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Research Article

GROWTH OPTIMIZATION AND COMPARATIVE ANALYSIS OF DIESEL OIL DEGRADATION POTENTIAL OF BACILLUS SP. ISOLATED FROM PETROLEUM CONTAMINATED SOIL OF BARMER, RAJASTHAN

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ABSTRACT

In the present work we have studied growth optimization of previously isolated diesel oil degrading *Bacillus sp.* i.e. *Bacillus coagulans 1A, Bacillus cereus 3E, Bacillus subtilis 4F and Bacillus megaterium 4H* from petroleum contaminated soil of Barmer, Rajasthan at different concentrations of diesel oil and at various pH and temperature. Analysis of extracted diesel oil from bacterial cultures was done gravimetrically and through GC-MS technique. The results of the study reveals that optimum hydrocarbon degrading activity of all the four bacterial isolates 1A, 3E, 4F and 4H was found at pH 7 and 37°C. After an incubation period of 7 days at 37°C biodegradation rates of 7.84%± 3.57%, 12.34% ± 2.9%, 29.79% ± 3.83% and 7.52% ± 2.8% were found for Strain 1A, 3E, 4F and 4H respectively, while biodegradation rates after an incubation of 15 days were found increased i.e. 60.00% ± .02%, 67.44% ± 4.5%, 82.39% ± 5.1% and 49.00% ± 4.8% for strains 1A, 3E, 4F and 4H respectively. Thus, the results obtained clearly shows that isolated strain *Bacillus cereus 3E* and *Bacillus subtilis had degraded 4F* was degraded more than 65% and 80% of diesel oil. Strain *Bacillus megaterium 4H* was found degrading less than 50% of diesel oil. GC-MS chromatogram showed reduction in the intensity of diesel oil peaks after the degradation with strains 1A, 3E, 4F and 4H. Therefore this study proved that the strain *Bacillus cereus3E* and strain *Bacillus subtilis4F* is the most potent diesel oil degrader among all four isolates.

INTRODUCTION

Human activities led to the introduction of potentially toxic substances into the environment. These substances cause dangerous detrimental and toxic effects to human health to variable degrees. Petroleum hydrocarbons have been considered as priority pollutants regarding their potential toxicity, mutagenicity and carcinogenicity. Globally countries are working at their best to control the release of hazardous pollutants from industries into the environment. The biodegradation of petroleum hydrocarbons in the environment is a complex process which depends on the nature and quantity of the oil or hydrocarbons present, the ambient seasonal environmental conditions and the composition of the indigenous microbial community (Leahy and Colwell, 1990; Atlas, 1995; van Hamme *et al*, 2003). Bacteria and fungi are the key agents of degradation. Adapted communities are those which have been previously exposed to hydrocarbons which exhibit more rates of degradation than communities with no history of petroleum hydrocarbon exposure. Petroleum hydrocarbon pollutants biodegradation by bacteria have been extensively studied and investigated (Oboirien *et al*, 2005;Ojumu *et al*, 2005).

MATERIALS AND METHODS

Growth optimization of diesel degrading bacterial isolates

Analysis of the effect of diesel oil concentrations on the growth kinetics of bacterial isolates

Effect of diesel concentrations on the growth of four bacterial isolates and their biodegradation capability was determined using Bushnell Haas medium supplemented with different concentrations of diesel oil i.e. 2.5 µl, 5 µl, 15 µl, 25µl and 50 µl in 50 ml of Bushnell Haas broth(45 ml BH broth + 5 ml

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primary culture) at pH 7 and 37°C. These culture vessels were incubated for 72 hrs in orbital incubator shaker. The growth patterns of four bacterial isolates were determined at 600nm using UV-VIS spectrophotometer Systronics 108.

Effect of different pH on the growth kinetics of bacterial isolates

Effect of pH on the growth of four bacterial isolates and their biodegradation capability was determined using Bushnell Haas medium with pH 5, 7 and 9 supplemented with 2.5 µl of diesel oil in 50 ml Bushnell Haas broth for strain 1A, 3E and 4H and 25µl for strain 4F. These culture vessels were incubated at 37°C for 72 hrs in orbital incubator shaker. The growth patterns of four bacterial isolates were determined at 600nm using UV-VIS spectrophotometer Systronics 108.

Effect of different temperature on the growth kinetics of bacterial isolates

Effect of temperature on the growth of four bacterial isolates and their biodegradation capability was determined using Bushnell Haas medium at temperature 27°C, 37°C and 47°C supplemented with 2.5 µl of diesel oil in 50 ml Bushnell Haas broth for 1A, 3E and 4H and 25µl for 4F. These culture vessels were incubated at 37°C for 72 hrs in orbital incubator shaker. The growth patterns of four bacterial isolates were determined at 600nm using UV-VIS spectrophotometer Systronics 108.

Measurement of diesel degradation capacity of the bacterial isolates by gravimetric analysis

Analysis of the percent degradation using gravimetric method

Preparation of Bushnell Haas Medium (BHM) was done at pH 5.6 ± 0.2 with inoculation of 1% Diesel. Then inoculate 1% of the bacterial inoculum from Nutrient Broth into the respective flasks containing BHM. Then proceed with the Gravimetric analysis on Day 0, Day 7, Day 10 and Day 15.

