

The Effect of Temperature on Possible Microorganisms Isolated and Identified in Freshwater and Saltwater Media from Mixed Crude Oil

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Abstract

The investigation of the effect of temperature on the characteristics and properties of microorganisms isolated and identified in freshwater and saltwater media mixed with crude oil demonstrates the potential impact of temperature variation under different bioreactor conditions. However, temperature was identified as an inhibitor of microbial growth, resulting in a decrease in the population of individual organisms sampled during the investigation. This trend was observed in both freshwater and saltwater environments. Other parameters may also cause a decline in microbial counts or population; however, this research focuses mainly on the significance of temperature on microbial activity during crude oil remediation or the treatment of effluents before their discharge into the environment. The investigation of the effect of temperature on the characteristics and properties of microorganisms isolated and identified in fresh- and saltwater media mixed with crude oil demonstrates the potential of microorganisms to degrade petroleum hydrocarbons at elevated temperatures under superthermophilic conditions. The microorganisms identified included Bacillus coagulase, Bacillus niacinic, and Micrococcus sp. as bacteria, whereas Penicillium sp., yeast sp., and Niger were identified as fungi. This investigation was able to identify the possible operational temperatures at which some microorganisms can effectively degrade petroleum hydrocarbons under the influence of temperature. However, the treatment of pollutants in some cases requires heating to disintegrate toxic substances that may adversely impact the environment. The application of heating in some cases enhances microbial activity, mitigates excessive microbial growth, and increases the rate of pollutant degradation or remediation. This investigation has demonstrated the effect of temperature on the growth mechanisms of some bacteria and fungi isolated and identified from the experimental setups involving freshwater polluted with crude oil and saltwater contaminated with crude oil.

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INTRODUCTION

Research on the factors that influence the biodegradation of contaminants has revealed that

the temperature of the system is one of the contributing components identified, and the role played indicates its significant contribution to biodegradation and bioremediation processes [1–4]. It is observed that some microorganisms are more active when the temperature is within <20°C to <45°C (mesophilic), <20°C to <75°C (thermophilic), and <20°C to <105°C (superthermophilic) [5]. In this case, one can say that the microorganisms' performance in biodegradation and bioremediation Journal of Modern Chemistry & Chemical Technology, depends on the operational temperature of the system. The optimum operating temperatures for mesophilic are 45°C, thermophilic are 75°C, and superthermophilic are 105°C [6]. It is observed that different microorganisms have different optimal temperatures, which can be attributed to the environment they are found in. In cold weather or climates, microorganisms serve well in hot climates Journal Modern Chemistry & Chemical Technology below 105°C) microorganism services even at room temperature and lower temperatures [7–12]. In most cases, thermophilic organisms do well at temperatures greater than mesophilic temperatures, and superthermophilic organisms do well at temperatures less than 75°C [13]. The attributes of thermophilic and superthermophilic organisms show their capability to withstand high operational temperature systems, as well as lower operational temperature systems for mesophilic organisms. In most cases, bioremediation systems are more effective at low operating temperatures (mesophilic conditions) than at thermophilic or superthermophilic [13–15]. To determine the activation energy of the process, it is necessary to recall the Arrhenius equation in the case where a decrease in metabolic activity Journal of Modern Chemistry & Chemical Technology was observed with a decrease in temperature [16–20]. Journal of Modern Chemistry and Chemical Technology

Temperature has a marked influence on the rate of biodegradation of petroleum hydrocarbons. The degradation rate is a direct function of temperature and obeys the relationship originally described by Arrhenius.

$$K = Ae^{-E/RT}, \quad (1)$$

Where A frequency factor, E is the activation energy, R is the gas constant, and T is the absolute temperature.

