DISCOVERY

58(323), November 2022

Comparison of Temperature Effect on Bacteria Growth in Crude Oil Degradation in Bioreactor of Water Media

Okirie FU, Ukpaka CP*, Dagde KK, Amadi SA

To Cite:
Okirie FU, Ukpaka CP, Dagde KK, Amadi SA. Comparison of Temperature Effect on Bacteria Growth in Crude Oil Degradation in Bioreactor of Water Media. *Discovery*, 2022, 58(323), 1194-1203

Author Affiliation:
Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, PMB 5080, Rivers State, Nigeria.

***Corresponding author:**
Department of Chemical/Petrochemical Engineering, Rivers State University Port Harcourt, PMB 5080, Rivers State, Nigeria. Email: peter.ukpaka@ust.edu.ng

Peer-Review History
Received: 14 August 2022
Reviewed & Revised: 17/August/2022 to 02/October/2022
Accepted: 05 October 2022
Published: November 2022

Peer-Review Model
External peer-review was done through double-blind method.

ABSTRACT

Comparison on the effect of temperature on bacteria growth upon crude oil degradation was monitored in water media (salt and fresh water media). The experimental investigation reveals the following organisms *Bacillus pumilus, Bacillus nealsoni, Bacillus Licheniformis, Klehsiella singaporenssis* and *Erwinio rhapnotic* isolated and identified from crude oil, *Edwardsiella ictaluri, Klebsiella aerogens, Brevibacillus laterosporus, Bacillus alcalophilus, Bacillus brevis, Tatumello ptyseos* and *Bacillus acidiceler* for fresh water medium and for salt water medium *Paenibocillus pectinilyticus, Bacillus niacin, Yersinia mollareti, Pectabacterium betavasculonm, Bacillus smithi, Bacillus siamensis* and *Bacillus cereus*. These organisms were cultured and inonculated into the bioreactor designed and fabricated at elevated height with the temperature of the process regulated within the range of 15^{o}C to 120^{o}C. The growth of each bacteria was monitored with increase on the functional temperature and research predicted no lag phase and decline phase within 15^{o}C to 45^{o}C for all rather increase in microbial was experienced. However, as temperature increased above 45^{o}C all the mesophilic organisms decline. This phenomena was experienced at 75Oc for bacteria optimum allowable operating temperature of thermophilic and super thermophilic of 105^{o}C, the investigation reveals that at temperature up to 120^{o}C bacteria as organism survives but the active site of the bacteria are highly inhibited to catalyzed the reaction process. This research predicted that bacteria of salt water medium performance was higher than the fresh water medium because of the ability to withstand the effect of temperature upon crude oil degradation.

Key words: Comparison, temperature, effect, bacteria growth, crude oil, degradation

1. INTRODUCTION

Bioreactors are dyed ponds equipped with aerated system and settling tank. The settling tank serves as a clarifier in which the biological solids effluent from the lagoon are removed [1-3]. The structure of the clarifier may be made of concrete equipped with mechanical rakes for continuous removal of the biological sludge and other materials. In some cases, stabilization ponds are used in place of

settling tank, where the biological solid in the pond are periodically drained and sludges removed are used for landfill as reported [4-7].

The aeration system employed in the Bioreactor is surface aeration using a floating device. Investigation conducted on aeration system revealed that the provision of oxygen in the upper zone for facultative while in some bioreactors perforated pipes are led at the bottom of the lagoon so as to release compressed air for the purpose of aeration [8-10]. The use of lagoon for effluent treatment has been found useful to be very economical compared to sludge treatment mechanism as reported by various researchers [11-12].

The major setback identified in the lagoon system is the large land area required for the construction of the lagoon and the clarifier. The Bioreactor functions as an activated sludge system without a recycle hence the biochemical kinetics for activated sludge could be used for design purposes in some cases [13]. Research conducted on stagnant pond revealed that pond can be defined as a stagnant pool of water contaminated with pollutant as well as often referred to as stabilization or oxidation ponds [14]. The stagnant ponds are used for de-mineralization of organic contaminant from individual effluent and during the process of bio-oxidation, carbon dioxide, ammonia and inorganic radicals may be given up as products in addition to the biomass [15-18].

