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Assessment of Biosurfactant Production and Petroleum Hydrocarbons Biodegradation Capability of Actinomycetes Isolated from Soils

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ABSTRACT

Production of biosurfactants and emulsifiers by bacteria has been suggested to enhance the biodegradability of hydrocarbons (HCs) in complex matrices like crude oil and its refined products. Recent studies have focused on the capability of actinobacteria to biodegrade HCs because of their abundance in soils and high tolerance to recalcitrant chemicals. Here, actinomycetes isolated from pesticide-impacted agricultural soils were assessed for their biosurfactant production and complex HC biodegradation capabilities. Five actinomycetes (*Lentzea albidocapillata*, *Actinomyces slackii*, *Actinomyces liubingyangii Rhodococcus erythropolis* and *Trueperella bernardiae*) identified based on morphological, physiological, and 16S rRNA gene sequencing were assessed for biosurfactant production using four tests. The biodegradation capability was assayed in aviation fuel, petrol, kerosene, diesel and crude oil using the standard CO_2 respirometry for 30 days. All isolates exhibited high emulsifying activity (35.71 - 65.12%) in all HCs but their ability to adhere to hydrophobic surfaces varied greatly (28.77-70.11%) with *L. albidocapillata* demonstrating the most significant hydrophobicity. With the exception of *T. bernardiae*, all actinomycetes' whole-cell suspensions and cell-free supernatants generated sizable biosurfactants that removed diesel film from water more effectively than crude oil. Biosurfactants produced by *A. liubingyangii* caused the greatest displacement of the oils. The complex HCs were mineralized to different extents, with the highest extent of mineralization by *L. albidocapillata* in petrol (53.40mgCO_2/mL). The findings imply that the ability of these actinomycetes to adhere to hydrophobic matrices and produce biosurfactants with strong emulsifying activity makes them good candidates for bioremediation of complex HCs like crude oil and its refined products.

***Keywords*:** Actinomycetes, biosurfactants, petroleum hydrocarbons, bioremediation

Introduction

Crude oil remains one of the major sources of energy for most countries of the world.[1] Oil exploration, production and distribution activities release petroleum hydrocarbons (PHCs) into the environment. [2] These PHCs are of great concern because in humans, as certain constituents exhibit carcinogenic and mutagenic effects. In the environment, they are recalcitrant and may accumulate especially in soils. [2] These organics also impose threats to animal and plant species thereby affecting the environment as a whole. Their hydrophobic characteristics hinder their easy clean up from the environment.[3] Several physical and chemical methods have been imbibed for the treatment of harmful contaminants in the environment [4] but they are mostly expensive, destructive and technologically-complex.[1,5] This has made more attention to be given to devising and applying safe and less expensive ways such as bioremediation to clean up these contaminants.[6] Bioremediation offers a green approach that relies on the ability of microorganisms such as actinomycetes to use hydrocarbons as their source of carbon and energy. It has proven to be more effective, safer and less expensive compared to other method of remediation. [7] Actinomycetes are Gram-positive organisms that belong to the order Actinomycetales within the phylum Actinobacteria.[8] They are mostly found in environments that are uncultivated.[1,9,10] The ability of these organisms to produce biosurfactants makes them one of the choicest organisms in the bioremediation of soils contaminated with PHCs. Biosurfactants aid solubilization of PHCs hence increasing the bioavailability of contaminants and enhancing crude oil biodegradation process.[1] Biosurfactants' amphiphilic nature enables them to interact at the boundary between the water and oil phases, reducing the surface tension between the two layers.[1] In comparison to chemical surfactants, biosurfactants have greater selectivity owing to the presence of specific functional groups ensuring specificity in the detoxification of particular pollutants and activity under situations of severe salinity, pH, and temperature.[1,13] Studies have reported the role of biosurfactant production in biodegradation. For example, the production of biosurfactant by bacteria *Pseudomonas aeruginosa* PG1 that enhanced hydrocarbon biodegradation was reported by Patowary *et al.* [14] The total petroleum hydrocarbons (TPH) were reduced to 81.8% by the strain PG1 after 5 weeks of culture when grown in mineral salt media (MSM) supplemented with 2% (v/v) crude oil as the sole carbon source. The unique properties actinomycetes have, aid their survival in extreme conditions such as dry environments, contaminated sites and nutrient starvation. Literature reveals that actinomycetes have series of aliphatic and aromatic HC degradation genes with overlapping HC substrate ranges [6] hence being potential biodegraders. The concern of this research is generated from the fact that most bioremediation processes from literature reviews were carried out on soils contaminated with simple hydrocarbons. In nature, hydrocarbons do not occur singly;

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crude oil contamination involves a combination of a lot of hydrocarbons. Furthermore, most literatures reported the isolation of bacteria of the genera *Pseudomonas*, *Bacillus* for bioremediation processes. Literatures are limited on the use of actinomycetes for bioremediation. Hence, the study aimed to assess the capability of actinomycetes isolated from soil to produce biosurfactant and biodegrade petroleum hydrocarbons. The specific objectives of the study are to isolate actinomycetes from soil, characterize the isolates, assess the biosurfactant production potentials of the isolates and to assess the biodegradation potentials of the isolates.

Materials and Methods

Culture media and chemicals

The culture media including starch casein agar (SCA) which contains sea water, agar, starch and casein powder; Mueller–Hilton agar (MHA) which contains beef extract, acid casein hydrolysate, starch and agar; Sabouraud's dextrose agar (SDA) which contains dextrose, peptone, agar and nutrient broth (which contains meat peptone and meat extract) were products of Oxiod Ltd., Basingstoke, UK. Analytical grade chemicals obtained from Merck, UK were used to compound culture media including International Streptomyces Project 2 (ISP-2: 4 g yeast extract; 10 g malt extract; 4 g dextrose; 20 g agar powder in 1000 mL distilled water; pH 7.2), glucose–peptone–yeast agar (GPYA: 5 g glucose; 5 g peptone; 3 g yeast extract; 20 g agar powder in 1000 mL distilled water; pH 6.8), mineral salt medium (MSM: 0.3 g NaCl; 0.6 g $(NH_4)_2SO_4$; 1.5 g $MgSO_4 \cdot H_2O$; 0.6 g KNO_3; 0.25 g KH_2PO_4; 0.75 g Na_2HPO_4 and 10 g glucose per 1000 mL distilled water), and Bushnell–Haas broth (BHB: 1 g NH_4NO_3; 0.05 g $FeCl_3$; 1 g KH_2PO_4; 0.2 g $MgSO_4$; 0.02 g $CaCl_2$; 16 g $FeCl_3 \cdot 6H_2O$; 20.1 g NaEDTA; 0.18 g $CoCl_2 \cdot 6H_2O$; 0.18 g $ZnSO_4 \cdot 7H_2O$; 0.16 g $CuSO_4 \cdot 5H_2O$; 0.1 g $MnSO_4 \cdot H_2O$ in 1000 mL distilled water). All media were sterilized by autoclaving at 121ºC for 15 min.

