Journal of Environmental Protection, 2014, 5, 1183-1196
Published Online September 2014 in SciRes. http://www.scirp.org/journal/jep
http://dx.doi.org/10.4236/jep.2014.512115

Scientific Research

Isolation and Characterization of Hydrocarbon-Degrading Bacteria from Wastewaters in Ouagadougou, Burkina Faso

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Received 2 April 2014; revised 1 May 2014; accepted 26 May 2014

Abstract

Lubricants are very often found in nature under waste-oil forms and represent for the environment a real danger of pollution due to the difficulty of their biodegradation. The situation is especially worrying in most developing countries in particular those of Sub-Saharan Africa due to the absence of regulation or control. The present work aims to isolate bacterial strains able to degrade hydrocarbons which can later be used in biotechnology for environments depollution. Oil-contaminated wastewater samples were collected in Ouagadougou city (Burkina Faso) and then used as source of bacterial isolation. Appropriate amounts of samples were inoculated to a mineral salt medium (MS) with Total Quartz 9000 oil as sole carbon and energy source and then incubated for enrichment, prior to microbe isolation. Two bacterial strains namely S_2 and S_7 were isolated from the enrichment cultures. The strains were tested for their ability to degrade other hydrocarbons (*i.e.* gasoline, diesel oil, brake oil) and for temperature, pH and salt concentration ranges for growth before their biochemical characteristics were defined. Based on their morphological, physiological and biochemical traits, strains S_2 and S_7 belong to *Acinetobacter* and *Pseudomonas* genera, respectively.

Keywords

Biodegradation, Hydrocarbon, Bacteria, Wastewater, Depollution, Burkina Faso

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How to cite this paper: Sawadogo, A., Harmonie, O.C., Sawadogo, J.B., Kaboré, A., Traoré, A.S. and Dianou, D. (2014) Isolation and Characterization of Hydrocarbon-Degrading Bacteria from Wastewaters in Ouagadougou, Burkina Faso. *Journal of Environmental Protection*, **5**, 1183-1196. http://dx.doi.org/10.4236/jep.2014.512115

1. Introduction

Lubricants are manufactured in various formulations for different applications. Most formulas generally consist of two fractions: chemicals additives and basic oils. Basic oils of lubricants are of mineral origin and derived from crude oil refining. They consist of three hydrocarbons families: paraffinic, naphthenic and aromatic [1]. Lubricants can also contain vegetable-base oils (triglycerides) in non-esterified forms as complex esters.

Release of hydrocarbons into the environment whether accidentally or due to human activities is a main cause of water and soil pollution [2] [3]. In many cases these lubricants are very often found in nature under used oil forms. Thus, in the European Union, 4.5 million tons of oil are consumed each year, along with 600,000 tons are lost in nature, leading to a real danger for environment pollution [4] [5]. The situation is especially worrying for most developing countries in general and those of Sub-Saharan Africa in particular because of the absence of policy for regulation and control. The presence of these products in the environment is a permanent threat, which can measure the extent of considering a liter of mineral oil polluting one million liters of water [6]. Soil contamination with hydrocarbons causes extensive damage of local system since accumulation of pollutants in animals and plant tissue may cause death or mutations [3] [7]. In the environment, non-volatile fractions of oils disperse in the aquatic environment or are absorbed into the ground creating a possible pollution of surface and ground waters [5]. These pollutions can affect a catchment area of drinking water. In addition, lubricants can cause a malfunction of biological sewage treatment plants and sewage sludge contamination. Finally, lubricants pose problems in agriculture by reducing the contaminated soil capacity of water retention [8]. Similarly, the spreading of polycyclic aromatic hydrocarbons (PAHs) on agricultural crops can result in a decrease of germination, stunting and yield reduction [9]. From the environment, hydrocarbons can also enter in food chains, leading to health problems. Thus, the soluble fractions of some of these compounds, in particular those containing mainly aromatic hydrocarbons and polar compounds are toxic (carcinogenic) for many species [10]. Similarly, oil particles inhaled in aerosol form can cause the occurrence of lipid pneumonia [11]. In the body, PAHs are mutagenic and can cause a decrease in the immune response with an increased risk of infections [12].

Fortunately, the degradation of these oils in the environment is possible through several techniques: physical [13], chemical [14] or biological [5] [15]-[19]. The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants. The process of bioremediation, defined as the use of microorganisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of petroleum industry [20]. In addition, bioremediation technology is believed to be noninvasive and relatively cost-effective [21]. Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment [22] and is cheaper than other remediation technologies and the most efficient for environment safe depollution [23].

