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A Comparative study on the degradation of crude oil contaminated water by Pseudomonas sp.

S Sharmila*, L Jeyanthi Rebecca, Merina Paul Das, Amit Jha, Arup Chakraborty, Anuranjan Kumar, and Shailesh Anand

Dept of Industrial Biotechnology, Bharath University, Chennai, Tamil Nadu, India-600073.

ABSTRACT

Water pollution is the contamination of water bodies by various pollutants discharged from household wastes, improper treatment of industrial waste water, storm runoff etc. Among these, Industrial effluent has significant effect on water pollution. In this study, water samples which were contaminated by crude oil released from oil industries located at Manali, Tamil Nadu, India were collected. These contaminated water samples were treated with Pseudomonas sp. that was isolated from oil contaminated soil present over there with and without additional inorganic nutrients like ammonium phosphate, magnesium sulphate, potassium phosphate and sodium chloride. Then the percentage of oil degradation was estimated up to 6 days. 100% degradation was achieved by Pseudomonas sp. with additional inorganic nutrients on 6^{th} day and in Pseudomonas sp. without additional nutrients showed 97.89% oil degradation.

Keywords: Pseudomonas sp, crude oil, bioremediation, oil degradation.

Corresponding author

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INTRODUCTION

Water is one of the important matter on earth. Every living being needs water for its survival. Of late water is highly polluted by industrial effluents. Extensive petroleum hydrocarbon exploration activities often result in the pollution of environment which will lead to disastrous consequences for the biotic and abiotic components of the ecosystem [1, 2]. Such release often pose severe, immediate as well as long term ecological and environmental problem [3], because of lot of hydrocarbon compounds present in petroleum effluent are highly toxic. Several physio-chemical methods for decontaminating the effluent have been employed [4]. These methods are usually expensive, labor intensive and often involve the risk of spreading pollution [5]. The traditional treatment of oily waste water cannot degrade the crude oil thoroughly [6].

A best way of degrading this oil would be bioremediation which is the degradation or stabilization of contaminants by microorganism is claimed as a safe, effective and also economic alternative method of environmental cleanup [5]. This mechanism has been studied and reviewed [7]. The biodegradation of oil pollutants is not a new concept as it has been intensively studied in controlled conditions [8, 9] and in open field experiments [10, 11]. The use of surfactants has been found to enhance degradation of crude oil [12,13] or the hydrocarbons [14]. Many organisms have the ability to utilize hydrocarbon as the sole source of carbon energy and such microorganisms are widely distributed in nature. The microbial utilization of hydrocarbon was highly dependent on the chemical nature of the components within the petroleum mixture and environmental determinants [15].

Many species of microorganisms such as bacteria, yeast and fungi obtain both energy and tissue binding material from petroleum. The fuel eating bacteria *Pseudomonas sp.* has major role in degrading oil content present in waste released from automobile sector [16].

MATERIALS AND METHODS

Collection of Samples

The crude oil contaminated water samples were collected from Manali Industrial area, Tamil Nadu, India.

Isolation and Identification of Microorganism

The soil samples were collected from the same place where the water sample collected and it was serially diluted and pour plate technique was followed for the growth of microorganisms. The organism was identified preliminarily by gram staining technique and biochemical tests.

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Experimental setup for oil contaminated water treatment

The *Pseudomonas sp.* was grown in 500 ml nutrient broth for the mass production. An aliquot containing 50 ml of crude contaminated water was taken in 18 sterilized conical flasks and the experiment was set up as shown in Table.1 [16].

All the flasks were tightly cotton plugged in order to avoid evaporation. The flasks were incubated for a week under room temperature. Results were recorded from each flasks everyday up to 6 days.

Table.1 Experimental setup

Flask No.	Water Sample (ml)	Culture (ml)	Chemical nutrients	Type
1-6	50	-	-	Control
7-12	50	1	-	Type A
13-18	50	1	ammonium phosphate, magnesium sulphate, potassium phosphate and sodium chloride	Type B

Table.2 Biochemical Test for *Pseudomonas sp*

S.No	Test	Result
1	Simmon's citrate test	+ve
2	Oxidase test	+ve
3	Catalase test	+ve
4	Indole test	+ve
5	H_2S production test	-ve
6	Starch hydrolysis test	+ve
7	Casein hydrolysis test	-ve

Estimation of oil degradation

Oil degradation was estimated by the process in which oil is converted to from that is no longer extractable by benzene [16].

RESULTS AND DISCUSSION

Pseudomonas sp. was isolated from oil contaminated soil collected from Manali and it was identified preliminarily by Gram staining technique and biochemical tests. (Table.2).

After the treatment, it was found that on 1st day type A showed 1.93% of degradation and type B showed 5.77. On 2nd day the degradation percentage was increased for both types and type A showed 7.7% degradation and type B had 11.54%. On third day the degradation

increased rapidly and type B degraded more oil (77%) than type A (71.16%). On 4th day, type B showed 98.08% degradation and type A showed 96.16%. On 5th day of degradation, type B degradation reached 99.81% while type A had 97.12%. On day 6, type B degraded all the oil contaminants which was present in water (100%) and type A showed 97.89%. This shows that the degradation is more when the additional inorganic nutrients were added (Table.3)

Table.3 Estimation of oil degradation

Day	Flask No.	X (ml)	Y (ml)	Z% (X/Y)	Degradation (%)
1st day	7	5.1	5.2	98.07	1.93
	13	4.9	5.2	94.23	5.77
2nd day	8	4.8	5.2	92.30	7.7
	14	4.6	5.2	88.46	11.54
3rd day	9	1.5	5.2	28.84	71.16
	15	1.2	5.2	23.00	77
4th day	10	0.2	5.2	3.84	96.16
	16	0.1	5.2	1.92	98.08
5th day	11	0.15	5.2	2.88	97.12
	17	0.001	5.2	0.19	99.81
6th day	12	0.11	5.2	2.11	97.89
	18	Nil	5.2	0	100

CONCLUSION

Bioremediation is a promising technology in the treatment of oil contaminated water bodies. The use of bacterial strains leads to fast and efficient degradation of oil. Hence further more work needs to be carried out to characterize the treated sample for checking its purity and quality.

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