Survey

Three different soils sampled at a depth of 5–15 cm (Ah horizon) were collected from beneath a decaying palm tree, a compost of leaves and straw debris, and sediment by a river bank. All these sites were within the Botanical Garden, University of Ilorin, Nigeria latitude 8.4799° N and longitude 4.5418° E.

Isolation of actinomycetes in soil

The physicochemical properties of the samples were assessed after which large particles and stones were removed from the soil, sieved (2 mm) and air-dried at room temperature. Afterwards, the soils were heat-dried at 120°C for 60 minutes. To isolate actinomycetes, 10 g of soil was dispensed into 100 ml of sterile distilled water and thoroughly mixed using a vortex. Aliquots of 0.1 ml removed from the 10^{-2} and 10^{-3} diluents were inoculated (using the spread plate method) onto SCA plates. The plates were prior impregnated with nistatine (20 mg/L) and nalidixic acid (100 mg/L) to prevent fungal and bacterial growth, respectively. The plates were incubated in a DNP-9052 SANFA laboratory incubator (China) at 30ºC for 10 d. Distinct colonies were re-inoculated onto freshly prepared SCA using the streak method to purify the isolates.

Growth characterization and molecular identification of isolates

The actinomycetes isolates were initially characterized and differentiated based on their colonial and cellular characteristics and then identified using molecular analysis. The morphological characteristics of the isolates were differentiated using five culture media: SCA, ISP-2 medium, MHA, SDA and GPYA. The colonies were assessed on the basis of their shape, colour, elevation and margin as well as their consistency and optical characteristics. Microscopic assessment to determine their Gram reaction and cellular morphology was also carried out.

Molecular analysis based on 16S rRNA was performed at the Biotechnology section of the Bioscience Center of International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria to identify the isolates. The processes involved DNA extraction, polymerase chain reaction (PCR) and nucleotide sequencing. Extraction of genomic DNA of isolates followed the procedure in QIAamp DNA Mini Kit (250) cat No. 51306. The forward primer (16SF: 5'GTGCCAGCAGCCGCGCTAA'3) and reverse primer (16SR: 5'AGACCCGGGAACGTATTCAC'3). The reaction mixture (10 µL) comprised 10× buffer, 1.0 µL; 2.5 mM dNTPs, 0.8 µL; 5.0 U/ µL Taq DNA polymerase, 0.1 µL; 5 pmol of each primer, 0.5 µL; 10 ng/µL DNA template, 2.0 µL; 25 mM $MgCl_2$, 1.0 µL; DMSO, 1.0 µL, sterile ultrapure water, 3.1 µL A Biorad thermal cycler was used for the amplification to obtain amplicon size of 1500 bp. The PCR conditions were: 94ºC for 5 min (initial denaturation); followed by 36 cycles of 95ºC for 30 sec (denaturation), 56ºC for 30 sec (annealing) and 72ºC for 45 sec (extension); then final extension at 72ºC for 7 min. The amplified products (50 µl) were separated on 1% agarose gel prepared in 1% TAE buffer containing 0.5 µl/ml ethidium bromide and photographed with the gel documentation 27 system (Biorad, USA). A 1000 bp DNA ladder (Genei, USA) was used as molecular weight size markers. The amplified PCR product was purified by electro-elution of the gel slice containing the excised desired fragments with Qiaquick gel extraction kit (Qiagen, USA). The purification of PCR product was enhanced by elution in 300 µL of nuclease free water. For sequencing, the PCR amplicons was diluted in Tris buffer (10 mM, pH 8.5) at 1:1000 to obtain DNA concentration of (30 ng/µL). The sequencing reaction required was 8 µL DNA. The primer used for sequencing reaction was 16SF (5'AGAGTTTGATCCTGGCTCAG'3) and performed at a concentration of 3 µM using an ABI PRISM 310 automated DNA sequencer (Applied Biosystems, U.S.A). BLASTN tool (www.ncbi.nlm.nih.gov:80/BLASTN/) was used to analyze for similarities between the translated nucleotide sequences coding for the isolates and used to design a phylogenetic tree.

Assessment of biosurfactant production in actinomycetes

The ability of the isolates to produce biosurfactants was assessed as a potential index of their capability to degrade and mineralize petroleum hydrocarbons. Biosurfactant production by the actinomycetes was evaluated using their emulsifying activity and cell surface hydrophobicity, as well as their ability to collapse oil on water film and spread/displace oil on water surface.

Emulsification index (E_{24} %)

Cell-free supernatants (CFSs) obtained by centrifugation (7000 ×*g* for 15 min) from cultures of actinomycetes grown in BHB supplemented with 1% (v/v) petrol for 7 d (35ºC, 150 rpm) were used for the estimation of emulsification index. BHB was spiked with 1% (v/v) of 100× trace element solution (500 mg $Na_2EDTA \cdot 2H_20$, 143 mg $FeCl_2 \cdot 4H_2O$, 100 mg $CaCl_2 \cdot 2H_2O$, 30 mg H_3BO_3, 20 mg $CoCl_2 \cdot 6H_2O$, 4.7 mg $ZnCl_2$, 3.0 mg $MnCl_2 \cdot 4H_2O$, 3.0 mg $Na_2MoO_4 \cdot 2H_2O$, 2.0 mg $NiCl_2 \cdot 6H_2O$, and 1.0 mg $CuCl_2 \cdot 2H_2O$ in 1000 ml distilled water). Equi-volumes (3 mL) of hydrocarbons and CFSs were homogenized at a high-speed using a vortex for 2 min. Five petroleum hydrocarbons, crude oil, diesel, petrol, aviation fuel and kerosene were used. The test tubes were of equal height and diameter. The suspensions were left at room temperature for 24 h to equilibrate. Emulsification index was calculated using equation 1 as indicated by Petrikov *et al*. [15]

$$E_{24}\,\% = 100 \times \left(\frac{\text{Total height of the emulsified layer}}{\text{Total height of the liquid Layer}}\right) \qquad \text{equation 1}$$