The temperature of the environment can affect the degradation rate, either directly or indirectly, on the activities of the microbial population in several ways. [21]. Most microorganisms, in terms of biomass formation, are always influenced when a sudden change in temperature is observed [22–26]. The degradation of pollutants in either water or soil environments can be influenced by temperature and other environmental factors [27–33]. Atlas investigated microbial activity on petroleum hydrocarbon degradation and found that the best operating temperature can occur at a minimum temperature of less than 0°C and a maximum temperature of about 105°C [34–41]. Some microorganisms obtain their maximum yield at temperatures within 20–35°C and are classified as mesophilic, whereas those within the temperature range of 35–75°C are called thermophilic, and finally, those that survive at temperatures above 75–105°C are classified as super thermophilic [42–47]. Temperature shifts can affect the microbial population at any given time. Biodegradation is highly dependent on the incubation temperature, with higher temperatures resulting in higher abiotic losses and greater susceptibility to biodegradation [48–53]. It is only a limiting factor when it is considered with other factors, and research carried out by various groups has demonstrated the significance of temperature in the treatment of contaminants [54–58].

MATERIALS AND METHODS

Analysis of Temperature Determination

The temperature of the accreted lagoon was measured by inserting a thermometer and thermocouple inside the reactor. The heat system was programmed at incremental temperatures until the set point value was obtained.

The temperature remained constant for the hourly incremental set point, and the temperature was measured to ensure that there was no variation from the set point. The thermometer was dropped into the aerated lagoon for five minutes, and the temperature in degrees centigrade was measured and recorded.

Characterization and Identification of Fungi Isolates

9 mL of normal saline was dispensed into two different test tubes, and 1 mL of each water sample was measured and dispensed into one of the test tubes labelled 10^{-1} . One milliliter was transferred with a 1 mL pipette into the second and third tubes to complete a serial tenfold dilution [39–44]. This method employed the use of sterile filter paper saturated with 1 mL of sterilized crude oil and aseptically placed on the inside cover of the inverted Petri dishes before incubation. Aliquots of 0.1 mL from dilutions of 10^{-1} were inoculated into the media in duplicate. To enumerate the hydrocarbon-degrading bacteria, the medium was supplemented with Fungusol to prevent the growth of fungal contaminants, while antibiotics were supplemented in a separate medium to prevent the growth of bacteria. The plates were incubated for 7 days before enumeration. Mineral salt agar was constituted as adopted, with little modification, as shown in the table below for the enumeration of hydrocarbon-utilizing microorganisms [45–50].

Petroleum Hydrocarbon Contaminated Water Heated with Temperature Control System

The flow diagram for the petroleum hydrocarbon contaminated water heated using a temperature control system is shown in Figures 1 and 2.

- Revised process outlet to the tank—230 mm
- Distance between overflow outlets to revised outlet—380 mm
- Distance between bottoms to overflow outlet—400 mm
- Distance between bottoms to revised outlet—780 mm
- Pack bed = distance between each other—310 mm
- Height of the pack bed—2100 mm
- Base of the stand to control panel—1600 mm
- From control panel to top of the tank—1550 mm
- From control to top base of the tank—570 mm
- Lower circulator flow to the top of the tank—710 mm
- Upper circulator flow to the top of the tank—440 mm
- Distance between lower and upper circulator flow—280 mm
- Bottom of the tank to the lower circulator flow—300 mm
- Bottom of the tank to the upper circulator flow—560 mm
- Distance of tank base to the heater—470 mm
- Distance from the heater to the top of the tank—520 mm

RESULTS AND DISCUSSION

The results obtained in this study are presented in tables and figures. Table 1 demonstrates the initial concentrations of the physicochemical properties of freshwater and saltwater media. The parameters analyzed revealed that the concentrations were within the acceptable limits, except for the dissolved oxygen of the saltwater sample.

Table 2 shows the possible microorganisms (bacteria) isolated and identified in freshwater and saltwater media after mixing with crude oil. These organisms were cultured and inoculated into the aerated lagoon bioreactor.

Table 3 shows the possible microorganisms (fungi) isolated and identified in freshwater media mixed with crude oil. The organisms listed in Table 4 were cultured and inoculated into the bioreactor with the configuration of an aerated lagoon.