Furthermore, their research reveals that the biological and chemical processes in the system, are both beneficial to one another and to other biological organisms. The presence of other organization which make use of the carbon dioxide and sunlight to generate oxygen necessary for aerobic biodegradation [19-20]. In most cases, individual hydrocarbon properties are found to play a vital role in terms of microbial activities and biodegradability of the substrate. Studies conducted revealed that petroleum as a contaminant consists of various complex mixtures of constituents or compounds which influence the microbial growth and degradation processes. The chemical characteristics of the hydrocarbon indeed influence the environment and everything that derives their source of energy within the area of contamination. These hydrocarbons can be grouped as paraffin, olefin, nathylene and aromatic and the alphatics groups are classified as saturated such as branched alkanes, cycloalkanes and n-alkane whereas the aromatics are unsaturated such as benzene, toluene and xylene. Research conducted revealed that degradability of petroleum hydrocarbon group is in the order of magnitude of n-alkanes >branched alkanes > aromatic cycloalkanes hydrocarbon of low molecular weight > cycloalkanes > asphaltic > resin [21-25].

2. MATERIALS AND METHODS

Materials and Equipment

The following materials and equipment were used in carrying out the thesis work on the effect of temperature on bacteria growth of crude oil degradation in an Bioreactor, which include; thermometer, thermocouple, conical flask, plastic container, compressor, fabricated bioreactor, salt water, fresh water, crude oil, heater, capillary tube, electrical power source, sampling bottle for microbial analysis.

Sample Collection

Fresh water sample was collected from Orashi River in Ahoada Community in Ahoada East Local Government Area of Rivers State, Nigeria and the salt water sample collected from Eagle Island in Port Harcourt Local Government Area of Rivers State Nigeria. The samples were transported to the department of Chemical/Petrochemical Engineering Laboratory for analysis as well as to set-up the experiment. Crude oil sample obtained from Ogbogu flow station in Ogba/Egbema/Ndoni Local Government Area of Rivers State, Nigeria was transported to the same department for analysis as well as to set-up the experiment.

The Bioreactor was fabricated by Tonny Engineering Limited and transported to Chemical/Petrochemical Engineering Laboratory for onward set-up to receive the required connection and materials for the startup of experimental procedures.

Microbiology of Samples

This involved enumeration and isolation of aerobic heterotrophic bacteria and fungi from the water samples. Serial ten-fold dilution was employed, in which the water samples were diluted serially up to 10^{-2}dilutions. Aliquot (0.1ml) of appropriate dilutions were spread plated, using a sterile bent glass rod, onto the surfaces of fresh sterile dried nutrient agar plates for bacteria and sabouraud dextrose agar plates for fungi. The inoculated plates were incubated at 37^0C for 24 hours for bacteria and 2-3days for fungi. After incubation, plates that had significant growth were counted and the population of bacteria was recorded in colony forming units per gramme (cfu/ml) while population of fungi was recorded in spore-forming units per gramme (sfu/ml) soil.

Representative discrete bacterial colonies were purified by sub culturing onto fresh sterile nutrient agar plates which were incubated at 37^0C for 24 hours and used as pure cultures for characterization of the isolates. Similarly, colonies of fungi were sub

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

cultured onto Sabouraud Dextrose Agar (SDA) plates which were incubated for 28^{0}C for 3–5 days and the pure cultures used for characterization of fungal isolates.

Characterization and identification of bacterial isolates

Pure cultures of bacteria were obtained and subjected to various characterization procedures. The standard characterization tests performed included: Gram stain, motility test, catalase, methyl red and Vogues Proskaver test. Others are urease, indole, protease, nitrate reduction, starch hydrolysis and sugar fermentation tests. The isolates were identified on the basis of their cultural, morphological and biochemical reactions, and by reference to Cowan, 1974; Buchanan and 1994; Linn, 2006. ABIS online identification tool was further used to identify bacteria to generic and/species levels.