Cell surface hydrophobicity

Cell surface hydrophobicity (CSH) is the ability of microbial cells to form an effective biofilm over a hydrophobic surface. The greater the adherence of HC to microbial cell surfaces the higher the hydrophobicity of the organism. [16] The different strains of actinomycetes were grown for 7 d (35ºC, 150 rpm) in 20 ml BHB supplemented with different hydrocarbons (crude oil, diesel, petrol, aviation fuel or kerosene) at 1% (v/v). After incubation, the broth cultures were centrifuged (7000 ×*g* for 15 min), and the harvested cells were washed (centrifugation 3 times at 7000 ×*g* for 10 min), and then re-suspended in sterile phosphate buffer solution (PBS: 8 g NaCl; 0.2 g KCl; 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 1000 ml distilled water). To standardize the cell suspension, its optical density at 600nm (OD_{600nm}) was adjusted to 0.5 using a UV-6300PC double beam

spectrophotometer VWR, USA. Thereafter, 200 µL of each hydrocarbon was introduced into 1.5 mL of washed cell suspension and vortexed using a vortex mixer XH-C, JIN YI, China at high speed for 3 min. Changes in OD_{600nm} were recorded after the separation of aqueous and organic oil phases at room temperature. Cellular hydrophobicity in terms of bacterial adherence was quantified using equation 2

$$CSH\ (\%) = 100 \times \left(1 - \frac{\text{Initial OD of cell suspension (without shaking)}}{\text{OD of vortexed suspension}}\right) \text{equation 2}$$

Drop collapse

Here, 10 µL of diesel was carefully added to wells of a 96-well microtitre plate. The plate was equilibrated for 60 min at 30°C to form thin coats of oil in the wells. After equilibration, 20 µl of 48-h old whole-cell culture (48-h WCC) of each isolate was added to the oil surface. The collapse of the droplet (film became convex/flat) within 2 min indicated a positive result of the presence of biosurfactant. [17]

Oil displacement/spread

A 48-h old culture grown in BHB supplemented with 1% (v/v) petrol was used. Clean grease-free Petri dish bases (85 mm diameter) were filled with 50 ml of distilled water. An amount (15 µL) of either crude oil or diesel was then layered uniformly on the water. Thereafter, 10 mL of CFS obtained from the culture (centrifugation at 7000×*g* for 15 minutes) was added at a spot on the oil coated on water surface. Occurrence of clear zone/displacement of the oil was an indication of biosurfactant production.[18,19] The length of oil displaced (mm) was determined as the difference between the diameter of the oil film before the addition of CFS and the diameter of the oil film after the addition of CFS as shown in Figure 5.

Assessment of biodegradation of petroleum hydrocarbons by actinomycetes

Actinomycetes cells growing at exponential phase were obtained after centrifugation (7000 ×*g* for 15 min) of 96-h cultures grown in nutrient broth (30ºC, 100 rpm). The concentration (10^8 cells/mL) of purified cell pellets re-suspended in PBS was obtained by standardization with 0.5 McFarland solution using a spectrophotometer. The biodegradation microcosm is a standard respirometer bottle containing 98 ml of sterile MSM, 1 mL of inoculum and 1 mL of hydrocarbon, which served as the sole source of carbon and energy. Trace element solution (1% v/v) was also added. Vials containing 2 mL NaOH to trap CO_2 evolved during hydrocarbon mineralisation were fixed to the respirometer bottles. Incubation was carried out on an incubator shaker (30ºC, 150 rpm) and monitored for 30 days. Sampling of the trapped CO_2 in the vials was after 1, 3, 5, 7, 9, 13, 16, 20, 25 and 30 days. An automatic potentiometric titrator (model ZD-2) was used to determine the amount of CO_2 captured in the NaOH as described by [20]

Statistical analysis

Nonlinear analysis (SigmaStat ver 3.5) was used to fit four kinetics models to the mineralization data: Gompertz (with and without linear component), sigmoid and deterministic three-half or mixed first-order and zero-order kinetics models.

Results and Discussion

The assessment of the physicochemical properties of the soil samples showed that they were mostly sandy in nature and have a slightly alkaline pH as shown in Table 1. The 16S rDNA analysis indicated the isolates belong to four genera including *Lentzea, Actinomyces, Rhodococcus* and *Trueperella* (Figure 1). The molecular relatedness of the isolates indicates that they have similar characteristics that make them suitable for the biodegradation process.

The isolates were initially characterized on the basis of their growth patterns on five different culture media (Table 2). The results showed that the isolates differed in their characteristic growth on the different culture media. This implies that identifying actinomycetes may require culturing on a number of media. The isolates had different characteristic growths on the various culture media used (Table 2). None of the isolates showed the same colonial properties on the culture media. For example, on SCA, *L. albidocapillata* were dark grey circular colonies with opaque and rough surface while its colonies were raised whitish circular colonies that are translucent and smooth surface on ISP-2. They had aerial mycelium with no melanin pigment. *A. liubingyangii* had distinctively different growth on the culture media. They were non-spore formers that had flat colonies, whitish with smooth, translucent surface on ISP-2, raised, blackish with opaque and wrinkled surface on SDA, whereas they were raised pinkish with smooth, translucent surface on GYPA. While *R. erythropolis* showed mostly similar characteristics on SDA and GYPA, the colonies on SDA were smooth but mucoid on GYPA. The isolate did not grow at all on ISP-2 neither did it produce any spore. The different growth characteristics of the isolates on different growth media is a pointer to the fact that although the organisms belong to the same phylum Actinobacteria, the dissimilar characters of the isolates will make them produce varying results in petroleum hydrocarbon biodegradation process. The characterization of the actinobacterial isolates that showed that ISP-2 agar did not support the growth of *R. erythropolis* could be due to the environmental conditions of the laboratory environment. This could also be because the agar contains certain components that inhibited the growth of the isolates.

