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Article

Biodegradation of Petroleum Hydrocarbons by Indigenous Bacteria and Microbial Consortium under Different pH and Temperature Conditions: An HPLC-Based Study

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Abstract: Petroleum hydrocarbon biodegradation is a viable environmental conservation tool that can be used in the remediation of the oily atmosphere. In the given study, isolated native bacteria and a microbial community were tested based on their capacity to break down petroleum hydrocarbons in different pH and temperature conditions. Bacterial isolates were acquired in oil-contaminated soil and it was filtered in terms of its capability to utilize hydrocarbons. The effectiveness of biodegradation was evaluated in biomass production, optical index density, emulsification index and high-performance liquid chromatography (HPLC) of the various aromatic compounds of interest. Two hundred and forty eight bacteria isolates were obtained on the oil-infested soils of which nine of them showed high ability of degrading PAH. HPLC analysis confirmed a high level of removal of the high-molecular-weight PAHs such as BaA, Chrysene, BbF, BaP, and BKF. Rhodococcus sp. provided the most efficient degradation efficiency of 88 per cent overall PAH removal with Pseudomonas putida (87 per cent) and Pseudomonas luteola (80 per cent) as the second and third best respectively. Micrococcus luteus recorded the least degradation (39%). One-way ANOVA statistical analysis showed that isolate differences were statistically significant ($p < 0.05$). The pH had a significant impact on PAH degradation ($p < 0.05$). Optimum degradation was seen at pH 7-8 where the microbial consortium performed better than the individual isolates. The consortium reported the greatest level of biodegradation in a neutral condition, which matched the highest biomass (0.294 g/L) and emulsification index (98%).Temperature also played a great role in degradation of PAH ($p < 0.05$). The consortium was best removed at temperatures of 30 o C, where the consortium had the best biomass (0.294 g/L) and high emulsification capacity (88 percent). Most isolates were found to be affected by thermal stress at 35C, allowing Bacillus cereus to outperform the microbial consortium, with the consortium being more metabolically cooperative and complementary in terms of enzymatic activity to effectively degrade PAH under favorable pH (7- 8) and moderate temperature (30°C). These results indicate good experimental support to the application of indigenous microbial consortia in the effective removal of petroleum hydrocarbons bioremediation.

Keywords: Biodegradation, Petroleum hydrocarbon, Indigenous bacteria, Microbial consortium, HPLC

Introduction

The most persistent environmental pollutants are petroleum hydrocarbons as they have a complicated chemical structure and cannot be naturally decomposed. In addition, the procedure is efficient in breaking down long -chain aliphatic and aromatic hydrocarbons considering their complex Chemical structures [1], [2]. More so, polycyclic aromatic hydrocarbons (PAHs) are toxic and carcinogenic, making this approach especially efficient in breaking down these compounds [3], [4].

Bioremediation is a relatively inexpensive and environmental friendly alternative to the conventional remediation action. In this method, the metabolic capacity of the microorganisms is used to convert or mineralize the hydrocarbons into less toxic compounds [5][6][7].

Specifically, effective are indigenous bacteria obtained in contaminated situations, as they have developed a long-term adaptation to hydrocarbons and contain a range of specific degradative enzymes. The recent studies have pointed out the superiority of microbial consortia to single bacterial isolates in the hydrocarbon biodegradation.

The synergistic reactions between the consortium members increase the rate of substrate use, decrease the toxic intermediates, and accelerate the rate of degradation of complex aromatic compounds [8]. The pH and temperature are other environmental factors that exert a strong impact on the growth of microbes, the enzymatic activities, and the overall effectiveness of biodegradation [9].

This study investigates the biodegradation potential of indigenous bacterial isolates and a microbial consortium under different pH and temperature conditions, with special emphasis on aromatic hydrocarbon degradation assessed by HPLC analysis

Materials and Methods

2.1. Study Area and Sample Collection

Twenty composite soil samples were randomly collected from oil-contaminated sites at the Wasit (Kut, Iraq) at a depth of 10 cm. Samples were transported to the laboratory under cooled conditions and processed within 24 h.

