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Effect of Physical Parameters and Amino acids on the Oil degradation Activity of Bacteria Isolated from Oil Contaminated Sites

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ABSTRACT- Mechanical workshops release huge levels of used engine oil into the surroundings. Many mechanical workshops do not have proper disposal mechanisms and eventually used engine oil reaches the surrounding soil. The hydrocarbons present in used engine oil damage the environment and human health. There are microbes in nature which have the ability to degrade the used engine oil. Such microbes can be isolated and used for the biodegradation of hazardous and recalcitrant hydrocarbons occurring in used engine oil. The present study deals with the four used engine oil degrading bacterial species isolated from oil contaminated sites of mechanical workshops. The bacterial species were *Bacillus, Acinetobacter, Pseudomonas,* and *Micrococcus*. The optimum pH and temperature on the oil degradation activity of bacterial species were determined. In addition, effect of various amino acids on the oil degradation ability of these bacterial species was studied and best amino acid for oil degrading activity was determined. All the four bacterial species exhibited highest oil degrading activity at pH 7.0. The optimal temperature required for maximum oil degradation activity by *Bacillus* and *Acinetobacter* species was 33^0C and 35^0C respectively. At 31^0C, *Pseudomonas* and *Micrococcus* species had shown maximum oil degradation activity. All the bacterial species had shown enhanced oil degradation activity in methionine supplemented medium. *Bacillus* species was found to be efficient among all the four species.

Key-words- Amino acids, Mechanical workshops, Oil degrading bacteria, Optimum, pH, Temperature, Used engine oil

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INTRODUCTION

Used engine oil causes potential damage to the environment. Engine oil is filled in the motor vehicles for the functioning of engine. The engine oil is combusted during its usage. At intervals used engine oil is replaced with fresh engine oil. The used engine oil is disposed into the environment from mechanical workshops. Used engine oil consists of hazardous aliphatic, polyaromatic, organosulfur compounds and heavy metals [1]. These compounds and heavy metals are mutagenic and carcinogenic to humans [2]. Nearly six million tons of crude oil is disposed into the environment from various sources [3].

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Many of the organic compounds present in used engine oil are recalcitrant. The soils polluted with used engine oil contain immobilized minerals, such soils lose water holding capacity, permeability and binding ability resulting in loss of soil fertility [4]. There are microbes which can degrade the hazardous organic compounds occurring in used engine oil into non-toxic simple molecules. This process is known as biodegradation and the microbes are called biodegrading microbes. The biodegradation process is eco-friendly and simple [5]. Microbes which can degrade oil hydrocarbons include *Flavobacterium, Pseudomonas, Arthrobacter, Alcaligenes* etc. [6]. In the present work four bacterial species were isolated from oil contaminated sites of mechanical workshops of Kaman region of Karimnagar town, Telangana, India and identified till genus level. The bacteria were identified as *Bacillus, Acinetobacteria, Pseudomonas,* and *Micrococcus* species. All the four isolated bacterial species were able to degrade the used engine oil by utilizing the hydrocarbons of used engine oil as carbon source. The present paper deals with the study of effect of physical parameters (pH and temperature) and

various amino acids on the biodegradation ability of these four bacterial species. The physical parameters required for oil biodegradation activity were optimized and best amino acid source for enhanced oil degradation activity was determined.

MATERIALS AND METHODS

The present work was carried out in the Department of Biotechnology, Vivekananda Degree and PG College, Karimnagar for a period of six months. Oil degradation activity studies were performed using minimal salt (MS) medium with composition, 0.2 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.076 g $Ca(NO_3)_2.4H_2O$, and 40 mM phosphate buffer per liter distilled water [7]. The minimal salt medium was supplemented with 5% used engine oil (obtained from light motor vehicles mechanical workshops). The hydrocarbons (organic compounds) present in used engine oil serves as a sole carbon source for the bacteria.