Among the microorganisms able to grow on hydrocarbons, bacteria remain qualitatively and quantitatively the most active agents [3] [24]-[29]. Based on the frequency of isolation, the predominant bacterial genera found on this issue are *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Vibrio*, *Flavobacterium*, *Achromobacter*, *Micrococcus*, *Nocardia and Corynebacteria* [23] [28]-[30].

However, a number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons along with, temperature, salinity, oxygen content, oil concentration, presence of nutrients and hydrocarbon chemical composition [23] [31]-[39]. Many of these factors have been discussed by Brusseau [40]. The composition and inherent biodegradability of the petroleum hydrocarbon pollutant is the first and foremost important consideration when the suitability of a remediation approach is to be assessed. Among physical factors, temperature plays an important role in biodegradation of hydrocarbons by directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora [41]-[45]. Atlas [41] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons [42]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. Venosa and Zhu [43] reported that ambient temperature of the environment affected both the properties of spilled oil and the activity of the microorganisms. Significant biodegradation of hydrocarbons have been reported in psychrophilic environments in temperate regions [44] [45].

For the implementation of bioremediation technique, one important requirement is the presence of microorganisms with the appropriate metabolic capabilities. If these microorganisms are present, then optimal rates of growth and hydrocarbon biodegradation can be sustained by ensuring that adequate concentrations of nutrients and oxygen are present and that the pH is between 6 and 9 [23]. Unfortunately, at Sub-Saharan African countries level in general and Burkina Faso in particular, few studies focused on bioremediation technology for the depollution of hydrocarbon contaminated environments. To our knowledge, only one study relied on the biodegradation of hydrocarbons by a mixed inoculum containing bacteria, yeasts and fungi [8]. Unfortunately, the identity of microbes in the inoculum, and the conditions to optimize the process as well were not investigated. As also mentioned Das and Chandran [3], the scope of current understanding of oil bioremediation is also limited because the emphasis of most of these field studies and reviews has been given on the evaluation of bioremediation technology for dealing with large-scale oil spills on marine shorelines. However, an understanding of the impacts of oil on indigenous microbial communities and identification of oil-degrading microbial groups are prerequisite for directing the management and cleanup of oil-contaminated ecosystems [46].

Thus, in order to get insight of the bioremediation process of hydrocarbons in oil-contaminated environments of Ouagadougou city, the present study focused on the isolation, characterization and identification of indigenous hydrocarbon-degrading bacterial strains with regards to the conditions for optimizing their activities and the efficient cleanup of the hydrocarbon pollutants.

2. Materials and Methods

2.1. Source of Isolation

The source for bacterial isolation consisted of wastewaters contaminated with used motor oil, diesel and lubricating oils. Wastewater samples were collected into 500 ml sterilized glass bottles from five sites in Ouagadougou (**Table 1**, **Figure 1**) on June 2011, immediately carried to the laboratory and stored at +4˚C in a refrigerator prior to use.

2.2. Culture Media and Strains Isolation

For strains isolation, enriched cultures were prepared according to Malatova [19], Mittal and Singh [5] and Kostka *et al.* [46]. Bushnell-Haas Broth was used in the enrichment technique. Tubes containing 9 ml of the nutrient broth supplemented with 3% (v/v) 0.22 µm pore size filter-sterilized used motor oil as hydrocarbon substrate were inoculated in triplicate with 1 ml of wastewater sample and then incubated on a horizontal shaker (100 rpm) at 37˚C for one month. After one week incubation period, 1 ml of sample from primary enrichment was transferred to a fresh Bushnell-Haas Broth containing the same hydrocarbon and incubated as above. The bacterial growth was monitored by culture turbidity and depletion of added oil at regular intervals compared to controls with 0.22 µm pore size filter-sterilized wastewater sample. After three subculturing steps in broth medium, 1 ml of enrichment culture was transferred into tubes containing 9 ml of mineral salt (MS) medium supplemented with 3% used motor oil as a single source of carbon and energy. The mineral salt (MS) medium, adapted from Mittal and Singh [5] and Olfa *et al.* [47] contained per liter: KNO_3, 1.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $CaCl_2 \cdot 6H_2O$, 0.1 g; $FeSO_4$, 0.05 g; trace

Table 1. Location of the wastewater sampling sites in Ouagadougou, Burkina Faso.

