lactobacter, Arthrobacter, Pseudomonas, Micrococcus, Zoopage,* and *Articulosporium* but *Bacillus* sp was found to show higher efficiency in degrading oils which may be due to the ability of these organisms to produce spores that would protect them from the toxicity of hydrocarbons[21] and high level of endurance[22] while the presence of oil compounds may impede the ability of biodegrading and environmental-friendly microorganisms to grow and reproduce in polluted soils[23]. Some studies have shown that the indigenous microorganisms which have been separated from oil-polluted soils, due to their adaptability with the environment, have a greater role in the biodegradation of oil-related pollutions which sometimes may be a rapid process[24,25]. A study by Oyetibo *et al*.[26] reported that two strains of *Bacilli* achieved more than 94% and 85% degradation of crude oil and phenanthrene, respectively. Two species of *Bacillus, B. thuringiensis B3* and *B. cereus* B6 were found to bring about 84% and 28% of total petroleum hydrocarbons from crude oil and spent lubricating oil-polluted soils, respectively[27]. Many of the hydrocarbons in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment[28,29]. Therefore harnessing such indigenous bacteria would be a wise option for the efficient degradation of hydrocarbons.

Screening for biosurfactant production

The utilization of hydrocarbons by microorganisms through the process of oxidation is hurdled by the solubility rate. Biosurfactant production by bacteria is a phenomenon that alters the surface properties and increases the rate of biodegradation by enhancing the solubility by emulsification. Bacteria that play a role in oil degradation release extracellular biosurfactant that aid the uptake of oil and helps in degradation by emulsifying the hydrocarbons and making them more accessible for the microorganisms. They being non-toxic, non-hazardous, biodegradable and environmentally friendly compounds[30] make their production beneficial for the degradation process.

In the present study, the ability of the bacterial isolate *Bacillus* sp *SEA* 18 to produce biosurfactant was confirmed by screening methods and identified as a biosurfactant producer. In particular, the strain used in this study gave positive results for all biosurfactant screening methods. Specifically, drops collapsed within 30 sec indicating a higher amount of the biosurfactant present in the solution. The emulsification index was recorded as 68% by screening (Fig. 1). A study by Parthipan *et al*.[31] successfully demonstrated the ability of *Bacillus subtilis* A1 to produce biosurfactant and degrade hydrocarbons efficiently to an extent of 87%.

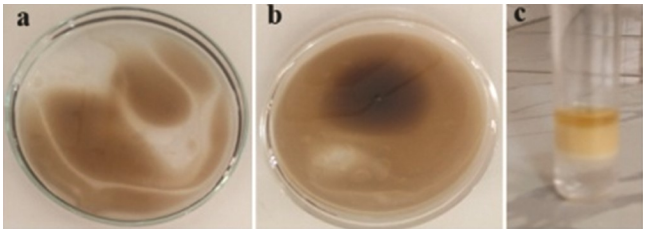

Fig. 1 — Biosurfactant production by drop collapse assay (A) Isolate; (B) 0^{th} day; and (C) Emulsification index

Table 1 — Biochemical tests for preliminary identification

S. No	Test	Result
1	Colony morphology	Round
2	Colony colour	White
3	Shape	Rod- shaped
4	Gram staining	Gram- positive
5	Catalase test	positive
6	Simmon citrate test	Negative
7	Acetate test	Negative
8	Motility	Negative
9	Urease test	Positive
10	MR-VP broth	Positive
11	Starch	Negative

Identification of the bacterium

The preliminary identification of the isolate was carried out by biochemical tests and the results were provided in (Table 1). The strain exhibited positive results for Gram's staining, motility, Voges Proskauer, catalase, and urease tests. Negative results were noted for Simmon citrate and Acetate test. The results of biochemical tests reveal that the bacterium could be a *Bacillus* sp. Further identification of the bacteria was carried out by 16S rDNA sequencing.

