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Research Article

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Screening and Identification of Petroleum-Degradation Bacteria in the Surface Seawater from the Red Sea Coast of Al-Hodeidah City, Yemen

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Abstract

Bacteria play a significant role in the biochemical cycling in aquatic environments. Environmental pollution from ports and jetties are complicated due to the various types of pollution, sources and their different characteristics. Although oil contaminants are weathered by photo-oxidation and evaporation complete degradation is dependent on the metabolic activities of the microbial population inherent to the area. The PDB in seawater from the Red Sea coast of Al-Hodeidah city were determined by serial dilution and plating on Bushnell Haas Agar and the isolated bacteria were identified by MicroScan Walkaway 96 plus system (BECKMAN - COULTER). The extracts of petroleum hydrocarbons n-alkanes were subsequently analyzed by gas chromatography-flame ionization detection. EPH aliphatic hydrocarbon standard 14 components (C9 – C36). Petroleum degrading bacterial counts in surface seawater (PDBCs) was from 1.10 x 103 CFU/ml and 2.94 x103 CFU/ml, high counts were obtained in summer. The bacteria isolated were belonged to an Aeromonas Hydrophila Complex. Aeromonas Hydrophila Complex was capable of using 10% Petroleum hydrocarbons n-alkanes of 1997.169 ppm concentration used crude oil, in 15 days under laboratory conditions at 25 ◦C with Bushnell-Haas media.

Keywords: Petroleum-Degradation Bacteria, Seawater, Red Sea coast, Al-Hodeidah City

Introduction

Crude oil/petroleum hydrocarbons present diverse physical properties as a result of their complex mixtures of several individual compounds classified based on polarity as saturates, aromatics, resins, and asphaltenes [1, 2]. The different crude oil components have varying susceptibility to microbial mineralization based on the structural arrangement. Alkanes are the most susceptible; following this are light aromatics (MAHs), cycloalkanes, heavy aromatics (PAH), resins, and asphaltenes. Generally, alkanes make up about 50% of crude oil although the source of the oil may alter the concentration [3, 4].

Oil and oil residues in coastal-marine environments can be acted upon by naturally occurring bacterial communities, ultimately leading to the disintegration of the oil components into carbon dioxide and water [5]. A variety of microorganisms are known to have hydrocarbon biodegradation capacity, e.g., hydrocarbon-clastic bacteria. These microorganisms are naturally present in coastal-marine environments, even before the occurrence of oil spills, but they are mostly in low abundance in natural, unpolluted environments [6]. They are widely distributed within surface water, sediments, and soil habitats, and the population of these bacteria rapidly increases after oil spills [7]. Hydrocarbons are readily degraded under favorable conditions, nevertheless, there is a clear preference for the catabolism of some molecules before others; hence the composition of fuel or crude oil changes as biodegradation proceeds [8, 9]. Oil biodegradation can occur, albeit slower, even in deep and in areas with extremely low temperatures [10]. A dominant process responsible for the removal of petroleum compounds in the oil residues from the environment is believed to be biodegradation [11]. As such, bioremediation of hydrocarbon pollutants is advantageous following an oil spill [12].

Study Area and Methodology

Study Area

The area under investigation is laying from the south eastern part of the Red Sea in the coast of Yemen, Hodeida City. It extends from Hodeida power plant which is located at latitude 15° 00' 20" N and longitude 42° 56' 02" E to Fishing port in the south at 14° 46' 54" N latitude and 42° 56' 50" E longitude. The distinctive areas are namely; Al-Hodeida port, Al-Hodeida power

plant, Fishing port and the Corniche of Al-Hodeida (Figure 1 and Table 1). The sampling stations were located by Global Positioning System (GPS).

Table 1: Geographical Locations of the Sampling Stations.

