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Original Article

Hydrocarbon-degrading Capability of Bacteria isolated from a Maize-Planted, Kerosene-contaminated Ilorin Alfisol

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ABSTRACT: In an effort at discovering autochthonous and active bacterial strains that could be of relevance in biodegradation and/or bioremediation of petroleum contaminated systems in Nigeria, twenty four bacterial species were isolated from kerosene treated Ilorin alfisol. The traditional method of identifying bacteria was complemented by using Microbact™ ID 24E system for the identification of Enterobacteriaceae and common miscellaneous Gram-negative bacilli (MGNB). The results show appreciable increase in optical densities and total viable counts contemporaneous with decrease in pH of the culture media. The most promising organisms in this study are *Leclercia adecarboxylata*, *Pseudomonas aeruginosa, Proteus mirabilis, Micrococcus luteus, Arthrobacter* sp. and *Streptococcus* sp. The results obtained in this study showed that kerosene spillage posses a great threat to the survival and development of *Zea mays*. It also revealed that some bacteria survive and even thrive in kerosene contaminated soil and hence have the potential to be used in biodegradation and/or bioremediation of oil contaminated soils and water.

KEYWORDS: Bacteria, biodegradation, alfisol, hydrocarbon, *Zea mays.*

INTRODUCTION

Kerosene is a thin, clear liquid formed from hydrocarbons, with a density of 0.78–0.81 g/cm^3. It is obtained from the fractional distillation of petroleum between 150 °C and 275 °C, resulting in a mixture of carbon chains that typically contain between 6 and 16 carbon atoms per molecule (Collins, 2007). Regardless of the crude oil source or processing history, the major components of all kerosenes are branched and straight chain alkanes and naphthenes (cycloalkanes), which normally account for at least 70% by volume (American Institute of Petroleum, 2010). Despite the several usefulness of kerosene, it also constitutes a major environmental concern globally. Based on the type and concentration of aromatic compound present in kerosene, its acute toxicity to living organisms vary from moderate to high. (Saratale *et al*., 2007).

Chronically, the effects of some of the constituents in kerosene (benzene,toluene, xylene, naphthalene, alkyl benzenes and various alkyl polycyclic aromatic hydrocarbons) include changes in the liver, harmful effects on the kidney, heart, lungs, and nervous system. (Irwin *et al*., 1997). In addition to economic damage and aesthetic problem caused by kerosene spills, plants, animals and microorganisms in both land and water are negatively affected (Blazquez *et al.,* 2004; Ikpeme *et al*., 2007). Industrialization, accidental and deliberate spill of petroleum products have increased the pollution of hydrocarbon compounds in the soil and water. Basically, kerosene enters the environment through different sources such as accidental spills, pipe leakage, pipe vandalization, deliberate disposal of oily wastes, corrosion of pipes, kerosene seeps and other operational deficiencies (Ikpeme *et al*., 2007; Kalme *et al*., 2008).

However, the unavoidable spills of kerosene arising from tank overflow, bunkering and poor vending facilities could be attributed to the importance of kerosene as a major source of

energy for cooking and lighting in all sectors of the society in Nigeria (Ikpeme *et al*., 2007). Bioremediation processes utilize naturally occurring microorganisms to treat specific environment polluted with chemicals (Wackett and Hershberger, 2001; Pelczar *et al.* 2002). Stimulation of the natural potentials of microorganisms to degrade and detoxify hazardous pollutants is a welcomed development as it brings about the biotransformation which reduces the complex mixture of noxious materials to simple nutrients in soil and water environments (Ikpeme *et al*., 2007).

Table 1: Physico-chemical characteristics of the soil sample. Values are presented as Mean ±SD (n=3).

Soil Parameter	
pH	6.88 ± 0.01
Moisture content (%)	0.20 ± 0.01
Water holding capacity (ml/g)	0.57 ± 0.01
Temperature (°C)	35.80 ± 0.01
TOC (%)	1.97 ± 0.01
Nitrogen (%)	1.14 ± 0.01
Phosphorus (mg/kg)	28.23 ± 0.01
Potassium (mg/kg)	1.19 ± 0.01
Calcium (mg/kg)	20.56 ± 0.02
Magnesium (mg/kg)	4.10 ± 0.00
Potassium (mg/kg)	1.19 ± 0.00
Sodium (mg/kg)	0.47 ± 0.01

The objectives of this study are to investigate the effect of different concentrations of kerosene oil on the germination of maize plants, to investigate, isolate and identify the types of bacteria present in maize planted-kerosene oil contaminated soil and to measure the level of biodegradation achieved.