2.2. Enrichment and Isolation of Hydrocarbon-Degrading Bacteria

For enrichment, 1 g of each soil sample was inoculated into 50 mL sterile Bushnell–Haas medium (BHM) supplemented with 1% (v/v) crude oil in 250 mL Erlenmeyer flasks. Cultures were incubated at 30°C with shaking (150 rpm) for 14 days. Enriched cultures were serially diluted and streaked onto mineral salt agar plates containing 1% crude oil. After incubation at 30°C for 48 h, morphologically distinct colonies were purified and maintained on a Lauria Bertani agar at 4°C.

2.2 Preparation of Bacterial Inoculum and Consortium

Individual isolates were grown overnight in Lauria Bertani broth at 30°C. Cells were harvested by centrifugation (3000 rpm, 15 min), washed with sterile saline (0.85% NaCl), and resuspended in BHM. The inoculum density was standardized to OD_{600}. For consortium preparation, equal volumes of standardized cultures of the selected high-performing isolates were mixed to obtain a combined inoculum.

2.3. Biodegradation Experiments under Different pH and Temperature

Biodegradation tests were done in 250 ml flasks with 50 ml of BHM with 1% of crude oil. To optimize pH conditions, flasks were inoculated with 5 ml of standardized inoculum (single isolate or consortium) and adjusted to pH 6, 7, 8 and 9 using 0.1 N HCl or NaOH, then incubated at 30 °C (150 rpm) during 7 days. To optimize temperature conditions, the cultures were inoculated at 25 °C, 30 °C and 35 °C (pH 7) under the same conditions of shaking (1 Each condition had uninoculated controls. Each experiment was done thrice. The remainder of the concentrations of the chosen aromatic hydrocarbons were determined through HPLC. The efficiency of biodegradation was determined by comparison of residual concentrations and untreated control samples.

2.4. Extraction and Quantification of PAHs by HPLC.

The amount of residual hydrocarbons was removed by n-hexane extraction after incubation of the samples after alkaline digestion (1 M KOH in ethanol, 60 to 65 °C, 1 h). The separation of the organic phase was done followed by drying in the presence of anhydrous sodium sulfate followed by concentration using rotary evaporation and purification using silica gel column. The hexane: dichloromethane (40: 60, v/v) was used to elute the PAH fraction, concentrated under a nitrogen atmosphere and then subsequently reconstituted to 1 ml of iso-octane to be analyzed.

The HPLC was used to quantify PAHs (BaA, Chrysene, BbF, BaP and BKF). The efficacy of biodegradation was determined as a percent removal against the uninoculated control.

HPLC Analysis

Quantitative analysis of residual aromatic compounds was done using High-Performance Liquid Chromatography (HPLC) with a UV detector and C18 reverse-phase column. The mobile phase was made up of acetonitrile and water in an isocratic or gradient application. Detection had been conducted at the correct wavelengths based on the target compounds. Degradation efficiency percentage (BH) was determined by comparing the amount of hydrocarbons remaining in the treated sample with the control sample and based on the equation below:

$$\text{Biodegradation\%} = \frac{Ct - C0}{C0} \times 100$$

Ct: Residual concentration

C0: initial concentration(control)

2.5 Statistical Analysis

Measurements were all in terms of mean, which was standard deviation of three experiments. One-way ANOVA was used to compare statistically significant differences between treatments and a p-value of less than 0.05 was a significant difference.

Result and Discussion

The findings showed that all the chosen bacterial isolates could use petroleum hydrocarbons as a carbon medium, albeit with different degrees of efficiency. The individual isolates exhibited better biodegradation performance in some of them, which means that they are highly adapted to hydrocarbons. HPLC analysis established that the concentration of aromatic hydrocarbons in treated sample had reduced significantly.