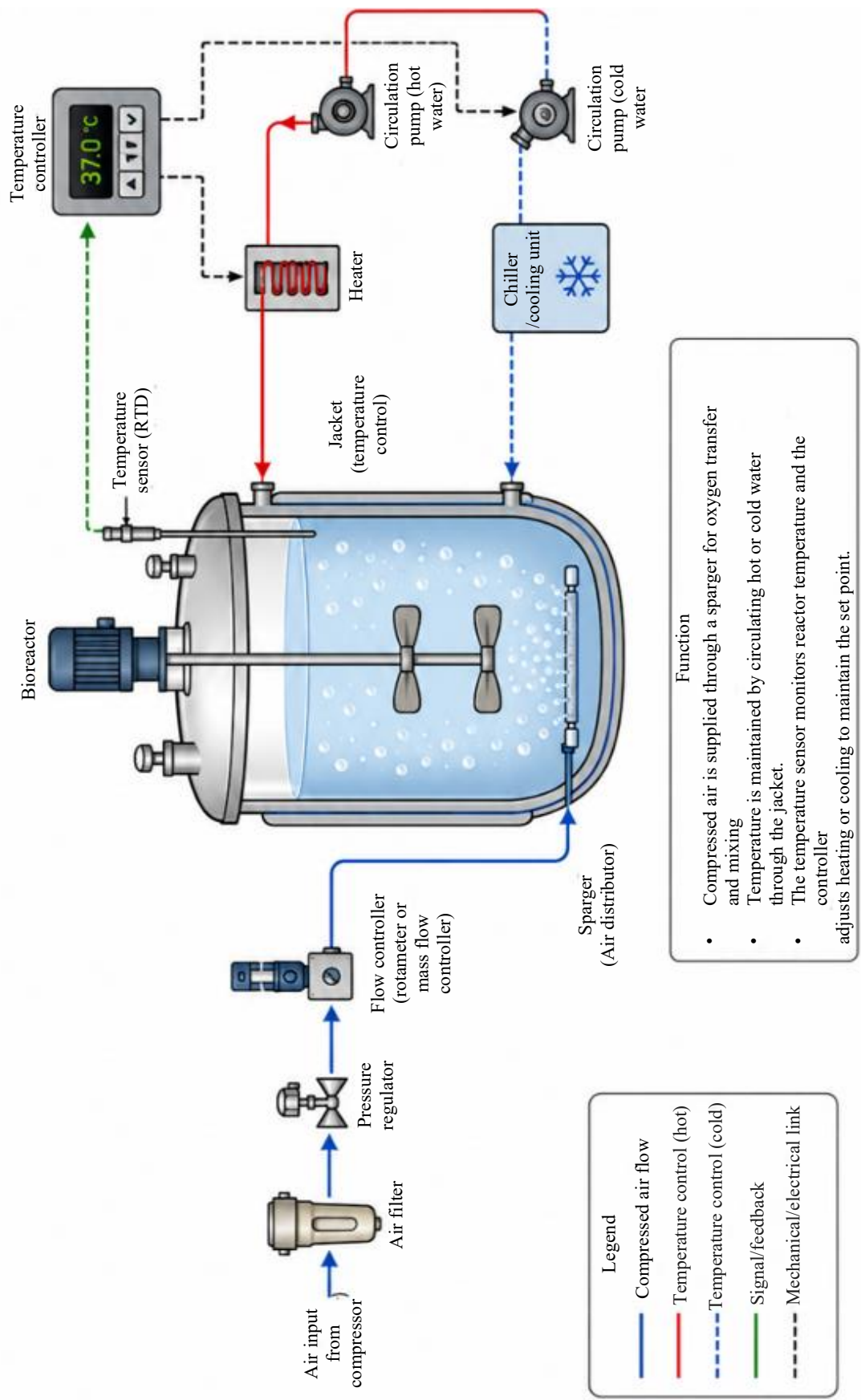


Figure 1. Schematic flow diagram of aeration and temperature control in a bioreactor.