Table 1: Physicochemical parameters of soil sample

Soil properties	mean value
Textural characteristics Sandy soil	75.26 ± 0.03%
Silt	14.00 ± 0.0%
Clay	10.75 ± 0.03 %
Soil pH	7.65 ± 0.25
Exchangeable acidity	0.52 ± 0.02cmolkg^{-1}
Organic Carbon	1.68 ± 0.12 %
Organic matter	2.89 ± 0.21%
Exchangeable bases Ca^{2+}	0.33 ±0.02 cmolkg^{-1}
Na^+	4.05± 0.05 cmolkg^{-1}
Mg^2	0.67 ± 0.02 cmolkg^{-1}
K^+	0.82 ± 0.02 cmolkg^{-1}
Cation Exchange Capacity (CEC)	6.38±0.01 cmolkg^{-1}
Moisture content	22.65 ± 0.12 %

Values are mean ± SEM of two replicate sub-samples

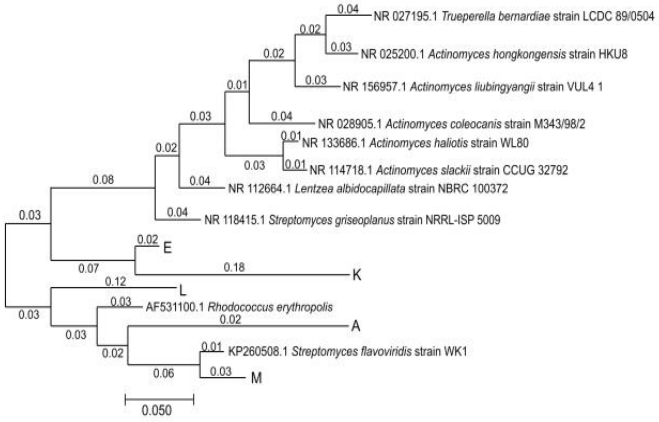

Figure 1: Phylogenetic tree of the molecular identity of the actinomycetes

The emulsification activity (E_{24}) of the biosurfactants produced by the organisms on various hydrocarbons are presented in Figure 2. *A. liubingyangii* has significantly high E_{24} in crude oil, and the highest mean E_{24} of all the organisms (Figure 2a). The mean values of E_{24} for *A. liubingyangii*, *A. slackii*, *R. erythropolis* and *T. bernardiae* were similar. E_{24} ranged 38.16–50.00% for *L. albidocapillata*, 39.47–50.00% for *A. slackii*, 38.09–65.12% for *A. liubingyangii*, 38.46–48.89% for *R. erythropolis* and 35.71–46.81% for *T. bernardiae*. Emulsification activity is influenced by the type of hydrocarbon used. Generally, E_{24} of the organisms are lowest in petrol (Figure 2b). The mean E_{24} was highest in crude oil and similar in diesel, aviation fuel and kerosene. The fact that *A. liubingyangii* has the highest emulsification activity in crude oil could be because the isolate produced large amount of emulsifier that could make it the choicest degrader of crude oil amongst the isolates.

Table 2: Characteristic growth of isolated actinomycetes on different culture media

Isolate	Colonial character	Growth pattern on culture media				
		SCA	**ISP-2**	**MHA**	**SDA**	**GPYA**
L. albidocapillata	Colour	DaGr	Wh	Cr	Cr	Cr
	Elevation	Um	Rs	Fl	Fl	Fl
	Shape	Ci	Ci	Ir	Ci	Ir
	Margin	Lo	En	En	En	Se
	Surface texture	Rg	Sm	Sm	Mu	Sm
	Light transmission	Op	Tr	Op	Tr	Tr
	Reverse side	Nc	Cr	Nc	Nc	Nc
A. slackii	Colour	Cr	Cr	Cr	Cr	Wh
	Elevation	Fl	Fl	Fl	Fl	Rs
	Shape	Ir	Ir	Ir	Ir	Ci
	Margin	Un	Un	Lo	Lo	En
	Surface texture	Sm	Sm	Sm	Sm	Sm
	Light transmission	Tr	Tr	Tr	Tr	Op
	Reverse colour	Nc	Nc	Nc	Nc	Nc
A. liubingyangii	Colour	Gr	Wh	LiGr	Bl	Pn
	Elevation	Fl	Fl	Fl	Rs	Rs
	Shape	Ir	Ir	Ir	Ir	Pf
	Margin	Lo	Se	Se	Se	Lo
	Surface texture	Wr	Rg	Rg	Wr	Sm
	Light transmission	Op	Tr	Op	Op	Tr
	Reverse colour	Pn	Pn	Gr	Bl	Nc
R. erythropolis	Colour	Wh	–	Cr	Cr	Cr
	Elevation	Fl	–	Fl	Fl	Fl
	Shape	Ci	–	Ir	Pf	Rh
	Margin	En	–	Er	En	Se
	Surface texture	Sm	–	Sm	Sm	Mu
	Light transmission	Tr	–	Op	Tr	Tr
	Reverse colour	Nc	–	Nc	Nc	Nc
T. bernardiae	Colour	LiGr	LiBr	Wh	Cr	Wh
	Elevation	Fl	Fl	Fl	Fl	Rs
	Shape	Ci	Pf	Ir	Pf	Ci
	Margin	En	Er	En	En	Rh
	Surface texture	Rg	Rg	Sm	Sm	Rg
	Light transmission	Op	Tr	Tr	Tp	Tp
	Reverse colour	Br	DpBr	DpBr	Nc	Nc

Key: (SCA) starch casein agar; (ISP-2) International Streptomyces Project-2; (MHA) Mueller-Hinton agar; (SDA) Sabouraud's dextrose agar; (GYPA) glucose peptone yeast agar; (Wh) white; (Cr) cream; (LiGr) light grey; (Gr) grey; (DaGr) dark grey; (Pn) pink; (LiBr) light brown; (Br) brown; (DpBr) deep brown; (Bl) black; (Nc) no colour; (Fl) flat; (Rs) raised; (Um) umbonate; (Pf) punctiform; (Ci) circular; (Un) undulate; (Rh) rhizoid; (Lo) lobate; (Ir) irregular; (Rg) rough; (En) entire; (Er) erose; (Se) serrated; (Sm) smooth; (Wr) wrinkle; (Mu) Mucoid; (Op) opaque; (Tr) translucent; (Tp) transparent; (–) no growth