Sampling site	Zone	Geographic coordinates
ABLASSE Garage	Tanghin	12˚23'31.8" LN 01˚31'43.1" LW
SONABEL Ouaga 1	Paspanga	12˚23'02.7" LN 01˚30'57.0" LW
TAN-ALIZ Factory	Kossodo	12˚24'55.4" LN 01˚28'33.4" LW
KOSSODO Abattoir	Kossodo	12˚25'01.5" LN 01˚28'31.1" LW
KARIM Garage	Koulouba	12˚21'58.7" LN 01˚30'40.2" LW

LN: Latitude North; **LW**: Longitude West.

Figure 1. Location of wastewater sampling sites in Ouagadougou, Burkina Faso Sampling site: 1—ABLASSE Garage; 2—SONABEL Ouaga 1; 3—TAN-ALIZ Factory; 4—KOSSODO Abattoir; 5—KARIM Garage.

elements of Pfennig *et al.* (1981), 250 ml; phosphate buffer (1 M, pH 6.8), 20 ml; and distilled water, 980 ml. Tubes were incubated at 37˚C on a horizontal shaker (100 rpm) for 7 days in the dark. Bacterial growth was monitored by measuring the optical density at 600 nm using a Milton Roy SPECTRONIC 601 spectrophotometer. After four successive transfers to fresh medium, 0.1 ml of culture was plated after appropriate dilution on PCA agar and incubated at 37˚C for one week as described by Mittal and Singh [5]. After 48 hours incubation period and later, single colonies appearing on the PCA agar were transferred on a fresh PCA agar and incubated. Pure colonies were obtained by using a single colony isolation procedure. The purity of cultures was confirmed by microscopic observations. Isolated colonies were stored at 4˚C on PCA agar slants and subcultured at 3-week intervals or mixed with 40% glycerol and stored at −70˚C for future utilization.

2.3. Growth Studies

Experiments were conducted in the liquid MS medium described above with used motor oil (SAE40) as sole carbon and energy source. Growth was monitored by measuring optical density at 600 nm with a Milton Roy SPECTRONIC 601 spectrophotometer.

2.4. Morphology

The Gram stain reaction was determined with 4-day-old cultures by the modified method of Hucker described by Doetsch [48]. Cell morphology of 4-day-old colonies on PCA agar cultures and 4-day-old colonies cultures in the MS liquid medium as well were determined by oil immersion phase-contrast microscopy. Colony morphology and pigmentation were determined using 4-day-old PCA agar cultures.

2.5. Physiological Tests

Growth at pH ranging from 4.5 to 9.0 was estimated in the MS liquid medium described above, where pH was adjusted with sterile 0.1 M NaOH or HCl solutions. The tested temperature range for growth was 30˚C - 44˚C. Tolerance to NaCl was monitored at concentrations ranging from 0.05% to 11.7% (w/v).

2.6. Hydrocarbons Utilization

The ability of strains to use the following hydrocarbons as sole carbon and energy source at a concentration of 3% (v/v) was investigated after 7 days incubation period at 37˚C: Diesel oil, Total Quartz 9000 motor oil, SAE40 used oil and gasoline. Controls without bacterial inoculation were prepared similarly. Experiments were performed in triplicate in 120 ml flasks. The biodegradation capability was evaluated by monitoring bacterial growth through optical density measurement at 600 nm and by a gravimetric analysis according to Fusey and Oudot [49] as follow: 40 ml of bacterial culture was added to 40 ml of chloroform in a separating 200 ml funnel which was then shaken vigorously to obtain two emulsified layers (a top layer consisting of a mixture of hydrocarbon and chloroform and a bottom layer containing water and biosurfactant in solution). The top layer was extracted and then passed through anhydrous sodium sulfate to remove moisture before the chloroform was evaporated on a water bath at 60˚C. The remaining residual hydrocarbon was estimated based on the weight of the empty funnel weight and the weight after chloroform evaporation and the % of biodegradation was then evaluated in comparison to the initial hydrocarbon amount in the control funnel according to Fusey and Oudot [49].

2.7. Biochemical Characteristics

Biochemical tests were performed on 24-h-old cultures grown on Plate Count Agar (PCA) medium at 30˚C. Oxidase, catalase and respiratory mode were investigated according to Kovacs [50] and Smibert and Krieg [51]. Metabolism of citrate was studied by inoculating bacterial colonies on a Simmons' citrate agar medium. Enzymes for sugars (glucose, lactose) fermentation and H_2S production were studied using Kligler Hajna medium. Tests of orthonitrophenyl-beta-D-galactopyranosidase (ONPG), arginine dihydrolase (ADH), ornithine decarboxylase (ODC), lysine decarboxylase (LDC) were performed using the API 20E gallery according to the supplier's recommendations (Biomerieux, France).