Station No.	Description	Location of Station	
		Latitudes (North)	Longitudes (East)
St-1	Al-Hodeida port	14° 49' 58"	42° 56' 02"
St-2	Al-Hodeida power plant	15° 00' 20"	42° 55' 15"
St-3	Fishing port	14° 46' 54"	42° 56' 50"
St-4	The Corniche of Al-Hodeida	14° 46' 49"	42° 56' 33"

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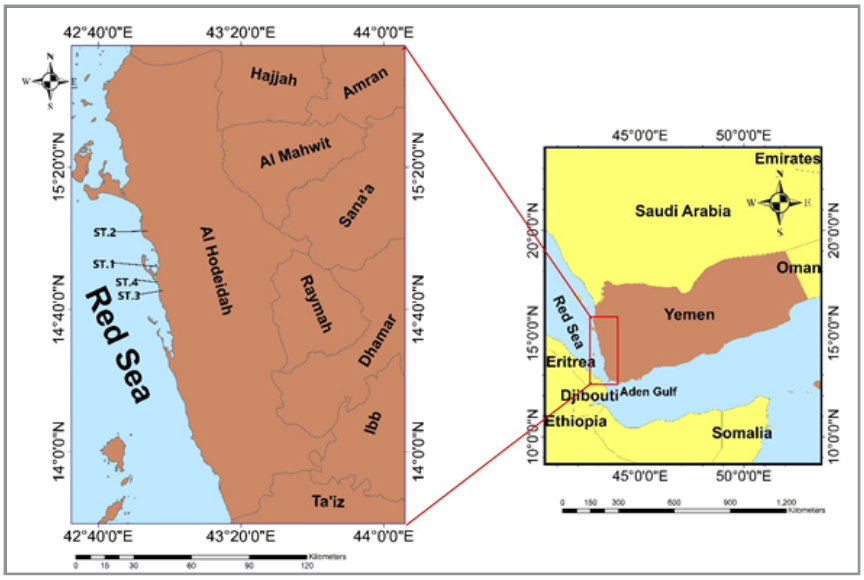

Figure 1: Sampling Stations of the Study Area

Collection of the Surface Seawater for Bacteriological Analysis

The water samples for bacteriological examinations were collected once seasonally aseptically to avoid contamination for each station of study. Detailed quality assurance and quality control procedures were followed for sample collection, holding [13]. The water samples were collected at a depth from surface to 10cm in the opposite direction of water current into commercial sterile universal bottles.

Media for the Isolation of Petroleum Degrading Bacteria

The isolation of petroleum-degrading bacteria was carried out using Bushnell Haas Agar (BH) agar medium containing 1% (v/v) sterile crude oil (Marib crude oil) used as the sole carbon source to isolate (PHDB). Medium contained MgSO4.7H2O (0.2 g/l), CaCl2 (0.02 g/l), KH2PO4 (1 g/l), K2HPO4 (1 g/l), and NH4NO3 (1 g/l), FeCl3 (0.05 g/l) and Agar (20 g/L) at pH 7.2. The media was sterilized by autoclaving at 121°C for 15minutes and dispensed into disposable Petri dishes, allowed to solidify [14, 15].

Isolation

The method of isolation used was the ten-fold dilution technique. Decimal dilution of the samples was made by adding 1 mL of the sample to 9.0 ml sterile normal saline to give an initial dilution of 1:10. Subsequent serial dilutions were made by adding 1 ml of the last dilution to 9.0 mL of fresh sterile saline. Lastly, 0.1 ml of appropriate dilution were plated out on BH agar medium with sterile crude oil and evenly spread with a sterile glass rod spreader. plates were incubated for 7 days at ambient temperature to observe and determine the colony-forming units (CFU g−1). One control (negative) was used, that is BH agar media with crude oil but without added the dilution sample. The enumerated colonies were isolated by streak plating onto fresh nutrient agar medium for more studies [16].