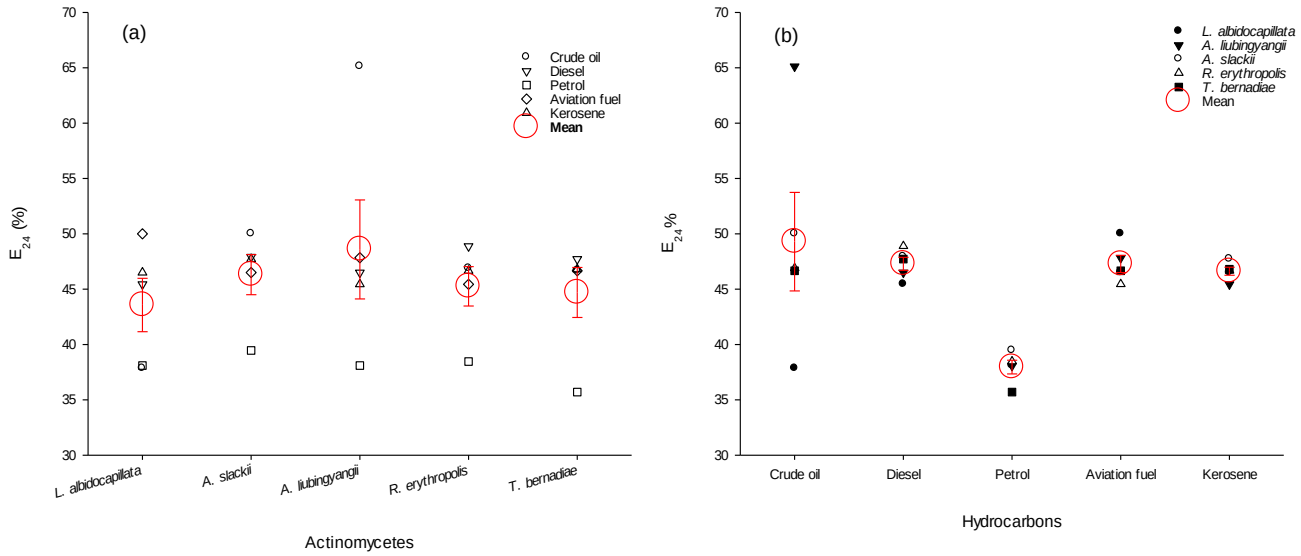

Figure 2: Emulsifying activity (E_{24}) of the actinomycetes on various petroleum hydrocarbons (a) compares E_{24} between various petroleum hydrocarbons, (b) compares E_{24} between different actinomycetes

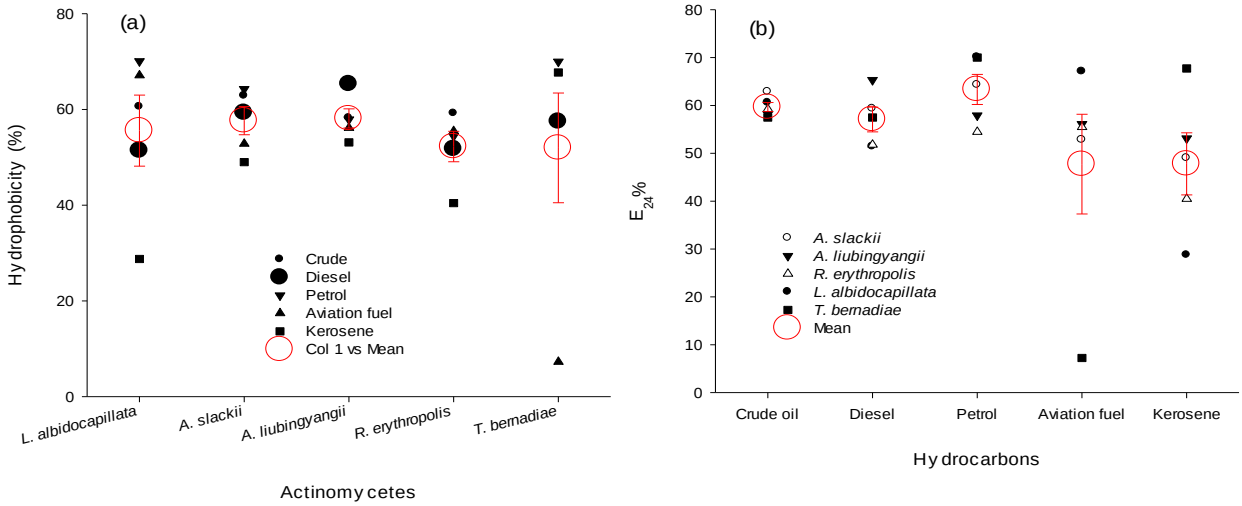

Figure 3: Hydrophobicity (%) of the actinomycetes on various petroleum hydrocarbons (a) compares hydrophobicity between various petroleum hydrocarbons, (b) compares hydrophobicity between different actinomycetes.

The results of the cell surface hydrophobicity as presented in Figure 3. CSH is lowest in kerosene, except for *T. bernardiae* that had significantly low CSH in aviation fuel (Figure 3a). CSH ranged 28.77–70.11% for *L. albidocapillata*, 49.00–64.29% for *A. slackii*, 53.15–65.33% for *A. liubingyangii*, 40.45–59.23% for *R. erythropolis* and 27.30–70.00% for *T. bernardiae*. However, the mean values of CSH are similar for all the organisms. Generally, CSH of the organisms is highest in petrol (Figure 2b). There are wider variations in CSH of the biosurfactants produced by the organisms in the light hydrocarbons aviation fuel and kerosene compared to the heavier hydrocarbons crude oil, diesel and petrol. *Lentzea albidocapillata* had the highest hydrophobicity in petrol. This is an indication of the ability of petrol molecules to adhere to the cell surfaces of the organism thereby, making the contaminant easily accessible. It implies that the isolate produced large biosurfactants in petrol thereby making it a choicest organism for biodegradation of petrol. It could also be traced to its ability to produce 3'-Demethoxy-3'-hydroxystaurosporine-*O*-methyltransferase as reported by Weidner *et al.*[21] Droplets of CFSs of the five isolates collapsed in the micro-wells coated with diesel or crude oil indicating positive drop collapse results as indicated in Table 3. Although *A. liubingyangii* collapsed molecules of oil the most, the other isolates demonstrated the ability to disperse crude oil and diesel on water surfaces; they recorded positive results for oil collapse. This indicates that they may produce enough emulsifiers that aid collapse of oil droplets into water.

Films of diesel and crude oil placed on water were displaced by whole cell suspensions (WCS) of all the organisms (Figures 4 and 5). Figure 4(a) indicates that, except for *T. bernardiae*, the biosurfactants produced by the organisms appreciably displaced diesel more than crude oil. Biosurfactants produced by *A. liubingyangii* caused the greatest displacement of the oils, followed by those of *R. erythropolis* and *T. bernardiae*. The displacement, expressed in term of the percentage increase relative to the original size of the oil film on water, shows *R. erythropolis* and *T. bernardiae* displaced crude oil to a greater extent than diesel (Figure 4b). Figure 5 presents visual presentations of the diesel oil films on water before and after the addition of WCS of the organisms.

The fact that most isolates displaced diesel more than crude oil indicates that they produced more biosurfactants sufficient to degrade diesel more than crude oil. This could be because hydrocarbons in diesel are less

heavy than those of crude oil making it easier for the isolates to produce large quantities of biosurfactants on the less heavy diesel. This tallies with the study of [6] who posited that amount of biosurfactant produced by isolates is dependent upon the molecular weight of the PAHs. They further stated that increase in molecular weight reduces amount of biosurfactant produced. However, *T. bernardiae* could have displaced crude oil better because of its high hydrophobicity rate.

