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Enzymatic Studies and Mineralization Potential of *Burkholderia cepacia* and *Corynebacterium kutscheri* Isolated from Refinery Sludge

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Abstract Environmental pollution with petroleum hydrocarbons has been recognized as a serious concern worldwide causing ecological imbalance. Mineralization is essential for the conversion of petroleum hydrocarbon and hazardous pollutants into less toxic compounds, mostly water and carbon dioxide. In this study, refinery sludge samples from Kaduna Refining and Petrochemical Company were collected. These were analyzed by selective enrichment technique, resulted in the isolation of two bacterial species which are Burkholderia cepacia and Corynebacterium sp. Crude oil degradation was performed by the pure and mixed culture of the isolates incubated in the shake flask culture. Optical density, pH, Protein, Dehydrogenase and DHA activities were used as bioindicators for the degradation processes while CO_2 production and GC-MS were used to analyze Mineralization potential. Dehydrogenase activities, Growth pattern, Protein estimation and FDA are strongly correlated with the rate of biodegradation therefore; these parameters can be used as indicators for aerobic biodegradation of crude oil while This research confirmed that Biodegradation of petroleum hydrocarbon generally relies upon the cooperation of more than a single bacterial species, and such cooperation that exists between the isolates is quorum sensing phenomenon. These findings have environmental implication towards developing a microbial consortium that could be exploited for cleaning oil polluted environment.

Keywords Mineralization, Bioindicator, Crude oil, GC-MS, DHA, FDA

1. Introduction

The impacts of crude petroleum, prosperity and production operation on oil producing countries of the world have resulted in enormous pollution of air, water and particularly agricultural land for food production [1,2]. Crude oil pollutant is a recalcitrant molecule toxic to biotic factor and persists in environment for many years depending on the natural and quality of oil spilled [3,4].

The removal of oil that has been accidentally or purposely spilled into the environment is therefore of great concern to the petroleum industry [23]. Previous investigations have shown that crude oil and other related organic pollutants can be degraded [5,6,7,8]. Hydrocarbon-degrading microorganis ms are widely distributed in marine, freshwater, and soil ecosystems [9]. The ability to isolate high numbers of certain oil-degrading microorganisms from an environment is commonly taken as evidence that those organisms are active degraders of that environment [10].

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There is an extensive body of knowledge on mineralization or degradation of hydrocarbons by microorganisms [11, 12]. In a recent study, Ojo [13] reported the capability of native bacterial population to mineralize petroleum hydrocarbons in wastewater.

Malik and Ahmed [14] reported that there is no single strain of bacteria with the metabolic capacity to degrade all the components found within crude oil. In nature, biodegradation of a crude oil typically involves a succession of species within the consortia of microbes present. A combination of bacterial strain with broad enzymatic capabilities is required for active extensive degradation of crude oil.

It is the process of using microorganisms to convert petroleum hydrocarbon and hazardous pollutants into less toxic compounds mostly water and carbon dioxide, a process called mineralization. It is a natural process, microbes work to break down practically all hydrocarbon contaminants, in the natural environment. It transforms pollutions, and not changes them from one medium to another. Also, it degrades a wide range of different pollutions. Relative to other methods of pollutant removal bioremediation is of reduced risk and huger degree of safety, reduced labour and very cost effective [15].

In many ecosystems there is already an adequate indigenous microbial community capable of extensive oil biodegradation, provided that environmental conditions are favorable for oil-degrading metabolic activity [16,17,18]. The ability of a biosurfactant to emulsify hydrocarbon water mixtures have been widely reported [19,20]. These emulsification properties have also been demonstrated to enhance hydrocarbon degradation in the environment; hence making them potentially useful tools for oil spill pollution control [21].

A number of investigation which focus on solving the problems of degradation of oil reported in the scientific literatures are purely biotransformation or co-metabolism rather than mineralization which can result into the formation of intermediate products that are not only toxic but also may remain recalcitrant and persistent in the ecosystem [22]. The immense potential of microorganisms to mineralize petroleum hydrocarbon does not only depend on the ability to provide energy or cell biomass, catabolic enzymes but also on their metabolic activities [23]. Mineralization is a combined activities of microbial consortia that does not produce intermediates pollutants in the environment, the final products are CO_2 and H_2O [24]. The present work has the aim of investigating the mineralization potential and characterization of Hydrolytic enzymes involved in biodegradation of crude oil by Autochthonous *Burkholderia cepacia* and *Corynebacterium kutscheri* in refinery sludge.

2. Materials and Methods

2.1. Collection of Sample

Refinery sludge was collected from under the storage tank in Kaduna Refining and Petrochemical Company (KRPC) Kaduna State, Nigeria using sterile trowel into polyethylene bags, stored at 4°C and kept in sterile containers. Analysis commenced immediately upon arrival in the department of Microbiology laboratory.

2.2. Isolation of Bacteria

A selective enrichment technique was used for the isolation of hydrocarbonoclastic bacteria from Kaduna refinery sludge. Bushnell-Haas broth was used as enrichment media supplemented with 5%v/v of Bonny light crude oil. Specifically, 10g of refinery sludge was incubated on rotary shaker at room temperature in 195ml of Bushnell-Haas medium. It was incubated for two weeks in a rotary shaker at 120rpm. Aliquot of 0.1mililiter from the set up was plated on Plate count agar after two weeks and incubated at 26°C for 48hours. The colonies form was stored as stock cultures before used [25,26].

2.3. Identification of Bacteria

The two isolates that survived 2weeks selective enrichment technique were subjected to Biochemical and Physiological Characterization. Identification of the isolates to the generic level was done following the scheme in Bergey's Manual of Systematic Bacteriology [27]. This was finally confirmed using an automated test system Vitek Analytical Profile Index (API20E) BioMerieux, inc) A manufacturer's specification for proper characterization and identification of the isolates was followed.

2.4. Mineralization Potential of Isolated Microorganisms

The ability of the bacterial isolates to mineralize Bonny light crude oil as the only source of carbon and energy was determined by the method of [6,13,10,29] with some modifications. The mineralization assay was carried out as follows: Each of the bacterial isolates was tested for their ability to mineralise crude oil using a liquid mineral medium, Bushnell-Haas (BH) medium [30]. A single colony of the isolate was inoculated into 10 ml nutrient broth (Merck) and incubated at 30°C overnight at 160 rpm. The overnight culture was centrifuged for 10 min at 5000 rpm. The cell pellet was washed twice in phosphate buffer saline (pH 7.6) and re-suspended in BH medium until OD_{600} nm was equivalent to 1.0ml of bacterial inoculum (1=OD_{600} nm equivalent) was added to 250ml Erlenmeyer flasks containing 100 ml of liquid BH medium supplemented with 2% carbon source (Bonny light crude oil), pH of the medium was adjusted to 7.0. Pure and mixed culture of the cells was cultivated separately. The control flasks contained the entire nutrient and the oil but no bacterial culture. All the flasks were incubated at 30℃ for 18days on an orbital shaker at 120rpm. Assay was carried out in duplicates.