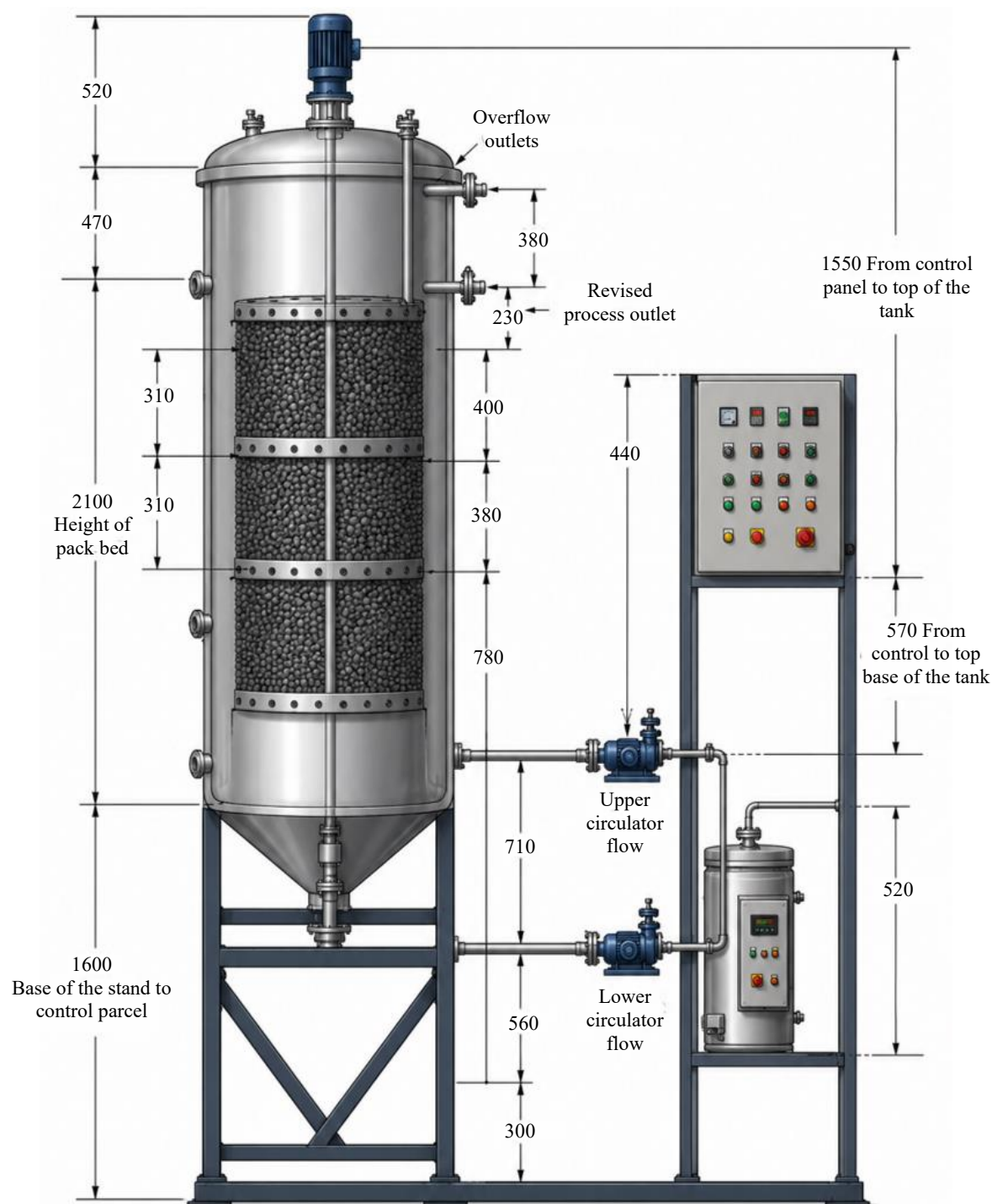


Figure 2. Systematic dimensions of bioreactors used for the investigation.