Species- specific signatures are provided in the hypervariable regions of 16S rDNA gene sequences of bacteria. Hence studying them has become more central in identifying the species as it is rapid and accurate compared to the phenotypic identification methods[32]. The bacterial DNA was isolated and the 16S rDNA sequence was amplified and sequenced and has been deposited in GENBANK with the accession number MH000669. The 16S rDNA sequence of the isolate SEA 18 obtained was compared with the non-redundant BLAST database to obtain the sequences that displayed maximum similarity. All the sequences reported by BLAST revealed that the bacterial species showed a very high percentage of similarity (100%) with the sequences of *Bacillus* sp with a reasonably high score and e-value being zero. The sequences showing the maximum similarity were used to arrive at the phylogenetic relationship represented by a phylogenetic tree (Fig. 2B) showing the evolutionary relationship that was constructed from the alignment using the neighbor-joining algorithm.

There exists a clear evolutionary relationship between all the 16S rDNA sequences as this is a highly conserved sequence. The tree derived by distance- based, the neighbor joining method is an unrooted tree inferring that the sequences do not come

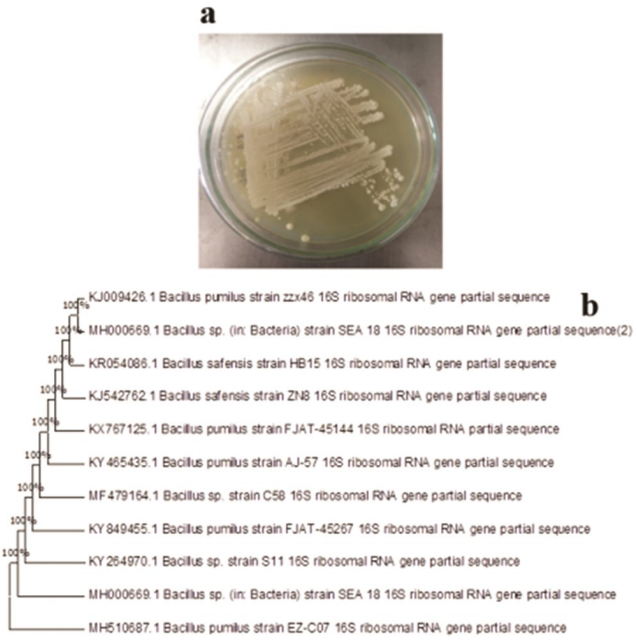

Fig. 2 — (A) Bacterial Isolate SEA 18; and (B) Phylogenetic analysis of Isolate SEA 18 identified as *Bacillus* sp

from a common ancestor. But they exhibit cladistic relationships which could be due to the similarities within the sequences. All the sequences under comparison belong to the genera *Bacillus*.

The occurrence of *Bacillus* sp in oil-contaminated soil is well documented by various studies[31,33,34]. Calvo *et al*.[35] reported the growth of *Bacillus pumilus* with 0.1% crude oil as carbon source along with naphthalene and was also found to be efficient in producing biosurfactant and therefore can be a potential candidate in remedial technologies. *Bacillus subtilis* is another species isolated by Tabari and Tabari[36] showed potential to degrade aliphatic alkanes. A study by Ubani *et al.*[37] testified that soil contaminated with oil is found to have bacteria from different genera namely *Bacillus, Arthrobacter, Staphylococcus, Brevibacterium, Variovorax, Paeni Bacillus, Ralstonia and Geo Bacillus* of which *Bacillus* sp was found to be the most predominant species in oil degradation.