Gravimetric analysis

25 ml culture from BHM was taken in a clean flask and add 1% 1N HCl into each flask. Then this 25 ml culture was added in the separating funnel with 25 ml of Petroleum ether: Acetone (1:1 ratio) and properly mixed. Then 1ml Acetone was added and the funnel remain still for 15 – 20 min. After 15 – 20 min, different layers (3 layers) were observed. Then in a clean beaker it was weighed (Initial weight).Then from the separating funnel, 1st and 2nd layers were discarded and the 3rd layer was collected in the weighed beaker. This beaker was kept in water bath at 100 °C for 10 – 15 min for complete evaporation of the 3rd layer. Once the evaporation was complete, the beaker was cleaned from outside properly to remove any water on the outer side and then the beaker was again weighed (Final weight).
The amount of diesel left in the beaker after evaporation is calculated as:

Amount of diesel = Final weight of beaker – Initial weight of beaker

Degradation can then be calculated by the following method:

Degradation = (Initial weight – Final weight)/ Initial weight × 100

GC-MS analysis of extracted diesel oil from bacterial cultures

GC-MS analysis of extracted diesel oil from bacterial cultures was done by mixing 5gm of soil sample with 50ml of dichloromethane in separating funnel. The organic phase was passed through Na_2SO_4 concentrated to 0.2ml. It was analyzed by GCMS.GC-MS was performed with Thermo GC 1300 and "TSQ 8000 "Triple Quadrupole GC-MS MS SYSTEM with auto sampler Al 1310. Gas Chromatography 1300 fused with a GC column TG-5MS AMINE. The column length was 30 m with internal diameter 250 µm; coated film 0.25µm. The conditions were as follows: PTV Temp. Program: 70 °C, hold 2.00 min, 10 °C/min to 270 °C, hold 10 min. Carrier gas helium flow rate 1ml/min, split ratio 1:50. GC is equipped with auto-sampler AI 1300 and sample volume was 1µ litre. The Elutes were automatically passed into a mass spectrometer. GC mass Spectrum analysis was conducted using TSQ8000 with transfer line temperature 280°C and ion source temperature 230°C in EI mode. Mass scan time was 4 min with full Scan MS. The mass spectrum was also equipped with a computer fed NIST mass Spectra data library. Chemical constituent components of the extracts were identified by matching the peaks with Computer NIST MS libraries and confirmed by comparing mass spectra of the peaks of literature.

RESULTS

Comparative Analysis of Effect of Different Concentrations on the Growth Kinetics of bacterial Isolates

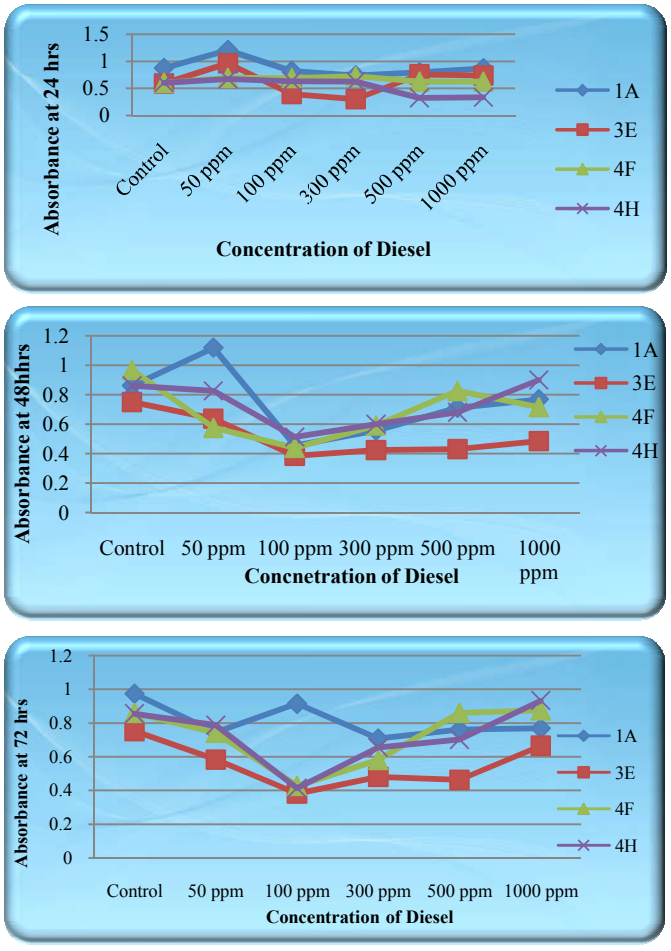


Figure 1 Comparative Analysis of Effect of Different Concentrations of Diesel Oil on the Growth Kinetics of bacterial Isolates (at 24 hrs, 48 hrs, 72 hrs)

Diesel is used as a carbon source but it can be toxic to microorganisms due to the solvent effect of diesel that could destroy bacterial cell membrane. Therefore, in order to increase the growth of bacterial isolates and hydrocarbon degradation efficiency it is essential to optimize the growth conditions. Hence in this study bacterial growth was optimized at different concentrations of diesel oil and it was found that 50ppm diesel oil supports excellent growth (maximum utilization of diesel) for strain 1A (*Bacillus coagulans*), 3E (*Bacillus cereus*), 4H (*Bacillus megaterium*)and 500ppm diesel oil supports growth of strain 4F (*Bacillus subtilis*). Cellular growth dramatically decreased at diesel degradation higher than this (Fig 1).

Comparative Analysis of Effect of Different Temperatures on the Growth kinetics of Bacterial Isolates