Table 4 predicts the allowable operating temperature of bacteria in crude oil degradation in the freshwater medium of an aerated lagoon in terms of mesophilic, thermophilic, and super thermophilic.

Table 5 showcases the characteristics of organisms (bacteria) at allowable operating temperatures for crude oil degradation in a saltwater medium of an aerated lagoon. In this case, most of the bacteria are more robust and able to withstand higher operating temperatures than the same organisms that were present in freshwater medium. This can be attributed to the physicochemical properties of the saltwater-to-crude oil mixture.

Table 1. The initial physicochemical properties of freshwater and saltwater media used for the investigation.

S.N.	Parameter	Test method	Result		Standard
			<i>Freshwater</i>	<i>Saltwater</i>	
1	Total dissolved solids (mg/c)	APHA 2510 B	31.0	28.11	500
2	Conductivity ($\mu\text{s}/\text{cm}$)	APHA 2510 B	36.90	45.33	
3	Temperature ($^{\circ}\text{C}$)	APHA 4500 T	26.18	27.30	27.0
4	pH	APHA 4500 HB	6.83	7.01	6.5-8.5
5	Chloride (mg/l)	APHA 4500 C	53.90	5136.13	250
6	Sulphate (mg/l)	APHA 4500 SO_4^{2-}	59.50	80.85	400
7	Nitrate (mg/l)	EPA 3521	0.38	2.90	40.0
8	Turbidity (NTU)	APHA 2130 B	1.36	3.55	5.0
9	Alkalinity (mg/l)	ASTMD 1067 B	58.74	365.13	400
10	Oil and grease (mg/l)	APHA 3111 B	1.30	1.42	10.0
11	Dissolved oxygen (mg/l)	ASTM D 3921	7.86	8.14	7.0
12	Iron (mg/l)	APHA 3111 B	18.6	0.39	0-0.30
13	Total hardness (mg/l)	APHA 2340 C	0.28	159.16	500

Table 2. Results of possible microorganisms (bacteria) are isolated and identified in freshwater and saltwater media mixed with crude oil.

S.N.	Sample medium	Organisms isolated and identified	Organism group	Sample medium	Organisms isolated and identified	Organism group
1	Freshwater and crude oil mixture	<i>Bacillus cereus</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus cereus</i>	Bacteria
2	Freshwater and crude oil mixture	<i>Bacillus coagulans</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus coagulans</i>	Bacteria
3	Freshwater and crude oil mixture	<i>Bacillus niacini</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus niacini</i>	Bacteria
4	Freshwater and crude oil mixture	<i>Bacillus subtilis</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus subtilis</i>	Bacteria
5	Freshwater and crude oil mixture	<i>Bacillus caubiriphilis</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus caubiriphilis</i>	Bacteria
6	Freshwater and crude oil mixture	<i>Micrococcus sp.</i>	Bacteria	Saltwater and crude oil mixture	<i>Micrococcus sp.</i>	Bacteria
7	Freshwater and crude oil mixture	<i>Alcaligenes faecalis</i>	Bacteria	Saltwater and crude oil mixture	<i>Alcaligenes faecalis</i> (NI)	Bacteria
8	Freshwater and crude oil mixture	<i>Staphylococcus sp.</i>	Bacteria	Saltwater and crude oil mixture	<i>Staphylococcus sp.</i> (NI)	Bacteria
9	Freshwater and crude oil mixture	<i>Bacillus badius</i>	Bacteria	Saltwater and crude oil mixture	<i>Bacillus badius</i> (NI)	Bacteria
10	Freshwater and crude oil mixture	<i>Bacillus megaterium</i> (NI)	Bacteria	Saltwater and crude oil mixture	<i>Bacillus megaterium</i>	Bacteria

Note: NI means microorganisms not isolated and identified.

