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SHORT COMMUNICATION

Biodegradation capability of bacterial species isolated from oil contaminated soil

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Abstract

Oil spills do so much more damage than just the initial havoc we see on the news and the effects can be very long lasting. Microorganisms are used to change harmful substances to non-toxic substances. This is a new technology for treating chemical spills and hazardous waste. This paper has studied the performance of different bacterial species in degrading the oil components. The degradation capability of isolated bacterial species were identified with solvent extraction method and confirmed by FT-IR analysis. *Pseudomonas* sp. found to degrade oil better than other isolated species. *Pseudomonas* degraded 90.2% of oil in 30 d followed by 82.3% of oil degraded by *Bacillus*, 78.8% of oil degraded by *Serratia* and 25.5% of oil degraded by *Staphylococcus.* Then, *Pseudomonas* was immobilized using sodium alginate method and no decline in biodegradation capacity of the immobilized consortium of bacterial cells was noted. The mixed bacterial culture degraded 97.2% of the oil components during 30 d of incubation, which is the maximum efficiency in this study.

Keywords: Oil spills, *Pseudomonas, Bacillus, Serratia,* sodium alginate, biodegradation.

Introduction

The place so many people calls home and the vacation destination for others are now being ruined by something the entire world needs: oil. The one product which is very vital for the mobility and to run all the man-made inventions is now destroying the beautiful ecosystem. Oil spill is one of the major man-made disasters in the developing and developed countries in both large and medium scale. When oil is spilled or leaked into in waterways and the ocean, it spreads very quickly with the help of wind and currents. A single gallon of oil can create an oil slick up to a couple of acres in size and these oil spill affect the region's environment and inhabitants, not only that it has also largely influenced the economy in the Gulf region and will continue to do so for many years.

One of the best approaches to restoring contaminated soil is to make use of bioremediation which is an attractive approach of cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to the complete destruction of the contaminant (Khan *et al.*, 2011). Crude oil, a mixture of many thousands of organic compounds, can vary in composition from one source to another. This suggests that the effects of crude oil spill will vary from source to source. However details of the potential biological damage will depend on the ecosystem where the spill occurred. Clean up and recovery from an oil spill is difficult and depends upon many factors, including the type of oil spilled, the temperature of the water (affecting evaporation and biodegradation), and the types of shorelines and beaches involved.

There are various methods that are available for cleaning the environment. Current methods available for cleaning the environment from the oil are namely bioremediation accelerator, controlled burning, dispersants acting as detergents, dredging, skimming, solidifying, vacuum and centrifuge and bioremediation. Biodegradation is a natural process by which microbes alter and breakdown oil into other substances. The resulting products can be carbon dioxide, water and simpler compounds that do not affect the environment (Bragg *et al.,* 1992).

To overcome these environmental problems, microbial bioremediation is only way to preserve our nature. Many species of microorganisms; bacteria, yeast and fungi-obtain both energy and tissue-building material from petroleum. Several species of fuel-eating bacteria have evolved a taste for hydrocarbons, the major component of fossil fuels which includes *Pseudomonas*, *Bacillus* and *Micrococcus* (Tani *et al.,* 2001; Arora *et al.,* 2008). Individual microbial populations can usually metabolize only a limited range of substrate, therefore the performance of mixed population with different degradation capabilities were studied.

Materials and methods

Sample collection: The contaminated soil samples were collected from Chennai Harbour near oil spill area and were subjected to serial dilution. Serially diluted samples from 10^{-1} to 10^{-7} were plated on nutrient agar using spread plate method and kept for incubation at 37°C for 24 to 48 h and the plates were observed for growth after the incubation time.

Table 1. Confirmation of bacterial isolates.