Fungal isolates were characterized by microscopy by observing the colonial morphology, colour of colony, texture, shape, surface appearance, and colour on the reverse plates; and microscopy using the wet preparation and slide culture by observing cultural characteristics to reveal the asexual and sexual reproductive structures.

3. RESULTS & DISCUSSION

Table 1: Distribution of Bacterial Isolated in Crude oil, Fresh and Salt Water for Preliminary Sample.

S/No	Sample	Description of bacteria isolated and identified in preliminary sample of crude oil and water media	Total Heterotrophilic Bacteria x10^3 cfu/g
1	Crude Oil	Bacillus pumilus	0.30
2		Bacillus nealsoni	
3		Bacillus Licheniformis	
4		Erwinio rhapnotic	
5		Klehsiella singaporenssis	
1	Fresh Water	Edwardsiella ictaluri	0.22
2		Klebsiella aerogens	
3		Brevibacillus laterosporus	
4		Bacillus alcalophilus	
5		Bracillus brevis	
6		Bacillus acidiceler	
7		Tatumello ptyseos	
1	Salt Water	Paenibacillus pectinilyticus	0.21
2		Bacillus niacin	
3		Yersinia mollareti	
4		Pectabacterium betavasculorum	
5		Bacillus smithi	
6		Bacillus siamensis	
7		Bacillus Cereus	

Table 1 demonstrates the preliminary sample of crude oil, water medium (fresh) and water medium (salt) in terms of possible microbes present, which was isolated and identified as bacteria. The microbial population was grouped as the colonies forming unit per gram (cfu/g) for the various organisms present in crude oil sample, fresh water and salt water media. Detail species of bacteria and fungi isolated and identified are presented in Table 1 for bacteria distribution.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Table 2: Identification of Bacteria in Different Samples of Salt and Fresh Water Media on the Effect of Temperature at Different Time (Temperature Range of 15 – 45°C)

Temperature (°C)	Time (Hr)	Distribution of Bacterial Isolates																		Total Heterotrophic Bacteria (cfu/mlg^{-1}) x10^3	
		Klebsiella singaporensis	*Paenibacillus pectinilyticus*	*Edwardsiella ictaluri*	*Bacillus niacin*	*Klebsiella aeroggers*	*Brevibacillus laterosporus*	*Erwinia rhapnotii*	*Yersinia mollaretii*	*Pectobaterium betavasculorum*	*Bacillus smithii*	*Bacilius licheniformis*	*Bacillus siamensis*	*Bacillus cereus*	*Bacillus alcalophilus*	*Bacillus pumilus*	*Bacillus brevis*	*Bacillus nealsonii*	*Bacillus acidiceler*	*Tatumella ptyseos*	Fresh Water	Salt Water
15	0	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, edwardsiella ictaluri, klebsiela aerogens, brevibacillus laterosporus, erwinia rhapnotii, Yersinia mollaretii, bacillus licheniformis, bacillus alcalophilus, bacillus pumilus, bacillus brevis, bacillus nealsonii, bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, bacillus niacin, erwinia rhapnotii, Yersinia mollaretii, Pectobacterium betavasculorum, bacillus smithii, bacillus licheniformis, bacillus siamensis, bacillus cereus, bacillus pumilus, bacillus nealsonii*																			0.5	0.5
	1																				5	0.3
	2																				8.1	0.6
	3																				25	0.7
	4																				21	1.2
	5																				9.6	1.7
	6																				0.5	0.5
30	0	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, edwardsiella ictaluri, klebsiela aerogens, brevibacillus laterosporus, erwinia rhapnotii, Yersinia mollaretii, bacillus licheniformis, bacillus alcalophilus, bacillus pumilus, bacillus brevis, bacillus nealsonii, bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, bacillus niacin, erwinia rhapnotii, Yersinia mollaretii, Pectobacterium betavasculorum, bacillus smithii, bacillus licheniformis, bacillus siamensis, bacillus cereus, bacillus pumilus, bacillus nealsonii,*																				7	2
	1																				12	3.1
	2																				20	5.6
	3																				9.3	6.8
	4																				5.6	6.3
	5																				3.7	4.1
	6																				0.5	0.5
45	0																				0.8	3.5
	1	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, edwardsiella ictaluri, klebsiela aerogens, brevibacillus laterosporus, erwinia rhapnotii, Yersinia mollaretii, bacillus licheniformis, bacillus alcalophilus, bacillus pumilus, bacillus brevis, bacillus nealsonii, bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, bacillus niacin, erwinia rhapnotii, Yersinia mollaretii, Pectobacterium betavasculorum, bacillus smithii, bacillus licheniformis, bacillus siamensis, bacillus cereus, bacillus pumilus, bacillus nealsonii,*																				1.1	7.5
	2																				15	16
	3																				43	25
	4																				8.3	14
	5																				5.0	7
	6																				4.1	5