Oil degradation studies by gravimetric analysis

Experiments were performed to study the biodegradation of crude oil by bacterium *Bacillus* sp SEA 18 and oil without the bacterium was used as control. The utilization of the hydrocarbons increased in cell densities that was evident by the turbidity of the medium and the degradation was initially observed visually wherein there is a gradual reduction

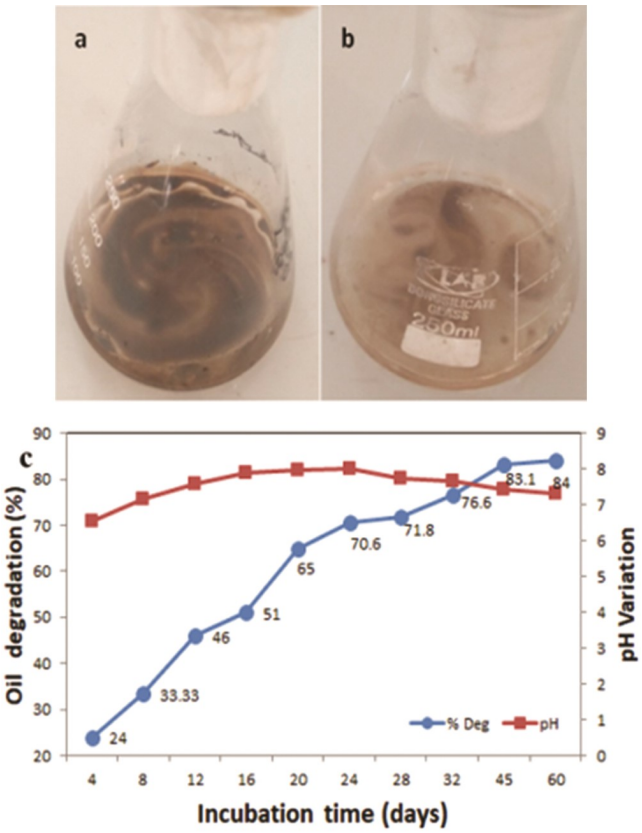

Fig 3 — Oil degradation by the isolate *Bacillus* SEA 18 (A) 0th day; (B) 60th day; and (C) Time Course of degradation

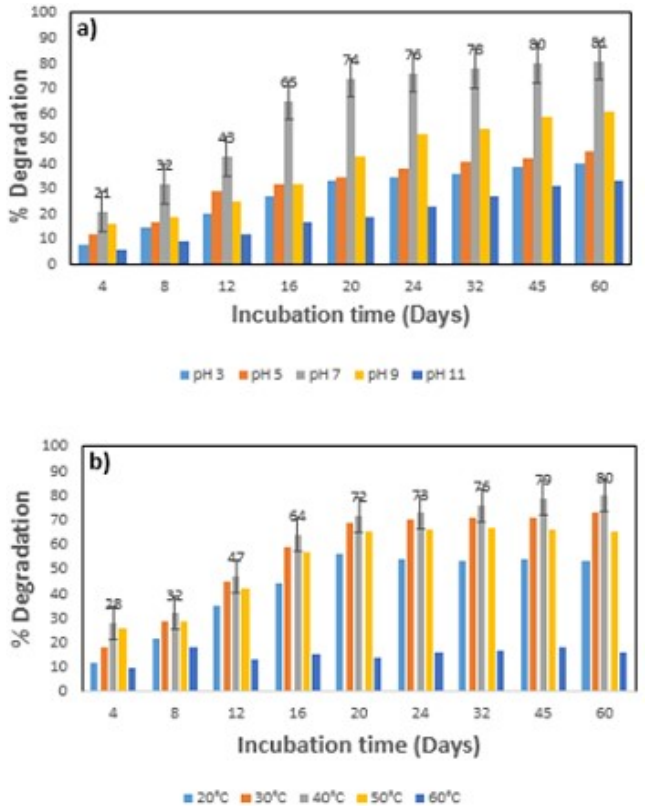

Fig 4 — (A) Optimization of pH; and (B) Optimization of Temperature

in the oil layer with complete disappearance of the oil upon prolonged incubation while there was no change in color in the control. The rate of oil degradation was determined *via* the gravimetric method, and the results indicated that 24% of the oil was degraded during the first four days, with the maximum rate of degradation of ~80% on Day 60 (Fig. 3). The degradation of oil by the bacteria was found to be a slow process and has taken 60 days to achieve 80% degradation.