Inoculum preparation

Each bacterial culture was inoculated into 10 ml of MS medium with 5% used engine oil and incubated overnight at 30^0C. After incubation, the culture was centrifuged at 3500 rpm to get cell pellet. After washing each cell culture's pellet was inoculated into MS medium with 5% used engine oil and grown till the optical density of each culture broth was 1 at 600 nm. One ml of such each bacterial culture served as inoculum source [8].

Optimization of Physical parameters

Each bacterial culture was grown at pH 5.0, 6.0, 7.0 & 8.0 at 30^0C to determine the optimum pH for maximum oil degradation activity. Then each bacterial culture was grown at its optimum pH within a range of temperatures 30^0C to 40^0C to determine the optimum temperature for oil degradation process.

Determination of optimum amino acid source

Each bacterium was grown at its optimum pH and temperature in different amino acids. Each amino acid concentration supplemented in the medium was 1 mg/ml [9]. The amino acids used for the study were Proline (Pro), Alanine (Ala), Phenylalanine (Phe), Tyrosine (Tyr), Arginine (Arg), Lysine (Lys), Cysteine (Cys), Methionine (Met) and Glutamine (Gln). Then optimum amino acid source required for each bacterium was determined.

For all the studies 1 ml of each bacterial culture was added to 100 ml of MS medium with 5% used engine oil and incubated in shakers. The growth was measured in terms of optical density (at 600 nm) of the biomass of each bacterial culture after 2 weeks. The growth of each bacterial culture was proportional to the oil degradation activity. More growth was an indication of higher oil degradation activity. All the studies were made in triplicates and standard deviation was calculated for each record.

RESULTS AND DISCUSSION

The four bacterial species, *Bacillus, Acinetobacter, Pseudomonas,* and *Micrococcus* isolated from oil contaminated sites of mechanical workshops were grown in MS medium with used engine oil as sole carbon source. The bacteria were grown at various pH and temperatures to determine the optimal physical parameters for the degradation of used engine oil. The optimal pH for the oil degradation activity (growth) of all the four bacterial species was pH 7.0 (Table 1). *B. subtilis* had shown maximum growth & protease activity and *Acinetobacter* sp. ISTPCP-3 exhibited maximum growth at pH 7.0 [10,11]. Similarly, pH 7.0 was found to be optimum for the growth of *Pseudomonas marginalis* and *Micrococcus* species [12,13].

The optimal temperature required for maximum oil degradation activity by *Bacillus* and *Acinetobacter* species was 33^0C and 35^0C respectively (Table 2). *B. subtilis* KC3 had shown maximum growth in 33^0C [14] and most of the *Acinetobacter* species showed the highest growth between temperatures 33–35^0C [15]. The optimal temperature for *Pseudomonas and Micrococcus* species was found to be 31^0C (Table 2). All the bacterial species were isolated from mesophilic environment and hence, the optimum temperature for all the isolated species was around 30^0C.

Maximum oil degradation activity was recorded in all the four bacterial species when used engine oil containing medium was amended with Methionine [9] followed by cysteine. All the four bacterial species had shown similar response to Methionine and Cysteine and varied response towards remaining amino acids (Table 3). The order of used engine oil degradation activity of the four bacterial species in response to various amino acids is given below:

***Bacillus*:** Methionine > Cysteine > Glutamine > Proline > Alanine > Tyrosine > Arginine > Phenylalanine > Lysine

***Acinetobacter*:** Methionine > Cysteine > Glutamine > Proline > Alanine > Arginine > Tyrosine > Phenylalanine > Lysine