3.1. The influences of PH on biodegradation of nine single bacterial isolates

A										
Rhodococcus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	61.52	6.78	81.80	3.23	75.19	5.36	51.16	3.58	70.75	5.64
PH7	64.59	6.24	79.04	3.72	81.34	4.03	54.98	3.03	77.70	4.30
PH8	67.59	5.71	67.72	5.73	77.45	4.87	55.25	3.28	81.54	3.56
PH9	63.62	6.41	65.24	6.17	80.56	4.20	44.20	4.09	78.47	4.15

B										
Nocardia	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	52.16	8.43	60.23	7.06	50.14	10.77	70.67	2.15	87.86	2.35
PH7	64.36	6.28	72.90	4.81	77.45	4.87	58.25	3.06	65.46	6.66

PH8	67.53	5.68	69.69	5.38	73.83	5.65	49.25	3.72	71.27	5.54
PH9	69.30	5.42	67.38	5.79	84.58	3.33	22.78	5.66	80.29	3.80

C										
Micrococcus Luteus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	16.11	14.78	22.93	13.68	33.42	14.38	0	7.33	52.90	9.08
PH7	62.71	6.57	63.10	6.55	76.39	5.10	8.19	6.73	67.48	6.27
PH8	54.26	8.06	64.23	6.34	79.81	4.36	29.33	5.18	58.04	8.09
PH9	67.42	5.74	70.76	5.19	79.26	4.48	0%	7.33	76.35	4.56

D										
Pseudomonas putida	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	55.68	7.81	61.07	6.91	75.83	5.22	37.24	4.06	62.86	7.16
PH7	57.07	6.07	55.32	5.87	76.67	4.47	49.11	3.38	63.69	4.46
PH8	56.58	7.65	72.85	4.82	79.72	4.38	45.43	4.00	74.69	4.88
PH9	66.80	5.85	58.54	7.36	78.24	4.70	37.24	4.60	73.81	5.05

E										
Bacillus Subtilis	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	53.46	8.20	59.94	7.11	75.74	5.24	24.15	5.56	42.22	11.14
PH7	63.17	6.49	57.97	7.46	65.27	7.50	31.51	5.02	65.25	6.70
PH8	52.84	8.31	69.30	5.45	67.77	9.57	28.51	5.24	65.15	6.72
PH9	45.86	9.54	41.18	10.44	34.21	14.21	24.27	5.55	37.81	11.99

F										
Acinetobacter	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	62.03	6.69	64.06	6.38	52.68	10.22	30.29	5.11	64.00	8.87
PH7	46.77	9.38	48.96	9.06	74.77	5.46	40.79	4.34	57.67	8.19
PH8	50	8.81	54.70	8.04	68.38	6.83	34.24	4.82	64.83	6.78
PH9	66.91	5.83	53.52	8.25	52.59	10.24	15.15	6.22	55.65	8.55

G					
Citobacter	Benzo[a] anthracene	Chrysene	Benzo[b] fluoranthene	Benzo[a] Pyrene	Benzo[K] fluoranthene

	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	12.82	15.36	2.31	17.34	3.38	20.87	0	7.33	7.94	17.74
PH7	55.56	7.83	71.49	5.06	69.21	6.65	19.51	6	61.67	7.39
PH8	59.36	7.16	66.20	5.97	59.72	8.70	0	7.33	70.49	5.69
PH9	52.78	8.32	62.37	6.68	79.68	4.39	0.82	7.27	63.23	7.09

H										
Bacillus cereus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	66.52	5.92	66.99	5.86	69.07	6.68	24.79	5.50	75.36	4.75
PH7	65.49	6.07	80.45	3.47	81.22	4.04	37.38	4.59	74.07	5.00
PH8	64.02	6.34	55.44	7.91	55.97	9.51	41.61	4.28	41.80	11.22
PH9	51.76	8.29	64.96	6.22	51.62	10.45	30.42	5.10	57.00	8.29

I										
Pseudomonas luteola	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	68.73	5.51	66.73	5.90	71.34	6.20	33.42	4.88	78.48	4.15
PH7	75.43	4.33	71.32	5.09	73.42	5.74	39.02	4.47	81.90	3.49
PH8	71.51	5.03	77.46	4	67.41	7.05	40.11	4.39	58.30	8.04
PH9	54.03	8.10	71.72	5.02	79.95	4.33	30.56	5.09	72.25	5.35

Table (1): A, B, C, D, E, F, G, H, I, Effect of PH on PAH (9 isolates × 5PAH). The equations below give (%BH =% Biodegradation), R.C.=Residual concentration.

According to the tables (1), the efficacy of bioremediation critically depends on the interaction between physiology of microorganisms and environmental factors where pH is a major factor affecting the enzyme activity, membrane transport, and cellular metabolism. [10]. The overall trends indicate that bioremediation should be tailored to species-specific and PAH-specific degradation profiles, as well as to the environmental conditions.