Table 2 illustrates the identification of bacteria in different samples of salt and fresh water media on the effect of temperature at different time (temperature range of 15 – 45 °C). The bacteria that withstand the temperature effects are well documented in this

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

research and the investigation reveals that temperature plays an active role on cell growth. The organisms that withstand this temperature is grouped as mesophilic.

Table 3: Identification of Bacteria in Different Samples of Salt and Fresh Water Media on the Effect of Temperature at Different Time (Temperature Range of 60 – 90 °C)

Temperature (°C)	Time (Hr)	Distribution of Bacterial Isolates																			Total Heterotrophic Bacteria (cfu/mlg^{-1}) x10^3	
		Klebsiella singaporensis	*Paenibacillus pectinilyticus*	*Edwardsiella ictaluri*	*Bacillus niacin*	*Klebsiella aeroggers*	*Brevibacillus laterosporus*	*Erwinia rhapnotii*	*Yersinia mollaretii*	*Pectobaterium betavasculorum*	*Bacillus smithii*	*Bacilius licheniformis*	*Bacillus siamensis*	*Bacillus cereus*	*Bacillus alcalophilus*	*Bacillus pumilus*	*Bacillus brevis*	*Bacillus nealsonii*	*Bacillus acidiceler*	*Tatumella ptyseos*	Fresh Water	Salt Water
60	0	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, Edwardsiella ictaluri, klebsiela aerogens, Brevibacillus laterosporus, Erwinia rhapnotii, Bacillus licheniformis, Bacillus alcalophilus, bacillus pumilus, Bacillus brevis, Bacillus nealsonii, Bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, erwinia rhapnotii, Pectobacterium betavasculorum, Bacillus smithii, Bacillus licheniformis, Bacillus pumilus, bacillus nealsonii.*																			0.5	0.5
	1																				10	5
	2																				21	10
	3																				30	18
	4																				22	20
	5																				17	11
	6																				14	7
75	0	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, Edwardsiella ictaluri, Klebsiela aerogens, Brevibacillus laterosporus, Erwinia rhapnotii, Bacillus licheniformis, Bacillus pumilus, Bacillus nealsonii, bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, bacillus niacin, Erwinia rhapnotii, Pectobacterium betavasculorum, Bacillus smithii, Bacillus licheniformis, bacillus pumilus, Bacillus nealsonii.*																			0.5	0.5
	1																				0.5	3
	2																				10	6
	3																				14	12
	4																				26	13
	5																				4	8.1
	6																				0.5	0.5
90	0																				0.8	3.5
	1	**Isolated Bacteria for water medium (fresh):** *Edwardsiella ictaluri, Klebsiela aerogens, Brevibacillus laterosporus, Erwinia rhapnotii, Bacillus licheniformis, Bacillus pumilus, Bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, bacillus niacin, Erwinia rhapnotii, Pectobacterium betavasculorum, Bacillus smithii, Bacillus licheniformis, bacillus pumilus, Bacillus nealsonii.*																			7	4
	2																				16	8

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

	3		21	15
	4		25	24
	5		27	13
	6		32	6

Table 4: Identification of Bacteria in Different Samples of Salt and Fresh Water Media on the Effect of Temperature at Different Time (Temperature Range of 105 – 120 °C)