The rate of crude oil degradation by the microorganisms depends on its ability to utilize hydrocarbon as a carbon source and varies from organism to organism. *Bacillus subtilis* A1 was found to degrade crude oil upto 87% along with the production of biosurfactant and enzymes namely alkane hydroxylase, and alcohol dehydrogenase[31]. Another study reported the efficiency of *Bacillus* sp along with *Pseudomonas* in degrading oils to an extent of 85%[38].

Simultaneously, the pH of the culture medium was also checked and it was observed that the pH of the medium initially was 6.55 which was slightly acidic. But as the bacterium grew, the pH remained close to neutral within the range of 7.74 – 7.65 as shown in (Fig. 3C) thereby suggesting that the bacteria preferred neutral pH for its growth as well as its activity. Tabari and Tabari[36] also reported that *Bacillus* sp grew well at pH>5.

Optimization of pH and temperature

The bacterial growth is generally influenced by the physical parameters like pH and temperature that is reflected in its growth and bioactivity. During the process of bacterial growth there may be a variation in the pH of the medium due to the assimilation of nutrients and accumulation of by products which would influence the growth of the bacteria positively or negatively. Hence, the pH of the medium has to be optimal for the growth of the bacteria. In the present study, different pH was maintained (3, 5, 7 and 9 and 11) and the results are given in (Fig. 4A).

It was observed that the growth of the bacteria was maximum at neutral pH compared to the acidic and basic conditions as reflected in the OD values. Correspondingly the degradation (74%) was also effective in neutral pH. This indicates that the bacteria

prefer a pH of 7 for its growth and bioactivity. These results were well supported by our previous experiment where it was observed that the pH during the course of oil degradation did not vary much and remained close to neutral. These results were well corroborated by the reports that state that *Bacillus* sp showed maximum growth at pH 7[39].

Whang *et al.*[40] suggested that the growth of bacteria and degradation of diesel was significant at pH 7.2 whereas any fluctuation in the pH towards acidic or basic showed a decrease in the growth as well as degradation. Similarly, degradation studies using a statistical approach like response surface methodology have shown that pH 7.4 was ideal for the degradation of [41]. Even the P*seudomonas* sp. strain F4 showed a preference for neutral pH for efficient degradation[42]. Interestingly even a bacterial consortium was capable of degrading oils at neutral pH[43]. It is generally stated that fat, oil, and grease degrading organisms have optimum growth between pH 5.5 and 8.0 with a maximum at 7.5[44].

Temperature considerably influences the growth of the microbes and their metabolism. Low to very low temperatures effect the cell membrane fluidity and the cells are damaged by ice crystal formation. Also, the diffusion slows down and the proteins become rigid to catalyze reactions. On the other extreme, high temperatures denature the proteins and nucleic acids[45]. Hence for the proper functioning of the microbes, the temperature has to be maintained at the optimum level. Therefore, in this study, the temperature was varied to understand its influence on the growth as well as the biodegradation of oils. The temperature range studied was 20 to 60°C and the results have been shown in (Fig 4B).

The results indicate that the growth of the bacteria was less at 20°C with low degradation capacity. 30°C was favouring the growth of the bacteria and degradation was also satisfactory to incubation time. Maximum growth and degradation were noted at 40°C above which there was a decline in the growth as well as degradation efficiency.

The temperature apart from playing a crucial role in the growth of the organism is also affecting the viscosity of the oil which increases under low temperatures delaying the onset of degradation[46]. The results in this study are substantiated by the report of Foght *et al.*[47] who stated that the decrease in temperature is unfavourable for degradation. Another report by Venosa and Zhu[48] documented that the temperature range of 30–40°C is generally the ideal range for soil microbes for the degradation of oil in the soil.

