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Degradation of bitumen by Burkholderia cepacia KT803965 isolated from heavy oil impacted tropical soil

Razgradnja bitumna z mikroorganizmom Burkholderia cepacia KT803965, izoliranim iz tropskih tal, prepojenih s težko nafto

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Abstract

Non-conventional heavy oil including bitumen is fast replacing conventional light ones due to depletion of conventional oil reserves and high demand. Environmental pollution from its exploration and exploitation is of great global concern. Microorganisms remain a force in the remediation of oil polluted environment. This study investigated the degradation of bitumen by *Burkholderia cepacia* KT803965. Experiments were conducted as a function of temperature (20–50 °C), pH (3–11) and incubation period (2–10 d). The optical density was used as index of degradation and was measured using a UV-VIS spectrophotometer. The residual bitumen was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The optimal process conditions of 40 °C, pH 7 after 13 d of incubation gave rise to the maximum biodegradation (optical density of 0.24). GC-MS result showed significant changes in chemical composition of the bitumen with generation of new compounds and elimination/ reduction of parent compounds owing to degradation. Some new degradation products identified were benzene (1-pentyloctyl), 23, 28-bisnor-17.β.(H)-hopane and 1,2-benzenedicarboxylic acid. This study revealed the excellent ability of *B. cepacia* KT803965 in degrading bitumen.

Key words: bitumen, *Burkholderia cepacia* KT803965, degradation, heavy oil, pollution

Izvleček

Zaradi izčrpanosti zalog in naraščajočega povpraševanja konvencionlno lahko nafto čedalje bolj nadomeščajo z nekonvencionalno težko nafto in bitumnom. Okoljsko onesnaževanje zaradi iskanja in črpanja teh surovin postaja že globalni problem. V rekultivaciji okolja, onesnaženega s težko nafto, se uveljavljajo tudi mikroorganizmi. V tem članku poročajo o raziskavi razgradnje bitumna z bacilom *Burkholderia cepacia* KT803965. V poskusih so preučevali odvisnost razgrajevanja od temperature (20–50 °C), pH (3–11) in inkubacijske dobe (2–10 d). Za indikator razgradnje so uporabili optično gostoto, ki so jo merili z UV-VIS-spektrofotometrom. Nerazpadli bitumen v tleh so določali s plinsko kromatografsko masno spektrometrično (GC-MS) metodo. Optimalne razmere pri postopku so ugotovili pri 40 °C in pH 7 po 13 d inkubacije, ko je bil dosežen maksimum biorazkroja pri optični gostoti 0,24. Določitve GC-MS kažejo v procesu razgradnje na izrazite spremembe kemizma bitumna z nastankom novih spojin in eliminiranjem/redukcijo izhodnih. Med novo nastalimi produkti razgradnje so ugotovili benzen (1-pentyloctyl), 23, 28-bisnor-17.β.(H)-hopan in 1,2-benzendikarboksilno kislino. S študijo so potrdili odlično uporabnost *B. cepacia* KT803965 za razgradnjo bitumna.

Ključne besede: bitumen, *Burkholderia cepacia* KT803965, razkroj, težka nafta, onesnaževanje

Introduction

The demand for crude oil worldwide has substantially increased which had resulted in straining the supply of conventional oil. This however, has led to consideration of alternative energy sources, among which are heavy oil and bitumen. These forms of oil are readily available to augment short and long-term needs [1].
The vast majority of heavy oils are a consequence of microbial alteration of oils in the reservoir [2] with more than 50 % of the world's oil occurring as biodegraded oils in heavy oil and tar sand accumulations [3]. These oils also represent a significant fraction of conventional oil reserves. According to Meyer et al. [1], there are about 3 396 billion barrels (539.6×10^{12} L) of heavy oil in known accumulations and about 5 505 billion barrels (874.7×10^{12} L) of bitumen resource in known accumulations in various basins around the world.
Gordon [4] described unconventional oils as heavy, complex, carbon laden, and locked up deep in the earth, tightly trapped between or bound to sand, tar, and rock. Strausz et al. [5] described bitumen as a thick, sticky form of crude oil, so heavy and viscous that it will not flow unless heated or diluted with lighter hydrocarbons. They may be immobile in the reservoir which typically requires upgrading to refinery feedstock grade [1].
High demand for petroleum and associated products has substantially increased during the last ten decades which has made oil spills inevitable consequences of oil production and refining [6]. As a result, the problem of pollution during production and transportation of oil would remain a major environmental issue of great global concern [7]. Environmental hazards of bitumen exploration include destruction of ecosystem and pollution from bituminous toxic wastes [8]. While complete elimination of these problems is difficult to achieve, the aim is often to minimize environmental degradation [9].
The processes of removal of hydrocarbon pollutants from the environment involve physical, chemical and biological methods [10]. The biological method gains the upper hand due to inherent advantages of microorganisms. Bioremediation which is defined as any process that uses microorganisms or their enzymes to return the environment altered by contaminants to its original condition is an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to carbon dioxide and water [11]. The ability of microorganisms to degrade pollutants and growth of cells are influenced by nutritional and environmental parameters such as carbon sources, nitrogen sources, inorganic salts, temperature, and pH [12]. Therefore, it is necessary to design an appropriate process of maximizing the degradation efficiency of bitumen by microorganisms.
Burkholderia cepacia formerly known as *Pseudomonas cepacia* [13] has been reported to degrade crude oil [14, 15]. It has been shown to be capable of remarkable growth on aromatic fractions of crude oil [16, 17]. The organism has also been reported to grow on Polycyclic Aromatic Hydrocarbon (PAH) [18, 19] but it has not been reported to degrade bitumen. Since *B. cepacia* has been known to degrade PAH compounds, hence this study investigates the ability of the organism to degrade bitumen which contains heavy molecular weight hydrocarbons not reported from previous studies.

Materials and Methods

Collection of samples

Soil impacted with refined bitumen were collected randomly from Odeda, Osiele and Isolu, all in Odeda Local Government of Ogun State, while the bitumen used was obtained from Agbabu bitumen deposit in Odigbo Local Government of Ondo State, Nigeria. All samples were collected in triplicates. The unimpacted soil samples (control samples) were collected from the campus of Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. Collection of soil samples was carried out as described by Balogun and Fagade [20]. Samples were collected in sterile aluminum foil and stored at 4 °C until used.

Total Heterotrophic Bacterial Count (THBC)

The total heterotrophic bacterial count was determined as described by Rahman et al. [21]. One gram of each of the samples was serially diluted five-fold in sterile distilled water and 1 mL

of the diluents was aseptically dispensed into sterile Petri-dishes. Using the pour plate method, Plate Count Agar (Lab M, UK) was poured aseptically on inoculated plates. The plates were then incubated at 28 °C for 48 h.

Total Oil Utilizing Bacterial Count (TOUBC)

Oil utilizing bacterial count was carried out on Mineral Salt Medium (MSM) agar on which Dual Purpose Kerosene (DPK) was used as the sole carbon source. Prior to use, DPK was filtered using a Whatman filter paper No1 [22]. Agar (2 %) was added to solidify the medium. The MSM composition as described by Balogun and Fagade [23] was made up of Basal Salt Medium (BSM) and Trace element solution. The BSM contained (g L^{-1}): K_2HPO_4, 1.8; KH_2PO_4, 1.2; NH_4CL, 4.0; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; yeast extract, 0.1 and $FeCl_2 \cdot 4H_2O$, 0.05. Trace elements solution contained (g L^{-1}): H_3BO_3, 0.1; $ZnSO_4 \cdot 7H_2O$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.05 and $MnSO_4 \cdot H_2O$, 0.04 with the pH of 6.5. Ten mililitres of the trace elements solution was added aseptically to the sterilized basal salt medium to make it up to 1 L. One mililitre of the serially diluted samples was aseptically dispensed into sterile Petri-dishes using the pour plate method.

Surface Active Bacterial Count (SABC)

Screening for surface-active bacteria was carried out on blood agar as described by Tabatabaee et al. [24]. The blood agar was made up of nutrient agar containing volume fraction 5 % defibrinated rabbit blood. One millilitre of the serially diluted sample was plated on the blood agar using the pour plate method. The plates were incubated at 28 °C for 48 h after which the colonies that showed clear zone of red blood β-heamolysis were counted as surface active agent producer.

Screening test for the utilization of bitumen

Bacterial isolates were tested for their potential to utilize bitumen as carbon source as described by Bidoia et al. (2010). Pure strain was inoculated into 7.5 mL of MSM incorporated with 1 g of bitumen. Then, 40 µL of 2, 6-Dichlorophenol Indophenol (DCPIP) was added and incubated at 37 °C for 60 h. Absorbance of the medium at 600 nm was measured at intervals of 24 h for 5 d using a digital colorimeter (Jenway 6051, UK).

Characterization of bacterial isolates

The isolate was identified on the basis of its colonial morphology, cellular morphology and biochemical characterization of the isolate was done using API 20E identification kit (Biomérieux, France). API 20E kit was used according to the manufacturer's instruction. The organism was further characterized using 16S rRNA. The PCR amplification of 16S rRNA gene, from the purified genomic DNA was carried out using universal primer sets. Sequencing of the gene was done and identification of the sequence was by Basic Local Alignment Search Tool (BLAST) with National Center for Biotechnology Information (NCBI) gene data base. The sequence was also deposited at the NCBI database.

Bitumen degradation experiment

The experiment was conducted according to the Critical Control Design (CCD) of Design Expert 7.0. (Stat-Ease Inc., USA). Three independent factors of temperature, pH and experimental time and optical density (dependent response variable) were considered. The complete design consisted of 20 experimental points which included 6 replications at the central point based on the pattern generated through the software. Growth of the isolate was monitored in 250 mL Erlenmeyer flasks containing 99 mL MSM with 1 g of bitumen as carbon substrate. The optical density at 600 nm of the inoculum was adjusted to 0.5 using a spectrophotometer (Uniscope SM 7504 model, UK) before seeding into each flask. The optical density (OD_{600}) of replicates readings was used as the degradation index [6, 25]. Control flasks were also set up in similar manner but without seeding with the isolate.

Gas chromatography- Mass Spectrometry (GC-MS) analysis

The residual and the undegraded (control) bitumen were extracted from the culture fluid with 50 mL aliquots of dichloromethane (DCM) in a separating funnel. The organic phase was

drawn off and concentrated. The concentrated extracts were fractionated on glass column packed with silica and alumina (2 : 1) into aliphatic, aromatic, and polar fractions by successive elution with 20 mL of hexane, 70 mL of hexane/DCM (7 : 3) and 25 mL of methanol respectively [26]. The aromatic fractions were further concentrated to 0.5 mL for GCMS analysis. The GC system (Agilent Technologies 7890A model) used for the analysis was equipped with Mass spectrometer detector 5975C (VLMSD) and injector (Auto) 7683B series. HP-5 Agilent technologies installed with an HP-DB5 column (length 30 m, 320 μL I.D., and 0.25 μm film thickness). Helium gas was used as carrier gas. The mass spectrometer was operated in the electron impact mode at 70 eV. The aromatic fractions were injected with an auto sampler in the splitless/split mode with a split time of 1 min after injection and the injector temperature was 280 °C. Column temperature was programmed from 50 °C (held for 1 min) to 150 °C at a rate of 10 °C/min, then to 210 °C at a rate of 2 °C/min and finally to 280 °C (held for 7 min) at a rate of 35 °C/min. Compound identification was based on the robust 2011 National Institute of Standards and Technology library by noting only compounds with over 50 % library matching.

Statistical analysis

A functional factorial design was applied to derive a statistical model for the effects of the cultural conditions on degradation of bitumen by *B. cepacia* using CCD and to identify the combination of factors that would lead to the enhancement of degradation. The statistical software package was used for regression analysis and graphical analysis of the data obtained during the experiment. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The significance of the model equation and model terms were evaluated by F-test. The quality of fit of the polynomial model equation was expressed by the coefficient of determination (R^2), adjusted R^2 and adequate precision. The fitted polynomial equation was expressed as three-dimensional surface plots to visualize the relationship between the responses and the experimental levels of each factor used in the design.

Results

The results of the total heterotrophic bacterial count (THBC) and total oil utilizing bacterial count (TOUBC) from bitumen impacted and unimpacted soil (Table 1) showed that the THBC ranged from 1.6×10^5 CFU g^{-1} to 2.0×10^6 CFU g^{-1} with Odeda having the highest bacterial counts. The sample from Odeda had the highest total oil utilizing bacterial count (TOUBC) of 3.6×10^5 CFU g^{-1} while the lowest counts of 1.5×10^4 CFU g^{-1} was recorded at FUNAAB. Surface active bacterial counts from Isolu had the highest of 6.5×10^4 CFU g^{-1} to the least count of 1.0×10^4 CFU g^{-1} obtained at Osiele.

It was obtained from the screening procedure that *B. cepacia* KT803965 was able to utilize bitumen in the presence of DCPIP in the shortest possible time and was therefore used for the degradation of bitumen. The rate and extent of colour change of DCPIP from blue to colourless with time revealed the potential of the isolates to utilize the hydrocarbon tested as a carbon source.

The bacterium *B. cepacia* isolated from bitumen impacted soil from Agbabu is a Gram-negative rod with ability to oxidize glucose, mannitol, inositol, sorbitol and rhamnose. It is oxidase positive, liquefied gelatin, grew on citrate and MacConkey agar and positive for arginine dihydrolase, and catalase. However the organism test negative for Voges–Proskauer, indole, urease, hydrogen sulphide, amygladin oxidation, ornithine decarboxylase and lysine decarboxylase. The 16S rRNA identification of the isolate revealed that, *B. cepacia* KT803965 is closely related (99 % similarity) to *Burkholderia cepacia* ATCC 25416 with accession number CP007746.1. The sequence of *B. cepacia* KT803965 has been deposited at the NCBI gene bank database.

The degradation experimental design is presented in Table 2. The optimum response of 0.24 was observed at temperature of 40 °C and pH 7 after 13 d of incubation. The central points have optical densities that ranged between 0.16 and 0.17 at 40 °C and pH 7 after 6 d of incubation. The lowest response of 0.004 was obtained at zero hour of incubation at 40 °C and pH of 7.

Table 1: *Bacterial counts from various sampling points*

S/N	Sample collection location	Coordinates	THBC (× 10^4 CFU g^{-1})	TOUBC (× 10^4 CFU g^{-1})	SABC (×10^4 CFU g^{-1})
	Heavy oil impacted soil				
1	Odeda	7.23119 N 3.52687 E	205.0 ± 7.1[b]	36.0 ± 5.7[b]	3.2 ± 0.3[bc]
2	Osiele	7.23278 N 3.52294 E	23.50 ± 2.1[a]	4.5 ± 0.7[a]	1.0 ± 0.0[a]
3	Isolu	7.20425 N 3.44136 E	19.3 ± 1.1[a]	7.0 ± 1.4[a]	6.5 ± 0.7[d]
	Natural bitumen deposit				
4	Agbabu	6.35253 N 4.49547 E	16.8 ± 1.8[a]	7.5 ± 0.7[a]	4.5 ± 0.7[cd]
	Heavy oil unimpacted soil				
5	Funaab	7.22844 N 3.43618 E	16.0 ± 2.8[a]	1.5 ± 0.7[a]	2.0 ± 1.4[ab]

Values are means of replicate readings ± standard error

Mean values with same letter within a column are not significantly different at $p < 0.05$

THBC: Total Heterotrophic Bacterial Count

TOUBC: Total Oil Utilizing Bacterial Count

SABC: Surface Active Bacterial Count

Table 2: *Central composite design matrix measured and predicted response*

Order	Factors			Response		
	A: Temperature /°C	B: pH	C: Time /Day	Experimental value (Actual)	Predicted	Residual (Actual – predicted)
1	30.00	3.00	2.00	0.025	0.022	3.051E-003
2	40.00	7.00	0.00	4.000E-003	0.044	–0.040
3	40.00	7.00	6.00	0.17	0.17	3.070E-003
4	40.00	7.00	6.00	0.16	0.17	–1.930E-003
5	40.00	7.00	6.00	0.17	0.17	2.070E-003
6	40.00	7.00	6.00	0.17	0.17	1.070E-003
7	30.00	3.00	10.00	0.095	0.11	–0.011
8	23.18	7.00	6.00	0.094	0.11	–0.015
9	30.00	11.00	10.00	0.12	0.13	–7.612E-003
10	50.00	3.00	2.00	7.000E-003	2.385E-003	4.615E-003
11	50.00	11.00	10.00	0.16	0.17	–6.048E-003
12	50.00	11.00	2.00	0.045	0.037	7.887E-003
13	40.00	7.00	6.00	0.17	0.17	2.070E-003
14	50.00	3.00	10.00	0.055	0.096	–0.041
15	40.00	13.73	6.00	9.000E-003	0.030	–0.021
16	40.00	0.27	6.00	4.000E-003	–0.021	0.025
17	40.00	7.00	6.00	0.17	0.17	1.070E-003
18	30.00	11.00	2.00	0.052	0.014	0.038
19	56.82	7.00	6.00	0.14	0.12	0.019
20	40.00	7.00	12.73	0.24	0.20	0.036

Values are means of replicate readings

E: Exponential

Table 3: *Identified products released during the degradation process by B. cepacia*

Compound Name
Benzene, (1-butyloctyl)
Benzene, (1-propylnonyl)
Benzene, (1-methyldodecyl)
Ambrosin
Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)- , [1s-(1.alpha.,4.alpha.,7.alpha.)
1,2-benzenedicarboxylic acid
2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
23,28-Bisnor-17.beta.(H)-hopane

Table 4: *ANOVA for response surface quadratic model*

Source	Sum of squares	Degree of freedom	Mean square	F value	P value Prob > F
Model	0.092	9	0.010	12.71	0.0002
A	1.643E-004	1	1.643E-004	0.20	0.6605
B	3.120E-003	1	3.120E-003	3.89	0.0768
C	0.036	1	0.036	45.31	< 0.0001*
AB	9.245E-004	1	9.245E-004	1.15	0.3082
AC	5.000E-005	1	5.000E-005	0.062	0.8079
BC	5.780E-004	1	5.780E-004	0.72	0.4158
A^2	4.714E-003	1	4.714E-003	5.88	0.0358*
B^2	0.047	1	0.047	58.87	< 0.0001*
C^2	4.437E-003	1	4.437E-003	5.53	0.0405*
Residual	8.020E-003	10	8.020E-004	-	-
Lack of Fit	8.005E-003	5	1.601E-003	539.65	< 0.0001*
Pure Error	1.483E-005	5	2.967E-006	-	-
Corrected Total	0.100	19	-	-	-
R^2	0.9196				
Adjusted R^2	0.8473				

** = significant model terms*

A: Temperature

B: pH

C: Time

R2: Coefficient of determination

E: Exponential

The response surface plot showed that the optical density increases with increase in temperature and pH. The highest optical density of 0.24 was obtained at 40 °C and pH 7 while the lowest optical density of 0.004 was obtained at the same temperature and pH after inoculation. Beyond this temperature and pH, there are further decreases in optical density (Figure 1).

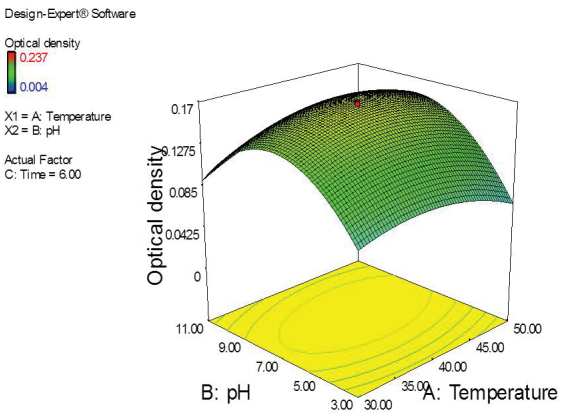

Figure 1: *Surface and contour plot showing effect of temperature and pH on bitumen degradation (optical density).*

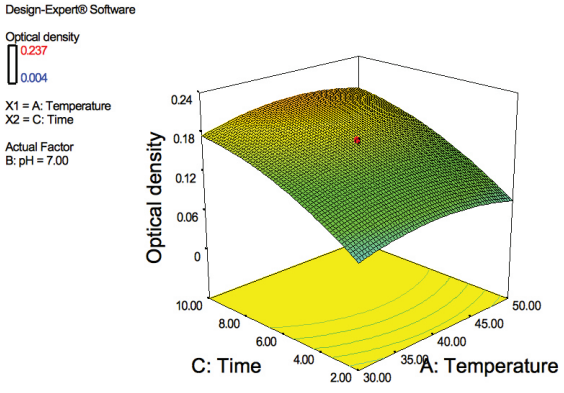

Figure 2: *Surface and contour plot showing the effect of temperature and time on bitumen degradation (optical density).*

Figure 2 show the plot of temperature and time against optical density. The optical density increases with time. The highest optical density of 0.24 was obtained after 13 d of incubation while the least optical density of 0.004 was obtained on the zero day of incubation.

Microorganisms serve as an important tool for the simultaneous biodegradation and release of valuable compounds during the degradation. Identified degradation products formed during the degradation process are shown in Figure 3. The analysis of variance was performed in order to verify the validity of the models and the results were presented in Figure 4. According to the analysis of variance, *F*-value (12.71) for the overall regression model is significant ($p > 0.05$) and the lack of fit is significant indicating that the model with interaction is inadequate in approximating the response surface of the experimental design. The regression analysis of the experimental design showed that C, A^2, B^2, C^2 are significant model term ($p < 0.05$). The predicted R^2 of 0.3947 is not as close to the adjusted R^2 of 0.8473 as one might normally expect. This may indicate a large block effect between the experimental and the predicted values. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable. In this case, a ratio of 11.099 was achieved indicating an adequate signal and so this model can be used to navigate the design space.

The GC-MS results showed significant variations among the studied degradation and control sample in the analyzed aromatic fractions. Table 3 showed the degraded compounds and new compounds that were detected. Benzene, (1-butyloctyl), benzene, (1-propylnonyl) and benzene, (1-methyldodecyl) were some of the degraded products while ambrosin and azulene are some of the new products detected.

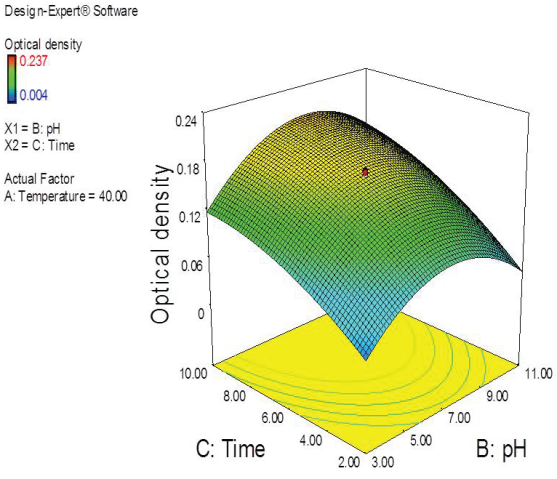

Figure 3: *Surface and contour plot showing the effect of pH and time on bitumen degradation (optical density).*

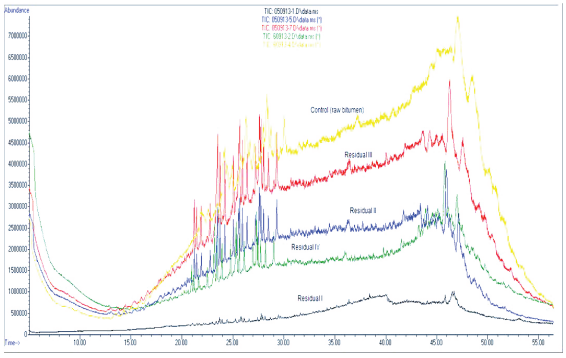

Figure 4: *The Total Ion Chromatogram (TIC) of the control sample and the extracts of the residual degraded bitumen.*

Discussion

This study showed high bacteria counts in all the locations. The counts are consistent with the works of Obayori et al. [15] and Wang et al. [27] that reported similar bacterial counts from hydrocarbon-polluted site. The range of THBC recorded in this study is consistent with the findings of Jennings and Tanner [28]. The high THBC recorded in unimpacted soil is due to the high level of organic matter usually present in fallow uncultivated soil which supports the growth of microorganisms. The enumeration of TOUBC is an important criterion for the determination of potential for microbial degradation of oil contaminated environments, and to assess the amount of oil pollution that has occurred [29].

The distribution of bacterial isolates obtained from various sampling sites indicates common occurrence of metabolically active strains contaminated with hydrocarbon suggesting the ability of the microorganisms to utilize these hydrocarbon as a carbon and energy source. The ability to utilize hydrocarbon substrate is exhibited by a wide variety of bacteria genera [30] that is widely distributed among oil polluted as well as pristine soils [31, 32]. Some general trends have indicated that Gram negative *Proteobacteria* group is major hydrocarbon utilizers [33, 34]. These groups were usually associated with degradation and their abundance was positively correlated to hydrocarbon utilization [34].

Screening of organisms that produce surface active agent had been done previously by several researchers using haemolytic activity test [24, 35]. The organism produced surface active agents and thus has the ability to produce biosurfactant which enhances degradation.

Environmental conditions such as temperature and pH have been observed to influence the rate of biodegradation [7, 8]. The effect of temperature and pH has also been found to influence the biodegradation of phenol [36, 37]. The physical conditions under which microorganisms acts on a substrate govern the speed and efficiency of its action [38], thus, the effect of temperature, pH and time were investigated in relation to the growth of the test organism on bitumen substrate. Differences in response of the organism with different conditions of temperature, pH and time also indicate fundamental differences in the mechanisms of bitumen oxidation [38]. The optimum temperature of 40 °C and pH 7 obtained in this study agrees with what was reported by Olabemiwo et al. [7] and Adebayo et al. [8] while working on solubilized bitumen. It is expected that biodegradation rates will be enhanced to a certain extent, typically in the range of 30 °C to 40 °C [39]. Above 40 °C, the membrane toxicity of hydrocarbon to microorganisms is increased, thus hindering biodegradation [40]. Degradation rates are highest at near neutral pH [41]. This implies that bitumen degradation is possibly more effective at neutral or slightly alkaline pH condition. This is in agreement with Sonawdekar [42] which found out that bacteria are able to grow on hydrocarbon at that pH range.

It was proven from this study that incubation time ($p < 0.05$) played crucial role in the degradation process. The convex response surface suggested well-defined optimum variables of temperature and pH and that the degradation increased with increase in temperature and pH up to 40 °C and 7 respectively and then declined with further increase of these two parameters.

The aromatic fraction was considered for analysis due to the fact that the fraction is expected to have much impact on degradation. Comparing the degradation results with the control sample, a significant number of the precursor compounds found in the bitumen have been degraded (Figure 4). In most cases the compounds were not found in the extracts of the residual bitumen. It was difficult to quantify the amount of individual compound degraded since individual standards of the compounds

were not available. However, using their relative abundance (peak areas) we were able to ascertain the level of degradation for most of the compounds (Figure 4).

The control sample has over two hundred compounds with a significant proportions identified with very high abundance. Nine major compounds were recorded as degradation products as recorded in the residual bitumen sample by *B. cepacia* KT803965. This is highly significant indicating the potency of this isolate in degrading and utilizing heavy molecular weight organic compounds [43]. Since bitumen contain Polycyclic Aromatic Hydrocarbons (PAH) [44], the aromatic fractions of the residual bitumen were significantly degraded by the organism. The control sample assisted in establishing the extent of degradation among the various degraded compounds. The results obtained showed that under conditions of reduced availability of a certain class of compounds, microorganisms opt for those that are, although less biodegradable but more accessible substrate. In this case, the reason for lower biodegradability might be the smaller amounts of *n*-alkanes in the pollutant [45].

However, some compounds such as (1-methylundecyl) benzene and (1-butylheptyl) benzene showed very high resistant to degradation as they were not amenable to degradation but with further timing and optimization, these compounds could be further degraded.

Conclusion

The method employed in this study can be successfully applied to determine the potential of organism in the degradation of bitumen. The optimal conditions obtained in this study laid a solid foundation for further use of this microorganism in the treatment of heavy oil polluted environment.

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