Growth was monitored by testing optical density (OD_{600}) at 600 nm after every 24hours. pH, Dehydrogenase Activities [31], Fluorescein Diacetate Analysis [32], Emulsification test [33]. CO_2 Production [34, 25, 35], and Estimation of Total protein [36].

2.5. Gas Chromatographic Analysis

Gas chromatographic analysis was made according to the methods of [37]. After the incubation period, 5ml of the cultures were extracted with 20ml volumes of n-hexane as a solvent by using separatory funnels to remove cellular material. The residues were transferred to tarred vials and the volume of each extract was adjusted to 100ml by adding further n-hexane. The vials were kept at 4°C until the gas chromatographic analysis was carried out. Uninoculated control was incubated in parallel to monitor abiotic losses of the substrate.

3. Results

The isolation and identification of crude oil degrading bacteria in Kaduna Refinery Petrochemical Company (KRPC) from oil sludge were identified based on colonial morphology biochemical and physiological characteristics of the bacteria were carried out. Automated test system vitek API 20NE and API Coryne. (BioMerieux, inc Hazelwood,

Mo, USA) was used for the confirmation and authentication of the genera belonging to *Burkholderia cepacia and Corynebacterium kutscheri* as shown in Table 1.

The degradation pattern of the isolates revealed a significant occurrence of hydrocarbon degrading bacteria in the refinery sludge after two weeks of selective enrichment in the bonny light crude oil. Two bacteria were identified as *Burkholderia cepacia* and *Corynebacterium kutscheri* demonstrated the ability to survive and proliferate in the presence of high concentration of hydrocarbons and used it as solely source of energy and carbon. Though, enrichment technique selected only those indigenous bacteria that have been acclimated to degrade crude oil. The pattern of dehydrogenase activities in each of the isolate was basically the same and steady. The highest dehydrogenase activities of (63µg/TPF/hr) and (72µg/TPF/hr) was produced in *Burkholderia cepacia* and *Corynebacterium* sp respectively after 216 hr of incubation, while in their co-culture, there was gentle increase in dehydrogenase activities after 24hrs of incubation to achieve (68µg/TPF/hr) and dip drop at 96hrs to (35µg/TPF/hr) before sharp increase to attain maximum dehydrogenase activities of (210µg/TPF/hr) and later decreased to (80µg/TPF/hr) in sinusoidal pattern as shown in Fig. 1.

The fluorescein diacetate activities in the culture filtrate of *Burkholderia cepacia* and *Corynebacterium kutscheri* was 40µg fluorescein mL^{-1}solvent$3h^{-1}$ and 46µg fluorescein mL^{-1} solvent$3h^{-1}$ respectively after 216hr of incubation, while their co-culture increased after 96hr of incubation to 111µg fluorescein mL^{-1}solvent$3h^{-1}$ and little decrease after 120hrs of incubation, later increased to reach the peak at 146 µg fluorescein mL^{-1}solvent$3h^{-1}$as shown in Fig. 2.

Protein utilization increased to 3.12mg/ml after 72hrs of incubation and declined before steady increase to reach the peak of 6.72mg/ml at 192hrs of incubation and dropped down to 4.86mg/ml in their co-culture medium, while; there was zig -zag production pattern in each of the isolates shown in Fig. 3.

The pH changes in the Bonny light crude oil was shown in Fig. 3. The pH of both isolates tend to decrease progressively from 7.0 to 6.38 and 6.45 in *Corynebacterium kutscheri* and *Burkholderia cepacia* respectively while, in their co-culture, it gently increased to 6.6 as shown in Fig. 4.

The CO_2 production was increased to 173µmol in 96hrs of incubation and later dropped to 100µmol after144hrs of incubation. There was sharp increase to reach the peak of 211µmol at 192hrs and later decreased progressively in co-culture of both *Burkholderia cepacia* and *Corynebacterium kutscheri*, while, production of CO_2 in *Corynebacterium kutscheri* was slightly higher than in *Burkholderia cepacia* culture filtrate as shown in Fig. 5.

Emulsification pattern was decreased in both pure culture and their co-culture. Highest emulsification was observed between 24-48hrs of incubation at their exponential phase. The possible hypothesis that might explain this phenomenon is that biosurfactant biosynthesis from crude oil degrading bacteria occurred predominantly during the exponential growth phase as shown in Fig. 6.

Growth pattern of *Corynebacterium kutscheri* and *Burkholderia cepacia* during mineralization of Bonny light crude oil measuring their optical density showed gentle increase from 24hrs to 72hrs and little drop in 96hrs before sharp increase to a peak at 0.75 and later decrease progressively in their co-culture while the growth pattern of *Corynebacterium kutscheri* was slightly higher than that of *Burkholderia cepacia*. However, exhibited synchronous pattern and increased steadily as shown in Fig. 7.

Gas Chromatography mass spectrometry resolved and elucidated various hydrocarbon component of Bonny light crude oil. The GC peaks were found to vary between n-C_1 to n-C_{35} in the control samples as shown in Fig. 8 as indicated by their GC-peak, n-C_{34} and n-C_{35} were observed to mineralize by *Burkholderia cepacia* after 5days of incubation as shown in Fig 9. While, n-C_{28} to n-C_{35}were completely mineralized in the *Corynebacterium kutscheri* and other GC peak were fainted as shown in Fig. 10.

In Fig 11, Co-culture of *Burkholderia cepacia* and *Corynebacterium kutsheri* completely degraded quite number of hydrocarbon components of the crude oil as indicated in GC-peaks. All the GC peaks were greatly reduced after 5days of incubation, except n-C_{13} and n-C_{14}.

The number of peak in GC finger prints in oil recovered from flask inoculated with *Burkholderia cepacia* incubated after 10days was the same while their sizes were slightly reduced as shown in Fig. 12. The GC peaks in *Corynebacterium kutscheri* culture filtrate after 10days of incubation showed that all the peaks were slightly reduced but still present in the medium as shown in Fig 13.

The n-C_{13} and n-C_{14} that were present in their co-culture filtrate after 7days of incubation were disappeared while, n-C_1 to n-C_{10} that were initially not found re appeared. It was assumed that their co-culture degraded n-C_{13} and n-C_{14} to n-C_1 to n-C_8 shown in Fig. 14.

In spite, of the fact that individual bacterial strain was isolated after 2 weeks enrichment in the presence of hydrocarbons as solely source of energy and carbon, ability to survive and mineralize crude oil therefore is significantly limited. This might be explained by the occurrence of mutualistic relationship in the enrichment process in this study.