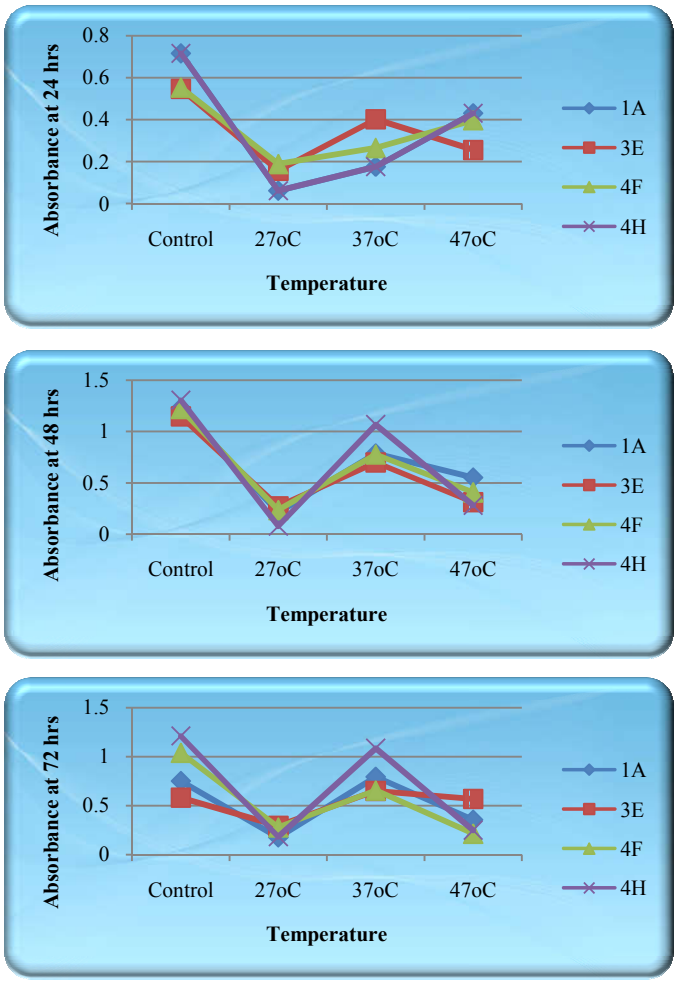


Figure 2 Comparative Analysis of Effect of Different Temperatures on the Growth kinetics of Bacterial Isolates (at 24 hrs, 48 hrs, 72 hrs)

Hydrocarbon degrading bacterial strains are able to grow in a wide range of temperature. The isolated bacterial strains 1A, 3E, 4F and 4H were able to grow at temperature 27^0C, 37^0C and 47^0C. The present study analyzed the optimum temperature for the degradation of diesel oil and highest microbial biomass was recorded at 37^0C. The minimum microbial growth was observed at 27^0C for all the four strains. Moderate growth was reported at 47^0C. Among these strains, 3E (*Bacillus cereus*) shows more growth at an incubation of 24 hrs and further it shows a decline while 4H (*Bacillus megaterium*) show more growth at an incubation of 48 hrs and 72 hrs as compared to other strains (Fig 2).

Comparative Analysis of Effect of Different pH on the Growth Kinetics of Diesel Oil Degrading Bacterial Isolates

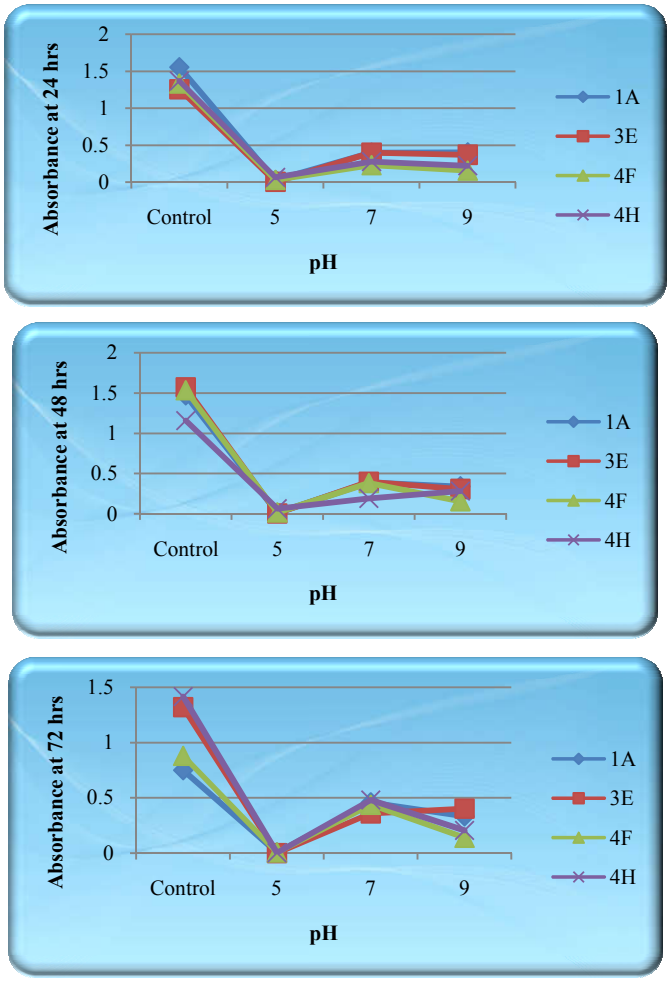
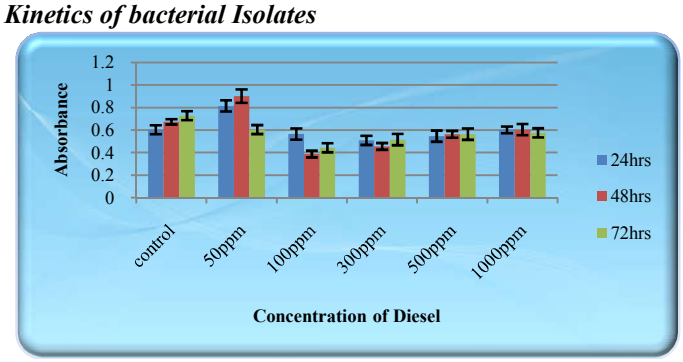