GC-MS analysis

The process of degradation and its products were identified by GC-MS analysis that was obtained for 0th day, sample after 24 days and 60 days of incubation (Fig 5). The GC-MS chromatogram of the engine oil gives us information on the composition of oil which is classified into saturated and unsaturated compounds with hydrogen, carbon, nitrogen, sulphur, oxygen *etc*. The length of carbon in these compounds ranges from 1 to 30. The various peaks obtained represent compounds which differ from one another in molecular weight, area, and retention time due to the variation in the number of carbons and hydrogens[49]. The main purpose of bioremediation is to convert highly toxic compounds to their lower toxic forms and if possible completely degrade the compounds having very low molecular weight compounds and non-toxic forms.

GC-MS analysis on 0th, 24th day, and the 60th day samples were carried out and the chromatograms are provided in (Fig. 5). The result indicates the presence of 30 peaks corresponding to different kinds of alkanes in 0th day sample. Of the 30 peaks, 12 peaks

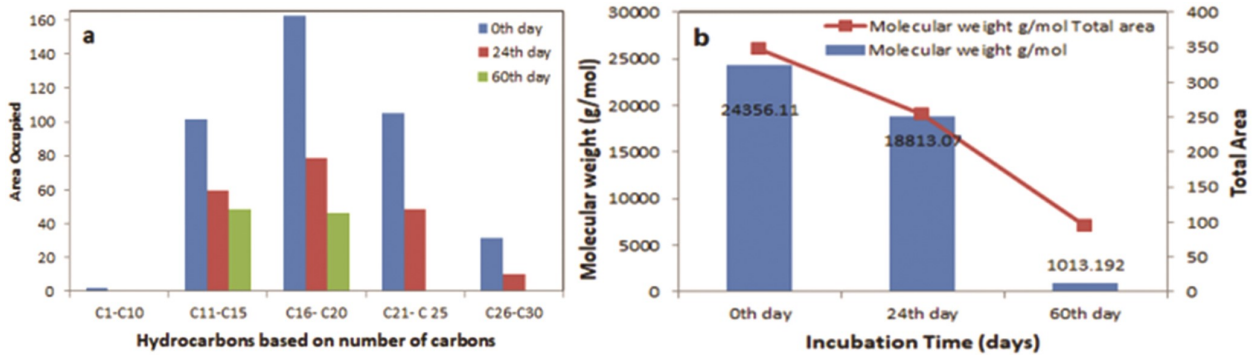

Fig 5 — Analysis after degradation (A) Number of carbon atoms; and (B) Molecular weight and area

were found to be more prominent. The chromatogram of the degraded oil after 24 days showed a decrease in the number of peaks from 30 (0^{th} day) to 22 indicating the possibility of reduction of compounds. Of the 22 peaks obtained only three peaks were found to be predominant while the other peaks clearly showed reduction in the area. The chromatogram on the 60^{th} day showed a drastic reduction in the number of peaks to 7 that successfully illustrates the degradation of crude oil.

The compounds identified after the degradation have been analyzed for their molecular weight variation and reduction in the peak area and provided in (Fig 6). It has been observed that the overall molecular weight of the compounds in the 0^{th} day sample amounted to 24356.11 (g/mol) which reduced slowly in the first 24 days to 18813.07 (g/mol). By 60^{th} day, it was noted that the molecular weight reduced drastically to 1013.192 (g/mol) signifying the good amount of reduction in the oil that was visually observed earlier (disappearance of oil Fig. 3B) during the process of degradation. The compounds have been categorized into different groups based on the number of carbon atoms namely C_1-C_{10}, C_{11}-C_{15}, C_{16}- C_{20}, C_{21}-