2.8. Kinetics of Strains Growth on Hydrocarbons

Tests were performed in triplicate at optimal growth conditions (pH, temperature, NaCl and substrate concentrations) in tubes containing 9 ml of the MS liquid medium supplemented with 3% (v/v) of gasoline, Diesel oil, SAE40 used oil and Total Quartz 9000 motor oil, respectively. Tubes were then inoculated with 1 ml of bacterial suspension before they were incubated at the optimal growth temperature as defined through the physiological tests described above. Bacterial growth was monitored per 24 h-incubation period up to 12 days by measuring the optical density at 600 nm. The maximum growth rate (μmax) of strains on the tested hydrocarbons was determined by the slope of the linear portion of the growth curve (exponential growth phase) as described by Smibert and Krieg [51].

2.9. Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) with regards to growth conditions and bacterial strain using XLSTAT-Pro 7.5 software. Mean variables were compared using the Newman Keuls test at probability level p = 5%.

3. Results and Discussion

3.1. Enrichment and Isolation

From the enrichment cultures, a total of six isolates able to use SAE40 used oil as carbon and energy source were obtained. Among the isolates, two strains namely S_2 and S_7 which showed the best growth (highest OD at 600 nm) were selected for further characterization.

3.2. Morphology

Colony morphology on agar medium revealed a translucent round form with 3 - 5 mm in size for strain S_2 while strain S_7 appeared yellowish, round and 2 - 3 mm in size. Both isolates stained Gram-negative and were non-motile and motile for S_2 and S_7, respectively. Cells of strain S_2 were short rods, while slightly coccobacillus-like cells were also observed (**Figure 2(a)**), contrasting with the long rods bacillus-like cells of strain S_7 (**Figure 2(b)**).

3.3. Physiology

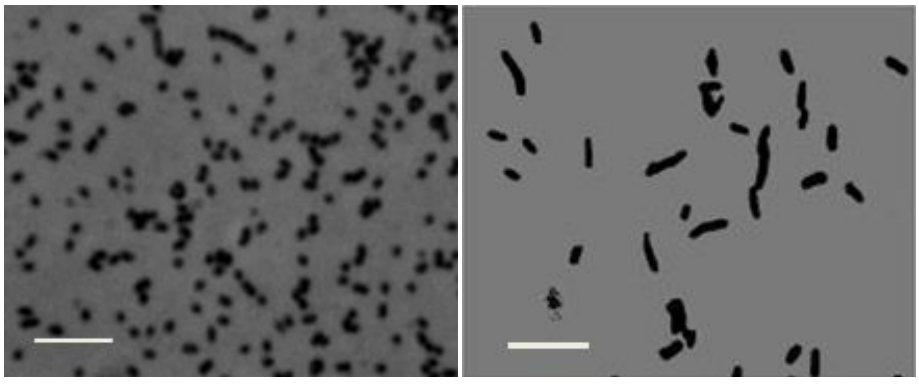
3.3.1. Temperature Range for Growth

For both strains, growth was evident between 30˚C and 39˚C with an optimum at 36˚C - 37˚C (**Figure 3(a)**).

From the results obtained, the best temperature range for growth of both strains S_2 and S_7 is 35˚C - 38˚C with an optimum at 37˚C (**Figure 3(a)**). Under and above this temperature range, strains growth is rather low. As underlined Atlas and Bartha [52], Atlas [53] and Leahy and Colwell [23], temperature influences hydrocarbon biodegradation by its effect on the physical nature and chemical composition of the oil and the rate of hydrocarbon metabolism by microorganisms. According to Atlas and Bartha [52], at low temperatures, the viscosity of the oil increases, the volatilization of toxic short-chain alkanes is reduced, and their water solubility is increased, delaying the onset of biodegradation. Higher temperatures increase the rates of hydrocarbon metabolism to a maximum, typically in the range of 30˚C to 40˚C, above which the membrane toxicity of hydrocarbons for microorganisms is increased leading to a decrease in metabolic activity [23] [37] [54]. The findings of these authors supported partially our results.