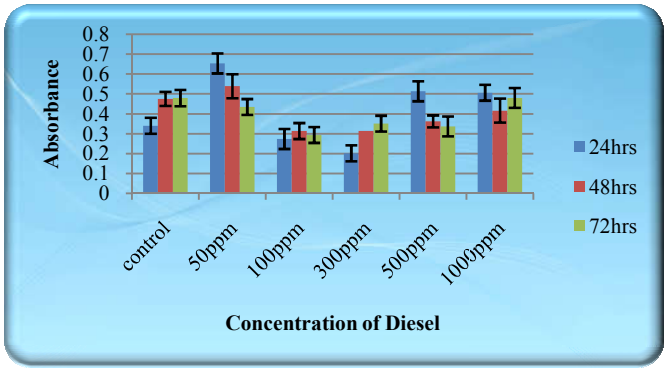
Figure 3 Comparative Analysis of Effect of Different pH on the Growth Kinetics of Bacterial Isolates (at 24 hrs, 48 hrs, 72 hrs)

The rate of biodegradation also depends on the pH of soil. Figure 3 shows the growth of bacterial strains 1A, 3E, 4F and 4H at different pH range i.e. pH 5, 7 and 9. The optimal growth of all the four strains was found at pH 7. Minimum growth was reported at pH 5 and pH 9. Among all these strains, 3E (*Bacillus cereus*) shows more growth as compare to other three strains after an incubation of 24 and 48 hrs while 4F (*Bacillus subtilis*) shows more growth after an incubation of 72 hrs as compare to other strains.

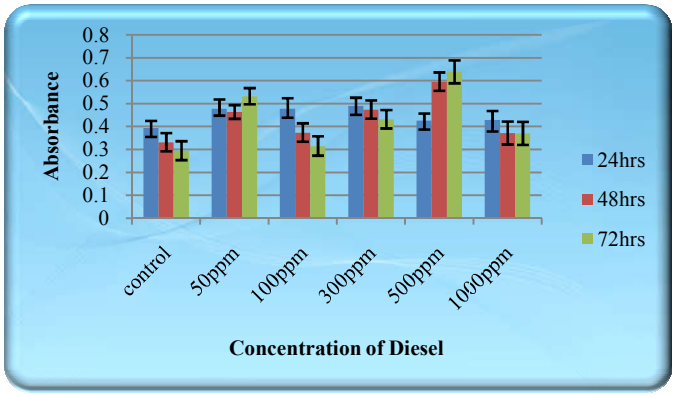
Comparative Analysis of Effect of Different Concentrations of Diesel Oil, different temperature and pH on the Growth Kinetics of bacterial Isolates



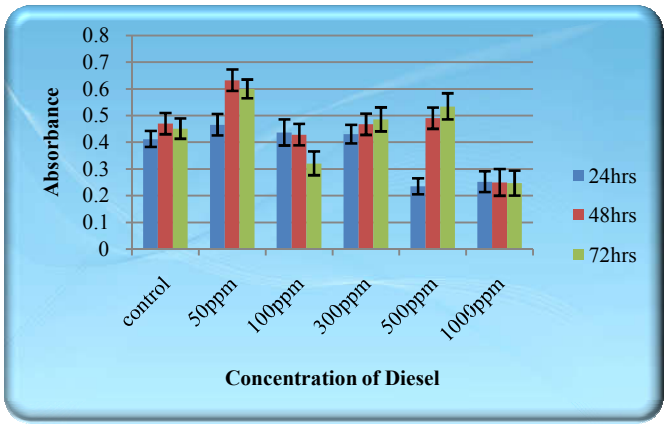
Bacterial Strain 1A



Bacterial Strain 3E

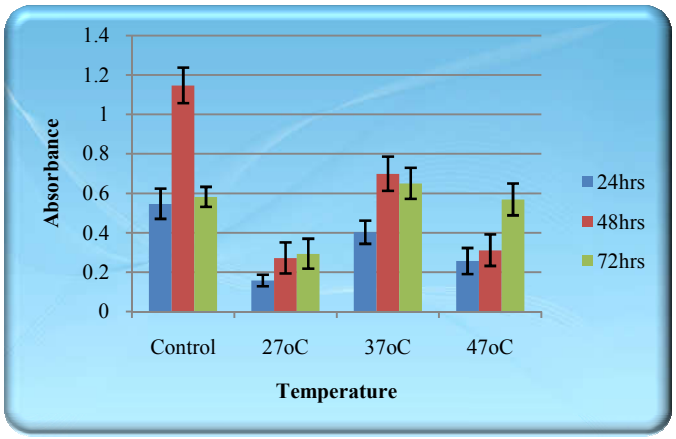


Bacterial Strain 4F

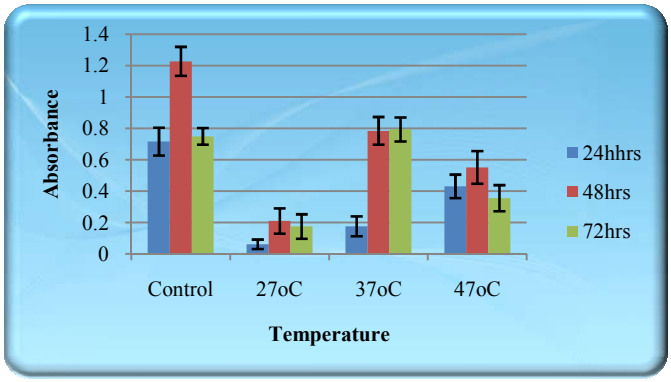


Bacterial Strain 4F

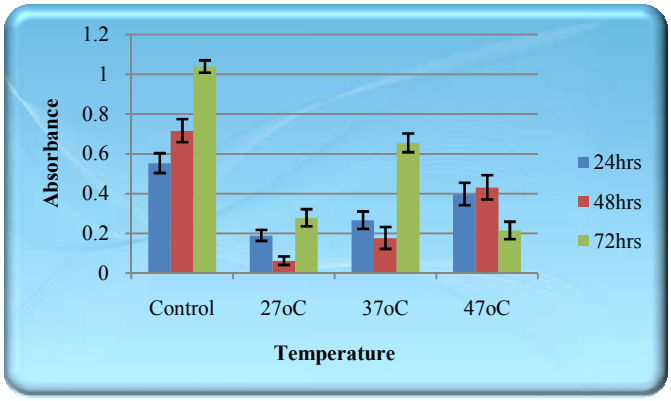
Figure 4 Comparative Analysis of Effect of Different Concentrations of Diesel Oil on the Growth Kinetics of bacterial Isolates