Strains	Gram's staining	Spore staining	Motility	Biochemical tests				Colonies on media	Result
				Indole Test	Methyl Red Test	Vogues Proskauer's Test	Citrate Test		
Green	-Rods	-	+	-	+	-	+	Green colour	*Pseudomonas* sp.
White	+Rods	+	+	-	-	-	-	White colour	*Bacillus* sp.
Orange	-Rods	-	-	-	-	+	+	Orange colour	*Serratia* sp.
Yellow	+Cocci	-	-	-	-	-	-	Yellow colour	*Staphylococcus* sp.

Identification of bacterial isolates

The bacteria strains were identified based on the microscopic (Gram staining, Motility test and Spore staining) and biochemical characteristics (Indole, methyl red, Vogues Proskauer's test, and citrate utilization) (Sundarraj, 2005).

Experimental set up for oil degradation

Minimal salt media was prepared by adding 1.2 g potassium dihydrogen phosphate, 1.2 g of dipotasium phosphate, 1.2 g of ammonium nitrate and 0.6 g of magnesium sulphate. The media was stirred on a magnetic stirrer and was poured in 5 sets of 30 flasks containing 6 flasks in each set. All the flasks were autoclaved at 121°C for 15 min. The 5 sets of flasks was labeled as mixed, yellow, orange, green, white and checked at the interval of 5 d, 10 d, 15 d, 20 d, 25 d and 30 d respectively. In all the 24 flasks, 2 g of lubricating oil was added, whereas 2 mL of culture broth was aseptically added in rest of the flasks in all the 4 sets and was left in incubator shaker for thorough mixing and microbial growth. The degradation capability of the bacterial species was checked after 5, 10, 15, 20, 25 and 30 d respectively for degradation efficiency of organisms. Using solvent extraction method the 30^{th} d result was confirmed with FT-IR analysis.

Solvent extraction method

Incubation mixture (50 mL) was mixed with 5 mL of benzene. Then the aqueous phase and the organic phase was separated by separating funnel. The separated aqueous phase was again mixed with 5 mL of benzene to extract the left out organic phase. Then, the total separated organic phase was centrifuged for 20 min for 1000 rpm. The supernatant was poured on to petriplates. The benzene was allowed to evaporate at 37°C and the rate of oil degradation was expressed in grams as well as in percentage. Percentage of residual oil and oil degradation is calculated by the below formula:

$$\% \text{ of residual oil} = \frac{\text{Weight of the remaining oil sample}}{\text{Weight of the control oil sample}} \times 100$$

$$\% \text{ of oil degraded} = \frac{\text{Weight of the used oil sample}}{\text{Weight of the control oil sample}} \times 100$$

Immobilization of Pseudomonas sp. for oil degradation

Sodium alginate (9 g) was dissolved in 300 mL of growth medium, to avoid clump formation. The final solution contained 3% alginate by weight. Wet cells (250 g) in the alginate solution were prepared and thoroughly suspended. Yeast-alginate mixture was dipped from a height of 20 cm into 1000 mL of cross linking solution. (The cross linking solution is prepared by adding 0.05 M of $CaCl_2$ to growth media). The calcium cross linking solution is agitated on a magnetic stirrer and gel formation was achieved at room temperature as soon as the sodium alginate drops come in direct contact with the calcium solution. Relatively small alginate beads are preferred to minimize the mass transfer resistance. A diameter of 0.5-2 mm was readily achieved with a syringe and a needle. The beads are fully hardened in 1-2 h. The concentration of the $CaCl_2$ is about one fourth of the strength used for enzyme immobilization. Finally the beads were washed with fresh calcium cross linking solution.

Results and discussion

The microorganisms which could be employed for the degradation of petroleum and its derivatives in minimising contamination due to oil leak and spill, has promoted a number of investigators to study the process in the laboratory (Zo Bell *et al*., 1946). Four different bacterial species were isolated from oil contaminated soil and their degradation capability was checked individually in the laboratory condition.