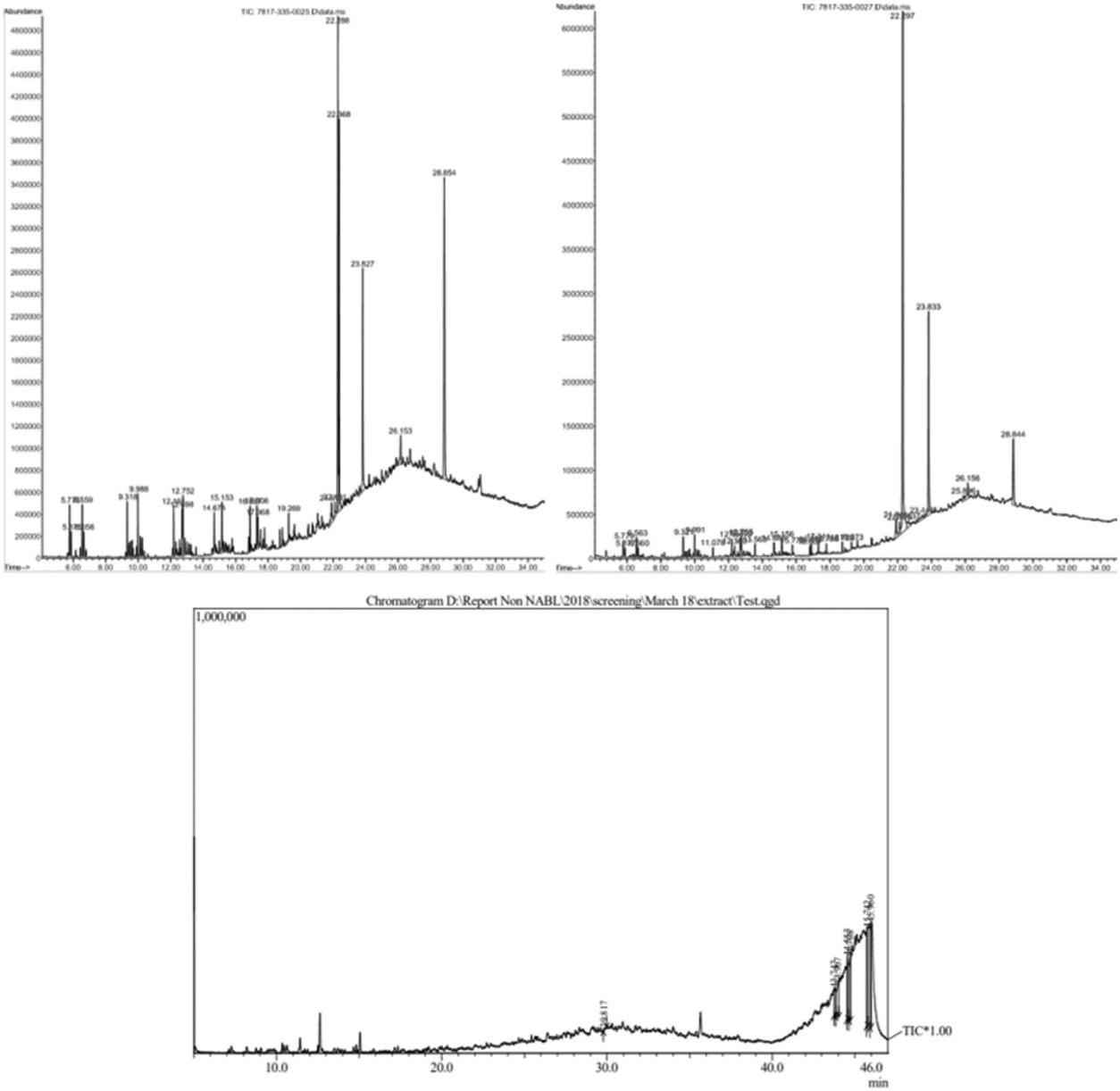

Fig 6 — GC-MS analysis spectra (A) 0^{th} day; (B) 24^{th} day; and (C) 60^{th} day

C_{25}, C_{26}-C_{30}. The peak area occupied by these groups of compounds showed a considerable reduction suggesting the lessening of the amount of these compounds. The 0^{th} day sample had higher amounts C_{11}-C_{15}, C_{16}-C_{20} compounds that showed a twofold and a fourfold reduction in the peak area suggesting a significant degradation. The compounds belonging to C_{21}- C_{25} group initially showed less area in the 0th day but after 24 days there was an increase in the total amount probably due to the breakdown of high carbon molecules (C_{26}-C_{30}) to low carbon molecules which is again evident in the reduction of C_{26}-C_{30} in the 0^{th} day to that of 24^{th} day. By 60^{th} day the high molecular weight compounds seem to be completely degraded with the presence of mainly C_{11}-C_{20} compounds.