Identification of Isolated Bacteria

Color and shape were examined and recorded as colony morphological characteristics according to. Microscopic features were recorded for all isolates via Gram stain protocol. The MicroScan Walkaway 96 plus system (BECKMAN- COULTER) was used for biochemical tests as catalase, coagulase, indole, citrate, methyl red, hydrogen sulphide production, Vorges Prauskouer, oxidase and carbohydrate fermentation tests etc. The manufacturer's instructions were followed for the in-depth experimental procedures and conditions. Using data from biochemical reactions, the biotype number was determined from the MicroScan Walk-Away processed panel data report. [17, 18].

The Biodegradation Test for Treatment Crude Oil Pollution by Isolated Bacteria

Preserved pure cultures were then subcultured and used as inoculants in 100ml of BH broth media with containing 1% (v/v) sterile crude oil (Marib crude oil) at 25°C for 15 days, and anther sample of 100ml of BH broth media with containing 1% (v/v) sterile crude oil but without bacteria as a controller sample [19, 20].

Gas Chromatography Analysis of The Residual Crude Oil

The procedure used for extraction and analysis of MassDEP EPH in the water samples was performed according to conventional procedures (21). A sample was subjected to liquid-liquid extraction method for hexane. The eluates were concentrated using an air blowdown apparatus under a gentle stream of air. The extracts were fractionated by alumina: Silica gel (80- 100 mesh) chromatography. The extracts were sequentially eluted from the column with n-hexane using a EPH Calibration Standards. Blank determinations were carried out by repeating the procedure with pre-extracted samples and analyzed by gas chromatography Shimadzu GC-2010 Plus.

Result and Discussion

Bacteria isolated from water from the study areas was: Aeromonas Hydrophila Complex. The results of Degrading Bacterial Counts from Surface Seawater in the study varied across the 4 locations ranged from 1.33 103 CFU at Al-Hodeidah Harbor to 1.10 103 CFU at Al-Hodeidah Power Plant in winter and from 2.94 103 CFU at Al-Hodeidah Harbor to 2.35 103 CFU at Al-Hodeidah Power Plant in summer Table 2 Figure 2. Generally, the petroleum degrading bacterial counts were lower in winter than in summer.

Table 2: Seasonal Petroleum Degrading Bacterial Counts in Surface Seawater (10^3 Cfu).

Stations	winter (PDBCs)	summer (PDBCs)
Fishing Port	1.25	2.85
Corniche Al-Hodeidah	1.12	2.68
Al-Hodeidah Harbor	1.33	2.94
Al-Hodeidah Power Plant	1.1	2.35

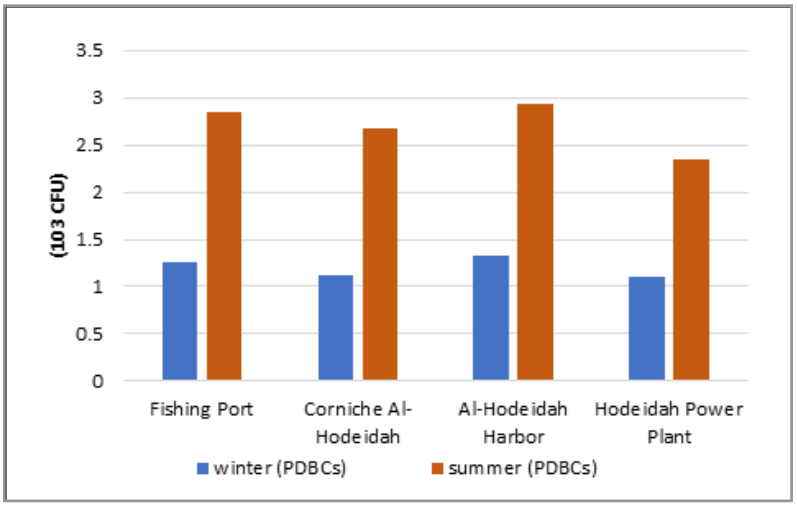

Figure 2: Seasonal Petroleum Degrading Bacterial Counts in Surface Seawater (10^3cfu).