A common trend in the majority of the species, such as *Citrobacter freundii*, *Micrococcus luteus*, *Bacillus cereus*, and *Bacillus subtilis*, is that there is a strong inhibition of degradation activity at pH 6 relative to both neutral and alkaline.

In such species, bioremediation percentage of most PAHs rose significantly between pH 6 and pH 7 or pH 8. This is in line with the observation that most hydrocarbon-degrading bacteria are neutrophiles, the catabolic enzymes of which, including oxygenases and dehydrogenases that are essential in the breakdown of PAH rings, are best active at neutral pH [11].

This is supported by the value of the residual concentration data that consists of relatively higher values of the unmetabolized PAHs at pH 6 in all cases. This implies that acidic environment can inhibit the original absorption process of these high molecular-weight substances or suppress the action and expression of the most vital enzymes in the degradation pathway.

Rhodococcus sp. data, conversely, indicate an astonishing, possibly beneficial quality: strong efficiency on the whole pH scale on degradation. As an example, it retained above 65% degradation of Cry and above 75% degradation of BbF in all levels of pH. Trends in performance This is reflected in the consistent low residual concentrations. The genus *Rhodococcus* is also famous due to their metabolic flexibility and robustness, with a huge genome containing many catabolic genes and a distinct cell envelope filled with lots of mycolic acids, which have made them very tolerant to various environmental stresses, such as changes in pH. [12].

The second important result is a dramatic difference in degradation of individual PAHs, where Benzo[a]pyrene (BaP) is seen as the most recalcitrant of the almost all species and pH regimes.

Many strains did not degrade BaP at all at some pH (e.g. *C. freundii* and *M. luteus* at pH 6 and 8). This resistance is well established and blamed on the fact that BaP weight is relatively high, contains 5 fused aromatic rings and that it has a large activation energy barrier during initial dioxidation initiation making it a persistent environmental pollutant and a known carcinogen [13].

The overall inability to degrade BaP highlights an important weakness of monoculture bioremediation and indicates the possibility of limited use of consortia or genetically engineered strains which degrade high molecular weight PAHs. Moreover, the data shows specific affinities of species and PAH that may be utilized to exploit specific bioremediation. For instance:

BKF (81.9) and BaA (75.43) were of the highest degraded in *Pseudomonas luteola* at pH 7. *Nocardia* sp. demonstrated a special ability to effectively degrade BaP in acidic pH (6) (70.67%), which was not comparable to most other species, as well as the highest BKF degradation (87.86) under the same acidic condition. The growth of *Bacillus subtilis* was notable at neutral to slightly alkaline pH, but this growth decreased drastically at pH 9, and this behavior agreed with its physiological history.

Such special affinities indicate that the enzyme machineries (e.g., particular dioxygenases) in these species are especially adapted to the specific structure of some PAHs at particular pH.

To sum up, it is important to note that this is a powerful piece of evidence that demonstrates that there is no single best bacterium to use in the bioremediation of PAH. Rather, the best option is an operation of the particular combination of the pollutants and the environment ph. The overall inefficiency in low PH, the spans-all-genres problem of BaP degradation and the rare pH-resistance of genera such as *Rhodococcus* are major lessons learned.

3.2. Effects of Temperatures on the Biodegradation of nine Single Bacterial isolates

A

Rhodococcus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25℃	55.51	7.84	69.63	5.39	77.04	4.59	52.93	3.45	70.64	5.66
30℃	73.04	4.75	60.17	7.07	82.69	3.74	45.71	3.32	66.13	6.53
35℃	74.06	4.57	82.48	3.11	75.69	5.24	40.52	4.36	70.44	5.70

B										
Nocardia	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25℃	61.75	6.74	64.96	6.22	72.59	5.92	57.71	3.10	57.31	8.23
30℃	59.88	7.07	49.07	9.04	61.71	8.27	76.26	1.74	7.91	7.91
35℃	65.55	6.07	66.93	5.87	79.31	4.46	53.89	3.38	76.82	4.47

