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Isolation and Enrichment of Microbes for Degradation of Crude Oil

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Abstract- Crude oil contamination creates a lot of hazard to the environment and remediation of crude oil is one the major area of interest for researchers. In the present study, microbes capable of crude oil degradation were isolated from crude oil contaminating site. Efficiency of these microbes for degradation of crude oil was enhanced and their degradation capacity was determined.

Keywords: enrichments of microbes, crude oil degradation, bioremediation.

I. INTRODUCTION

Petroleum based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. [1] The amount of natural crude oil spoilage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year. Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution. [1-2] Soil contamination with hydrocarbons causes extensive damage of local systems since accumulation of pollutants in animals and plant tissue may cause death or mutations. The technology commonly used for soil remediation includes mechanical burying, evaporation, dispersion, washing and bioremediation. [1-6]

The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry. [2],[5],[6]In addition, bioremediation technology is believed to be non invasive and relatively cost-effective. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and is cheaper than other remediation technologies. A wide array of microorganisms including fungi, algae and bacteria are known to degrade PAHs. [3-10] However, bacteria play by far the most important role in complete mineralization. Fungi on the other hand mainly biotransformation PAHs, namely, detoxification to less or nontoxic metabolites which can then be acted upon by other organisms.

This study focuses on isolation of potential microbes capable of degrading crude oil and transformation of their plasmid to non-degrading microbes to enable them for crude oil degradation.

II. MATERIALS AND METHOD

A. Sample Collection

Crude oil and crude oil contaminated soil were collected from ONGC oil well near Adraj village, Ahmedabad, India in sterile bottles and store at low temperature. Samples were immediately taken to the laboratory for further analysis.

B. Isolation of Microbes

Bushnell Hass (BH) media containing crude oil (less viscous in nature) as sole carbon source (1.0 %) was used as selective media for screening of crude oil degrading microbes. Plates were allowed to incubate at 37ºC until visible colonies form.

C. Enhancement of crude oil degradation capacity

To enhance the crude oil degradation capacity of microbes, they were transferred to the BH media containing increasing concentration of crude oil. At least four transferred were given to the each microbe.

D. Determination of crude oil degradation

At regular interval, aliquots from the media were collected and centrifuged to remove the microbial biomass. Optical density of remaining supernatant was measured to determine the crude oil degradation keeping non-degraded sample as control.

E. Determination of Biomass

Biomass was collected by centrifugation at 10,000 rpm and dried in oven until constant weight is obtained. Weight is taken in milligrams using Metter Toledo weighing balance having accuracy of 1 mg.

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III. RESULTS AND DISCUSSION

Six strains were selected from the isolated microbes which have potential to degrade crude oil with faster rate. These strains were further used for the experiment. The strains were designated xrfgp, xrfg1, xrfg4, xrfg11, xrfg12 and xrfg13. These strains will be identified using 16s rRNA sequencing.

It is known that certain microbes are capable of degradation of compound like PAHs because of presence of degradative plasmid. [11-12] Degradation capacity and rate of degradation of these compounds are highly depends on the number of plasmids in the cell. [11] It is known that the copy number of plasmid could be increased by transferring them to another media containing the same substrate for more than one time. [6] This process enabled microbes to grow very rapidly in the media within smallest lag phase. In this experiment, it was notice that as microbes were grown repetitively in crude oil containing media; their efficiency to grow in crude oil containing media was enhanced. In the first set of experiment it took longer incubation time to degrade 90.0% crude oil (78 – 98hrs), when a transferred was given to another same media, it took comparatively lesser time (26 -72) and at the end of 3 successive transfers, it was found that the microbes can degrade 90.0% of crude oil within 24 - 48 hrs.(Table 1.) It was also noticed that the as the number of transfer increases the lag phase of microbial growth curve decreases. We also assume that it is also possible that the initial lag phase could be reduced further by giving repetitive transfer to the respective media for several times.

Table 1. Results obtained from serial transfer of microbes for degradation of crude oil

Strain	Time in hrs			
	Initial Transfer	1st Transfer	2nd Transfer	3rd Transfer
xrfg1	96	78	50	24
xrfg4	98	98	48	38
xrfgp	75	72	26	26
xrfg11	98	96	52	48
xrfg12	78	75	48	28
xrfg13	120	98	72	48

Results indicate that as the strains were given transfer to another media their degradation rate increases gradually. Higher the number of transfer results into faster degradation with shorter lag phase.