Table 3. Results of possible microorganisms (fungi) are isolated and identified in freshwater and saltwater media mixed with crude oil.

S.N.	Sample medium	Organisms isolated and identified	Organism group	Sample medium	Organisms isolated and identified	Organism group
1	Freshwater and crude oil mixture	<i>Penicillium sp.</i>	Fungi	Saltwater and crude oil mixture	<i>Penicillium sp.</i>	Fungi
2	Freshwater and crude oil mixture	<i>Yeast sp.</i>	Fungi	Saltwater and crude oil mixture	<i>Yeast sp.</i>	Fungi
3	Freshwater and crude oil mixture	<i>Niger</i>	Fungi	Saltwater and crude oil mixture	<i>Niger</i>	Fungi
4	Freshwater and crude oil mixture	<i>Fusarium sp.</i>	Fungi	Saltwater and crude oil mixture	<i>Fusarium sp.</i>	Fungi
5	Freshwater and crude oil mixture	<i>Alternaria sp.</i>	Fungi	Saltwater and crude oil mixture	<i>Alternaria sp. (NI)</i>	Fungi
6	Freshwater and crude oil mixture	<i>Fumigatus</i>	Fungi	Saltwater and crude oil mixture	<i>Fumigatus (NI)</i>	Fungi
7	Freshwater and crude oil mixture	<i>Tereus</i>	Fungi	Saltwater and crude oil mixture	<i>Tereus</i>	Fungi

Note: NI means microorganisms not isolated and identified.

Table 4. Characteristics of organisms (bacteria) at allowable operating temperatures for crude oil degradation in freshwater medium of an aerated lagoon.

S.N.	Organisms (bacteria)	Temperature		
		Mesophilic	Thermophilic	Super thermophilic
		<15°C to <45°C	>45°C to <75°C	>75°C to <120°C
1	<i>Bacillus cereus</i>	$T_{max} = 60^{\circ}\text{C}$ ←————→		
2	<i>Bacillus coagulans</i>	$T_{max} = 45^{\circ}\text{C}$ ←————→		
3	<i>Bacillus niacini</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
4	<i>Bacillus subtilis</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
5	<i>Bacillus caubiniphilus</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
6	<i>Micrococcus sp.</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
7	<i>Alcaligenes faecalis</i>	$T_{max} = 30^{\circ}\text{C}$ ←————→		
8	<i>Staphylococcus sp.</i>	$T_{max} = 30^{\circ}\text{C}$ ←————→		

Table 6 demonstrates the characteristics of organisms (fungi) and the allowable operating temperature for crude oil degradation in the freshwater medium of an aerated lagoon. The allowable operating temperature was classified in terms of mesophilic, thermophilic, and superthermophilic, and the operating temperature of each organism was well illustrated.

Table 7 illustrates the characteristics of organisms (fungi) and the allowable operating temperature for crude oil degradation in the saltwater medium of an aerated lagoon. The results obtained revealed the operating temperatures of *Penicillium sp.*, *Yeast sp.*, *Niger*, and *Fusarium sp.*

Table 5. Characteristics of organisms (bacteria) on allowable operating temperature for crude oil degradation in saltwater medium of an aerated lagoon.

Temperature			
S.N.	Organisms (bacteria)	Mesophilic $<15^{\circ}\text{C}$ to $<45^{\circ}\text{C}$	Thermophilic $>45^{\circ}\text{C}$ to $<75^{\circ}\text{C}$ Super thermophilic $>75^{\circ}\text{C}$ to $<120^{\circ}\text{C}$
1	<i>Bacillus cereus</i>	$T_{max} = 120^{\circ}\text{C}$ 	
2	<i>Bacillus coagulans</i>	$T_{max} = 120^{\circ}\text{C}$ 	
3	<i>Bacillus niacini</i>	$T_{max} = 120^{\circ}\text{C}$ 	
4	<i>Bacillus subtilis</i>	$T_{max} = 105^{\circ}\text{C}$ 	
5	<i>Bacillus caubiniphilus</i>	$T_{max} = 105^{\circ}\text{C}$ 	
6	<i>Micrococcus sp.</i>	$T_{max} = 120^{\circ}\text{C}$ 	
7	<i>Bacillus megaterium</i>	$T_{max} = 60^{\circ}\text{C}$ 	

Table 6. Characteristics of organisms (fungi) on allowable operating temperature for crude oil degradation in freshwater medium of an aerated lagoon.