3.3.2. pH Range

The isolates grew at pH ranges of 7 - 8 for strain S_7 and 7 - 9 for strain S_2 with an optimum growth at pH 7.5 and 8.5, respectively (**Figure 3(b)**). The pH ranges for growth of isolates S_2 and S_7 are in agreement with the results on hydrocarbon-degrading strains of Dibble and Bartha [39] and Hambrick *et al.* [38] who found that bacterial degradation of hydrocarbons is higher under slightly basic conditions and with those of Leahy and Colwell [23] who indicated that this activity is rather greater in neutral medium. However, in contrast to most aquatic ecosystems, soil pH can be highly variable, ranging from 2.5 in mine spoils to 11.0 in alkaline deserts [54]. According to Atlas [55], most heterotrophic bacteria and fungi favor a pH near neutrality, with fungi being more tolerant to acidic conditions and thus, extremes in pH, as can be observed in some soils, would therefore be expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons. Verstraete *et al.* [56] reported a near doubling of biodegradation rates for gasoline in an acidic (pH 4.5) soil by adjusting the pH to 7.4. They however noticed that biodegradation rates dropped significantly when the pH was further raised to 8.5. Similarly, Dibble and Bartha [39] observed an optimum pH of 7.8 for the mineralization of oily sludge in soil. As underlined Patrick *et al.* [57], the pH of sediments in special environments such as salt marshes may be as low as 5.0 in some cases. However, Hambrick *et al.* [38] found the rates of microbial mineralization of octade-



(a) (b)

Figure 2. Cells' photomicrographs after Gram staining of hydrocarbon-degrading strains S_2 (a) and S_7 (b) isolated from wastewaters in Ouagadougou, Burkina Faso. The bar on (a) and (b) indicates 10 µm (Photo: Sawadogo 2012).

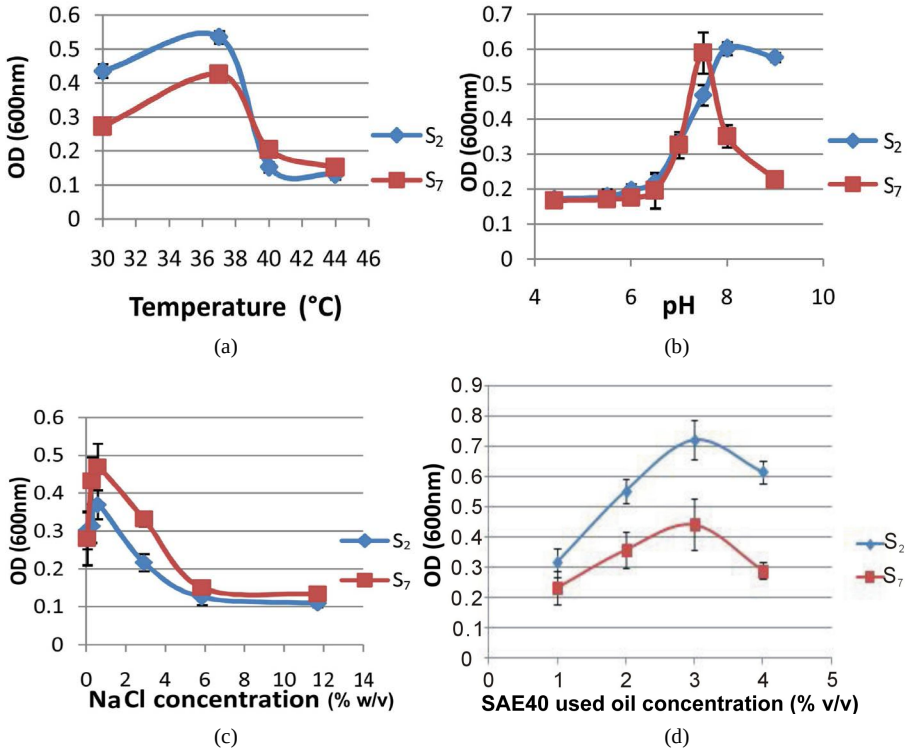


Figure 3. Temperature (a), pH (b), NaCl concentration (c) and SAE40 used oil concentration (d) ranges for growth of strains S_2 and S_7 (means of 3 replicates).

cane and napthalene to be depressed at this pH compared with pH 6.5. They also noticed that octadecane mineralization rates increased further when the pH was raised from 6.5 to 8.0, whereas naphthalene mineralization rates did not. All the findings of these studies indicated clearly that the optimum pH for biodegradation may strongly be related to the nature and composition of the hydrocarbon in a given environment.

