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Effect of Metal Ions on Oil Degradation Activity of *Bacillus and Acinetobacter* Isolated from Oil Contaminated Sites of Mechanical Workshops

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Abstract: ***Used engine oils released from various automobile mechanical workshops pollute the environment. The various toxic chemical compounds present in the used engine oil cause deleterious effects to environment and living beings. In nature, there are some microbes which have the ability to degrade organic pollutants present in used engine oils. Such microbes can be isolated, identified and can be used for biodegradation of organic pollutants. In the present paper, effect of different metal ions on oil degradation activity of two bacterial species viz., Bacillus and Acinetobacter was determined using minimal salt broth supplemented with used engine oil and different metal ions. Majority of the metal ions selected for the study were found to be important for the enhancement of oil degradation activity of both the bacteria. Presence of only two ions viz., Fe^{3+} and Mn^{2+} decreased the oil degradation ability of the bacteria.***

Keywords: Used engine oil, organic pollutants, oil degradation, *Bacillus*, *Acinetobacter*, metal ions

1. Introduction

Automobile mechanical workshops are the main sources of used engine oils. Used engine oils contain heavy metals, wide variety of aliphatic and polyaromatic compounds. Used engine oils when disposed from mechanical workshops and other sources reach surroundings and cause environmental pollution. Especially, some of the polyaromatic compounds present in used engine oil are highly mutagenic and carcinogenic. Naphthalene, one of the most toxic polyaromatic compounds adversely affects the various human organs like kidney, heart, lungs etc. The chemical compounds present in the used engine oil change the physico-chemical conditions of soil and immobilize plant nutrients [1,2,3]. Some microorganisms in the environment have the ability to metabolize the hydrocarbons present in used engine oils. These microbes can be employed for the degradation of toxic hydrocarbons. The process of degradation of organic pollutants by employing microbes is called biodegradation. Microbes which can degrade organic pollutants include *Mycobacterium, Streptococcus, Bacillus* etc. Biodegradation process is the main technique employed in bioremediation [4]. In the present work twenty four oil degrading bacteria were isolated from oil contaminated soils of mechanical workshops of Autonagar, Kaman region of Karimnagar town, Telangana and identified till genus level. Thirteen were identified as *Bacillus* species, four were *Acinetobacter* species, three were *Pseudomonas* and remaining three were *Micrococcus* species. Bacterial species belonging to four genera (viz., *Bacillus, Acinetobacter, Pseudomonas* and *Micrococcus*) were isolated. In the present paper, two bacterial species each one from two bacterial genera (viz., *Bacillus* and *Acinetobacter*) exhibiting highest oil degrading activity were selected and effect of different metal ions on the oil degradation of activity these two bacteria was studied.

2. Materials and Methods

Medium and metal ions used

Effect of different metal ions on oil degradation activity of *Bacillus* and *Acinetobacter* was carried out using minimal salt medium (MS medium) supplemented with used engine oil (5%) as sole carbon for the growth of bacteria [5]. Effect of seven metal ions on oil degradation activity of bacteria was determined. The metal ions selected were $Fe3^{+}$, Co^{2+}, Cu^{2+}, Ca^{2+}, Mn^{2+}, MoO_4^{2-} and Zn^{2+}. The MS medium with 5% used engine oil and all seven metal ions is considered as negative control and MS medium with 5% used engine oil without any ions as positive control. The effect of absence of each ion on oil degradation ability of bacteria was studied in the presence of remaining all ions in the medium [6].

Inoculum preparation

The bacterial cultures were inoculated separately into 10 ml of MS broths supplemented with used engine oil (5%) as sole carbon source and incubated at 30^0C for one day. After one day each bacterial culture broth is subjected to centrifugation at 3500 rpm and cell pellets of bacteria were obtained. The bacterial cell pellets were washed and grown in MS broth supplemented with used engine oil till the optical density (OD) of each culture was read 1.0 at 600nm in colorimeter [7.] Each such 0.5 ml of bacterial culture was used as inoculum and transferred to 10 ml of MS medium (with used engine oil and different metal ions combinations prepared separately) and incubated for seven days. After incubation period the OD of the bacterial cultures biomass was measured at 600 nm.