MATERIALS AND METHODS

Soil Investigation

The garden soil used for the planting was obtained from fallow land located at the botanical garden of the University of Ilorin. It was analyzed before treating with kerosene. The methods of Walkley & Black (1943), and Ekpo & Thomas (2007) was used for organic carbon determination (dichrometer-oxidation). The total nitrogen was determined by the micro-kjeldahl digestion method and the nitrogen content in the digest was measured calorimetrically (Odu *et al*., 1986). Available phosphorus (P) in the soil sample was determined by the Bray's P method (Bray and Kurtz, 1945). Exchangeable Ca, Na, Mg and K were extracted with 1N neutral ammonium acetate solution. Thereafter K, Na and Ca were determined by flame photometer while Mg was read from Atomic Absorption Spectrophotometer (AAS).

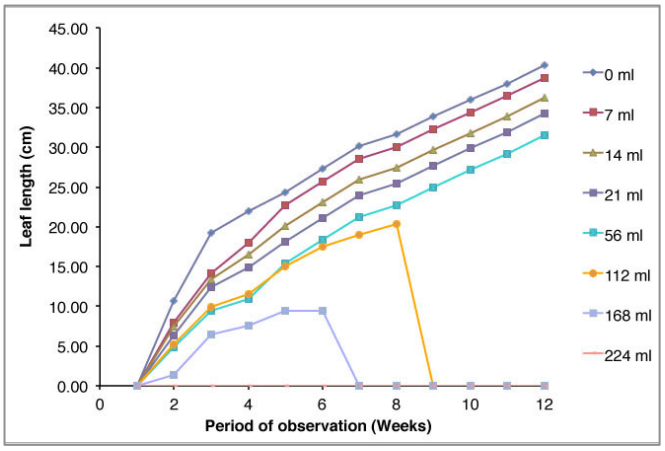

Figure 1: Length of the leaves of *Zea mays* during the period of observation.

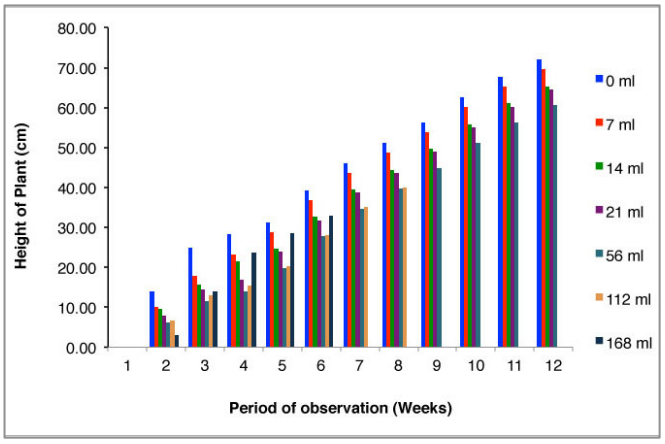

Figure 2: Height of *Zea mays* during the period of monitoring.

The loamy alfisol was collected in sterile polythene bags. The experimental set up comprised eight (8) treatment options including the control, each option had three replicates. Each planting pot contained 2000g of soil and was treated with eight (8) different concentrations of kerosene; 0 (control), 7, 14, 21, 56, 112, 168 and 224 ml according to the method of Ekpo and Thomas (2007). A day after contamination, three healthy maize seeds were planted in each pot and thinned after germination. The arrangement of the pots was then randomized according to standard methods. The planting pots were perforated at the bottom and sides to allow for aeration and drainage of excess water. The set up was watered throughout the investigation period.

Characterization and Identification of Isolates

Pure cultures of bacterial isolates were identified on the basis of their colonial morphology, cellular morphology and biochemical characteristics according to the scheme of Cowan and Steel (Barrow and Feltham, 1995). This traditional method of identification was complemented by using Microbact™ ID 24E system for the identification of Enterobacteriaceae and common miscellaneous Gram-negative bacilli (MGNB). The Microbact™ ID 24E kit was used according to manufacturer's specifications (Oxoid Ltd., Basingstoke,Hants, UK).