3.3.3. Salinity

Both isolates S_2 and S_7 support NaCl for growth at a concentration range of 0% - 3% (w/v) with an optimum growth at 0.6% - 0.7% (**Figure 3(c)**). There are few published studies which deal with the effects of salinity on the microbial degradation of hydrocarbons. Shiaris [58] reported a positive correlation between salinity and mineralization rate of phenanthrene and naphthalene in estuarine sediments. Kerr and Capone [59] also observed a relationship between the naphthalene mineralization rate and salinity in sediments of the Hudson river that was dependent upon the ambient salinity regime, with estuarine sites exhibiting a lack of mineralization inhibition over a wider range of salinities than was the case for the less saline upstream site. In a study of hypersaline salt evaporation ponds, Ward and Brock [60] showed that rates of hydrocarbon metabolism decreased with increasing salinity in the range 3.3% to 28.4% and attributed the results to a general reduction in microbial metabolic rates. Our result is supported by Csonka and Epstein [61] who reported that high concentration of salt in the growth medium may decrease the water activity of the surrounding environment, which decrease can disrupt the normal cellular activities and the growth of bacteria. However, our finding contrasts with the data of Bertrand *et al.* [35] who found an optimum NaCl concentration of 2.34% (0.4 M) for two hydrocarbon-degrading microbial communities and with those of Ward and Brock [60]. One reason of the difference with their data could be explained by the fact that these authors focused on marine bacteria, known to support high salt concentration and hypersaline environments as well.

3.3.4. Hydrocarbon Concentration

For all the hydrocarbons tested, 3% (v/v) although rather low appeared the best substrate concentration providing the optimum growth for both strains S_2 and S_7 (**Figure 3(d)**). The rates of uptake and mineralization of many

organic compounds by microbial populations in the aquatic environment are proportional to the concentration of the compound, generally conforming to Michaelis-Menten kinetics [23] [62] [63]. However, according to Button and Robertson [64] and Robertson and Button [65], Michaelian kinetics have been demonstrated for the microbial uptake and oxidation of low molecular-weight aromatic hydrocarbons of relatively high water solubility, but may not apply to the more insoluble hydrocarbons. In line with that, Wodzinski and Bertolini [66], Wodzinski and Coyle [67] and Thomas *et al.* [68] showed that the rates of mineralization of high molecular-weight aromatic hydrocarbons, such as naphthalene and phenanthrene, are related to aqueous solubility rather than total substrate concentrations. The microbial degradation of long (C12) alkanes, for which solubility is less than 0.01 mg/liter, occurs at rates which exceed the rates of hydrocarbon dissolution [68]-[70] and is a function of the hydrocarbon surface area available for emulsification or physical attachment by cells [71]-[73]. Therefore, biodegradation rates for many hydrocarbons will not display the dependence on concentration which is typically observed with more soluble organic substrates. High concentrations of hydrocarbons can be associated with heavy undispersed oil slicks in water, causing inhibition of biodegradation by nutrient or oxygen limitation or through toxic effects exerted by volatile hydrocarbons [23]. Fusey and Oudot [49] reported that contamination of seashore sediments with crude oil above a threshold concentration prevented biodegradation of the oil because of oxygen and/or nutrient limitation. As also mentioned Soltani [54], a high hydrocarbon concentration leads to the formation of large masses having a low contact surface area with the culture medium and thus, results in microbial growth inhibition. The concept of a maximum or threshold concentration for microbial degradation of hydrocarbons may apply also to soil ecosystems. Dibble and Bartha [39] reported increases in CO_2 evolution over the range of 1.25% to 5% hydrocarbon mass per dry weight of soil, when oil sludge was applied to soil. No increase was observed at a level of 10% and the rates declined at 15%. They concluded that decreases in activity at high oil loading concentrations were ascribed to inhibition of microbial activity by toxic components of the oil sludge [39]. All these findings suggest that the concept of a maximum or threshold concentration for microbial degradation of hydrocarbons may be related to the pollutant type and environmental factors.

3.3.5. Hydrocarbon Utilization

The growth variance in MS medium with hydrocarbon as sole carbon and energy source at 37˚C, pH 8.5 and 7.5 for strains S_2 and S_7, respectively is presented in **Table 2**. After 12 days incubation period, strains S_2 and S_7 showed a significant difference for the hydrocarbons tested ($p = 0.05$, **Table 2**). However, no significant difference was found among hydrocarbons for strain S_2 and strain S_7 as well ($p = 0.461$). The combined effects of hydrocarbon source and strain showed also no significant difference ($p = 0.235$). The maximum growth rates (μmax) of strains S_2 and S_7 were determined on different hydrocarbons: SAE 40 used oil, Total Quartz 9000, Diesel oil and gasoline. From the results obtained (**Figure 4**), strain S_2 showed the highest growth rates on SAE 40 used oil, Total Quartz 9000, Diesel oil and gasoline (0.169 ± 0.067, 0.140 ± 0.051, 0.128 ± 0.011 and 0.109 ± 0.026, respectively) compared to the ones of strain S_7 (0.130 ± 0.045, 0.101 ± 0.015, 0.096 ± 0.015 and 0.093 ± 0.015, respectively). For all the hydrocarbons tested, statistical analysis showed no significant difference between the growth rates of strains S_2 and S_7 ($p > 0.05$, **Figure 4**). However, a significant difference between both strains was observed for each hydrocarbon tested ($p = 0.05$, **Figure 4**).