Four bacterial species were identified as *Pseudomonas* sp., *Bacillus* sp., *Serratia* sp. and *Staphylococcus* sp. by using standard biochemical tests (Table 1). The degradation capability of isolated bacterial species was checked after 5, 10, 15, 20, 25 and 30 d respectively using solvent extraction method and the 30^{th} d result was confirmed with FT-IR analysis (Fig. 1). The *Pseudomonas* sp. found to degrade oil better than other isolated species (Table 3). *Pseudomonas* degraded 90.2% of oil in 30 d followed by 82.3% of oil degraded by *Bacillus*, 78.8% of oil degraded by *Serratia* and 25.5% of oil degraded by *Staphylococcus* (Van Hamme *et al.,* 2003).

Fig. 1. FT-IR graph of untreated oil sample followed by bacterial treated samples and immobilized *Pseudomonas* sp.

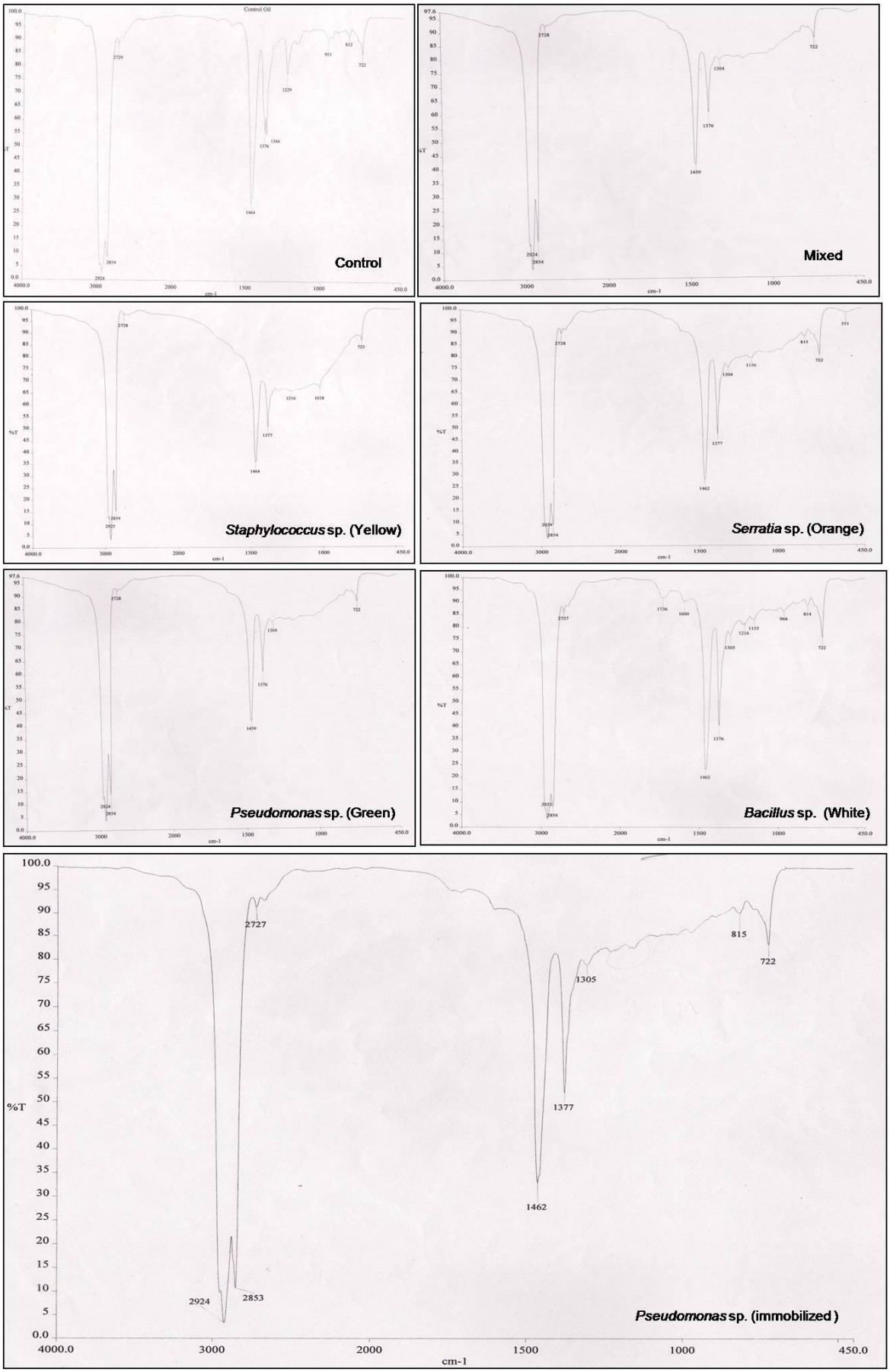

Table 2. FT-IR peaks of the control sample.

Frequency range (cm^{-1})	Compound type
2924	Alkanes
2854	Alkanes
2729	Carboxylic acids
1464	Alkanes
1376	Alkanes
1366	Alkanes
1229	Nitro compounds
951	Alkenes
812	Alkenes
722	Alkenes

Table 3. The degradation capability of isolated bacterial species in various incubation periods.

Colour	Oil added (g)	Oil remained (g)	Oil used (g)	% Degraded
		5th day		
Mixed	2	1.4	0.6	30
Yellow	2	1.8	0.2	10
Orange	2	1.64	0.36	18
Green	2	1.4	0.6	30
White	2	1.7	0.3	15
		Control- 1.96 g of oil remained		
		10th day		
Mixed	2	1.2	0.8	40
Yellow	2	1.75	0.25	12.5
Orange	2	1.44	0.56	28
Green	2	1.14	0.86	43
White	2	1.4	0.6	30
		Control- 1.97 g of oil remained		
		15th day		
Mixed	2	1.2	0.8	40
Yellow	2	1.75	0.25	12.5
Orange	2	1.44	0.56	28
Green	2	1.14	0.86	43
White	2	1.06	0.94	47