The growth of bacteria was measured in terms of OD of their cell mass in culture broth. As the broth contains used engine as the only source of carbon the growth of bacteria is the direct measure of oil degradation activity. All the experiments were carried out with triplicates and standard deviation (SD) value was calculated for each result.

3. Results and Discussion

In the present work effect of absence of selected metal ions on biodegradation ability (in terms of growth) of *Bacillus* and *Acinetobacter* was carried out. Majority of the cellular enzymes activity is under the influence of metal ions. Metal ions act as enzyme activators. Metal ions change flow of electrons in either substrates or enzymes and control the biocatalysis [8]. On the other hand some metal ions inhibit the activity of certain enzymes. One of the key roles of certain metal ions is involvement in the formation of protein synthetic machinery [9]. In the absence of Fe^{3+} and Mn^{2+} ions the biodegradation ability (growth) of the bacteria increased when compared to growth observed in negative control and positive control (Table-1). Jernejc and Legisa (2002) reported that Fe^{3+} inhibited the activity of malic dehydrogenase [10]. In *Lysobacter enzymogenes* Mn^{2+} ions (at concentration more than 0.01 mM) inhibited the formation of nuclease, RNAase and alkaline phophatase [11]. In the absence of Ca^{2+}, Co^{2+}, Cu^{2+} and MoO_4^{2-} ions the bacteria showed decreased oil degradation activity (growth) (Table-1). In *Myriococcum thermophilum* and *Humicola insolens* the Ca^{2+} ions enhanced the cellobiose dehydrogenase activity [12]. In the presence of Co^{2+} ions the activity of alkaline phosphatase was increased in *Bacillus licheniformis* [13]. Cu^{2+} ions (in the form of $CuSO_4$) increased the activity of ascorbate oxidase in callus of Cucumber [14]. Al-Issawi reported from his studies on two wheat cultivars that presence of MoO_4^{2-} ions had enhanced the activity of antioxidant enzymes [15]. In both the bacteria the growth was adversely affected in the absence of Zn^{2+} ions in the medium (Table-1). Similar results were obtained for Alves (2007) while working on dibenzothiophene desulfurizing *Gordonia alkanivorans* strain 1B [6].

Table 1: Effect of Metal ions on growth (oil degradation activity) of *Bacillus* and *Acinetobacter*

Sl. No	Metal ion	OD (Growth) of *Bacillus* at 600 nm	OD (Growth) of *Acinetobacter* at 600 nm
1	Negative control (contains all ions	3.35 ± 0.14	3.03 ± 0.05
2	Positive control (contains no ions)	1.81 ± 0.09	1.60 ±0.12
3	Fe^{3+} absence	3.75 ± 0.08	2.40 ± 0.08
4	Co^{2+} absence	3.05 ± 0.05	2.73 ± 0.05
5	Cu^{2+} absence	2.61 ± 0.12	3.17 ± 0.14
6	Mn^{2+} absence	3.65 ± 0.17	2.60± 0.09
7	MoO_4^{2-} absence	2.91 ± 0.09	3.27 ± 0.05
8	Zn^{2+} absence	3.35 ± 0.14	1.83 ± 0.17
9	Ca^{2+} absence	1.81 ± 0.05	2.93 ± 0.05

4. Conclusion

The oil degrading bacteria *Bacillus* and *Acinetobacter* exhibited decreased oil degradation activity (growth) in the absence of Ca^{2+}, Co^{2+}, Cu^{2+} MoO_4^{2-} and Zn^{2+} ions indicating their role in growth and metabolic activities. Only two metal ions viz., Fe^{3+} and Mn^{2+} decreased the growth of both the oil degrading bacteria. Overall, Zn^{2+} ions can be regarded as key metal ions for growth and various cellular activities as its absence adversely affected the oil degradation activity (growth) of both the bacteria.

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