In general, the highest concentration was observed at Al-Hodeidah Harbor, followed by Fishing Port and Corniche of Al-Hodeidah as shown in Table 3. The difference in Petroleum Degrading Bacterial Counts observed in this study may be related to the presence levels of TPH in the locations, also an increase in oil degradation was associated with an increase in microbial cell number [22-24]. On the other hand, the seasonal variations of petroleum degrading bacterial counts suggests that the populations of bacteria were actually influenced by the physio-chemical parameters during the seasons [22].

Table 3: Identification Result of Isolated Bacteria

Bacteria			% Probability		
Aeromonas Hydrophila Complex			99.99		
Color	Pale	H2S	-	TO4	-
Shape	Rod	Indole	-	TAR	-
Gram	-	Lysine	-	ACE	-
Glucose	+	Arginine	+	CET	-
Sucrose	+	Ornithine	-	OF-glucose	+
Sorbitol	+	TDA	-	P4	+
Raffinose	-	Esculin	+	K4	-
Rhamnose	-	VP	+	NIT	+

Arabinose	+	Citrate	-	FD64	-
Inositol	+	MAL	-	OXI	+
Adonitol	-	ONPG	-	CF8	-
Melibiose	-	CL4	+	Urease	-

The result of the biodegradation test of crude oil by Isolated bacteria shown in Table 4 and Figure 3 and 4. From the result we can say that Aeromonas Hydrophila Complex has the ability to degrade the petroleum hydrocarbons n-alkanes. The chromatography study showed that Aeromonas Hydrophila Complex was capable of using 10% of 1997.169 ppm concentration used crude oil, in 15 days under laboratory conditions at 25 ◦C with Bushnell-Haas media.

Table 4: Concentrations of Aliphatic Hydrocarbons N-Alkanes in a Liquid Broth Medium: Sample a Without Isolated Bacteria, Sample B with Isolated Bacteria (Ppm).

Carbon Number	Name	Sample A	Sample B
9	n-Nonane	95.66	76.84
10	n-Decane	162.23	139.17
12	n-Dodecane	203.07	173.20
14	n-Tetradecane	276.73	189.31
16	n-Hexadecane	280.04	263.18
18	n-Octadecane	156.81	155.80
19	n-Nonadecane	178.36	175.28
20	n-Eicosane	151.47	148.08
22	n-Docosane	123.43	122.00
24	n-Tetracosane	128.91	127.33
26	n-Hexacosane	102.13	101.17
28	n-Octacosane	83.18	81.09
30	n-Triacotane	53.30	48.68
36	n-Hexatriacotane	1.84	1.74
	Total	1997.16	1802.87

Initial oil concentration affects the rate of oil biodegradation in the coastal-marine ecosystems. For example, in a study by Bacosa the alkane degradation rate constant was highest at 50 ppm (0.25 ppm/day) initial oil concentration, followed by 100 ppm and 200 ppm (0.13 ppm – 0.15 ppm/day) [25]. The lowest degradation rate constants (0.05 ppm/day and 0.08 ppm/day) were observed in the high concentration 400 and 800 ppm treatments. The bacterial community structures at high oil concentrations differ from those at low concentrations (25). However, it was barely detected at high concentrations. In general, it has been shown that oil degradation decreases as oil concentration increases. Whereas, various hydrocarbons such as alkanes degrade at different rates.