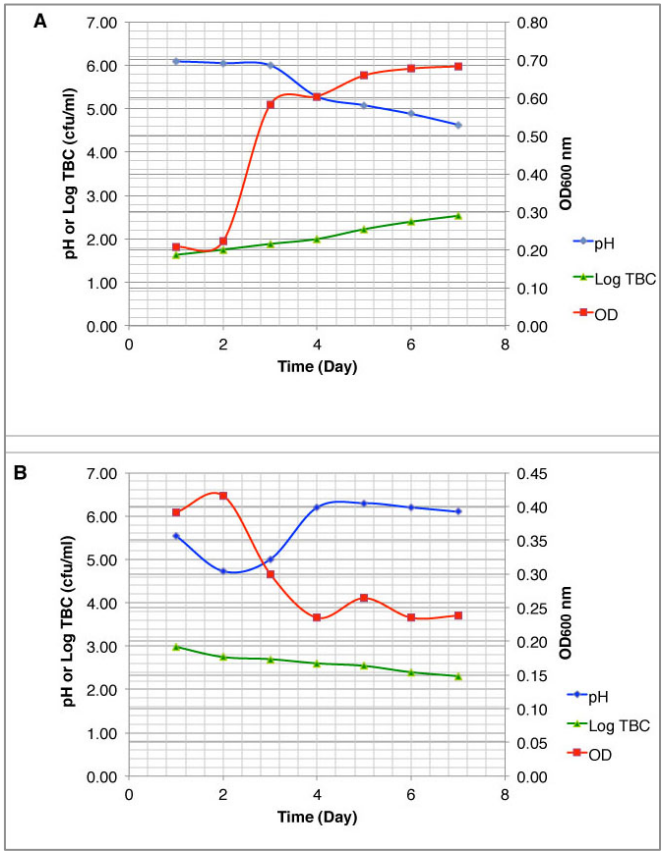

Figure 3: Growth configuration of *Leclercia adecarboxylata* on kerosene. (A) with Kerosene; (B) without Kerosene.

Growth of Bacterial Isolates on Hydrocarbon Substrate

Time course degradation of the oil was performed using mineral salts medium described by Vecchioli *et al,* (1990). Growth of the bacterial species on hydrocarbon substrates was carried out by the inoculation of each of the bacterial cultures into a 250 ml Erlenmeyer flask as described by Oboh *et al* (2006), containing 99 ml of mineral salt medium with a composition of (g/L): 0.5 KH_2PO_4; 1.4 Na_2HPO_4; 0.2 $MgSO_4.7H_2O$; 0.3 KNO_3; 1 $(NH_4)_2SO_4$. The pH of the mineral salt medium was 7.0. Each flask was supplemented with 1ml of kerosene which was obtained from a filling station and added to this medium as the only carbon source. Control samples on hydrocarbon free basis were run in parallel. The culture flasks were incubated for 168 hours at 37°C with continuous agitation in a rotatory incubator shaker. The optical density (OD 600nm), total viable count (TVC) and pH of the culture fluids were monitored every 24 hours as indicators of biodegradation.

Statistical analyses

One way analysis of variance (ANOVA) test was used to determine whether Time course hydrocarbon degradation of the oil and other measured parameters differed significantly according to type of inocula. *P* value of less than 0.05 was considered to indicate statistical significance.