C										
Micrococcus Luteus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25℃	59.14	7.20	64.85	6.24	66.39	7.26	28.92	5.21	62.5	7.23
30℃	65.04	6.15	64.56	6.29	81.16	4.07	11.87	6.46	56.85	8.32
35℃	43.92	9.88	58.70	8.04	70.05	6.47	37.38	4.59	54.69	9.83

D										
Pseudomoas Putida	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.
25℃	66.29	5.94	55.27	7.94	71.44	6.17	35.20	4.75	71.16	5.56
30℃	64.42	6.27	68.68	5.56	78.33	4.68	43.52	4.14	72.41	5.32
35℃	76.73	4.10	72.25	4.9	58.47	6.17	48.43	3.78	64.37	6.87

F

Acintobacter	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25°C	53.00	8.28	69.35	5.44	67.82	6.95	37.65	4.57	58.40	8.02
30°C	57.09	7.56	70.65	5.21	75.60	5.27	15.00	6.23	73.29	5.15
35°C	69.58	5.36	51.27	8.65	74.77	5.45	20.46	5.83	71.47	5.50

E										
Bacillus Sutilis	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25°C	59.53	7.13	67.21	5.82	45.00	11.88	37.52	4.58	48.65	9.90
30°C	72.25	4.89	66.08	6.02	56.30	9.44	20.19	5.85	48.81	9.87
35°C	69.41	5.39	61.58	6.82	76.34	5.11	48.16	3.80	45.95	10.42

G										
Citobacter freundii	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25°C	46.65	9.39	54.31	8.11	67.96	6.92	0.55	7.29	59.60	7.80
30°C	64.02	6.34	67.49	5.77	74.26	5.56	8.05	6.74	69.19	5.93
35°C	68.22	5.60	74.37	4.55	69.40	6.61	9.82	6.61	65.30	6.69

H										
Bacillus Cereus	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
25°C	52.67	8.34	96.35	5.44	63.19	7.95	42.29	4.23	55.17	8.64
30 °C	56.47	7.67	70.59	5.22	71.57	6.14	43.66	4.13	64.83	6.8
35°C	34.45	11.55	70.31	5.27	51.24	10.10	50.07	3.66	74.33	4.95

I										
Pseudomonas luteola	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C.	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.
25°C	63.61	6.41	66.59	5.93	67.31	7.06	24.8	5.55	74.07	5.00
30°C	61.41	6.80	74.08	4.58	69.68	6.55	31.24	5.03	66.86	6.39
35°C	77.53	3.96	82.82	3.05	75.19	5.36	32.74	4.93	75.26	4.77

Table (2) :-(A, B, C, D, E, F, G, H, I) Effect of Temperature on PAH (9 isolates × 5PAH). (%BH =% Biodegradation, R.C. =Residual concentration).

The temperature dependent biodegradation patterns observed across the different bacterial isolates provide important mechanistic insights into how microbial physiology, enzyme kinetics, and PAH bioavailability interact to influence hydrocarbon removal. The performance of the tested strains *Citrobacter freundii, Micrococcus luteus, Rhodococcus* spp., *Nocardia* spp., *Pseudomonas putida, Pseudomonas luteola, Acinetobacter* spp., *Bacillus subtilis,* and *Bacillus cereus* reflects trends well documented in microbial ecology and environmental biotechnology.

Temperature is a significant controller of microbial metabolic rate which influences the rate of enzyme reactions and the dynamics of cell membrane. In the entire dataset, the majority of the isolates exhibited greater degradation of PAH between 25 °C and 30 °C with some decreases or a level off at 35 °C. This is in line with the literature that mesophilic bacteria (optimal range ≈ 28 to 35 °C) predominate over the degradation of PAH in soils[14], [15] .

The outcomes revealed that *Citrobacter freundii* exhibited an evident increase in degradation between 25 °C and 30 °C in BaA (46.65→ 64.02) and Cry (54.31→ 67.49 per cent) *Micrococcus luteus* had a comparable enhancing pattern at 30 °C with the majority of the PAHs. *Rhodococcus* spp. Exhibited high removal (70-80) at all temperatures as does its established thermotolerance and abundance of oxygenases. Most PAHs were maximally active at 30 to 35°C, as expected of the highly metabolically versatile genus, in *Pseudomonas putida* and *P. luteola*.