Temperature (°C)	Time (Hr)	Distribution of Bacterial Isolates																		Total Heterotrophic Bacteria (cfu/mlg^{-1}) x10^3	
		Klebsiella singaporensis	*Paenibacillus pectinilyticus*	*Edwardsiella ictaluri*	*Bacillus niacin*	*Klebsiella aeroggers*	*Brevibacillus laterosporus*	*Erwinia rhapnotii*	*Yersinia mollaretii*	*Pectobaterium betavasculorum*	*Bacillus smithii*	*Bacilius licheniformis*	*Bacillus siamensis*	*Bacillus cereus*	*Bacillus alcalophilus*	*Bacillus pumilus*	*Bacillus brevis*	*Bacillius nealsonii*	*Bacillius acidiceler*	*Tatumella ptyseos*	Fresh Water	Salt Water
105	0	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, Edwardsiella ictaluri, Klebsiela aerogens, Bacilius licheniformis, Bacillus pumilus, Bacillus nealsonii, Bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Paenibacillus pectinilyticus, Bacillus niacin, Bacillus licheniformis, Bacillus pumilus, Bacillus nealsonii.*																		0.5	0.5
	1																			10	6
	2																			15	14
	3																			35	24
	4																			8.4	30
	5																			4.3	23
	6																			5.9	16
120	0																			0.5	0.5
	1																			0.6	0.6
	2	**Isolated Bacteria for water medium (fresh):** *Klebsiella singaporensis, Edwardsiella ictaluri, Klebsiela aerogens, Brevibacillus laterosporus, Erwinia rhapnotii, Yersinia mollaretii, Bacillus licheniformis, Bacillus alcalophilus, bacillus pumilus, Bacillus brevis, Bacillus nealsonii, Bacillus acidiceler, Tamumella ptyseos.* **Isolated bacteria for water medium (salt):** *Klebsiella singaporensis, Paenibacillus pectinilyticus, Bacillus niacin, erwinia rhapnotii, Yersinia mollaretii, Pectobacterium betavasculorum, Bacillus smithii, Bacillus licheniformis, bacillus siamensis, Bacillus cereus, Bacillus pumilus, Bacillus nealsonii,*																		1.2	1.4

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

	3		1.7	2.2
	4		1.9	3.3
	5		1	2.8
	6		0.6	2.5

Table 3 shows the identification of bacteria in different samples of salt and fresh water media on the effect of temperature at different time (temperature range of 60 – 90 °C). The bacteria that withstand the temperature effects are well documented in this research and the investigation reveals that temperature plays an active role on cell growth. The organisms that withstand temperature of above 45°C to 75°C are grouped as thermophilic.

Table 4 demonstrates the identification of bacteria in different samples of salt and fresh water media on the effect of temperature at different time (temperature range of 105 – 120 °C). The bacteria that withstand the temperature effects are well documented in this research and the investigation reveals that temperature plays an active role on cell growth.

Temperature Effect on Total Heterotrophic Bacteria in Salt and Fresh Water Media

The effect of temperature on the microbial growth of the bacteria and fungi in fresh and salt water media are presented in Figure 1 to 2.