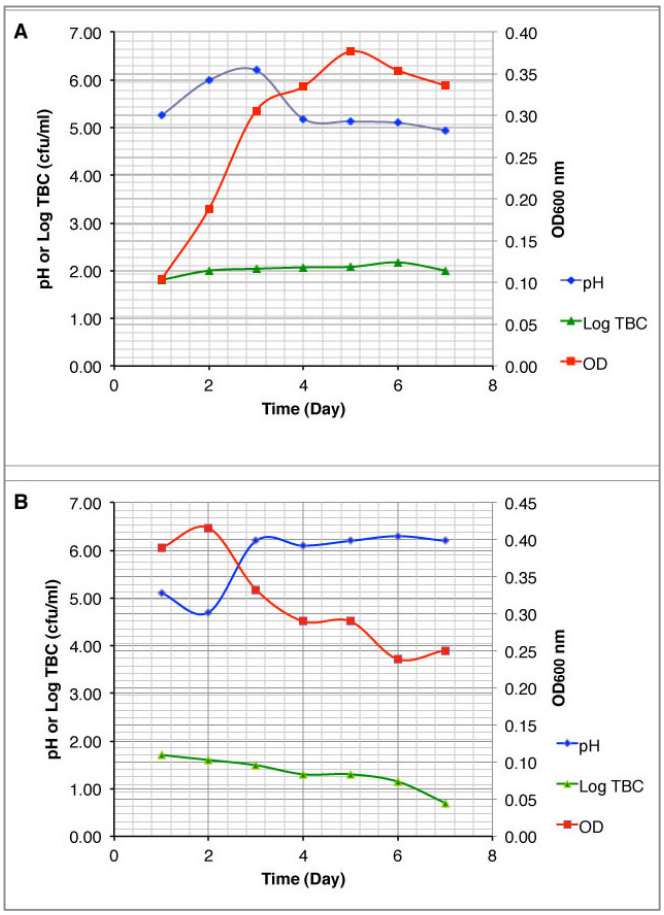

Figure 4: Growth configuration of *Pseudomonas aeruginosa* on kerosene. (A) with Kerosene; (B) without Kerosene.

RESULTS & DISCUSSION

The physico-chemical characteristics of the experimental soil revealed that the pH was neutral. Other physicochemical soil parameters are indicated in table 1. Some of the parameters measured have the same value with that of Agarry *et al.* (2010) while some variations in values existed. Figure 1 shows the length of the leaves of *Zea mays* during the period of observation while figure 2 shows the height of *Zea mays* during the period of monitoring. From figures 1 and 2 it can be observed that there is a progressive increase in the measured maize parameters. However, the maize plants died

off after some weeks in the higher concentrations of kerosene soil. The physicochemical properties of the soil had effect on the microorganisms. pH is one of the most indicative measurements of the general status of the soil. Generally, bacteria are more prevalent in alkaline soils.

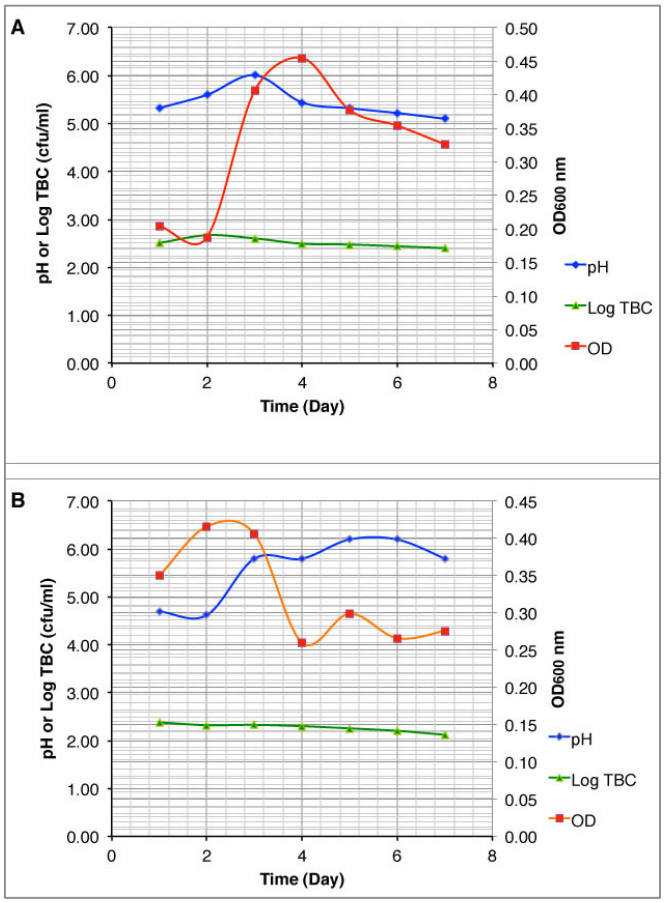

Figure 5: Growth configuration of *Proteus mirabilis* on kerosene. (A) with Kerosene; (B) without Kerosene.

Zea mays with the highest kerosene pollutant showed the least growth while the unpolluted alfisol produced the best growth. It was observed that the growth of maize reduced as the concentration of kerosene pollutant increased. This reduction in plant growth with increase in volume of kerosene pollutant was similar to what Ekpo and Ebeagwu (2009) reported in their work on *Telfairia occidentalis*. The most diverse and numerous populations are found in near neutral soils and this is observed in the pH range (table 2), which is between 6.58 to 6.94 for the experimental control and 6.13 to 7.21 for the treated soil. This may be responsible for the high bacterial number observed in the study, as pH plays an important role in the growth rate of microorganisms (Salle, 1973).