Table 1. Morphological, Biochemical and Physiological Characterization of Bonny Light Candle Oil Degrading Bacteria

	Isolate A	**Isolate B**
Colonial Characteristics	Small colonies, convex, regular margin, translucent gelatinous consistency	Cream, entire, convex and circular
Gram reaction	-ve rod	+ rod
Spore/capsule staining	-	-
Motility	-	-
Catalase	+	+
Oxidase	+	+
Urease	+	-
Indole	-	-
Gelatin hydrolysis	+	+
Methyl red	+	-
Voges – proskauer	-	+
Citrate utilization	+	+
Glucose fermentation	+	+
Lactose fermentation	+	+
Starch hydrolysis	-	-
Mannitol	+	+
Nitrate reduction	-	+
Maltose	+	+
Probable organisms	*Burkholderia cepacia*	*Corynebacterium kutscheri*

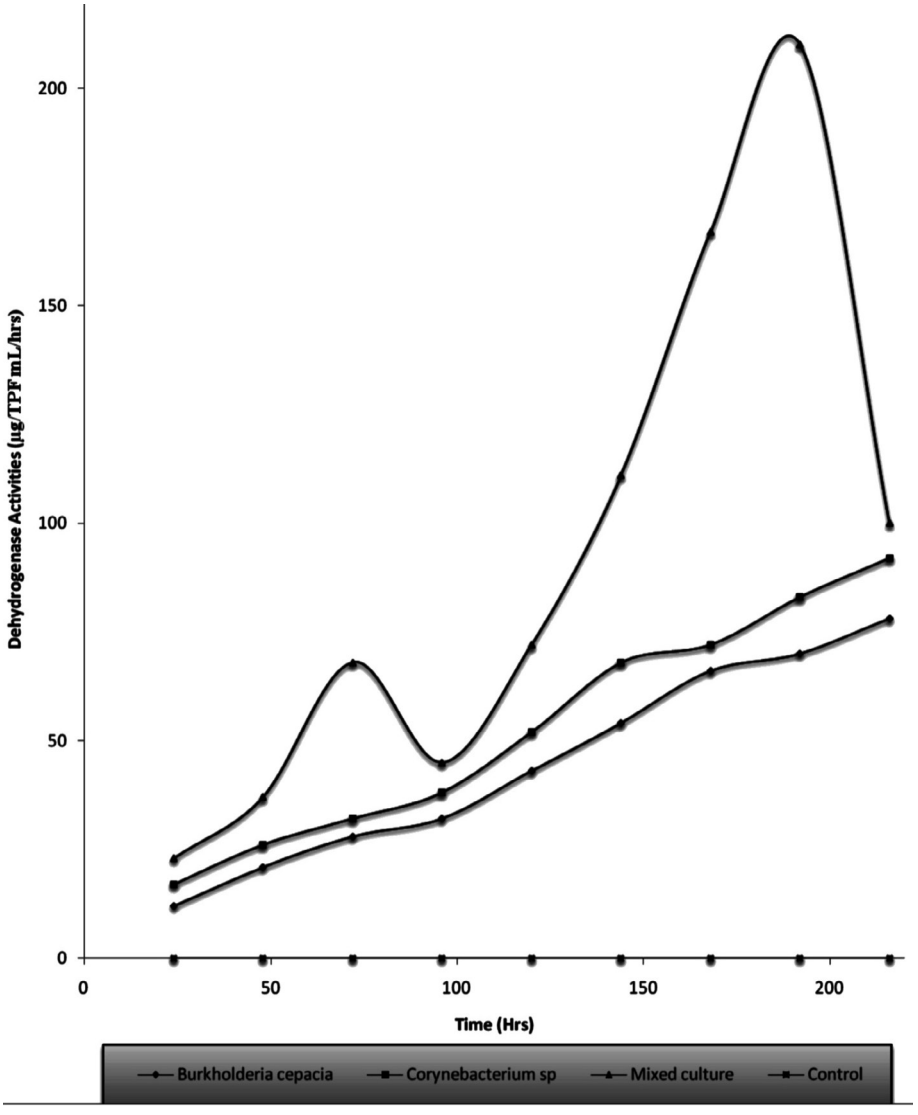

Figure 1. Changes in Dehydrogenase Activities during mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

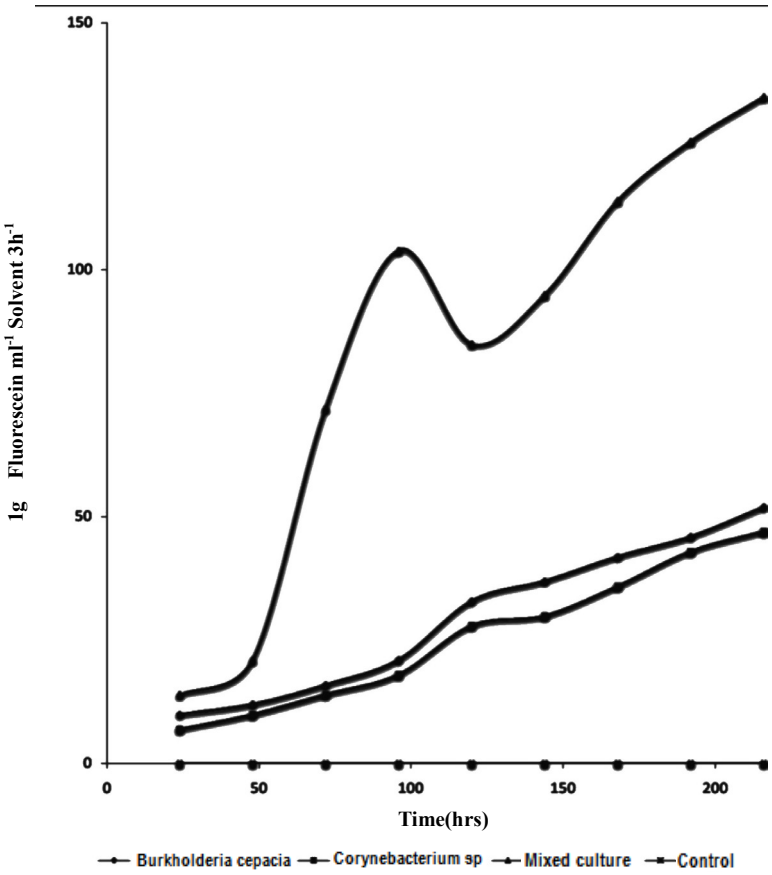

Figure 2. Changes in Fingerprints Diacetate during mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

