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Biological reprocessing of water contaminated by refinery effluents collected from Manali, Tamil Nadu

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ABSTRACT

Increasing industrialization trends worldwide has generated effluents in large quantities with high organic content which are toxic to the soil and water. In this study, water samples that were contaminated by refinery effluents were collected from Manali, Tamil Nadu, India and were treated with *Pseudomonas sp.* isolated from soil present over there with different carbon sources such as glycerol (Type A) and glycerol, sucrose and maltose (Type B). The percentage of oil degradation was analyzed up to 6 days. On 5th day maximum degradation of oil was achieved by Type A (98.08%) while Type B showed 97.70%. But on 6th day Type B reached 98.08% degradation and Type A remained the same.

Keywords: *Pseudomonas sp.*, Oil Degradation, Microbial degradation, Carbon source.

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INTRODUCTION

The three most important things for human survival are air, water and food. Among these, second main priority is given to water, since without water nothing exists. This essential water is nowadays, largely polluted by industrial waste water. Petrochemical industries and petroleum refineries generate large amounts of major pollutants. These mainly include hydrocarbon specifically aliphatic hydrocarbons. Hydrocarbon degradation has been widely reported in laboratory scale batch studies [1-3]. Several physical-chemical methods have been used for treating this contaminated water [4, 5]. These methods are usually expensive and involve risk spreading pollution [6]. The traditional treatment of oily waste water cannot degrade the crude oil completely [7].

Microbial degradation is one of the foremost ways in the removal of these oily pollutants from the water. Several researchers have studied the use of microbes to decompose petroleum products and have demonstrated this to be a promising technological alternative [6, 8, 9, 10, and 11]. Advances in biotechnology have confirmed that several compounds present in petroleum hydrocarbons are consumed by microorganisms as a sole source of carbon. It has been proved that addition of nitrogen and phosphorous substantially enhances the growth of hydrocarbon degrading microbes [12].

Microbial degradation targets the aliphatic or light aromatic fractions of oil. Several microbial species live on hydrocarbons and are responsible for the biodegradation of crude oil [13, 14]. For microbes to act on oil, it needs to be brought to a soluble state. Fungi and bacteria are the key agents which decompose oil and oil products. Isolation and identification of microorganisms responsible for hydrocarbon transformations have long been acknowledged as pivotal from both fundamental and applied points of view and various hydrocarbon degrading organisms like bacteria, yeast, fungi and algae are available [12, 15]. 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 sample was collected from Manali Industrial area, Tamil Nadu, India.

Isolation and Identification of Microorganism

The soil sample was 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].

Table 1: Experimental Setup

Flask No.	Water Sample (ml)	Culture (ml)	Chemical nutrients	Type
1-6	50	-	-	Control
7-12	50	1	Glycerol	Type A
13-18	50	1	Glycerol, Sucrose, Maltose	Type B

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.

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).

Table 2: Biochemical Test.

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

After the treatment, it was found that on 1st day, there is no significant difference in both types and both the types showed 3.85% of degradation. On 2nd day the degradation percentage was increased for both types and type A showed 5.77% degradation and type B had 9.62%. On third day the degradation increased rapidly and type A degraded more oil (55.77%) than type B (19.24%). On 4th day, type A showed 94.24% degradation and type B showed 92.31%. On 5th day of degradation, type A degradation reached maximum of 98.08% while type B had 97.70%. On day 6, type B degraded 98.08% oil while type A remains the same. This

indicates instead of adding too many carbon sources, glycerol alone may be used as a carbon source along with Pseudomonas sp. (Table.3) for better degradation.

Table 3: Analysis of oil degradation

Day	Flask No.	X (ml)	Y (ml)	Z% (X/Y)	Degradation (%)
1st day	7	5.0	5.2	96.15	3.85
	13	5.0	5.2	96.15	3.85
2nd day	8	4.9	5.2	94.23	5.77
	14	4.7	5.2	90.38	9.62
3rd day	9	2.3	5.2	44.23	55.77
	15	4.2	5.2	80.76	19.24
4th day	10	0.3	5.2	5.76	94.24
	16	0.4	5.2	7.69	92.31
5th day	11	0.1	5.2	1.92	98.08
	17	0.12	5.2	2.30	97.70
6th day	12	0.1	5.2	1.92	98.08
	18	0.1	5.2	1.92	98.08

CONCLUSION

Microbial degradation is an advanced technology in the treatment of water contaminated by the refinery effluents. 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 water sample for reuse.

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