Optimization of Growth Parameters of Naphthalene Degrading Bacteria Isolated From Oil Contaminated Soils

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ABSTRACT:

Polycyclic aromatic hydrocarbons (PAHs) released from various sources are reaching the environment. They are hazardous to vegetation and human health. The PAHs are carcinogenic and mutagenic. They cause various disorders among humans. Bioremediation which makes use of microbes to degrade such PAHs is a safe approach to remove PAHs from environment. The naphthalene is considered as model compound for PAHs biodegradation studies. In the present work three naphthalene degrading bacterial isolates viz., Bacillus, Micrococcus and Pseudomonas were grown in BSM broth supplemented with naphthalene and physical parameters were optimized. All the bacteria showed maximum naphthalene degrading ability at pH 7.0. Bacillus exhibited highest naphthalene degrading activity at 330 C whereas Micrococcus and Pseudomonas at 310 C.

Keywords: Polyclic aromatic hydrocarbons, Naphthalene, Bioremediation, Bacteria, Physical parameters, Bacillus, Micrococcus, Psuedomonas

I. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely found in fossil petroleum products and coal. These compounds are released into the environment from industries, mechanical workshops and other human activities. These compounds adversely affect the plants, nutrient cycles and human health. They are toxic and mutagenic. Department of Health and Human Services of United States of America declared fifteen polycyclic aromatic compounds as carcinogenic. They enter into the food chain and magnify. They cause skin related problems and other disorders in humans [1,2,3]. Bioremediation is the process which makes use of microorganisms to degrade/detoxify the PAHs. Bioremediation is an environmental friendly and safe practice to clean up the environment [1]. Naphthalene is considered as model compound for the studies of PAHs biodegradation [4]. In the present work fourteen naphthalene degrading bacteria were isolated from oil contaminated soils of four mechanical workshops of Kaman area of Karimnagar, Telangana and identified till their genus level. Eight were identified as *Bacillus*, three were *Pseudomonas* and remaining three were identified as *Micrococcus*. In the present paper optimization of physical parameters of growth and naphthalene degrading process are studied.

II. MATERIALS AND METHODS

Effect of physical parameters (pH and temperature) on naphthalene degrading ability of the bacteria was studied using Basal salt mineral (BSM) medium (K_2HPO_4, 0.38g; $MgSO_4.7H_2O$, 0.2g; NH_4Cl, 1.0g; $FeCl_3$, 0.05g; Distilled water, 1.0 liter with pH, 7.0) liquid medium supplemented with 1.0g peptone and 0.1% naphthalene [2]. The naphthalene is the only carbon source present in the medium. Hence, growth of the bacteria is the indicator of naphthalene degradation ability of the bacteria (more growth more naphthalene degrading ability).

Inoculum source

Each bacterial culture was transferred into 10 ml of BSM broth supplemented with 0.1% naphthalene and incubated for one day at 30^0C. After incubation period the culture was centrifuged at 3500 rpm and a cell pellet is obtained. Then each cell culture pellet was inoculated into BSM broth supplemented with 0.1% naphthalene and cultivated till the optical density of each culture broth was 1.0 at 600 nm. Such 1 ml bacterial cultures were used as source of inoculum [5].

Optimization of physical parameters (pH and temperature) for naphthalene biodegradation

Each bacterial culture was cultivated at different pH ranging from 5.0 to 8.0 at 30^0C to determine the optimum pH for maximum naphthalene degradation activity. Then each bacterial culture was grown at different temperatures ranging from 30^0C to 40^0C at its determined optimum pH. Triplicates were maintained for all the experiments.

III. RESULTS AND DISCUSSION

The three naphthalene degrading bacterial isolates, *Bacillus, Pseudomonas and Micrococcus* isolated from oil contaminated sites of four mechanical workshops were cultivated in BSM broth supplemented with 1% peptone and with 0.1% naphthalene as sole source of carbon. The bacteria were grown different pH conditions to determine the optimum pH and temperatures to optimize the physical parameters required for maximum naphthalene biodegradation. The growth was measured in terms of cell density which is measured at 600 nm in a colorimeter. The growth is the measure of naphthalene degradation activity. The optimum pH for naphthalene biodegradation (growth) required for all the three bacteria was pH 7.0. The optimum temperature required by *Bacillus* for maximum naphthalene degradation activity was 33^0C where as *Micrococcus and Pseudomonas* exhibited highest naphthalene degradation activity at 31^0C [6].

Table-1: Effect of pH on naphthalene degradation activity (growth) of bacteria

S. No.	Bacterial Isolate	OD values at 600 nm at different pH conditions			
		5.0	6.0	7.0	8.0
1.	*Bacillus*	0.89±0.12	0.82±0.05	1.21±0.09	1.05±0.09
2.	*Micrococcus*	0.68±0.08	0.71±0.12	0.75±0.05	0.70±0.05
3.	*Pseudomonas*	0.73±0.09	0.78±0.05	0.89±0.12	0.82±0.05

Table-2: Effect of temperature on the naphthalene degrading activity (growth) of bacteria

Sl. No	Bacterial Isolate	OD at 600 nm at different temperatures										
		30^0C	31^0C	32^0C	33^0C	34^0C	35^0C	36^0C	37^0C	38^0C	39^0C	40^0C
1.	*Bacillus*	1.23±0.05	1.26±0.09	1.29±0.12	1.35±0.05	1.32±0.12	1.30±0.12	1.27±0.08	1.22±0.12	1.18±0.08	1.12±0.12	1.0±0.05
2.	*Micrococcus*	0.74±0.05	0.80±0.12	0.78±0.08	0.72±0.09	0.70±0.09	0.67±0.12	0.62±0.05	0.58±0.08	0.53±0.05	0.51±0.09	0.47±0.05
3.	*Pseudomonas*	0.90±0.05	0.96±0.005	0.92±0.12	0.89±0.05	0.85±0.12	0.81±0.09	0.78±0.05	0.73±0.09	0.69±0.08	0.63±0.09	0.59±0.12

IV CONCLUSION

Naphthalene and similar PAHs are released from industries, refineries, mechanical workshops etc. These compounds are deleterious to plants, animals and humans. Biodegradation is the safe and eco-friendly approach to clean such PAHs. Naphthalene is the model compound for the study of biodegradaton of PAHs. In the present work the the physical parameteres of naphthalene degrading ability of three

bacteria *viz., Bacillus, Micrococcus* and *Pseudomonas* isolated from oil contaminated soils were optimized. Further these bacteria can be identified till species level, genetically improved and used for bioremediation. The *Bacillus* species exhibited maxmium naphthalene degradation ability when compared to other two naphthalene degrading bacteria. Hence, more emphasis can be made on *Bacillus* isolate.

V. REFERENCES

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