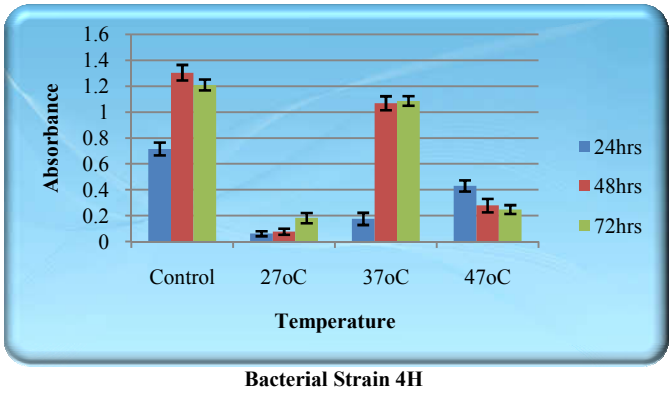
Bacterial Strain 1A



Bacterial Strain 3E

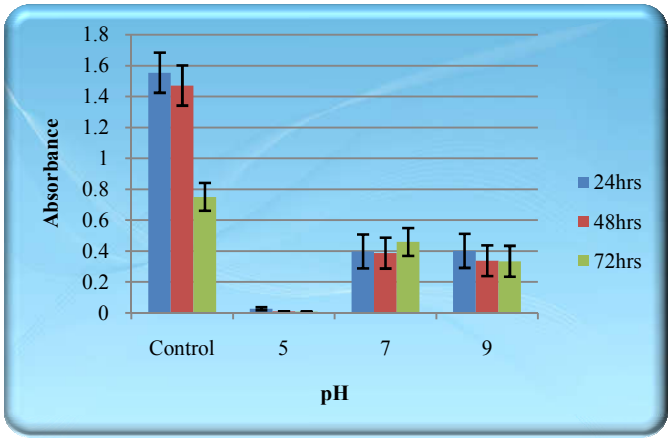


Bacterial Strain 4F

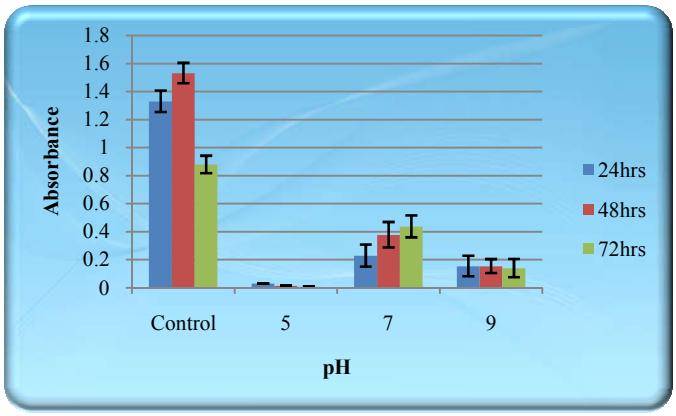


Bacterial Strain 4H

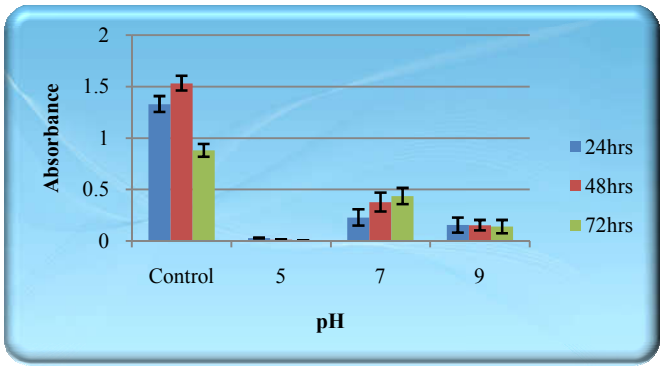
Figures 5 Comparative Analysis of Effect of Different Temperatures on the Growth kinetics of Bacterial Isolates



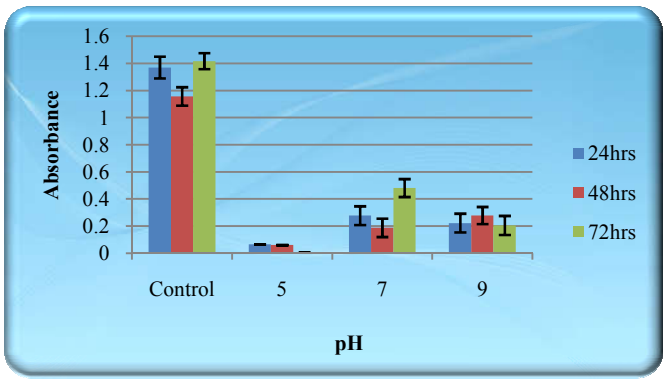
Bacterial Strain 1A



Bacterial Strain 3E



Bacterial Strain 4F



Bacterial Strain 4H

Figure 6 Comparative Analysis of Effect of Different pH on the Growth Kinetics of Bacterial Isolates

Degradation Analysis of Diesel Oil by the Isolated Bacteria Using Gravimetric Method