***Pseudomonas*:** Methionine > Cysteine > Glutamine > Proline > Alanine > Tyrosine > Arginine > Lysine > Phenylalanine

***Micrococcus*:** Methionine > Cysteine > Proline > Glutamine > Alanine > Tyrosine > Phenylalanine > Arginine > Lysine

Table 1: Effect of pH on oil biodegradation activity of bacterial species

S. No	Bacterial Sp.	OD values at 600 nm at different pH conditions			
		5.0	6.0	7.0	8.0
1	*Ba*	0.90±0.05	1.20±0.09	1.41±0.05	1.10±0.12
2	*Ac*	0.61±0.12	0.64±0.09	0.71±0.08	0.62±0.05
3	*Ps*	0.84±0.05	1.00±0.05	1.26±0.08	0.98±0.09
4	*Mi*	0.72±0.08	0.91±0.05	1.10±0.09	0.87±0.09

Ba- Bacillus, Ac- Acinetobacter, Ps- seudomonas, Mi- Micrococcus

Table 2: Effect of temperature on oil biodegradation activity of bacterial species

S. No	Bacterial Sp.	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	*Ba*	1.43±0.12	1.49±0.05	1.56±0.05	1.63±0.09	1.5±0.05	1.44±0.09	1.38±0.05	1.30±0.09	1.22±0.08	1.03±0.08	0.92±0.05
2	*Ac*	0.70±0.08	0.77±0.12	0.84±0.05	0.91±0.08	0.9±0.12	1.18±0.05	1.10±0.08	1.06±0.05	1.03±0.05	1.00±0.12	0.97±0.12
3	*Ps*	1.19±0.09	1.28±0.05	1.11±0.09	1.03±0.05	0.96±0.09	0.89±0.12	0.80±0.08	0.72±0.05	0.67±0.09	0.61±0.12	0.55±0.08
4	*Mi*	1.06±0.05	1.11±0.08	1.01±0.08	0.97±0.05	0.91±0.08	0.84±0.08	0.77±0.08	0.69±0.08	0.62±0.05	0.57±0.05	0.50±0.08

Ba- Bacillus, Ac- Acinetobacter, Ps- seudomonas, Mi- Micrococcus

Table 3: Effect of various amino acids on oil biodegradation activity of bacterial species

S.No	Bacterial Sp.	OD_{600} at different amino acids								
		Pro	Ala	Phe	Tyr	Arg	Lys	Cys	Met	Gln
1	*Ba*	1.73±0.12	1.69±0.09	1.59±0.09	1.66± 0.05	1.62±0.05	1.57±0.09	1.80±0.05	1.84±0.12	1.76±0.09
2	*Ac*	1.34±0.05	1.31±0.12	1.22±0.12	1.25± 0.09	1.28±0.12	1.20±0.12	1.40±0.14	1.44±0.09	1.37±0.05
3	*Ps*	1.47±0.08	1.44±0.09	1.30±0.08	1.40±0.16	1.37±0.09	1.33±0.16	1.54±0.05	1.59±0.12	1.50±0.05
4	*Mi*	1.40±0.09	1.31±0.05	1.24±0.05	1.28±0.05	1.20±0.09	1.15±0.08	1.46±0.12	1.50±0.05	1.36 ±0.12

All the bacterial species oil degrading activity was enhanced when they were grown in amino acid supplemented media. No amino acid had been decreased the oil biodegradation activity. The *Bacillus* species was found to be efficient oil degrading species, when compared to other three oil degrading bacterial species [16].

CONCLUSIONS

Used engine oil is disposed from various mechanical workshops reach the surrounding environment. Some hydrocarbons present in used engine oil are recalcitrant, hazardous and cause deleterious effects to environment and human health. These hazardous hydrocarbons present in used engine oils can be degraded by microbes to clean up

the environment. This present work used engine oil degrading ability of four bacterial species viz., *Bacillus, Acinetobacter, Pseudomonas,* and *Micrococcus* isolated from oil contaminated sites of mechanical workshops in the Kaman area of Karimnagar town under different parameters was studied. The bacteria were grown on MS medium with 5% used engine oil to study their oil degradation activity under different parameters. The physical parameters pH and temperature for each bacterium were optimized and best amino acid to be amended to increase the oil degrading activity was determined. *Bacillus* species was found to be efficient among all the four species. Further, these bacteria can be identified till species level, genetically improved and used for bioremediation. As *Bacillus* found to be the efficient species among the four, more emphasis can be made on its oil degradation activity.