The colonial, morphological and biochemical characterization of the bacterial isolates obtained from the different kerosene contaminated pots revealed the following nineteen genera: *Leclercia, Stenotrophomonas, Acinetobacter, Pseudomonas, Proteus,, Actinomyces, Chromobacterium,, Corynebacterium,* B*acillus, Cytophaga*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Arthrobacter*, *Staphylococcus, Sarcina, Staphylococcus, Streptococcus*, and *Serratia.* Fifteen of the isolates were Gram negative while nine were Gram positive. Nineteen of the isolates were rod shaped while only five were cocci shaped. The predominant species were mainly *Pseudomonas* and *Acinetobacter.*

Previous reports by some workers (Akhavan *et al*., 2008) have shown that native bacterial population have the potential to degrade hydrocarbons in soil and water.

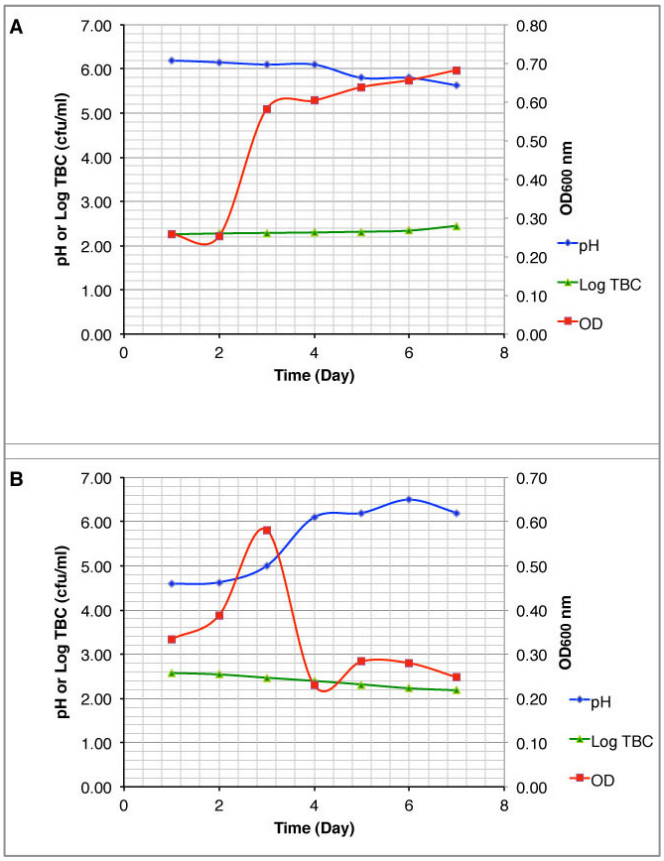

Figure 6: Growth configuration of *Proteus mirabilis* on kerosene. (A) with Kerosene; (B) without Kerosene.

All the isolated and identified bacteria were able to grow on kerosene as the sole energy and carbon source at different extents when assessed for hydrocarbon consumption. However, *Leclercia adecarboxylata* (Figure 3), *Pseudomonas aeruginosa* (Figure 4), *Proteus mirabilis* (Figure 5), *Micrococcus luteus* (Figure 6), *Arthrobacter* sp (Figure 7) and *Streptococcus* sp (Figure 8), grew maximally on the kerosene substrate when supplied as the sole carbon and energy source. Data from control experiments in which the isolates were grown on kerosene free mineral salt medium show that the isolates did not do as well in the absence of kerosene.

Some of these organisms have been shown to be hydrocarbon degraders by workers such as Moneke and Nwangwu (2011), Nwachukwu (2001), Oboh *et al.,* (2006), Nwachukwu and Ugoji (1995), Atlas (1992) and Amund and Adebiyi (1991) and can be attributed to the genetic makeup due to the constitutive expression of hydrocarbon catalysing enzymes or physiological owing to exposure to exogenous hydrocarbons present in the kerosene.