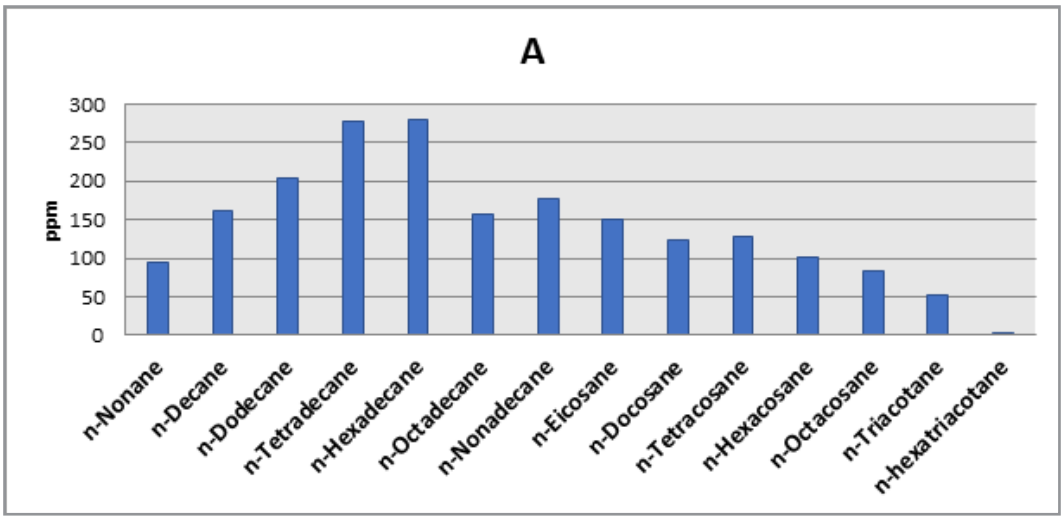

Figure 3: The Concentrations of Individual (N-Alkanes) from the Crude Oil, A = without Isolated Bacteria.

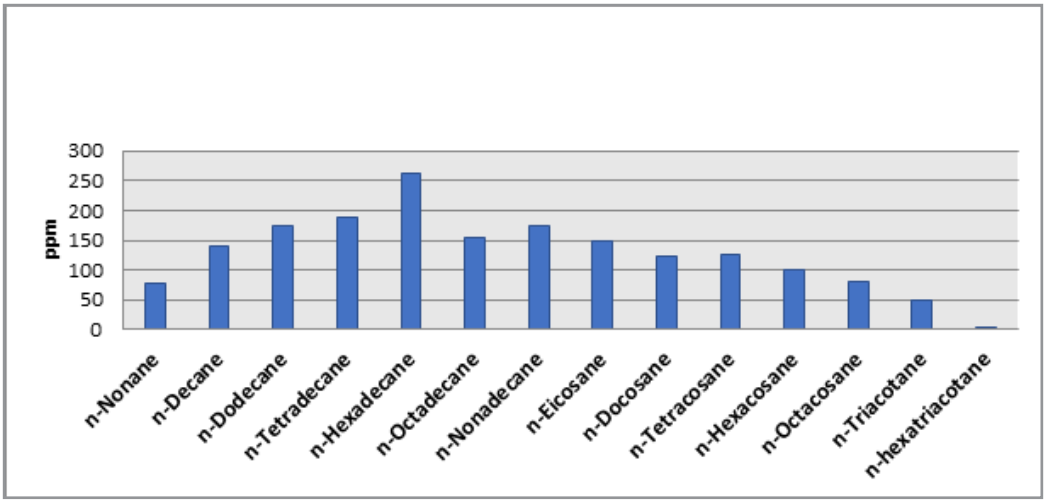

Figure 4: The Concentrations of Individual (N-Alkanes) from the Crude Oil, B = with Isolated Bacteria.

Conclusions and Recommendations

A variety of microorganisms are known to have hydrocarbon biodegradation capacity bacteria. These microorganisms are naturally present in coastal-marine environments, even before the occurrence of oil spills, but they are mostly in low abundance in natural, unpolluted environments. They are widely distributed within surface seawater, sediments, and soil habitats, and the population of these bacteria rapidly increases after oil spills. PDBC were actually influenced by the physio-chemical parameters. The activities of PDBC in a polluted environment is the most efficient and eco-friendly means of riding off pollutants from (detoxifying) the environment.

It is recommended that, continuous Researches to study on the Hydrocarbons-Degrading Bacteria in seawater for the Red Sea cost of Yemen as a great eco-friendly to mineralization of hydrocarbons by microorganisms for detoxifying the environment from these pollutants.

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