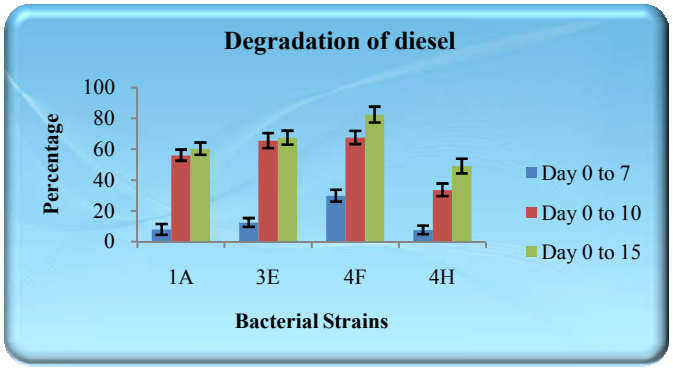
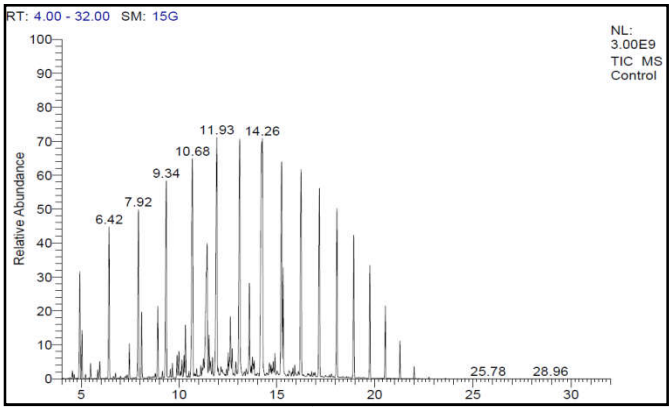


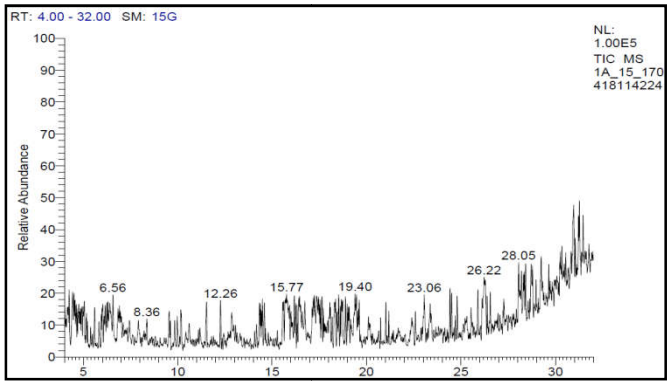
Figure 7 Percentage degradation of Diesel oil by Bacterial strains 1A, 3E, 4F and 4H by Gravimetric Analysis

After an incubation period of 7 days at 37^0C biodegradation rates of 7.84%± 3.57%, 12.34% ± 2.9%, 29.79% ± 3.83% and 7.52% ± 2.8% were found for Strain 1A, 3E, 4F and 4H respectively, while biodegradation rates after an incubation of 15 days were found increased i.e. 60.00% ± .02%, 67.44% ± 4.5%, 82.39% ± 5.1% and 49.00% ± 4.8% for strains 1A, 3E, 4F and 4H respectively (Fig 7). Thus, the results obtained clearly shows that isolated strain 3E (*Bacillus cereus*) and 4F (*Bacillus subtilis*) was degraded more than 65% and 80% of diesel oil. Therefore they can be considered as a potent diesel oil degrader. Strain 4F (*Bacillus megaterium*) was found degrading less than 50% of diesel oil.

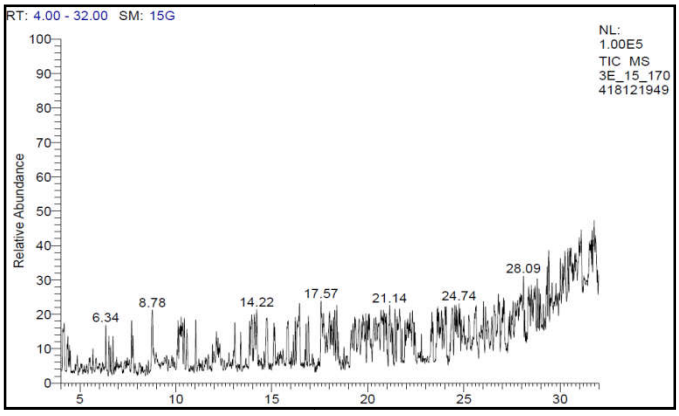
GC-MS Analysis



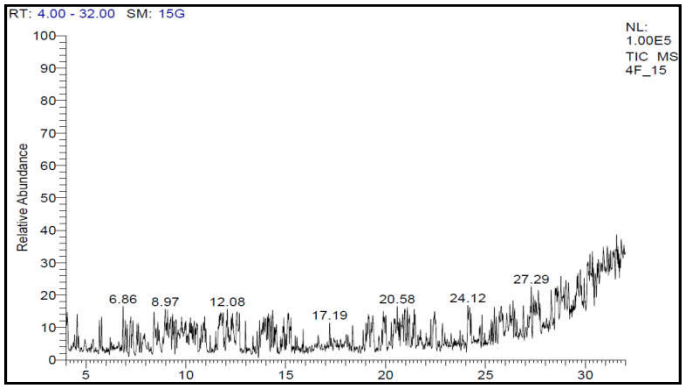
A Control (abiotic)



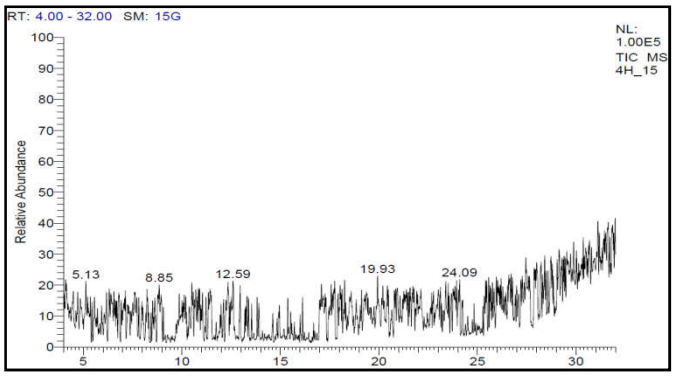
B Strain 1A



C Strain 3E



D Strain 4F



E Strain 4H

Figure 8 A. GC-MS profiles for control (abiotic) diesel oil. GC-MS profiles of degraded diesel oil after biodegradation by strains 1A, 3E, 4F and 4H after 15 days of cultivation