		Control- 1.96 g of oil remained		
		20th day		
Mixed	2	0.9	1.1	55
Yellow	2	1.64	0.36	18
Orange	2	0.86	1.14	57
Green	2	0.62	1.38	69
White	2	0.80	1.20	60
		Control- 1.97 g of oil remained		
		25th day		
Mixed	2	0.5	1.5	75
Yellow	2	1.58	0.42	21
Orange	2	0.72	1.28	64
Green	2	0.4	1.6	80
White	2	0.46	1.54	72
		Control- 1.96 g of oil remained		
		30th day		
Mixed	2	0.06	1.94	97.2
Yellow	2	1.49	0.51	25.5
Orange	2	0.44	1.56	78.8
Green	2	0.2	1.80	90.2
White	2	0.36	1.64	82.3
		Control- 1.96 g of oil remained		

The mixed bacterial culture degraded 97.2% of the oil components during 30 d of incubation, which is the maximum efficiency in this study (Fig. 1) (Bagherzadeh-Namazi *et al*., 2008). Since Pseudomonas sp. recorded highest percentage of degradation compared to all other species, it was immobilized using sodium alginate and checked for oil degradation. But no decline in biodegradation capacity of the immobilized consortium of bacterial cells was noted.

Conclusion

Four different bacterial species were isolated from oil contaminated soil and their degradation capability was checked individually in the laboratory condition among which *Pseudomonas* sp. showed highest degradation efficiency followed by *Bacillus* sp., *Serratia* sp. and *Staphylococcus* sp. *Pseudomonas* which showed highest individual degradation efficiency in this study was immobilized in alginate and its degradation efficiency does not varied notably after immobilization, so from this study we conclude that immobilized bacterial cells could be used to degrade the hydrocarbon contaminated area rather than the free suspended cell.

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