



INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X

Impact factor: 4.295

(Volume2, Issue6)

Available online at: www.Ijariit.com

RESPONSE OF BACTERIAL ISOLATES TO VARIOUS ANTIBIOTICS ISOLATED FROM PETROLEUM SPILLED SOIL

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Abstract:-All the bacterial isolates were studied for their responses to various antibiotics. Out of 28 bacterial forms, 12 forms were resistant to ampicillin, 10 forms to penicillin, 3 forms to erythromycin, 5 forms to streptomycin and only one isolate was resistant to tetracycline. Indicating that tetracycline was the most effective antibiotic for these bacteria.

Key Words: Antibiotics, Petroleum, Response hydrocarbon bioremediation sensitivity resistance.

I. INTRODUCTION

Petroleum microbiology is the study of interaction of microbiology with petroleum. One such environment is hydrocarbon systems which include petroleum and their products, a complex chemical mixture that contains predominantly hydrocarbons. Diverse microbial population can metabolize the hydrocarbons found in petroleum. The metabolism of hydrocarbons by micro organisms ultimately forms CO₂, H₂O carbon dioxide water and release of energy.

Microbial hydrocarbon degradation can be used for the disposal of oily wastes and for the bioremediation of oil polluted sites.

On the other hand microbial metabolism must be controlled and regulated to prevent corrosion of tanks and bio deterioration of fuels. Microorganisms are used in the petroleum industry to enhance oil recovery and in the production of alternate fuels.

II. MATERIALS AND METHODS

The nutrient agar with 1% dextrose dissolved by streaming was dispensed into tubes and flasks. Sterilized at 15 PSI for 15 minutes and then placed in water bath at 40-50⁰C. The medium (12-15ml) was poured into petriplates form flasks as 'basal layer' and allowed to solidify at room temperature.

One drop of 18-24 hours nutrient broth culture was added to tube of nutrient agar with dextrose medium, it was mixed by shaking, was poured on to evenly distribute over previously solidified basal layer. The layer of medium plus inoculum was the "seed layer".

Petri plates were immediately placed in the refrigerator for half hour, solidifying the seed layer and to retard the growth of the inoculum.

Aseptically place commercially prepared antibiotics discs (Ampicillin, Erythromycin, Penicillin, Streptomycin and Tetracycline) on the surface of the seed layer. Incubated at $28\pm^0\text{C}$ for 24-48 hrs. A zone of inhibition around discs was recorded as positive for sensitivity (Schaad, 1988) and no zone as negative.

III. OBSERVATION

All the bacterial isolated were examined for their sensitivity and resistant to various antibiotics. Formation of zone of inhibition (ZI) of various sizes was taken as sensitive and its total absence as resistant. Commercially available discs of five antibiotics (Ampicillin, Erythromycin, Penicillin, Streptomycin and Tetracycline) were used.

The result obtain are as under: -

Isolate I	A>S>T>E	Resistant to penicillin
Isolate II	E>T=S	Resistant to penicillin and ampicillin
Isolate III	T=S>E>A	Resistant to penicillin
Isolate IV	T>E=A	Resistant to penicillin and streptomycin
Isolate V	T>P>E>S	Resistant to ampicillin
Isolate VI	T>E=S	Resistant to ampicillin and penicillin
Isolate VII	T>E>P=A	Resistant to streptomycin
Isolate VIII	E>T>P=S	Resistant to ampicillin
Isolate IX	T>S>A=E	Resistant to penicillin
Isolate X	T>A>E>S	Resistant to penicillin
Isolate XI	T>E=A>S	Resistant to penicillin
Isolate XII	A>S>P>E>T	
Isolate XIII	A>S>E>T	Resistant to penicillin
Isolate XIV	E>P>T	Resistant to ampicillin and Streptomycin
Isolate XV	T>S>P	Resistant to erythromycin and ampicillin
Isolate XVI	E>T>A>S	Resistant to penicillin
Isolate XVII		

Isolate XVIII	T>E>S>P	Resistant to ampicillin
Isolate XIX	E>T>P	Resistant to Streptomycin and ampicillin
Isolate XX	E>S=P>T	Resistant to ampicillin
Isolate XXI	E>T>S>P=A	
Isolate XXII	S>T>A=P	Resistant to erythromycin
Isolate XXIII	A>P>E>S	Resistant to tetracycline
Isolate XXIV	T>E>P=S	Resistant to ampicillin
Isolate XXV	S>A>P>T	Resistant to erythromycin
Isolate XXVI	E>T>S>P	Resistant to ampicillin
Isolate XXVII	T>E>S=P	Resistant to erythromycin
Isolate XXVIII	T>E>P=S=A	
	E>P>T>A	Resistant to Streptomycin

The response of the bacterial isolates obtained from the hydrocarbon spilled habitats to various antibiotics this experiment was designed. All bacterial forms were examined for their sensitivity/resistant to various antibiotics. Formation of zone of inhibition (ZI) of various sizes was taken as sensitive and its total absence as resistant.

The antibiotics were used in the form of discs and as constituents of media. The use of disc is more convenient since in one petriplate it is possible to study the sensitivity of several antibiotics against a single bacterium at a time. In the present time, antibiotics sensitivity is being extensively used to separate various species. Janse and Split (1989) used this character for the separation of various species of the genus *Erwinia*. The antibiotics were also used in the media for the isolation of specific strains of bacteria by Kado and Heskett (1970) and Sands and Hankin (1975).

In the present study five antibiotics discs of ampicillin, penicillin, erythromycin, streptomycin and tetracycline were used. It was observed that out of 28 bacterial isolates, 12 forms were resistant to ampicillin, 10 forms to penicillin, 3 forms to erythromycin, 5 forms to streptomycin and only one isolate was resistant to tetracycline. Indicating that tetracycline is the most potent antibiotic.

TABLE: SUSCEPTIBILITY / RESISTANCE OF BACTERIAL ISOLATIONS TO VARIOUS ANTIBIOTICS

S.No.	Isolates No.	Ampicillin	Erythromycin	Penicillin	Streptomycin	Tetracycline
1	I	5	1	-	4	3
2	II	-	6	-	1	1
3	III	1	2	-	3	3
4	IV	4	5	-	-	5
5	V	-	1	2	0.9	3
6	VI	-	2	-	2	3
7	VII	2	5	2	-	6
8	VIII	-	5	1	1	2
9	IX	4	4	-	5	6
10	X	2	1	-	0.9	3
11	XI	2	2	-	1	3
12	XII	4	1	2	3	0.9
13	XIII	4	2	-	3	1
14	XIV	-	5	2	-	1
15	XV	-	-	5	6	10
16	XVI	8	18	-	4	15
17	XVII	-	3	1	2	5
18	XVIII	-	6	1	-	2
19	XIX	-	3	2	2	1
20	XX	1	6	1	2	4
21	XXI	1	-	1	4	2
22	XXII	12	3	11	2	-
23	XXIII	-	6	2	2	10
24	XXIV	4	-	3	5	2
25	XXV	-	15	1	3	4
26	XXVI	-	4	1	1	5
27	XXVII	1	3	1	1	4
28	XXVIII	1	8	4	-	2

Figures indicate the size of zone of inhibition in mm.

- = Resistance

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