Gas chromatograms of Diesel Oil (abiotic control) revealed that diesel oil contain fractions certain hydrocarbon compound which might be mineralized by bacterial treatment. For comparison, chromatogram of GC-MS analysis between control and four treatments (1A, 3E, 4F, 4H) at an incubation period of 15days are shown in Fig 8. GC-MS chromatogram showed reduction in the intensity of diesel oil peaks after the degradation with strains 1A, 3E, 4F and 4H. Therefore, the biodegradation of diesel oil by isolated strains was confirmed by GC-MS analysis. The study showed that strain 3E (*Bacillus cereus*) and strain 4F (*Bacillus subtilis*) was able to degrade more than 70% and 80% respectively of the diesel oil within 15 days of incubation at initial pH 7 and 37^{0}C. Therefore this study proved that the strain 3E (*Bacillus cereus*) and strain 4F (*Bacillus subtilis*) are the most potent diesel oil degrader among all four isolates.

DISCUSSION

Under normal growth conditions every organism reveals a characteristics growth pattern. A typical sigmoid curve is normally obtained when bacterial growth is quantified in terms of optical density. The addition of hydrocarbon to an ecosystem, due to accident oil spills may selectively increase or decrease size of microbial population depending on various factors such as temperature, pH, chemical composition of the contaminant and the species of microorganism present within the microbial community of the particular ecosystem (Bharathi and Vasudevan, 2001).

Hence in this study bacterial growth was optimized at different concentrations of diesel oil and it was found that 50ppm diesel oil supports excellent growth (maximum utilization of diesel) for strain 1A (*Bacillus coagulans*), 3E (*Bacillus cereus*), 4H (*Bacillus megaterium*) and 500ppm diesel oil supports growth of strain 4F (*Bacillus subtilis*). Cellular growth dramatically decreased at diesel degradation higher than this (Fig 1).

According to Pantelelis *et al,* (2006) half-life of hydrocarbons is much higher in acidic soil than the alkaline soil. As microbial population of the contaminated area cannot grow properly in acidic environment and it affects the biodegradation rate.

Figure 3 shows the growth of bacterial strains 1A, 3E, 4F and 4H at different pH range i.e. pH 5, 7 and 9. The optimal growth of all the four strains was found at pH 7. Minimum growth was reported at pH 5 and pH 9. Among all these strains, 3E (*Bacillus cereus*) shows more growth as compare to other three strains after an incubation of 24 hrs and 48 hrs while 4F (*Bacillus subtilis*) shows more growth after an incubation of 72 hrs as compare to other strains.

According to Whang*et al*, (2009) microbial growth and diesel biodegradation was found to be at a pH 7.2, while decreasing or increasing the pH reduced the degradation efficiency considerably. Likewise Luo *et al,* (2013) at pH level of 7 *Pseudomonas sp*. strain F4 showed efficient diesel oil degradation potential. Hence, the optimization of pH is very important for the enhanced growth of bacteria and also for selection of effective bioremediation strategy. Kumar *et al*, (2008) also reported that the optimum pH for the degradation of crude oil by individual bacterial strains and a mixed bacterial consortium was found to be 7.

The isolated bacterial strains 1A, 3E, 4F and 4H were able to grow at temperature 27^{0}C, 37^{0}C and 47^{0}C. The present study analyzed the optimum temperature for the degradation of diesel oil and highest microbial biomass was recorded at 37^{0}C. The minimum microbial growth was observed at 27^{0}C for all the four strains. Moderate growth was reported at 47^{0}C. Among these strains, 3E (*Bacillus cereus*) shows more growth at an incubation of 24 hrs and further it shows a decline while 4H (*Bacillus megaterium*) show more growth at an incubation of 48 hrs and 72 hrs as compared to other strains (Fig 2).

Similarly, Ma and Herson, (2000) reported growth of diesel degrading *Burkholderia sp*. at 37^{0}C. Diesel degradation at higher temperature by a bacterium has been described by Ma´rquez-Rocha *et al*, (2005). *Rhodococcusruber* and *Rhodococcuserythropolis* also have been shown to be able to degrade diesel at 37^{0}C (Bicca*et al*, 1999). While Mnif*et al*, (2014) found contrary to our study that 30°C was the optimum condition for the degradation of diesel by *Bacillus subtilis* SPB1. Same time, the diesel oil degrading ability of *Pseudomonas sp*. strain F4 was reported to be 37°C (Luo*et al*, 2013).

In our study we used gravimetric method for analysis of diesel oil degradation and found that after an incubation period of 7 days at 37^{0}C and pH 7 biodegradation rates of 7.84% ± 3.57%, 12.34% ± 2.9%, 29.79% ± 3.83% and 7.52% ± 2.8% were found for Strain 1A, 3E, 4F and 4H respectively, while biodegradation rates after an incubation of 14 days were found

increased i.e. 60.00% ± .02%, 67.44% ± 4.5%, 82.39% ± 5.1% and 49.00% ± 4.8% for strains 1A, 3E, 4F and 4H respectively (Fig 7). Thus, the results obtained clearly shows that isolated strain 3E (*Bacillus cereus*) and 4F (*Bacillus subtilis*) was degraded more than 65% and 80% of diesel oil. Therefore they can be considered as a potent diesel oil degrader. Strain 4F (*Bacillus megaterium*) was found degrading less than 50% of diesel oil.