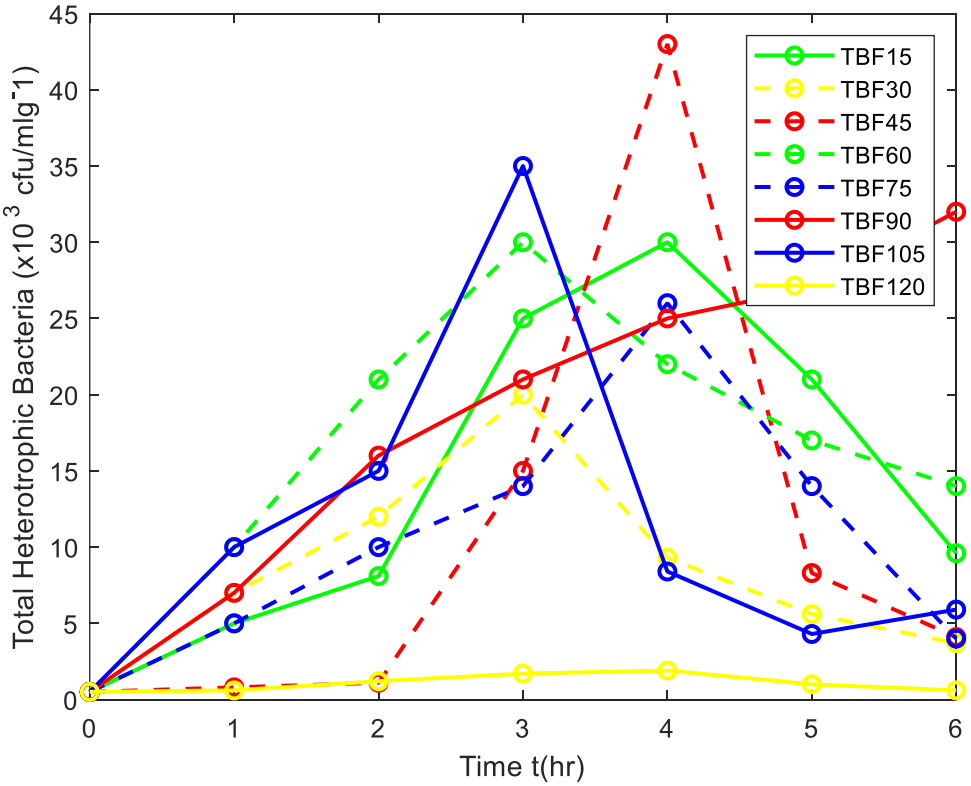

Figure 1: Temperature effect on Total Heterotrophic Bacteria Growth versus Time for Fresh Water Medium.

Figure 1 showcases the effect of temperature on total heterotrophic bacteria growth on water medium (fresh) with increase in time. Increase in microbial growth was experienced with increase in time for the various operating temperatures before sudden decrease as the operating time decreases. The organisms isolated and identified as total heterotrophic bacteria are *Edwardsiella ictaluri, Bacillius pumilus, klebsiella aerogens, bacillius brevis, klebsiella singaporensis, brevibacillus laterosporus, Erwinia rhapnotii, Bacillius licheniformis, Bacillius alcalophilus, Bacillius mealsonie, Erwinia rhapnotic.* The research work reveals that the temperature influence the microbial growth negatively in some cases as described in Figure 1.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