Mineralization of different petroleum hydrocarbons by actinomycetes

Mineralisation of the five petroleum hydrocarbons by the actinomycetes was monitored in a liquid phase over a period of 30 d (Figure 6; Table 4). The mineralization curves were mostly sigmoidal typical of microbial-mediated dissipation of HCs with relatively short lag phases and rapid rates of mineralization. This indicates that the actinomycetes readily utilized the components of the HCs. The evolution of CO_2 established the fact that the actinomycetes possess the catabolic capabilities to completely biodegrade constituents of the HCs (Figure 6). Further, the rapidity of CO_2 evolution (≥18 $mgCO_2$/ml by 10 d) suggests that biodegradation by these actinomycetes could have been catalyzed by inherent enzymes rather than inducible enzymes as the organisms were isolated from soil environments not previously exposed to HC contaminants.

The extents of mineralization of a given HC, with the exception of diesel and aviation fuel, were significantly different ($p < 0.05$) for the various actinomycetes. For instance, *L. albidocapillata* mineralized crude oil (53.36 ± 2.93 $mgCO_2$/ml) to a greater extent than *A. liubingyangii* (37.24 ± 2.03 $mgCO_2$/ml) while the converse was the case when kerosene was used as the HC (Table 4).

For the two *Actinomyces* species, the extents of mineralization were not significantly affected ($p < 0.05$) by the nature of the HCs (35.89 ± 1.59–51.31 ± 2.36 $mgCO_2$/ml for *A. slackii*; 42.12 ± 1.86–51.31 ± 4.43 $mgCO_2$/ml for *A. liubingyangii*); with the exception of kerosene. However, the extents of mineralization by the other actinomycetes were significantly influenced by the nature of the HCs (Table 4). For example, *L. albidocapillata* mineralized the heavier HCs (crude oil and petrol) to significantly greater ($p < 0.05$) extents than the lighter HC (kerosene). Similarly, *R. erythropolis* mineralized the heavier HC crude oil better ($p < 0.05$) than the lighter HCs petrol or kerosene. These results indicate that the extent of mineralization is largely dependent on the nature of the HCs.

Kinetic modeling

Various models were applied to evaluate the best model fitting for HC biodegradation by actinomycetes. The modified Gompertz model[13] well fit the mineralisation data was selected on the basis of lowest root mean square error, uppermost R^2 and highest F statistic values. The original Gompertz model was modified by adding a linear term, which correspond to the more slowly-occurring mineralisation of microbially-incorporated or microbial-bound C (i.e. turn-over of biomass-C) and/or the mineralisation of HC in non-aqueous phase. Modification of model helps in the estimation of HC mineralization in the rapid aqueous phase, A_o, and the derivation of the rate of turnover of biomass-C and/or the rate of mineralization of HC, C, in the slower non-aqueous phase, respectively.

For data treatment and parameter calculation, the SigmaPlot© 14 was used. The main parameters are shown in Table 4.

Kinetic characterization of actinomycetes strains utilizing crude oil as sole carbon source fit the modified Gompertz model with an R^2 of 0.xx – 0.yy, a maximum biodegradation rate (μ_{max}) ranged xx – yy achieved within aa – bb d. When the lighter HC aviation fuel was used, the enhanced degradation also fit very well with the modified Gompertz model with a higher maximum biodegradation rate of cc – dd attained within ff – gg d.

While the maximum rates of mineralization (μ_{max}) by the actinomycetes were different for the various HCs (Table 4), the differences were not significantly different ($p > 0.05$). However, the time to reach maximum rate (t_0) differed significantly ($p < 0.05$) depending on the actinomycetes and HC being degraded (Table 4). This implies that the limitations of bioavailability and mass transfer might have played a major role in the biodegradation of the HCs by the actinomycetes. The 3-parameter sigmodal model fit the mineralization data very well ($R^2 = 0.954–0.995$; average) and described an initial short lag period preceding rapid mineralization which often peaked between 3.26±0.52 – 8.61±1.68 d.

Table 3: Drop collapse test

Oil	Isolate	Drop collapse
Diesel	*L. albidocapillata*	+
	A. slackii	+
	A. liubingyangii	+++
	R. erythropolis	++
	T. bernardiae	++
Crude	*L. albidocapillata*	+
	A. slackii	+
	A. liubingyangii	+++
	R. erythropolis	++
	T. bernardiae	+

Key: +++: <1 min; ++: 1–2 mins; +: 2–3 mins; –: no collapse

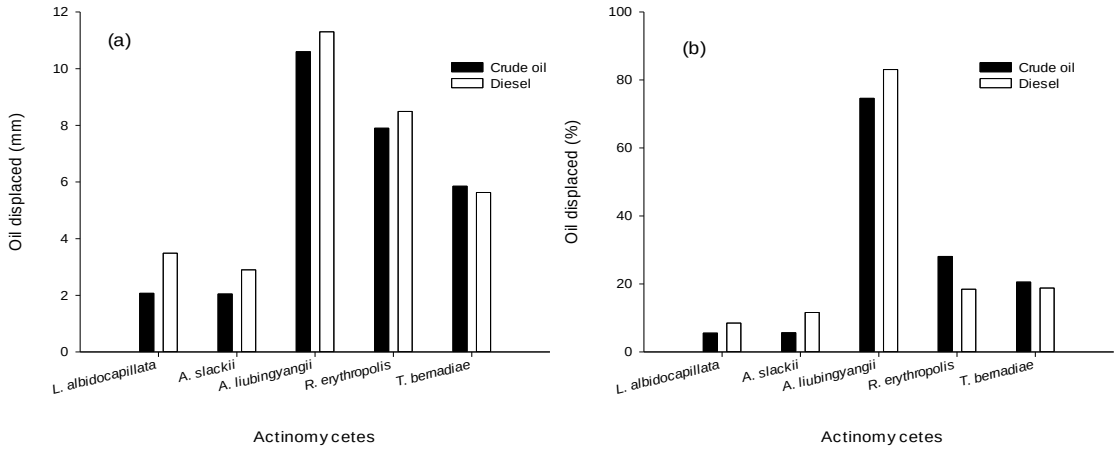

Figure 4: Oil displacement assay on water surface by whole cell suspension of actinomycetes (a) compares oil displaced measured in mm between crude oil and diesel, (b) compares oil displaced reported as percentage between crude oil and diesel.