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REFERENCES

[1] Ugoh SC, Moneke LU. Isolation of bacteria from Engine Oil contaminated Soils in Auto-mechanic Workshops in Gwagwalada, Abuja, FCT-Nigeria. Acad. Arena, 2011; 3(5)**:** 28-33.

[2] Ogunbayo AO, Bello RA, Nwagbara U. Bioremediaton of engine oil contaminated site. J. Emerging Trends Eng. Appl. Sci., 2012; 3(3**)**: 483-89.

[3] Kafilzadeh F, Sahragard P, Jamali H,Tahery Y. Isolation and identification of hydrocarbons degrading bacteria in soil around Shiraz Refinery. African J. Microbiol. Res., 2011; 4(19): 3084-89.

[4] Khan JA, Rizvi SHA. Isolation and characterization of Micro-organisms from oil contaminated sites. Adv. Appl. Sci. Res., 2011; 2(3): 445-60.

[5] Umar H, Umar A, Ujah UJ, Hauwa B, Sumayya BI, et al. Biodegradation of waste lubricating oil by bacteria isolated from soil. J. Environ. Sci. Toxicol. Food Technol., 2013; 3(6): 32-37.

[6] Ahmad M, Sajjad W, Rehman ZU, Hayat M, Imran Khan I. Identification and characterization of Intrinsic Petrophilic bacteria from oil contaminated soil and water. Int. J. Curr. Microbiol. Appl. Sci., 2015; 4(2): 338-46.

[7] Nwinyi OC, Ajaja O, Nwinyi Chibuzo. Growth dynamics of bacteria isolated from spent engine oil contaminated tropical soil. Res. J. Environ. Earth Sci., 2014; 6(9): 430-36.

[8] Thenmozhi R, Nagasathya A, Thajuddin N. Studies on biodegradation of used engine oil by consortium cultures. Adv. Environ. Biol., 2011, 5(6): 1051-57.

[9] Saurav K, Kannabiran K. Diversit optimization of process parameters for the growth of Streptomyces VITSVK9 spp. isolated from Bay of Bengal, India. J. Natural Environ. Sci., 2010; 1(2): 56-65.

[10] Vijayalakshmi TM, Murali R. Isolation and screening of Bacillus subtilis isolated from the dairy effluent for the production of protease. Int. J. Curr. Microbiol. Appl. Sci., 2015; 4(12): 820-27.

[11] Sharma A, Thakur IS, Dureja P. Enrichment, Isolation and Characterization of pentachlorophenol degrading bacterium Acinetobacter sp. ISTPCP-3 from effluent discharge site. Biodegrad., 2009; 20(5): 643-50.

[12] Membre JM, Burlot PM. Effect of temperature, pH and Nacl on growth and pectinolytic activity of *Pseudomonas marginalis*. Appl. Environ. Microbiol., 1994; 60(6): 2017-22.

[13] Jegan J, Vijayaraghavan K, Senthilkumar K, Manickam V. Naphthalene degradation kinetics of Micrococcus sp. isolated from Activated Sludge. Clean Soil Air Water, 2010; 33(9): 837-42.

[14] Vijayalaxmi- Sushma K, Abha S, Chander P. Isolation and characterization of Bacillus subtilis KC3 for amylolytic activity. Int. J. Biosci., Biochem. Bioinfo., 2012; 2(5): 336-41.

[15] James H, Doughari. Patrick Alois Ndakidemi, Izanne Susan Human and Spinney Benade. The Ecology, Biology and Pathogenesis Acinetobacter spp. An Overview. Microbiol. Environ., 2011; 26(2): 1-12.

[16] Kaustuvmani P, Rupchikha P, Mohan C, et al. Developments of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated soils. Frontiers Microbiol., 2016; 7: 01-14.

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