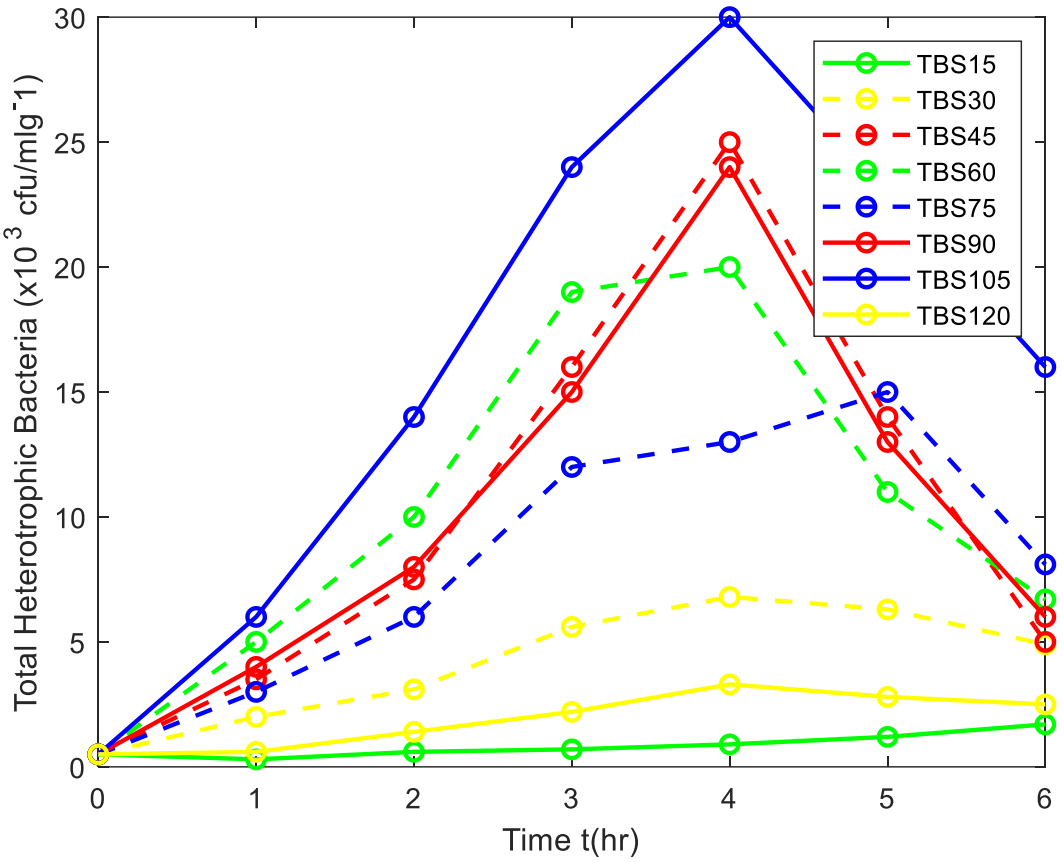

Figure 2 Temperature effect on Total Heterotrophic Bacteria Growth versus Time for Salt Water Medium.

Figure 2 reveals the effect of temperature on the total heterotrophic growth in water medium (salt) with increase in contact time in a bioreactor. The total heterotrophic bacteria isolated and identified are *Rectobacterium betarasculorum, Bacillus Smithii, klebsiella singaporensis, Wrwinia rhapnotii, Bacillius licheniformis, Bacillius alcalophilus, pnaenibacillus pectinilyticus, Bacillus niacin, Bacillius pumilus.*

Acceleration, progressive and decline phase was experienced in all phase of temperature effect of TBS 15, TBS 30, TBS 45, TBS 60, TBS 75, TBS 90, TBS 105 and TBS 120. The result reveals the effect of temperature on the bacteria growth in terms of crude oil degradation. In some cases, the temperature acted as an inhibitor and also as activator. The result predicts the allowable time on the effect of temperature for accelerating the microbial growth in the bioreactor as demonstrated in the experimental sampling.

4. CONCLUSION

This research was able to demonstrate the following:

a. The temperature above 105℃ reveals that some organisms served with low population and at high temperature range of above 75℃ (super thermophilic organisms) plays less active role in enhancing bioremediation or degradation of crude oil in Bioreactor rather than thermal degradation or remediation was experienced to be more effective because of constant evaporation of the methane gas.
b. The research work reveals some of the possible microorganisms capable of degradating petroleum hydrocarbon in an elevated temperature of super thermophilic.
c. The research work revealed that organism isolated and identified from Water medium (salt) withstand higher temperature with low decline than those obtained from water medium (fresh) yielding higher hydrocarbon degradation.