It was observed that there was a reduction in the numbers of the individual compounds from 0^{th} day to 24^{th} day namely Nonane reduced from 4 to 1; octane 2 to 1; Tetradecane 2 to 0; Hexadecane 5 to 3, Nonadecane 3 to 1; Pentadecane 4 to 1; heneicosane 6 to 2; and pentacosane 1 to 0. While some hydrocarbons showed a decrease in number, few of them showed a slight reduction in peak area like Dodecane, decane, Tridecane, Octane, heptadecane, undecane, octadecane, and hexacosane. But by 60^{th} day most of the compounds were found to reduce completely thereby demonstrating the ability of the bacteria *Bacillus* isolate SEA18 to degrade the oil.

Usually, nonacosane, tetracosane, docosane, tetrapentacontane, and hexacontane are defined as High Molecular Weight compounds and are observed to be converted into their lower forms such as to octacosane, eicosane, heneicosane, tetracontane, tetracontane, and tetratriacontane *etc*[49].

Reports are supporting the ability of *Bacillus* sp to degrade oils. Report by Lin and Ganesh[49] demonstrated the ability of *Bacillus cereus* isolated from polluted sites to degrade oils by 41%. Also, another sp *B. thuringiensis* was shown to degrade oils by 80%[43]. Similarly, *Bacillus pumilus* (MTCC 1002) isolated from crude oil- contaminated soil was shown to grow in the presence of pyrene by degrading this into 9-methoxyphenanthrene and phthalate[50]. The aromatic compounds found in the 0th day were 8 in number which reduced to 3 after 60 days which suggests the degradation of a few of the ring compounds. This may be due to the initial oxidation of the benzene ring by the action of dioxygenase enzymes to form cis-dihydrodiol, which are dehydrogenated to form dihydroxylated intermediates that can be metabolized further *via* catechols to CO_2 and H_2O. Cis-dihydrodiols of PAHs are potentially soluble and more bioavailable[51]. The higher alkanes were found to be degraded to lower alkanes which may be due to the production of alkane hydroxylase by the microorganism[52].

Reports have shown that most bacterial species have individual capabilities to degrade hydrocarbons completely or carry out their transformation and mineralization to different extents[53-58]. *Bacillus* sp. have been isolated and identified from soil contaminated with oil refinery wastewaters, which have the potential to degrade and utilize organic compounds such as PAHs of both low and high molecular weight as the sole source of carbon[59]. *Bacillus subtilis*, which is one of the dominant bacteria species identified in a study have been reported to have the potential to utilize several PAHs including benzo(a)pyrene, anthracene, naphthalene and dibenzothiophene as the sole source of carbon energy[60].

Conclusion

The role of indigenous microorganisms in degradation of oil is recognized as a viable alternative for the efficient cleaning up method as they possess the required enzyme system to utilize the hydrocarbons and survive. In this study, a bacterial strain having good potential for hydrocarbon degradation was successfully isolated. The bacterium isolated was identified as *Bacillus* sp based on the biochemical tests and 16S rDNA sequencing and named as *Bacillus* sp SEA18. This strain was able to degrade oils to an extent of 80% though it was a slow process. The degradation process was efficient under neutral pH and at 40°C. The GC-MS chromatogram of oil before and after degradation clearly indicates a difference in the area and molecular weight of the compounds with respect to retention time during the course of degradation. Though not a rapid cleanup process, based on the present study, it may be concluded that microbial degradation using *Bacillus* sp SEA 18 can be considered as a key component in the cleanup of hydrocarbon.

Conflict of interest

All authors declare no conflict of interest.

Acknowledgment

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