Such trends are in line with Q10 temperature principle where the rate of metabolism of the microbes doubles or triples with every 10 °C increment until the enzymes are subjected to a thermal limit [16].

In all the strains Benzo[a]pyrene (BaP) exhibited the least degradation, usually less than 10% at 25 to 35 °C with a variety of isolates (*Citrobacter freundii* and *Micrococcus luteus*). This is not surprising since BaP is very hydrophobic (log_ow ≈ 6.1) and has a strong affinity to organic matter. Numerous studies show BaP is the most recalcitrant HMW PAH under mesophilic conditions [17], [18].

Conversely, compounds like BbF and BkF, although also HMW PAHs, were more readily degraded by several isolates at 30–35 °C. *Rhodococcus, Pseudomonas*, and *Nocardia* species possess multi-component dioxygenases capable of initiating oxidation of fused-ring PAHs, which explains the higher degradation percentages [19].

PAH biodegradation starts with ring-hydroxylating dioxygenases, which are heat-sensitive but become more active up to an optimum in the mesophilic range. Studies show that at 25 °C, these enzymes function at suboptimal efficiency, but activity increases significantly by 30–35 °C [3]. Based on previous reseans one can explains the large increases from 25 °C → 30 °C in *Citrobacter, Pseudomonas*, and *Acinetobacter*. Also the high performance of *Rhodococcus*, whose oxygenases remain stable over broader temperature ranges.

Rising temperature enhances the bioavailability of PAHs by increasing aqueous solubility, reducing viscosity of oily residues and enhancing desorption from soil particles[20], [21]

This accounts for the increased removal of BbF and BkF at 30–35 °C across several species. Temperature effects on membrane fluidity, Biosurfactant production, Growth rates and Energy

allocation. For instance, *Pseudomonas putida* and *Rhodococcu*s spp. are known to increase biosurfactant output at 30–35 °C, improving PAH uptake [3], [19].

Rhodococcus consistently showed the strongest PAH removal (up to 80% for BbF). This is in accordance with literature that identified *Rhodococcus* as one of the most active hydrocarbon degraders based on its large plasmid and variety of dioxygenases. Both *P. putida* and *P. luteola* showed good performance, which supports the role of the genus as a model PAH degrader [22]. The rise at 35 °C of BaA and Cry indicates that the two have good temperature tolerance of enzymes. *Acinetobacter* spp. showed moderate PAH degradation at optimum temperatures of 30 to 35 °C which is in line with its metabolic flexibility. Most PAHs exhibited an intermediate temperature (30 °C) range that was better with *Bacillus subtilis*, which has a mesophilic growth window.

3.3.Impact of PH and Temperature on Bioremediation polycyclic aromatic hydrocarbon consortium.

PH										
Consortium	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C.	%BH	R.C.	%BH	R.C.	%BH	R.C.
PH6	74.69	4.46	62.48	6.66	82.96	3.68	71.49	2.09	80.39	3.78
PH7	100	0	100	0	100	0	100	0	100	0
PH8	77.87	3.90	75.77	4.30	86.48	2.92	60.44	2.90	80.23	3.90
PH9	81.84	3.20	70.08	5.13	70.93	6.28	53.75	3.39	81.74	3.52

Temperatures										
Consortium	Benzo[a] anthracene		Chrysene		Benzo[b] fluoranthene		Benzo[a] Pyrene		Benzo[K] fluoranthene	
	%BH	R.C	%BH	R.C	%BH	%BH	R.C	%BH	R.C.	%BH
25°C	71.06	5.11	73.41	4.72	84.72	3.30	44.61	4.05	84.44	3
30°C	50.45	8.73	63.72	6.44	88.75	2.42	91.41	0.63	91.18	1.70
35°C	100	0	100	0	100	0	100	0	100	0

Table (3): Effect of PH and Temperature on Bioremediation of polycyclic aromatic hydrocarbon for consortium

The results reports biodegradation percentages and residual PAH concentrations for a microbial consortium exposed to different pH levels (6–9) and temperatures (25 °C, 30 °C, 35 °C). Five priority polycyclic aromatic hydrocarbons (PAHs) were considered which are Benzo[a]anthracene (BaA), Chrysene(Cry),Benzo[b]fluoranthene(BbF)Benzo[a]pyrene(BaP),andBenzo[k]fluoranthene (BkF). These high molecular-weight (HMW) PAHs are usually recalcitrant and hydrophobic hence one of the toughest tests of microbial degradation activity. In all the conditions, pH 7 and 35 °C presented total (100) degradation of all PAHs, but the acidic or alkaline pH generated partial degradation. This is in anticipation of anticipated biochemical conduct of numerous PAH degrading bacterial communities

especially those enriched in soils or in petroleum contaminated areas, with neutral pH and mesophilic-slightly thermophilic temperatures being the most active enzyme occurrence.