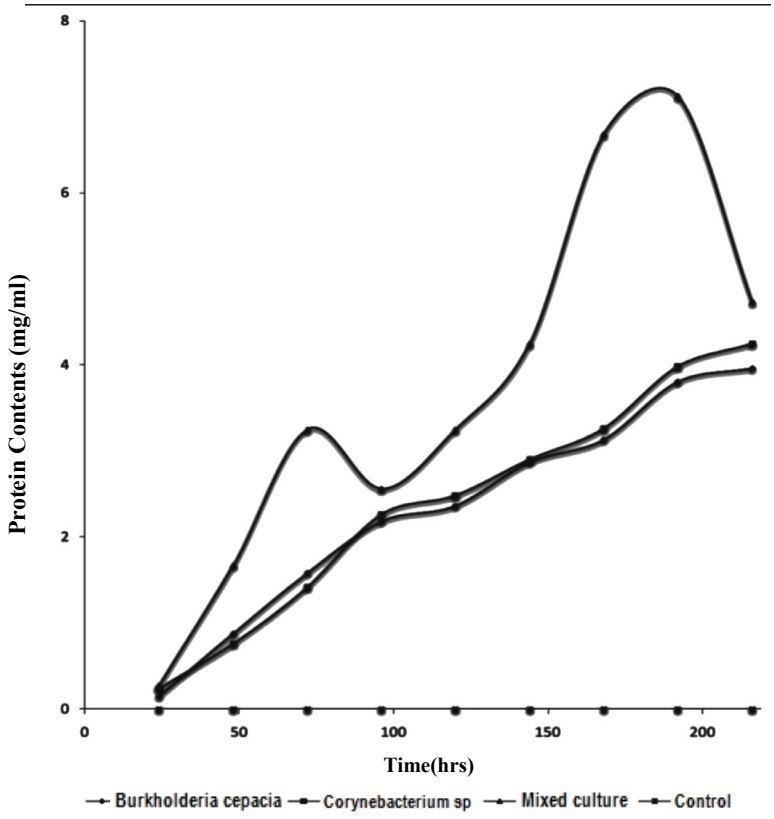

Figure 3. Changes in Protein contents during mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

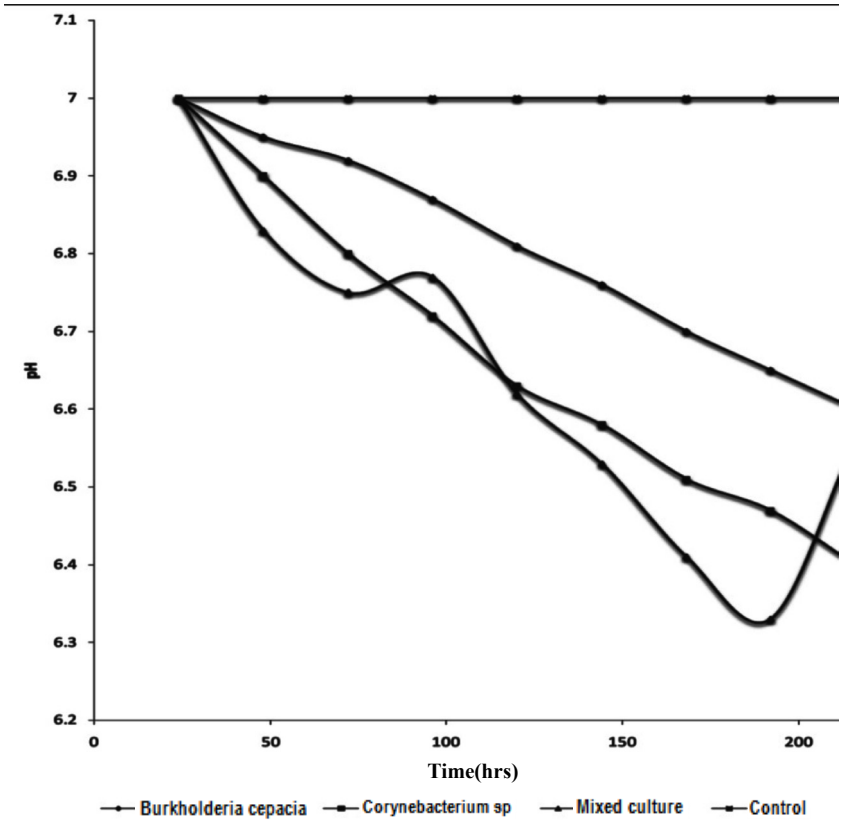

Figure 4. Changes in PH during mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

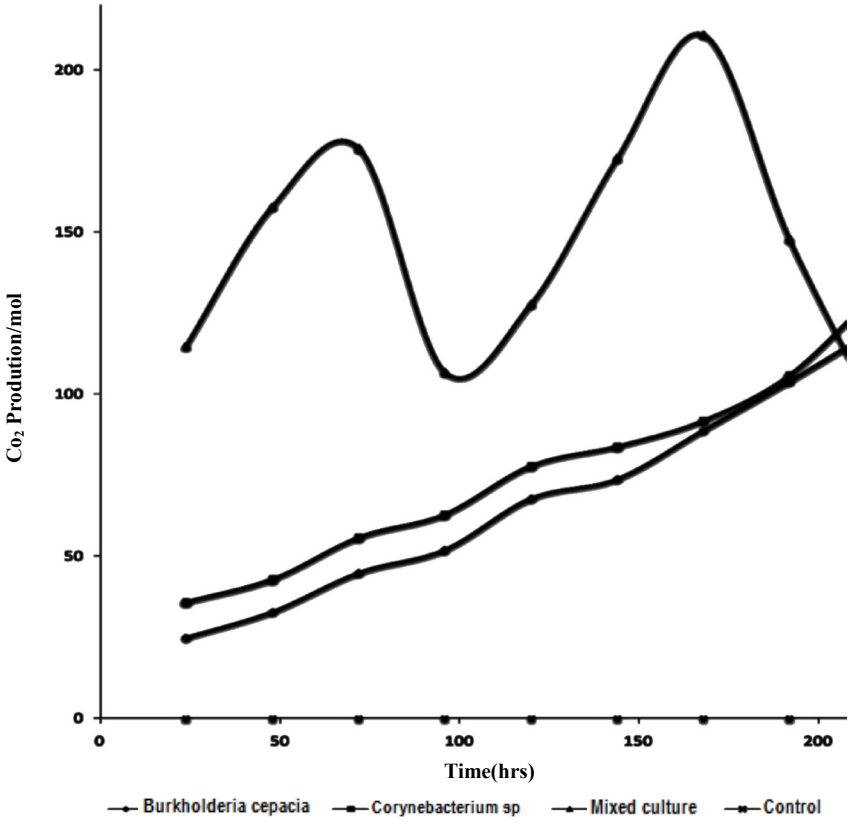

Figure 5. Changes in CO_2 production during mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