Ethical issues

Not applicable.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

Informed consent

Not applicable.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Al-Hawash, A.B., Alkppranee, J.T., Abbood, H.A., Zhang, Sun, J, Zhang, X.. & Ma, F. (2018). Isolation and characterization of two crude oil degrading fungi strains from Rumaila oil field. *Iraq Biotechnology, Rep.*, 17, 104-109.
2. Al-Hawash, A.B., Zhang, X., & Ma, F. (2018a). Removal and biodegradation of different petroleum hydrocarbon using the filamentous fungus Aspergillus sp. *RFC-1 Microbiology Open*. 8(1), 619-630.
3. Silk, S. (2015). Biodegradation and transport of crude oil in sand and gravel beaches of Aretic Alaska. *Coastal Marine Institute (CMI), Department of Civil and Environmental Engineering University of Alaska Fairbanks*, OCS study BOEM 2015-041. 1-54.
4. Sun, J. and Lu, G. (2017). Applied contanimant transport modeling (2nd edition) [M] *Beijing Higher Educaiton, Chinese press*, 51-56.
5. Ukpaka, C. P . (2016a). Studying the depuration time on changes in biomarkers profile on Nigeria crude oil. *Applied Science Reports*. 13(2), 69 – 74.
6. Ukpaka, C. P. (2012). The effect of functional parameters on microbial characteristics in crude oil degradation. *Journal of Research in Environmental Science and technology*. 1(4), 66-90.
7. Ukpaka, C. P. (2013). The concept of chemical and biochemical oxygen demand in inhibiting crude oil degradation in fresh water pond system. *Merit Research Journal of Environmental Science and Toxicology*. 1(7), 136 – 146.
8. Ukpaka, C. P. (2015). Investigation into the effect of momentum transfer on de-oxygenation of wastewater treatment in pond system for wet seas. *International Journal of Novel Research in Engineering & Pharmaceutical Sciences*. 2(4) 85 – 106.
9. Ukpaka, C. P. (2015a). Effect of pH in biodegradation of crude oil upon the application of moringa extract. Bioreactor. *International Journal of Novel Research in Engineering & Pharmaceutical Sciences*. 2(4) 43 - 70.
10. Ukpaka, C. P. (2016). Development of model for bioremediation of crude oil using moringa extract. *International Scientific Organization: Chemistry International*. 2(1), 19 – 28.
11. Ukpaka, C. P. (2017). Modeling the methodology for bioremediation decision tree for an integrated environmental management system. *Journal of Chemical Engineering and Process Technology*. 4(1), 300-325.
12. Ukpaka, C. P. (2018). Modeling of Mesophilic Characteristics of Hydrocarbon Degradation in a Fluidized Bed Reactor, Unpublished work.
13. Ukpaka, C. P. (2020). Biokinetic Model of Crude Oil degradation: The Integration of Moringa-Alcohol-Water Root Extracts, *International Journal of Environmental Chemistry*. 6(2), 1-12.
14. Ukpaka, C.P. (2006). Microbial growth and decay rate kinetics on biodegradation of crude oil. *Association for the Advancement of Modelling & Simulation Techniques in Enterprises (AMSE)*, 67(1&2), 70-75.
15. Ukpaka, C.P. (2011). Biodegradation model on effect of some physiochemical parameters on aromatic compounds in water medium (fresh). *Journal of Engineering and Technology Research*, 3(3), 4-55.
16. Ukpaka, C.P. (2013a). Evaluating the effect of conductivity on crude oil degradation in salt water pond. *International Journal of Applied Chemical Sciences Research*, 1(9), 144-158.
17. Ukpaka, C.P. (2014). Modeling of petroleum hydrocarbon obtained in Niger Delta area of Nigeria in a pond system. *International Journal of Novel Research in Engineering & Pharmaceutical Sciences*, 1(5), 25-34.
18. Ukpaka, C.P. (2015). Evaluation of microbiological corrosion of carbon steel in salt water environment of Niger Delta region. *Physicochemical Chemistry Pakistan*, 17(1), 21-26.
19. Ukpaka, C.P. (2016b). Comparison of degradation of benzene, toluene and phenol in both fresh and salt water media. *International Scientific Organization: Current Science Perspective*, 2(4), 105-115.

20. Ukpaka, C.P. (2016c). BTX degradation: The concept of microbial integration. *International Scientific Organization: Chemistry International*, 3(1), 8-16.
21. Ukpaka, C.P. (2017). Examination of plant growth on improved soil environment polluted with crude oil bioremediation. *International Scientific Organization: Current Science Perspectives*, 3(2), 67-78.
22. Ukpaka, C.P. (2018a). Matlab techniques for evaluation of crude oil degradation at various functional parameters. *Current Science Perspective*, 4(1), 7-12.
23. Ukpaka, C.P. (2018b). Comparison of theoretical and experimental approach to predict crude oil spreading rate in Niger Delta Area, Nigeria. *International Journal of Chemical Engineering and Processing*, 4(2), 27-37.
24. Ukpaka, C.P. & Otabiri, B. (2013). Responses application to monitor and predict crude oil disfillation rate using pneumatic control system on a furnace. *Journal of Engineering and Technology Research*, 5(7), 217-229
25. Ukpaka, C.P., Ogoni, H.A., Amadi, A.S. & Adebayo, T.A. (2005). Mathematical modeling of the microbuial growth and decay rate of pseudomonas species on biodegradation of Bonny light and oil. *Global Journal of Pure and Applied Science*, 11(3), 423-431.

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS