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Enhancing Hydrocarbon Degrading Property by Assemblage of Effective Microbial Consortium by Potential Degraders Isolated from Polluted Site

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Abstract Consortium application in bioremediation is well documented. A group of microorganism being capable of utilizing and breaking down complex compounds into intermediate derivatives, which is then consumed and/or broken down to simpler compounds by another group of microorganisms. This is a synergistic conversion which renders pollutants harmless. This is especially helpful approach with pollutants that are complex and recalcitrant in nature such as petroleum or pesticides based pollutants. In nature the group of microorganisms come together in this synergistic biodegradation process. But so as to make this bioremediation process faster and more vital, effective group of microorganisms are used to prepare the consortium. This is done by isolating hydrocarbon utilizing microorganisms from a polluted site and scrutinizing them by series of tests. These isolates are then combined in a group and a consortium is prepared. The degradative properties is thus enhanced by using this consortium instead of individual isolates.

Keywords: *effective microbial consortia, hydrocarbon utilizing microorganisms, synergistic mechanism, bioremediation*

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1. Introduction

Petroleum is a complex mixture of hydrocarbons and other organic compounds. Petroleum recovered from different reservoirs varies widely in composition and physical properties. It is a heterogeneous mixture of hydrocarbons including aliphatic, alicyclic and aromatic in varying concentration depending upon the origin and nature. [12] Long recognized as substrates supporting microbial growth, these hydrocarbons are both a target and a product of microbial metabolism. Microorganisms are equipped with metabolic machinery to use petroleum as a carbon and energy source. [7] Being hydrophobic, petroleum derivatives are recalcitrant in nature. Limited range of petroleum compounds is metabolized by individual microorganisms. Mixed populations with variety of enzymatic capacities shows effective hydrocarbon degradation. Microbial consortia that consist of different strains have been detected in hydrocarbon-contaminated soil, water or sediment samples. This strongly suggests that each strain has specific role in the degradation processes. Thus using these indigenous microbial population, having hydrocarbon degrading capacity, to construct a consortia can result into an effective microbial consortia, which is able to degrade complex hydrocarbons into simpler compounds in an efficient manner. Thus screening of individual microbe for hydrocarbon degradation is an important step to develop the bioremediation processes.

The present study represents isolation of hydrocarbon degrading individual microorganisms, scrutinizing each for their degradative properties and constructing an effective microbial consortium with enhanced hydrocarbon degrading capacity.

2. Material & Method

2.1. Indigenous microorganism isolation and screening

A hydrocarbon polluted site was identified from south Gujarat region and soil sample was collected. This soil sample was then suspended in Mineral Salt Medium (MSM) for enrichment. Then further isolation was carried out using petrol as hydrocarbon based substrate. After 48 hours of incubation, colonies were observed. The colonies so formed were further scrutinised for their degradative properties by Blue Agar plate, Blood agar method to check the haemolytic properties and confirmatory Phenol – Sulphuric acid test [13].

2.2. Blue Agar Plate

This method was developed by Wegnar *et al.,* (1991). The microbes of interest are inoculated on a light blue

mineral salt agar plate containing cationic surfactant Cetyl-trimethyl-ammonium bromide (CTAB) and the basic dye methylene blue. If microbes are able to degrade hydrocarbons, they secret anionic surfactants which form a dark blue, insoluble ion pair with cetyl-trimethyl-ammonium bromide and methylene blue. The positive isolates will thus form dark blue coloured colonies [13].

2.3. Blood Agar Haemolysis

This method was developed by Mulligan *et al*., (1985) ; and is based on the fact that hydrocarbon degrading microbes are able to haemolyse the red blood, observe for zone of hemolysis [13].

2.4. Confirmatory Test: Phenol- Sulphuric Acid Test

The positive isolates were further scrutinized by Phenol – Sulphuric acid test [13].

Only 6 isolates gave these tests positive and thus were studied further.

2.5. Hydrocarbon Degradation

The positive isolates so formed were checked for their individual hydrocarbon degradation properties by using 0.5 % petrol as hydrocarbon substrate for 5 days of incubation. The Optical Density (OD) was then measured to determine their growth. Increased OD suggested increase in growth i.e. consumption of petrol by the individual isolate. This way, all the individual isolates were scrutinised.

2.6. Microbial Consortia Preparation and Comparative Study

The isolates so formed were clubbed into group of two isolates: Consortium - I and three isolates to prepare a consortium, Consortium-II. These consortium was checked for their degradative properties, in the same way as the individual isolates, i.e. using 0.5% Petrol and the results were compared.

3. Result and Discussion

Hydrocarbon utilization property of microorganisms is well studied and discussed. Role of consortium in bioremediation of hydrocarbon based pollution is also studied and reported widely. Hydrocarbons are complex compounds made up of various components, are hydrophobic and recalcitrant. Many times due to accumulation of recalcitrant compounds and low availability of hydrocarbons, complete hydrocarbon degradation is not possible in field conditions [14]. Hydrocarbons such as petrol are thus not biodegraded by a single microorganism. Hence a consortium; a group of microorganisms is required to fulfil this task effectively. Here the consortium is prepared by combining effective microorganisms which are successfully identified as potential hydrocarbon degraders.

In the following Figure 1, the OD of individual isolates and constructed consortiums are shown.

Figure 1.a. shows the OD of individual isolates. The maximum OD shown is below 0.3, shown by isolate D. Figure 1.b and Figure 1.c shows the OD of consortiums.

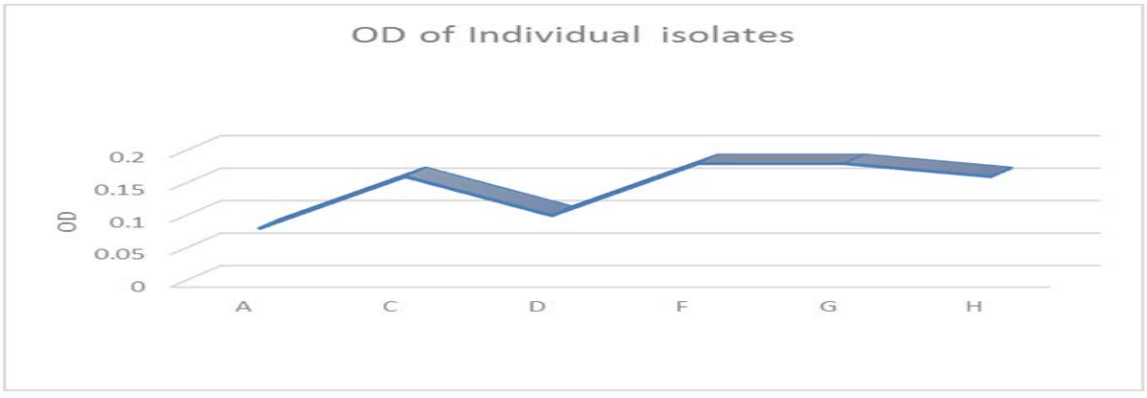

Figure 1.a. OD of individual isolates

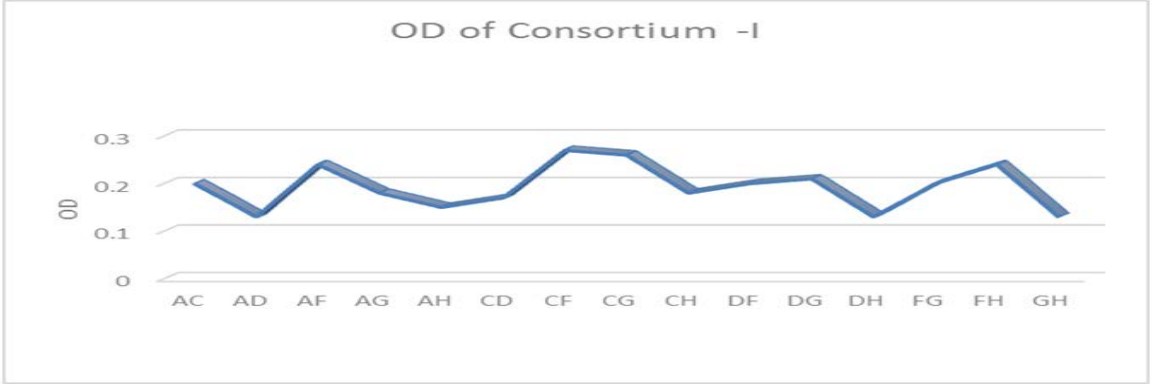

Figure 1.b. OD of Consortium – I

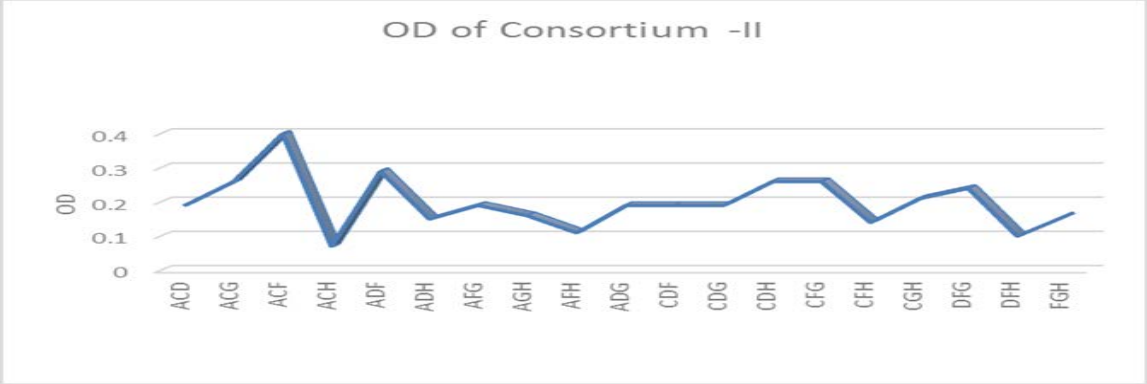

Figure 1.c. OD of consortium –II

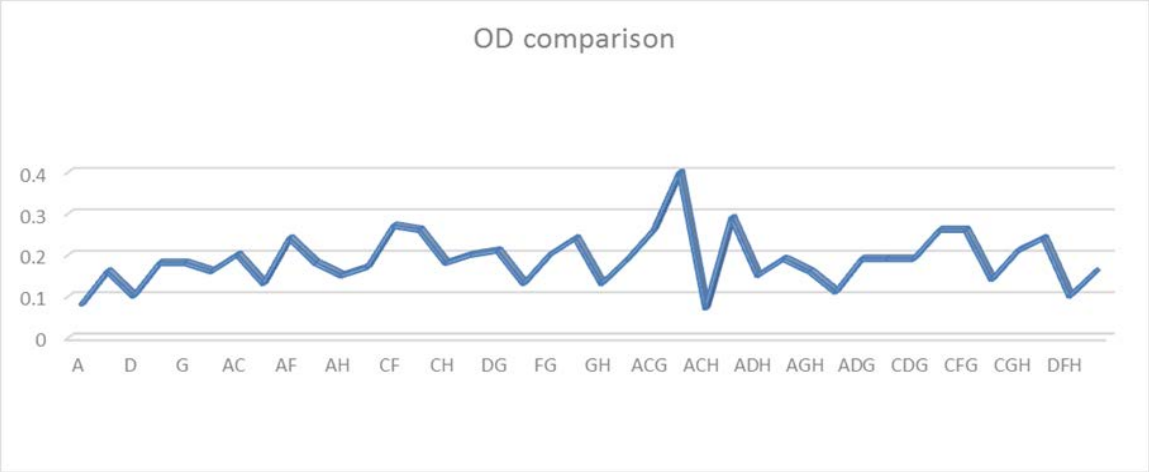

Figure 2. Comparison of OD of Individual Isolates and consortium

The Figure 2 shows comparison of OD of individual isolates and Consortium. It clearly shows that the OD shown by consortiums is higher than that of individual isolates. The highest OD shown by ACF consortium of 0.4.

Identification of Microorganisms: Isolated microorganisms has been identified by gene sequencing (Saffron's Gene Laboratory) and further submitted to NCBI. The submitted sequences got accession numbers as below:

Table 1

Isolate	Strain	Accession number
A	*Pseudomonas aeruginosa* strain RRLP1	KU314415
C	*Pseudomonas aeruginosa* Strain AS-1	KU314416
D	*Pseudomonas aeruginosa* Strain SI5(1)3	KU314417
F	*Pseudomonas spp.* Strain 14-1	KU314418
G	*Pseudomonas aeruginosa* RRLP1	KU314419
H	*Pseudomonas aeruginosa* Strain JQ-41	KU314420

4. Conclusion

The study represented enhanced Hydrocarbon degrading property by using a consortium, using petrol as a hydrocarbon based substrate. This suggests that consortium prepared by using effective microorganisms has better degradative capacity as compared to individual isolates. Further study using different substrate and on a larger scale is recommended.

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