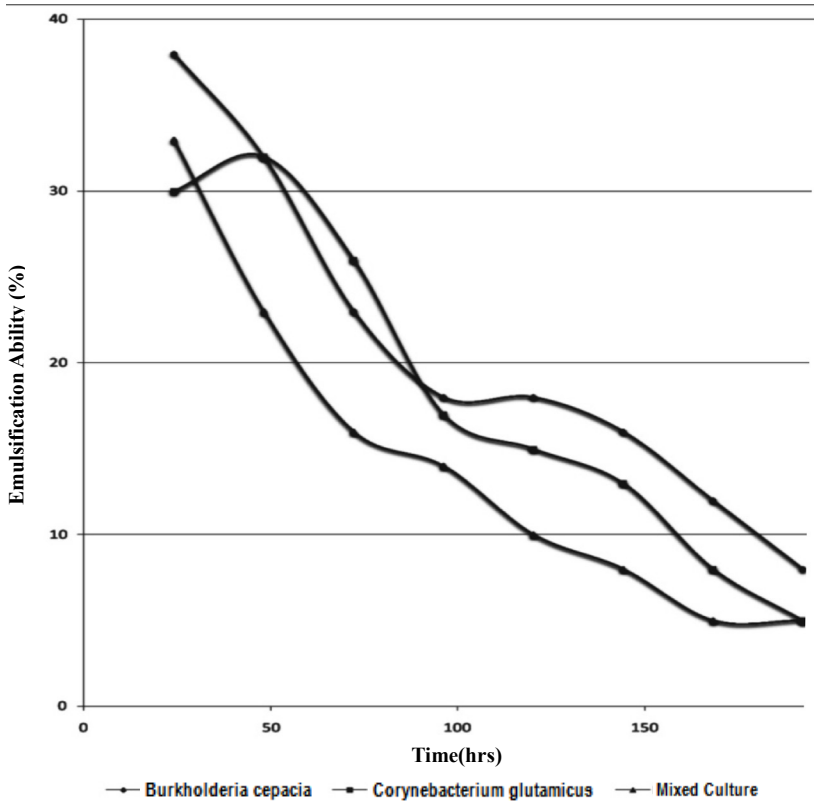

Figure 6. Changes in Emulsification Ability of Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

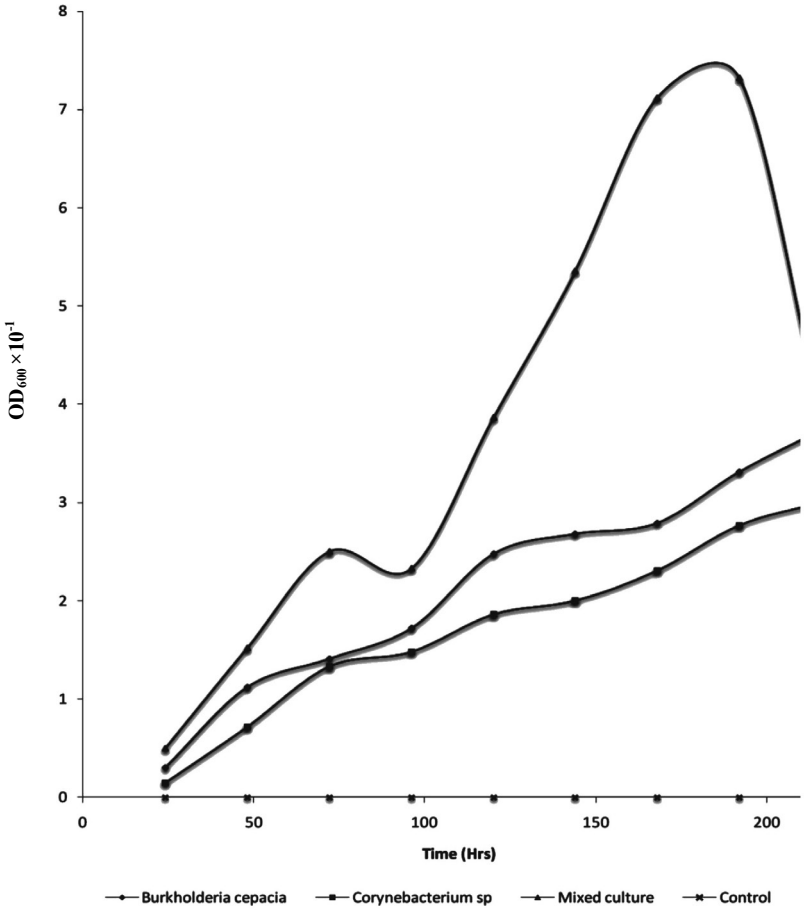

Figure 7. Changes in Bacterial Growth during Mineralization of Crude Oil by Pure and Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri

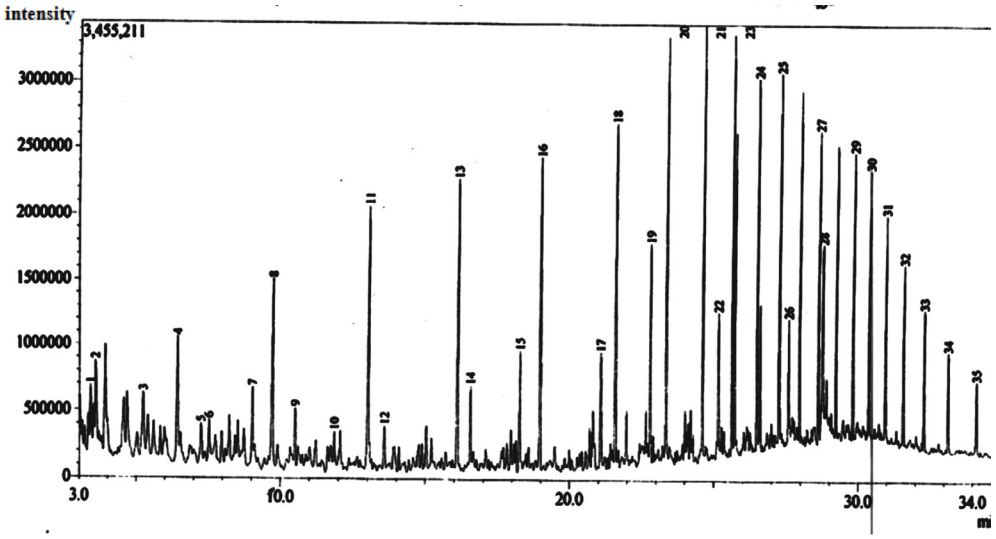

Figure 8. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Uninoculated with Bacterial Isolates (Control)

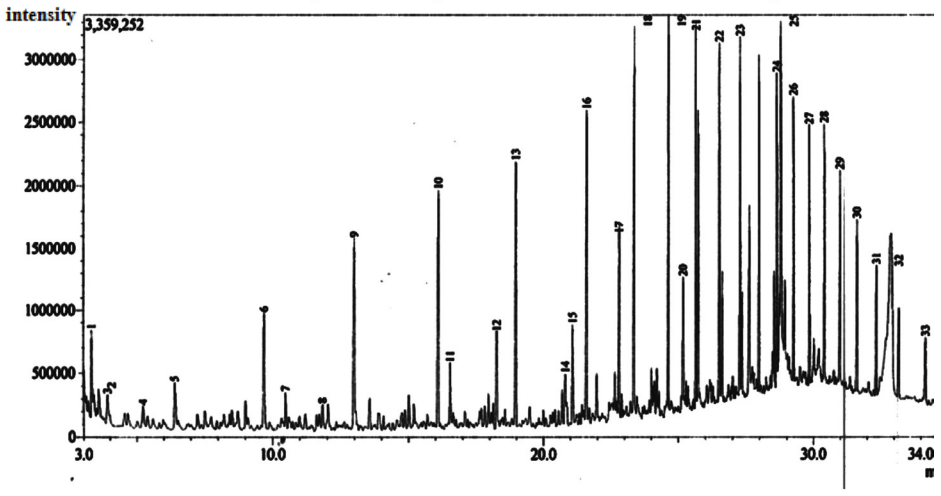

Figure 9. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Inoculated with Burkholderia Cepacia after 5 days of Incubation

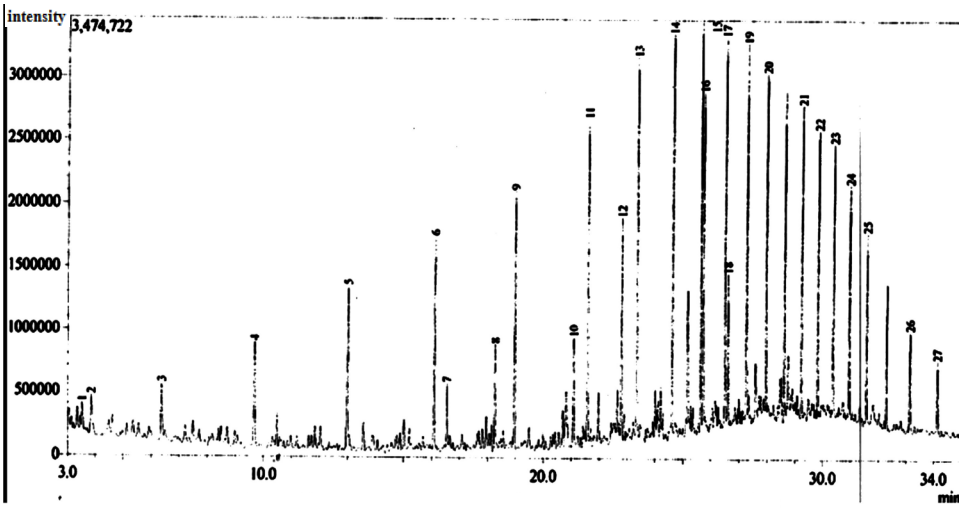

Figure 10. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Inoculated with Burkholderia Cepacia after 10days of Incubation

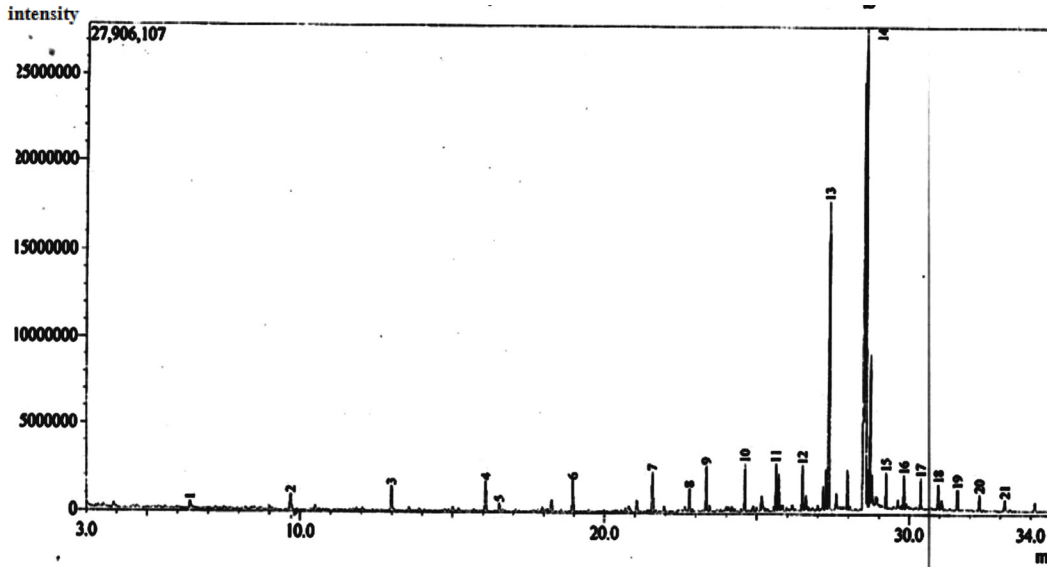

Figure 11. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks inoculated with Mixed Culture of Burkholderia Cepacia and Corynebacterium Kutscheri, after 5 days of Incubation

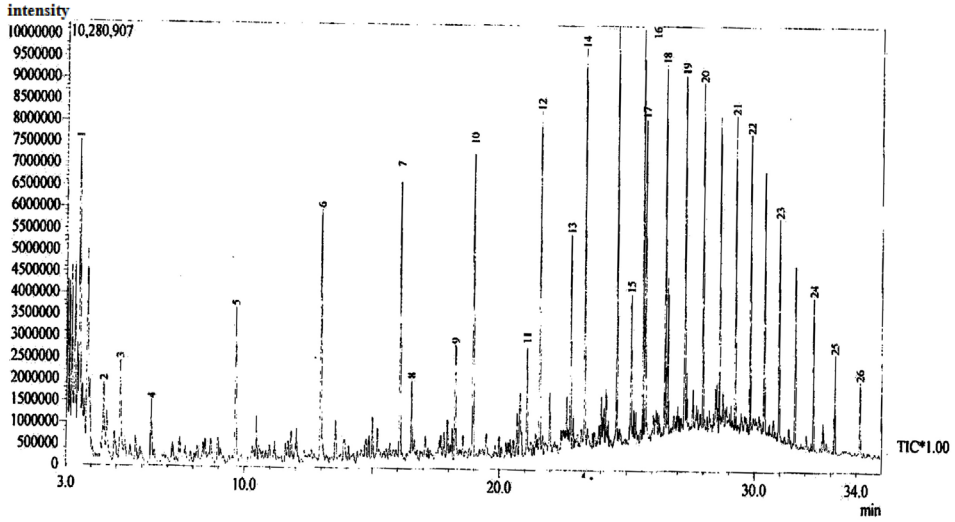

Figure 12. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Inoculated with Burkholderia Cepacia after 10 days of Incubation

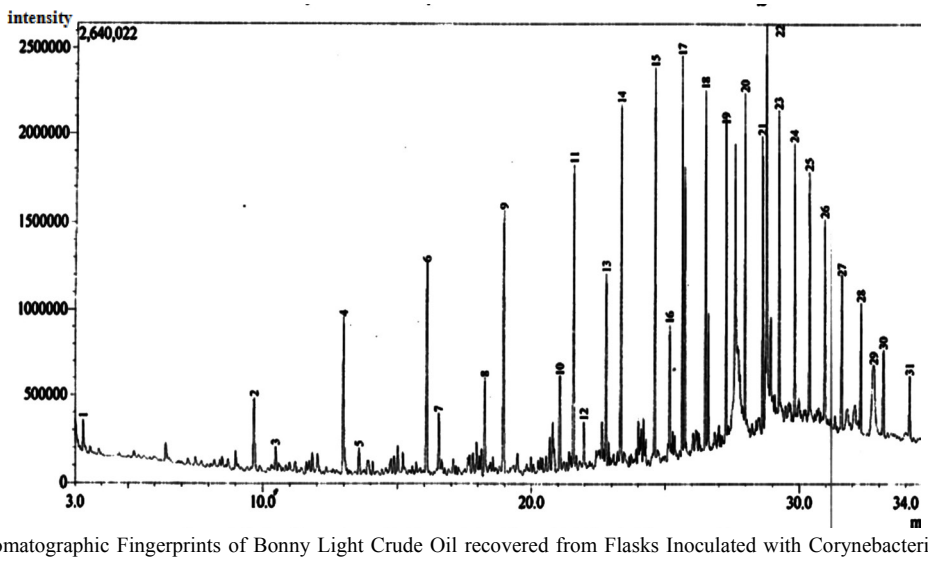

Figure 13. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Inoculated with Corynebacterium Kutscheri, after 10 days of Incubation

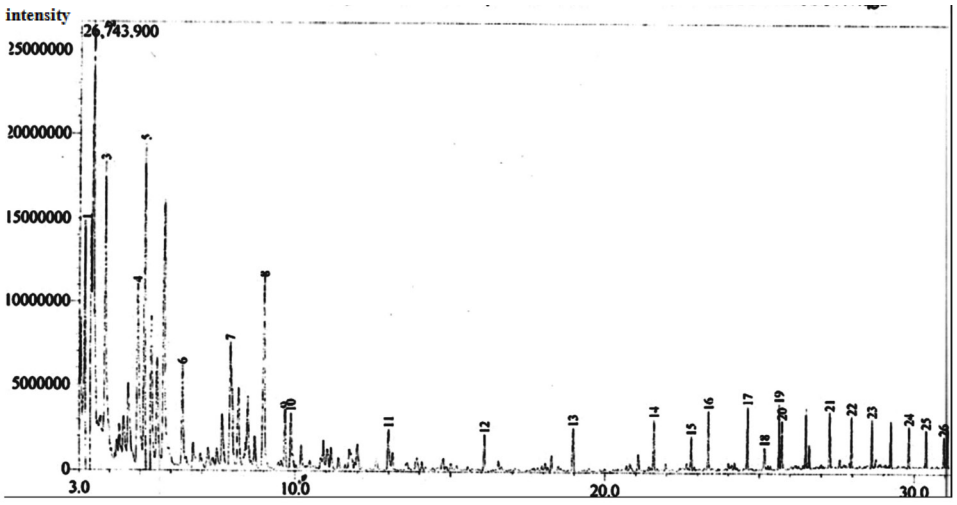

Figure 14. Gas Chromatographic Fingerprints of Bonny Light Crude Oil recovered from Flasks Inoculated with Culture of Burkholderia Cepacia and Corynebacterium Kutscheri, after 10 days of Incubation

4. Discussion

The presence or ability of the two isolates to survive 5% concentration of crude oil during two weeks of enrichment technique is important which seemed to have led to the synthesis of enzymes involved in crude oil metabolism or changes in the genetic capacity of microbial species to maintain their ability to degrade crude oil [38]. This findings showed that enrichment technique is useful for the isolation of various culturable oil degrading bacteria from contaminated sites for making consortia use in bioremediation [40]. The mechanisms employed by the autochthonous microorganisms to achieve this feat includes synthesis of inducible enzymes, mutations such as single nucleotide change or DNA re-arrangement that results in degradation of the compound and acquisition of genetic information from closely related or phylogenetically distinct population within the hydrocarbon-challenged community [39].

The occurrence of *Burkholderia cepacia* and *Corynebacterium kutscheri* in oil sludge is in line with the work of [41] who reported the degradation of heavy crude oil (maya) by *B. cepacia* strain RQI. There are other reports of Burkhoderia sp RQ1 capable of remarkable growth on aromatic fractions of crude oil [42, 43, 44]. The biodegradation potentials of *Corynebacteium* sp have also been reported by [45, 46, 47].

The slight increase after 72hours of incubation of Dehydrogenase activity in the culture filtrate can be attributed to the repression and induction of both inducible and constitutive enzyme involved in the biodegradation due to the presence of utilizable hydrocarbon substrates, while dip drop in 96hours may be as a result of non availability of utilizable hydrocarbon substrates that led to co-metabolism and increase in enzymatic activities.

As optical density and protein content increased dehydrogenase activity would also increase, this hypothesis is supported by the smaller increase in dehydrogenase which led to increase in bacteria growth and protein content this is in agreement with the work of [48] who reported that the extracellular enzymes of organisms are produced in response to their growth phases. Wang *et. al*., [49] reported that consumption of easily-available organic-C in the experimental medium may directly result into high content of biomass and dehydrogenase activity.

A gradual increase in FDA observed in 96 hours can be attributed to high enzymatic activity, which may be as a result of the presence of non-degradable hydrocarbon compounds that led to decrease in both DHA and CO_2 production. Such increase in FDA can result into co-metabolism leading bioconversion of the substrate without releasing energy. It is reported that FDA is closely related to total C, total N, and total P and other nutrient indices in experimental medium and that it has close correlation between conversion status of organic matter and overall microbial activity [32, 50]. Slight drop in 120hrs of incubation may be attributed to availability of utilizable hydrocarbon substrate as later increased progressively, such FDA production pattern not considered a representative measure of actual biodegradation because FDA did not decrease when all other parameters had decreased after mineralization, this is in agreement with work of Lee *et al.,* [51].

Protein content represents the total enzyme in the medium both inducible and constitutive enzymes. Sepahi *et al.,* [52] reported that the protein estimation is an effective method for monitoring the microbial population in an experimental medium.

It can be deduced that the growth dynamic of the isolate, dehydrogenase activities and protein contents are proportionate when there is availability of utilizable hydrocarbon compounds, therefore, the increase or decrease in protein concentration, Dehydrogenase activities and optical density during incubation period revealed that the isolates can utilize Bonny light crude oil as source of carbon and energy [45,52,53] also in their work, they showed that

the same pattern of relationship existing between the protein content and microbial growth during mineralization of crude oil.

The reduction in pH of the culture filtrate in the experimental flasks within the incubation period further confirmed chemical changes of the hydrocarbon substrates which must have been precipitated by microbial enzymes [9]. It has been reported that microbial degradation of hydrocarbon often leads to production of organic acids and other metabolic products [54,55]. Thus, the acids probably produced accounted for the reduction in the pH levels. This is important in view of the microbial survival and adaptation in terms of suppressing effect on the synthesis of enzymes involved in crude oil metabolism or by changes in the genetic capacity of microbial species to maintain their ability to degrade crude oil [38].

Biosurfactant biosynthesis occurred mainly between 24hrs to 48hrs of suggesting that biosurfactant is produced as primary metabolites accompanying cellular biomass formation which accounted for the flunctuation in pH during degradation, similar to [56]. Ayanwu, [57] who reported that biosurfactant production is bacterial specific.

High emulsification index and low surface tension facilitate the bioavailability of hydrophobic compounds to bacteria cells and thus enhance crude oil degradation [58,53]. The rate of biodegradation is dependent on the chemico-physical properties of the biosurfactants and not by the effects on microbial metabolism [59,60,61].

Biometric flask experiments indicated that the CO_2 production rates correlate with GC data, which is somewhat complex and time-consuming to produce thus the CO_2 evolution rate provided an essential criterion for evaluating the optional methods for determination of crude oil biodegradation. Gas-Chromatography and mass spectrometry (GC-MS) elucidated degradation pattern of Bonny light crude oil. Before the degradation the main peaks were C_1-C_{35} in Burkhoderia *cepacia* degraded n-C_{34}-n-C_{35} within 7days at 37% in culture broth while *Corynebacterium sp* degraded n-C_{28}-n-C_{35} and co-culture degraded n-C_1 to $_{n}$-C_{12} n-C_{22}-n-C_{35} and n-C_{15} to n-C_{21} within the same day. Little or no reduction in total petroleum hydrocarbon was noticed in both isolate while n-C_{13} and n-C_{14} was degraded to n-C_1 and n-C_8 in their co-culture filtrate. Sathiskumar *et al*., [47] and Bello [62] reported that microbes preferably degrade and metabolise n-C_8 to $_{n}$-C_{15} n-akane followed by C_{16}-C_{36} n-alkanes due to the simplicity of these hydrocarbon but this was contrary to this present finding, C_{34}-C_{35} was noticed to degrade within 7days while other lower hydrocarbon were still present, this can be attributed to the autochthonous adaptation that allows microorganisms to be physically compatible with their habitat [63].

This present finding revealed that *Corynebacterium kutscheri* has slight higher ability to degrade Bonny light crude oil than *Burkhoderia cepacia* such difference in the rate of hydrocarbon degradation may be due to presence of different catabolic genes involved in hydrocarbon degradation in the bacterial species [64,33].

It is widely believed that individual organisms could only metabolize limited range of hydrocarbon substrates [65,66]. This has led to the assertion that mixed culture exhibited superior degradative potential than pure culture strains [38, 66]. This often results in adaptation of the autochthonous organisms to the pollutants due to selective pressure and acquisition of degradative abilities [67] as reported by Salam *et al*., [39].

Several reports [68,69,70,71,72] have confirmed microbial consortia as better degraders than pure isolates. In a culture, some species utilize intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil [1,71]. Thus, a mixed culture is a better innoculum for bioremediation. The observation of diauxic growth phenomenon in the complex mixture of petroleum is expected as petroleum consists of linear as well as aromatic compounds which usually required different enzymes and biodegradation pathways.

The utilization of the oil resulted in increase in both Optical density ($OD_{6oo)}$, protein content and dehydrogenase activities evidenced from the reduction in pH of the culture confirmed by chemical changes of the hydrocarbon substrates which must have caused by microbial enzymes [9,73,52]. Dehydrogenase was found to be synthesized in proportionate to growth and protein content.The enzyme activity is regulated through product inhibition, covalent modification and feedback inhibition [74]. Biodegradation of petroleum hydrocarbon generally relies upon the cooperation of more than a single bacterial species. This is particularly true when complete mineralization of the hydrocarbons to CO_2 and H_2O is desired. A pure bacterial culture may not have the metabolic capability to readily degrade certain compounds or to have the biomass necessary to degrade the compounds rapidly enough consortium of mixed populations with overall broad enzymatic capacities may be required to achieved total degradation of the petroleum.

The higher biodegradation rate and microbial activity observed in the mixed culture may be related to shift in importance of one metabolic pathway over another possibly as a result of microbial synergism. The assumption that, more than one hydrocarbon compound degradation pathway exists in different microbial species and therefore, it is possible that individual bacteria able to degrade more than one aromatic substrate will have more than one pathway for their metabolism [75] (Shringfellow and Artken, 1995) This assertion is contrary to the present phenomenon. Williams, [76] reported that *B.cepacia* produced N-acetylhomoserine lactone an inducer that can stimulate quorum sensing mechanism in response to environmental challenges [77]. Since number of group of enzymes involved in complete mineralization of crude oil cannot be quantified, in this present finding the kind of synergism exhibited is neither protocooperation nor succession. GC- finger prints showed the same number of hydrocarbons was degraded throughout the experiment in their axenic culture while in their mixed culture degree of degradation was higher.

However, the hypothesis that might explain this synergistical activities of these bacteria is quorum sensing mechanism such that the *Corynebacterium kutscheri* was able to intercept and transduce the quorum sensing signal produced by *Burkholderia cepacia* to control physiological interaction leading to gene expression that resulted into synthesis of various enzymes involved in mineralization of Bonny light crude oil.

Both isolates produced surface active agents which might have very positively affected the uptake of substances by other hydrocarbons. For these reasons, mixed culture exhibited high degradation potential. There is no single strain of bacteria with the metabolic capacity to degrade all the components found within crude oil. In nature, biodegradation of a crude oil typically involves a succession of species within the consortia of microbes present. A combination of bacterial strain with broad enzymatic capabilities is required for active extensive degradation of crude oil. Oil degrading mixed cultures can be constructed by combining a number of strains with known degradative capabilities [79, 78], from the same ecological niche such that all the interaction that exist between them in their ecosystem can be maintained to enhance mineralization.

5. Conclusions

These findings indicate that each bacterial strain possesses lower ability to utilize particular compounds but in their mixed culture, all the hydrocarbons were degraded within 10 days of incubation. The kind of interactions that influenced their degradative potential has been attributed to quorum sensing mechanism. Such combination can be formulated for the construction of bacterial consortia useful in bioremediation of refinery effluent

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