		Temperature		
S.N.	Organisms (bacteria)	Mesophilic <15°C to <45°C	Thermophilic >45°C to <75°C	Super thermophilic >75°C to <120°C
1	<i>Penicillium sp.</i>	$T_{max} = 120^{\circ}\text{C}$ ←————→		
2	<i>Yeast sp.</i>	$T_{max} = 60^{\circ}\text{C}$ ←————→		
3	<i>Niger</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
4	<i>Fusarium sp.</i>	$T_{max} = 60^{\circ}\text{C}$ ←————→		
5	<i>Alternaria sp.</i>	$T_{max} = 45^{\circ}\text{C}$ ←————→		
6	<i>Fumigatus</i>	$T_{max} = 75^{\circ}\text{C}$ ←————→		
7	<i>Tereus</i>	$T_{max} = 45^{\circ}\text{C}$ ←————→		

Table 7. Characteristics of organisms (fungi) on allowable operating temperature for crude oil degradation in saltwater medium of an aerated lagoon.

Temperature				
S.N.	Organisms (bacteria)	Mesophilic $<15^{\circ}\text{C}$ to $<45^{\circ}\text{C}$	Thermophilic $>45^{\circ}\text{C}$ to $<75^{\circ}\text{C}$	Super thermophilic $>75^{\circ}\text{C}$ to $<120^{\circ}\text{C}$
1	<i>Penicillium sp.</i>	$T_{\max} = 120^{\circ}\text{C}$ 		
2	<i>Yeast sp.</i>	$T_{\max} = 120^{\circ}\text{C}$ 		
3	<i>Niger</i>	$T_{\max} = 120^{\circ}\text{C}$ 		
4	<i>Fusarium sp.</i>	$T_{\max} = 45^{\circ}\text{C}$ 		

CONCLUSION

A study on the effect of temperature on the biokinetics of crude oil degradation in an aerated lagoon was carried out to examine the significance of temperature on crude oil remediation or degradation with the action of microbes that were isolated, identified, cultured, and inoculated in the bioreactor.

This thesis demonstrates the significance of temperature in the biodegradation of crude oil in an aerated lagoon in the following manner:

1. The ability of microbes to enhance crude oil degradation in an aerated lagoon is controlled by temperature. In most cases, the temperature acts as an activator.
2. The research revealed that temperature influences microbial growth, especially with the optimum population of microbes in an aerated lagoon being mesophilic organisms with a temperature range of $T_{mm} < 20^{\circ}\text{C}$ to $T_{max} < 45^{\circ}\text{C}$. An increase in temperature above 45°C influences the performance of mesophilic organisms in terms of growth and activity, which leads to a phase of decline. Similarly, at this temperature, thermophilic organisms will enhance crude oil degradation and remediation in an aerated lagoon, but above a temperature of 75°C , the active sites of thermophilic organisms will be denatured, and a decline in performance will be observed. However, temperatures above $T_{mm} > 75^{\circ}\text{C}$ to $T_{max} < 120^{\circ}\text{C}$ are classified as superthermophilic organisms. At temperatures above 75°C redeemed the active sites of mesophilic and thermophilic organisms were redeemed (the active sites of the organisms were inhibited); therefore, the superthermophilic organisms were found to be active in the biodegradation and remediation of the crude oil present in an aerated lagoon.
3. The effect of temperature was examined to monitor and predict the characteristics of the microbes (bacteria and fungi) in terms of mean count and colony-forming units per milliliter (cfu/mL) at the initial and final stages of operating temperature.

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