Although no significant difference in growth of both strains was observed between the hydrocarbons tested (**Table 2**, **Figure 4**), the growth on SAE 40 used oil appeared relatively the highest and the one on gasoline the lowest compared to the growth on the other hydrocarbons (**Figure 4**). According to Ratledge [74], the short aliphatic hydrocarbon chains abundant in gasoline can dissolve in the culture medium and become toxic to bacteria, thus justifying the low growth observed with gasoline. Wrenn and Venosa [75] also underlined that many polyaromatic hydrocarbon rings present in used oil can lead to the formation of colored compounds during their degradation. These colored compounds can increase the turbidity of the culture medium and thus leading to an overestimation of the OD and μmax. However, since no significant difference was observed between the hydrocarbons tested, this point may influence very weakly or not the results obtained in the present study.

The gravimetric analysis according to Fusey and Oudot [49] for Diesel oil, SAE 40 used oil and Total Quartz 9000 motor oil revealed after 7 days incubation period, biodegradation rates of $50.62\% \pm 1.55\%$, $20.41\% \pm 3.88\%$ and $16.26\% \pm 0.38\%$, respectively with strain S_2, while $10.33\% \pm 2.33\%$, $6.28\% \pm 1.61\%$, and $3.31\% \pm 1.56\%$, respectively with strain S_7. The discrepancy of these data with those of the growth studies (**Figure 4**) can be related to the gravimetric estimation used. As underlined Bossert and Bartha [37], the presence of particulate matter, affects the physical and chemical nature of the oil and hence its susceptibility to microbial degradation. In

Table 2. Variance of hydrocarbons utilization with regards to bacterial strain and hydrocarbon type (means of 3 replicates).

Source of variation	df	p	F
Strain	1	0.05	s
Hydrocarbon	3	0.461	ns
Strain × Hydrocarbon	7	0.235	ns

ns: not significant; **s**: significant; **df**: degree of freedom; **p** = probability at a risk of 5%; **F**: Fischer' test.

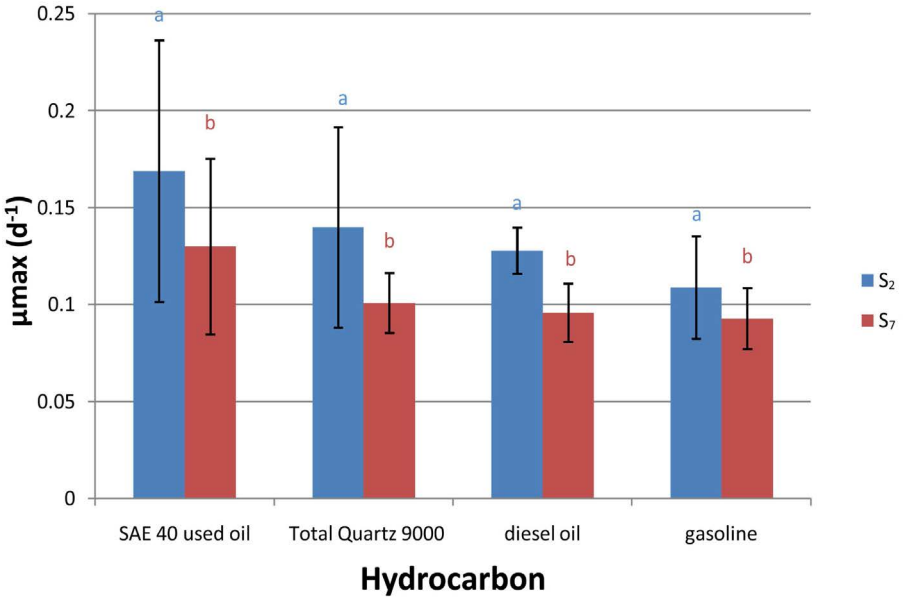


Figure 4. Maximum growth rate (μmax) of strains S_2 and S_7 at optimum growth conditions (37˚C, pH 7.5 and 8.5, 0.5% - 0.6% (w/v) and 3% (v/v) NaCl and hydrocarbon concentrations, respectively) on SAE 40 used oil, Total Quartz 9000, diesel oil and gasoline. For both strains and for all the hydrocarbons experienced, values sharing the same letter are not significantly different according to the Newman-Keuls test at p = 5%.

the present study, the probable presence of particulate matter in Diesel oil and SAE 40 used oil may lead to an overestimation of the biodegradation rates with these oils compared to Total Quartz 9000 motor oil one when using the gravimetric method.