Mineralization of different petroleum hydrocarbons showed varying results by the different actinomycetes. The high mineralisation of crude

oil by *L. albidocapillata* could be as a result of its ability to produce inherent enzyme called 3'-Demethoxy-3'-hydroxystaurosporine-*O*-methyltransferase which hastened the mineralisation process as suggested in the study by Weidner *et al*. [21] that stated that production of enzymes could be enhanced mineralization process by microbes. Crude oil mineralisation was closely followed by *R. erythropolis*. The mineralisation process might have been aided by the secretion of the enzyme nitrile hydratase by the isolate or because of its ability to produce trehalose lipids as reported by Shekhar *et al.* [22] who reported that production of biosurfactants by an organism is indicative of its mineralization capability.

T. bernadiae could have mineralised diesel the most because the isolates produced higher biosurfactants in diesel compared to other contaminants. This tallies with the study of Prez-Armendriz *et al* [23] who, although worked on bacteria, identified that PAHs degradation rates were high as a result of the high biosurfactant production by their isolates. The reason for *A. slackii* mineralizing aviation fuel the most is not farfetched; aviation fuel contains the least aromatic hydrocarbons when compared to other contaminants. The hydrocarbons in the contaminants could have been more readily available for the isolate to assess them.

Conclusion

This study assessed the capability of five actinomycetes named *Lentzea albidocapillata*, *Actinomyces slackii*, *Actinomyces liubingyangii Rhodococcus erythropolis* and *Trueperella bernardiae* to produce biosurfactants and ultimately biodegrade petroleum hydrocarbon. Although the production of biosurfactants and emulsifiers by the isolates is in varying amounts, it is an indication that actinomycetes are one of the choicest organisms for bioremediation process and they have great potentials for cleanup of contaminated soils. Their ability to survive and thrive in adverse conditions also makes them great prospects for restoration of contaminated sites. Amongst all the isolates in this study, *T. bernardiae* mineralized the hydrocarbons to a greater extent; hence, we recommend that further study be carried out on the isolate to harness its potential in the biodegradation of petroleum hydrocarbons.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

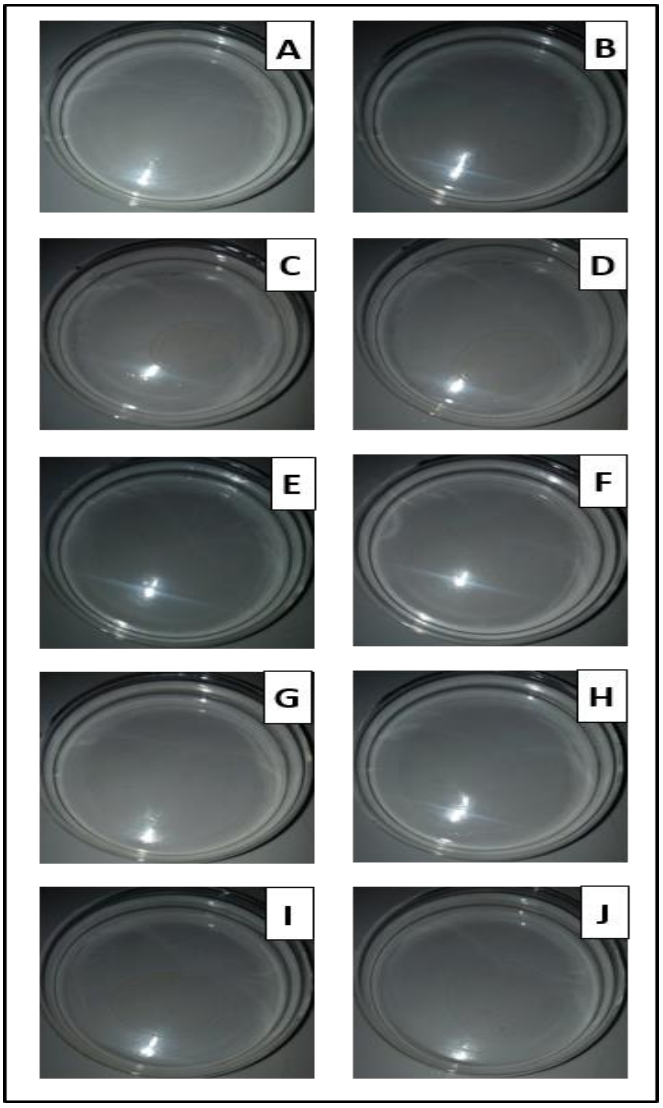

Figure 5: Diesel films displaced on water surface by whole cell suspension of actinomycetes isolates. Diesel oil films on water surface before (A, C, E, G and I) and after (B, D, F, H and J) addition of whole cell suspension of isolates *L. albidocapillata*, *A. slackii*, *A. liubingyangii*, *R. erythropolis* and *T. bernardiae*, respectively.

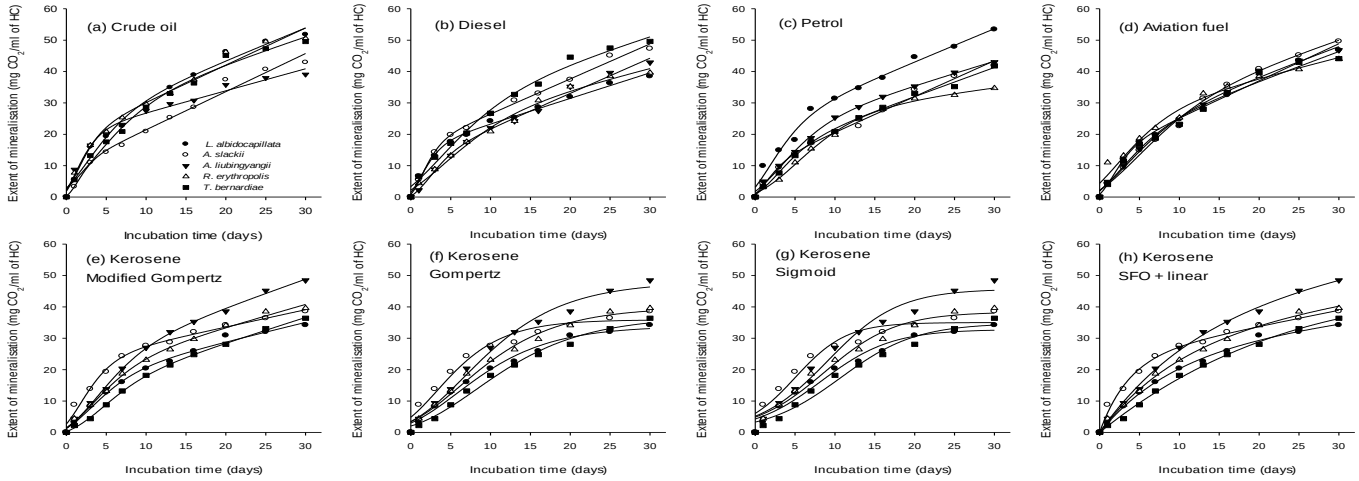

Figure 6: Biodegradation of various petroleum hydrocarbons by (●) *Lentzea albidocapillata*, (○) *Actinomyces slackii*, (▼) *Actinomyces liubingyangii* (Δ) *Rhodococcus erythropolis* and (■) *Trueperella bernardiae*.