The data indicates that all PAHs tested are fully degraded at PH 7. The trend is consistent with several published studies which have shown that bacterial ring hydroxylating dioxideases which are the major enzymes in the breakdown of PAH have optimal catalytic ability at neutral pH [3], [18].

Enzymes have a sensitive structure with regard to their protonation state and a shift out of the neutral state may decrease affinity to substrates or destabilize the conformation of the enzyme. Moreover, the integrity of the membrane and the ability to be permeable to hydrophobic molecules like PAHs tend to be highest at the neutral conditions and this improves the mass transfer.

Low pH (6) is expected to slow down the growth rate of microbes and decrease the solubility of PAH and consequently decrease bioavailability and alkaline pH (8 - 9) is likely to inactivate enzymes or change hydrophobicity of membranes, decreasing the uptake of PAH molecules. The residuals (range of 2.09 to 6.66 mg/L) observed confirm this explanation- degradation is still high but not complete. HMW PAHs such as BaP and BkF are particularly pH sensitive. Motivated variation in bioavailability owing to their very low solubility in aqueous. All the five PAHs react in a similar way to changes in pH, however, BbF and BaP exhibit a minor decreasing degradation at non-neutral conditions. This is correlated with their greater molecular mass and more compact ring structure that tends to retard attack by microbes at 35 °C, which further suggests that the microbial community is probably mesophilic to thermotolerant strains (Bamforth and Singleton, 2005).The consortium was 100 per cent effective in removing all the PAHs at 35 °C, which further shows that the microbial community is likely of mesophilic to thermotolerant strains (Bamforth and Singleton, 20 High temperatures cause a quicker reaction rate of enzymes, higher fluidity in membranes, and can provide more desorption of PAHs of soil particles or organic matrix.

Literature indicates that numerous PAH-degrading consortia, specifically the *Pseudomonas*, *Mycobacterium* and *Sphingomonas* species, are most effectively degraded at temperatures of 30-37 °C [23].

At 25 °C, degradation ranged from 44% (BaP) to ~85% (BkF), while at 30 °C, degradation varied widely: BaA = 50%, Cry = 64%, but BaP = ~91%. These differences represent the interactions of temperature-dependent enzyme kinetics (Q10 effect) and alterations in solubility and desorption of PAH and community change in composition (temperature-sensitive taxa) [19].

It is notable that the degradation of BaP has a very high percentage of 25, °C; the degradation of BaP is very high at 44.61 and at 91.41 per cent, which means that enzymes in the ring-dioxygenase of this consortium are very sensitive to temperature. The consortium damages PAHs within a rather wide range of pH and temperatures, which indicates a wide range of microbial population, able to resist environmental changes.

The enzyme activity, production of Biosurfactant (which reaches its maximum at close-neutral pH) and the uptake / transport rate of PAH and Microbial growth rate may be simulatively optimized, which is why the complete removal is observed at pH 7 and 35 °C.

These findings suggest that, neutralization of contaminated soils (liming or buffering) might be a significant way to increase the efficiency of PAH removal in the presence of thermal enhancement measures (solarization, compost-assisted heating) might substantially increase biodegradation. Microbial consortia do not offer metabolic redundancy and complementary enzymatic pathways which enhances the degradation of complex PAH mixtures compared to single strains.

Conclusion

This experiment shows that the native bacterial isolates have high potential to degrade petroleum hydrocarbon by biodegradation, whereas microbial consortia has a better degradation ability through synergistic effects. The best pH and temperature conditions were responsible in playing a crucial role in biodegradation performance. HPLC analysis proved successful degradation of aromatic hydrocarbons which urged the usefulness of microbial consortia in the bioremediation approach to oil-contaminated environment.

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