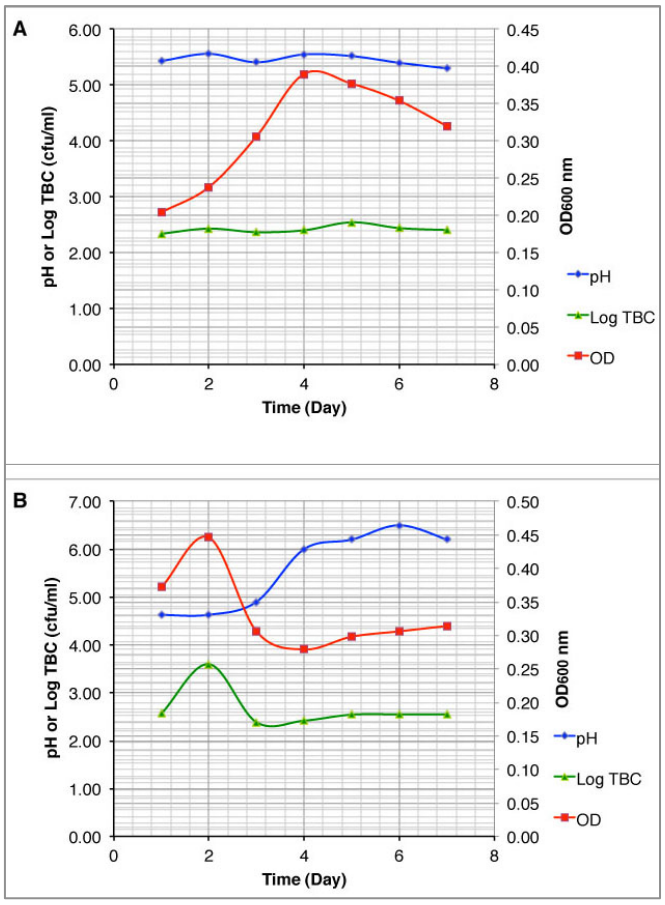

Figure 7: Growth configuration of *Arthrobacter* sp on kerosene. (A) with Kerosene; (B) without Kerosene.

Desouky (2003) have reported that bacteria of the genus *Acinetobacter* are known to be involved in biodegradation, leaching and removal of several organic and inorganic man-made hazardous wastes. This agrees with the findings of this work.

It is clear from this work that oil degrading microorganisms could be isolated from kerosene contaminated soil and water without the need for time consuming genetically engineered microorganisms and the traditional enrichment protocols. Further understanding of the metabolic process of these organisms on the hydrocarbons will increase possibilities of developing models and strategies for removing hydrocarbon pollutants from hydrocarbon contaminated ecosystems. The results obtained in this study showed that kerosene spillage posses a great threat to the survival and development of *Zea mays*. It also revealed that some bacteria survive and even thrive in the kerosene contaminated soil and hence have the potential to be used in biodegradation and/or bioremediation of oil contaminated soils and water.

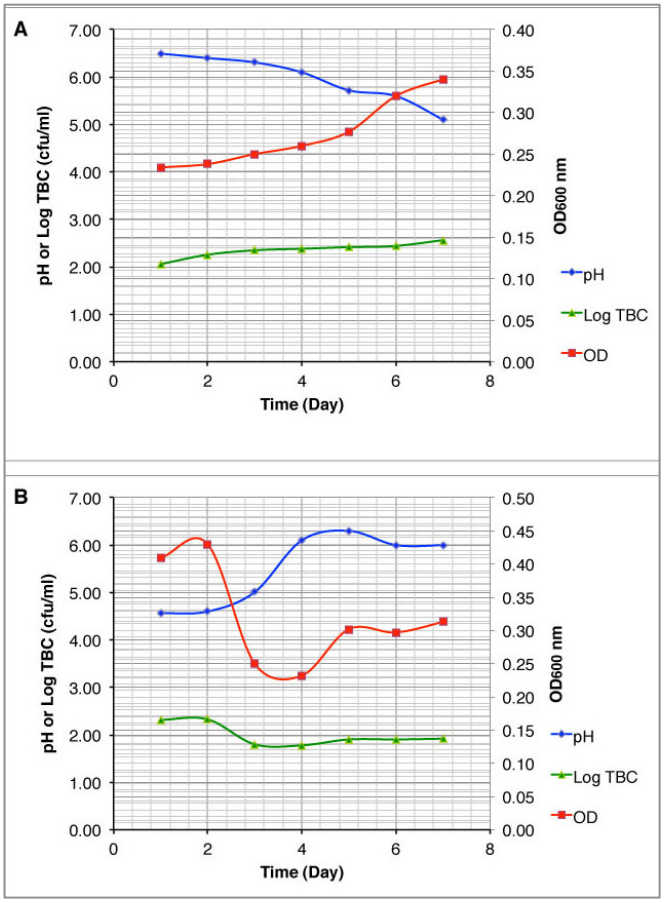

Figure 8: Growth configuration of *Streptococcus* sp on kerosene. (A) with Kerosene; (B) without Kerosene.

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