A. Determination of crude oil degradation

From the results, it was found that for initial 8 hrs, microbes were not able to degrade crude oil. This is because unavailability of enzymes required for degradation of crude oils and also less population of microbes. [5],[9] This results into longer lag phase of microbial growth curve. Once the enzymes were synthesized, they started degradation of crude oil at an exponential rate and degraded more than 50.0% of crude oil within 12 hrs of incubation in all the cases (Figure 1.). It was also noticed that xrfg1, xrfg11 and xrfg12 have degraded almost 90.0% of crude oil in the same period of time after which it became constant. This shows that these strains have potential ability for degradation. While the maximum efficiency of xrfgp, xrfg13 and xrfg4 was found less than 70.0% even after 24 hrs which indicates the limitation of degradation efficiency of these microbes. These results indicate that each microbe has its own capacity of degradation beyond which it cannot act further even if favorable conditions are provided. [3][4]

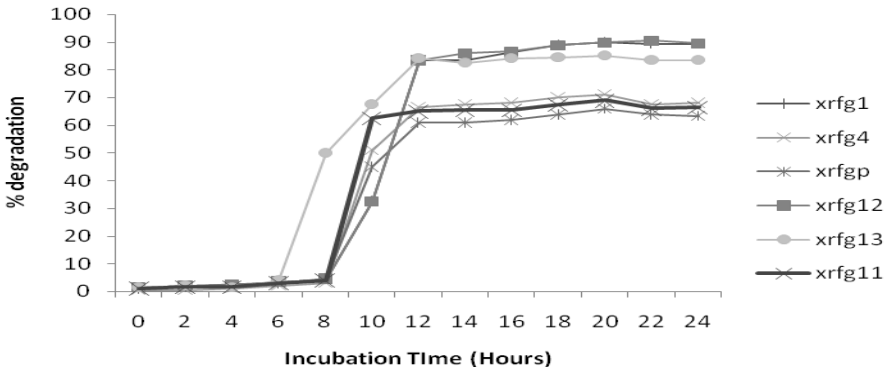

Fig 1. Rate of crude of degradation by different microbes

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Figure indicates that for the initial 8 hrs incubation no significant degradation was observed but after this degradation increases in an exponential way and reached maximum at 12 hrs.

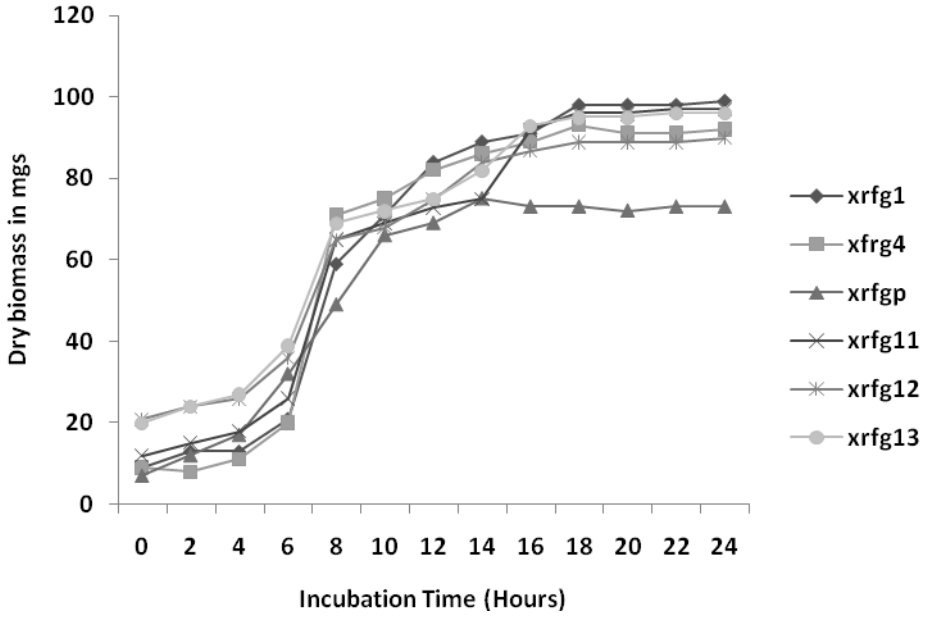

Fig 2. Biomass production: Figure indicates that in the initial period no net production of biomass was seen. It increases exponentially and became maximum at 18 hrs of incubation after which it became stable.

B. Determination of Biomass

There was no significant change in biomass concentration was observed during first 8 hrs of incubation, after this biomass increases exponentially and reached maximum at 18 hrs in most of the cases. Longer lag phase is because of adaptation of microbes for their growth in the media containing crude oil as sole carbon source. This forces microbes to produce degradative enzymes to degrade crude oil to utilize it as a source of energy.[6],[10] Not only this it also produces certain enzymes which were capable of producing certain secondary metabolites. [10] Except xrfgp all the other strains have produced more than 80mgs of dry biomass per 100 ml of broth in 24 hrs.(Figure 2.) When the produced biomasses were correlated with degradation rate, a direct correlation was observed between biomass and rate of degradation as the biomass increases the degradation also increases. This is because higher the number of microbes in the media leads to faster rate of degradation. [2],[4]

IV. CONCLUSION

From this study it is concluded that serial transfer of microbes in similar media increases their efficiency of degradation and it also favours higher growth rate in the same media by reducing the lag phase. It was also noticed that as the biomass increases degradation rate also increases.

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