3.4. Biochemical Characteristics

The tests carried out revealed that cells of strain S_2 were oxidase-negative, ONPG-negative, ADH-negative, ODC-negative, LDC-negative, H_2S-negative and indole-negative, while Cells of strain S_7 appeared catalase-positive, oxidase-positive, ONPG-negative, ADH-positive, ODC-negative, LDC-negative, H_2S-negative and indole-negative (**Table 3**). Both strains are strict aerobes and able to ferment citrate and glucose but not lactose.

3.5. Taxonomy

The morphological, physiological and biochemical characteristics (**Table 3**) indicate that strains S_7 and S_2 are very closely related to *Pseudomonas* and *Acinetobacter* genera, respectively. According to some authors [3] [5] [13] [15] [17]-[19] [23] [30], bacterial strains belonging to these two genera are generally able to degrade hydrocarbons. Our isolates can grow in MS medium supplemented with hydrocarbons as sole carbon and energy source and thus, sustaining their capability to degrade hydrocarbons in agreement with the finding of these authors. However, the determination of the genetic characteristics of our isolates remains of utmost importance to confirm their affiliation to these genera and for their full characterization and identification at species level.

Table 3. Phenotypic characteristics of strains S_2 and S_7 and related genera.

Characteristic	S_2	S_7	Pseudomonas[1]	Acinetobacter[2]
Colony morphology				
Form	Round	Round	Round	Round
Size (mm)	3 - 5	2 - 3	Small	0.5 - 2
Color	Translucent	Yellowish	Yellow	Translucent, opaque
Cell morphology	Short rod Coccobacillus-like	Long rod Bacillus-like	Long rod Bacillus-like	Short rod Coccobacillus-like
Motility	−	+	+	+/−
Physiology Hydrocarbons utilization	+	+	+	+
Substrates fermented				
Glucose	+	+	+	+/−
Lactose	−	−	−	+/−
Citrate	+	+	+	+/−
Respiratory metabolism	Strictly aerobic	Strictly aerobic	Strictly aerobic	Strictly aerobic
Optimal growth temperature	37˚C	37˚C	30˚C - 35˚C	30˚C - 37˚C
Biochemical characteristics				
Gram stain	−	−	−	−
Oxidase	−	+	+	−
Catalase	+	+	+	+
ONPG	−	−	−	−
ADH	−	+	+	−
LDC	−	−	−	−
ODC	−	−	−	−
H_2S	−	−	−	−
Indole	−	−	−	−

+: positive, −: negative, [1]Franzetti and Scarpellini [76]; [2]Constantiniu *et al.* [77].

4. Conclusion

The ability of various indigenous bacteria (especially those isolated from contaminated sites) to metabolize crude oil or aliphatic hydrocarbons is well known. In this study, bacterial strains that are able to grow on used oil as a carbon and energy source were isolated from hydrocarbon-contaminated wastewaters in Ouagadougou, Burkina Faso. The enrichment technique was used for selecting hydrocarbon degraders. Growth on various hydrocarbons revealed the presence of hydrocarbon- and oil-degrading activities in the isolated bacterial strains. Based on the morphological, biochemical and physiological characteristics defined, the isolated hydrocarbon-degrading strains S_7 and S_2 appear to be related to *Pseudomonas* and *Acinetobacter* genera, respectively. However, to confirm their affiliation to these genera, and for a full characterization and identification, the determination of genetic characteristics (16S rDNA and key enzymes encoding genes sequences analysis) and refining their physiology for their ability to use high/low molecular hydrocarbons are required. The optimization of the bioremediation ability of the strains may also require a full elucidation of the metabolic pathways and the key enzymes involves in both strains during hydrocarbons degradation. In order to increase the feasibility of the bacterial isolates as potential commercial strains, future studies need to clarify the factors affecting the ability and efficiency of hydrocarbon and crude oil degradation, such as nutrient concentration, oxygen content and physical state of the oil.

Acknowledgements

The authors would like to express profound gratitude to ISP-SUEDE, PACER-UEMOA, CRSBAN-RABIO-

TECH, UFR-SVT/UO and CNRST/IRSS, for financial and technical supports.

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