Table 4: Model (3-parameter sigmodal) kinetics parameters for the 30-d mineralisation of different hydrocarbons by actinomycetes

Hydrocarbon	Organism	A ($mgCO_2$/ml)	A_0 ($mgCO_2$/ml)	μ_{max} ($mgCO_2$/ml /d)	t_0 (d)	k_o ($mgCO_2$/ml /d)	R^2
Crude oil	*L. albidocapillata*	51.80	22.60 ± 5.02^{bC}	2.43 ± 1.01^{aA}	1.04 ± 0.21^{abA}	2.55 ± 0.87^{aA}	0.987
	A. slackii	42.90	9.29 ± 1.79^{abA}	2.81 ± 1.65^{aA}	1.22 ± 0.09^{bAB}	1.48 ± 0.83^{aA}	0.988
	A. liubingyangii	39.10	19.82 ± 1.84^{aA}	4.45 ± 1.00^{aA}	0.70 ± 0.09^{aA}	1.24 ± 0.46^{aA}	0.986
	R. erythropolis	50.60	18.37 ± 2.56^{abB}	3.95 ± 1.30^{aA}	1.18 ± 0.13^{abA}	1.40 ± 0.69^{aA}	0.986
	T. bernardiae	49.50	25.38 ± 6.64^{abAB}	2.13 ± 0.75^{aA}	0.86 ± 0.26^{abA}	3.47 ± 1.34^{aA}	0.989
Diesel	*L. albidocapillata*	38.50	15.17 ± 1.05^{aAB}	3.71 ± 0.66^{aA}	0.81 ± 0.05^{aA}	1.28 ± 0.33^{aA}	0.995
	A. slackii	47.30	15.34 ± 1.08^{aA}	4.09 ± 0.99^{aA}	1.12 ± 0.06^{aAB}	1.45 ± 0.25^{aA}	0.996
	A. liubingyangii	42.90	10.63 ± 1.54^{aA}	2.02 ± 0.62^{aA}	1.12 ± 0.07^{aB}	2.47 ± 0.69^{aA}	0.995
	R. erythropolis	39.60	21.84 ± 7.39^{aAB}	1.55 ± 0.67^{aA}	0.64 ± 0.27^{aA}	4.26 ± 1.62^{aA}	0.989
	T. bernardiae	49.60	26.96 ± 9.20^{aB}	1.88 ± 0.88^{aA}	0.81 ± 0.33^{aA}	3.88 ± 1.46^{aA}	0.989
Petrol	*L. albidocapillata*	53.40	21.85 ± 3.70^{bC}	2.88 ± 0.84^{aA}	1.06 ± 0.17^{aA}	1.86 ± 0.95^{aA}	0.987
	A. slackii	41.90	9.19 ± 1.35^{abA}	2.07 ± 0.74^{aA}	1.14 ± 0.07^{aB}	1.43 ± 0.71^{aA}	0.994
	A. liubingyangii	43.00	20.04 ± 2.19^{abA}	2.03 ± 0.32^{aA}	0.78 ± 0.09^{aAB}	3.23 ± 0.56^{aA}	0.997
	R. erythropolis	34.70	25.28 ± 3.48^{aA}	1.97 ± 0.33^{aA}	0.32 ± 0.13^{aA}	5.26 ± 0.61^{aA}	0.997
	T. bernardiae	41.80	13.46 ± 1.50^{abAB}	1.82 ± 0.34^{aA}	0.93 ± 0.07^{aA}	2.91 ± 0.55^{aA}	0.997
Aviation fuel	*L. albidocapillata*	46.80	13.38 ± 1.76^{aBC}	2.89 ± 0.91^{aA}	1.18 ± 0.09^{aA}	1.74 ± 0.62^{aA}	0.993
	A. slackii	49.60	24.80 ± 7.52^{aA}	1.65 ± 0.62^{aA}	0.85 ± 0.27^{aAB}	4.96 ± 1.62^{aA}	0.995
	A. liubingyangii	46.80	19.50 ± 4.79^{aA}	1.68 ± 0.64^{aA}	0.95 ± 0.19^{aB}	3.42 ± 0.97^{aA}	0.993
	R. erythropolis	44.00	24.90 ± 6.87^{aAB}	2.22 ± 0.88^{aA}	0.65 ± 0.28^{aA}	2.27 ± 1.63^{aA}	0.976
	T. bernardiae	44.10	17.86 ± 4.69^{aAB}	1.87 ± 0.89^{aA}	0.95 ± 0.20^{aA}	2.79 ± 1.02^{aA}	0.987
Kerosene	*L. albidocapillata*	34.20	16.46 ± 2.44^{aA}	1.80 ± 0.40^{aA}	0.62 ± 0.10^{abA}	3.15 ± 0.75^{aA}	0.994
	A. slackii	38.60	22.54 ± 2.18^{aA}	3.49 ± 0.64^{aA}	0.55 ± 0.10^{aA}	1.78 ± 0.51^{aA}	0.989
	A. liubingyangii	48.50	21.75 ± 2.52^{bA}	2.11 ± 0.33^{aA}	0.90 ± 0.10^{bAB}	3.93 ± 0.56^{aA}	0.998
	R. erythropolis	39.60	18.95 ± 2.67^{abA}	1.91 ± 0.38^{aA}	0.73 ± 0.11^{bA}	3.39 ± 0.71^{aA}	0.996
	T. bernardiae	36.40	12.20 ± 1.20^{aA}	1.31 ± 0.17^{aA}	0.82 ± 0.05^{bA}	4.72 ± 0.46^{aA}	0.999

Key: A = measured extent of mineralisation; A_0 = upper asymptote or modelled extent of mineralisation; μ_{max} = maximum rate of mineralisation; t_0 = time (at inflection) to reach the maximum rate; R^2 = root mean square; different superscripts indicate mean values that are significantly different from each other while same superscripts indicate mean vales that are not significantly different from each other @ $p < 0.05$

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