Likewise, Tanzadeh and Haghighat, (2014) used diesel oil, left standing in a laboratory for six months, as source for the isolation of *Bacillus subtilis, Bacillus cereus, Trichodermaharzanium* and *Trichotherciumroseum*. They found these organisms to be hydrocarbon degraders. On further testing, they found that *B. subtilis* had higher potential to utilize diesel oil as carbon source. They introduced diesel oil in soil samples at a loading rate of 5% (v/w) (oil/soil). These soil samples, together with the unpolluted control samples, were seeded with the *B. subtilis* isolate. The rates of degradation of diesel oil by the isolate at the end of day one, day twelve and day twenty-seven were gravimetrically found 6.8×10^{-4}, 1.73×10^{-3} and 1.04×10^{-3} g/h, respectively.

In present investigation GC-MS chromatogram showed reduction in the intensity of diesel oil peaks after the degradation with strains 1A, 3E, 4F and 4H (Fig 8). The study showed that strain 3E (*Bacillus cereus*) and strain 4F (*Bacillus subtilis*) was able to degrade more than 70% and 80% respectively of the diesel oil within 15 days of incubation at initial pH 7 and 37^0C.

Sharma *et al,* (2014) also examined the diesel oil degrading capacity of an indigenous isolate of *Pseudomonas aeruginosa*to remediate the diesel contaminated soil. Their results obtained revealed that 66 % diesel degradation was observed during the incubation period of 30 days after bioaugmentation with *P. aeruginosa* in diesel contaminated soil.

The previous study achieved degradation of 95.01% diesel by *Trichosporonasahii* (Iloriet *al*, 2008). Similarly Das and Chandran, (2011) reported that free cells of *C. tropicalis* able to degrade 80% of the diesel oil over a period of one week.

Therefore this study proved that the strain 3E (*Bacillus cereus*) and strain 4F (*Bacillus subtilis*) are the most potent diesel oil degrader among all four isolates.

Acknowledgment

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References

Atlas, R. M. (1995). Bioremediation. *Chem. Eng. News*., 73: 32-42.

Barathi, S. and Vasudevan, N. (2001). Utilization of petroleum hydrocarbons by Pseudomonas fluorescensisolated from a petroleum-contaminated soil. *Environment International*., 26(5-6): 413-416.

Bicca, F. C., Fleck, L. C., Antonio, M. (1999). Production of biosurfactant by hydrocarbon degrading Rhodococcusruber and Rhodococcuserythropolis., *Rev Microbiol.* 30:231-236.

Das, N., Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. *Biotechnol. Res. Int*., 2: 1-13.

Ilori, M. O., Adebusoye, S. A., Ojo, A. C. (2008). Isolation and characterization of hydrocarbon degrading and biosurfactant producing yeast strains obtained from lagoon water. *World J MicrobiolBiotechnol*., 24:2539-2545.

Kumar, S., Binupriya, A. R., Baik, S. H., Yun, S. E. (2008). Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. *Clean-Soil Air Water*., 36:92-96.

Leahy, J. G. and Colwell, R. R. (1990). Microbial Degradation of Hydrocarbons in the Environment. *Microbiological Reviews*., 54(3): 305-315.

Luo, Q., Zhang, J. G., Shen, X. R., Sui, X., Fan, Z. Q. (2013). Characterization of a novel diesel oil-degrading pseudomonas sp. strain F4. *Fresenius Environ Bullet*., 22:689-697.

Ma, Y., Herson, D. S. (2000). The cathecol 2, 3-deoxygenase gene and toluene monooxygenase genes from burkholderia sp. AA1, an isolate capable of degrading aliphatic hydrocarbons and toluene. *J IndMicrobiolBiotechnol*., 25:127-131.

Márquez-Rocha, F. J., Rodríguez, V. H. and Lamela, M. T. (2001)..Biodegradation of diesel oil in soil by a microbial consortium. *Water, Air, and Soil Pollution*.,128: 313-320.

Mnif, I., Sahnoun, R., Ellouze-Chaabouni, S., Ghribi, D. (2014). Evaluation of B. subtilis SPB1 biosurfactants' potency for diesel-contaminated soil washing: optimization of oil desorption using Taguchi design. *Environ SciPollut Res*., 21:851-861.

Oboirien, B. O., Amigun, B., Ojumu, T. V., Ogunkunle, O. A., Adetunji, O. A., Betiku, E. and Solomon, B. O. (2005). Substrate inhibition kinetics of phenol degradation by Pseudomonas aeruginosaand Pseudomonas fluorescens. *Biotechnology*.,4: 56-61.

Ojumu, T. V., Bello, O. O., Sonibare, J. A. and Solomon, B. O. (2005). Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria. *AfrJ Biotechnol*., 4(1):31-35.

Pantelelis, I., Dimitrios, M. S. and Nikolas, T. (2006): Influence of soil physiocochemical and Biological properties on the degradation and adsorption of the nematicidefosthiazate. *J. Agric. Food Chem*.,54(18): 6783-6789.

Sharma, A., Kumar, P. and BudholiaRehman, M. (2014). Biodegradation of Diesel Hydrocarbon in Soil by Bioaugmentation of Pseudomonas aeruginosa: A Laboratory Scale Study. *International Journal of Environmental Bioremediation & Biodegradation*., 2(4): 202-212.

Tanzadeh, J., Haghighat, A. (2014). Application of Bacillus Subtilis in Degradation of Diesel Oil at Polluted Soil in Gilan.. *Journal of Current Research in Science*.,2(6): 971-976.

Van Hamme, J. D., Singh, A. and Ward, O. P. (2003). Recent advances in petroleum microbiology. *Microbiology and Molecular Biology Reviews.*, 67(4): 503-549.

Whang L. M., Liu, P. W. G., Ma, C. C., Cheng, S, S. (2009). Application of rhamnolipid and surfactin for enhanced diesel biodegradation-Effects of pH and ammonium addition. *J Hazard Mater.,* 164:1045-1050.

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