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Biostability of Synthetic Crankcase Oils

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Authors' contributions

This work was carried out in collaboration between all authors. Author GCO designed the study. Authors NKU and CEI carried out the laboratory work. Authors CEI and AUO analysed the data. Author AUO reviewed relevant literature and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The biostability of used and fresh samples of Lenoil GTX 15W/40 synthetic motor oil and Lenoil GLX 20W/50 synthetic motor oil were examined to investigate potential biodeteriogens and the stability of synthetic crankcase oils to degradation by microbial isolates obtained from them. The degrading bacteria were isolated via enrichment culture technique. Flow rates were established by determining drop rates and the growth profiles were established by monitoring the degree of turbidity, pH, optical densities and total viable counts. It was determined that used oils dropped faster than fresh oils at both low and high temperatures. Isolates from both groups of oils belonged to the genera *Bacillus, Actinomyces, Edwardsiella, Pseudomonas, Micrococcus* and *Citrobacter* among the bacteria and *Aspergillus, Cladosporium, Cephalosporium, Penicillium* and *Mucor* amongst the fungi. The used oil proved to be a better substrate for microorganisms. Fungi had higher counts than their bacterial counterparts. Used samples had higher microbial counts than fresh samples. The mixed culture of *Actinomyces, Edwardsiella,* and *Pseudomonas*, as expected, utilized the oil samples more readily than single cultures. For the single cultures, *Pseudomonas* showed the greatest degrading capacity for the used crankcase oils while *Edwardsiella* excelled with regards to the fresh oil. Observed pH dropped from 7.2–7.0 to 6.88–6.0 on average across all the samples. Synthetic crankcase oils are susceptible to attack both prior to use and when in use; it is, therefore, advisable to change the oil regularly to preserve the motor engine.

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1. INTRODUCTION

Crankcase oil, like other motors oils, is considered the most widely used product of crude oil refinement. It generally contains long-chain saturated hydrocarbons. Its key quality is its viscosity as it defines its functionality [1]. All materials are subjected to some level of degradation by microorganisms, however, in certain instances, this degradation may be unwelcome. Several studies have shown that microorganisms cause the malfunction of crankcase oils [2–4]. Most crankcase oils contain dispersants, antioxidants, and anti-rust agents. They are not pure mineral oils but have some of their components replaced by water soluble components like glycol to control rusting and oxidation, neutralize acids, carry heavy applied loads, act as dispersants and detergents and pacify heavy metals. Natural oils, dyes, odor masks and biocides are also commonly added. Fresh and used oils differ in chemical composition. The fresh oil contains a higher percentage of lighter hydrocarbons. The base oil contains C_{16}–C_{36} hydrocarbons and more than 75% c-alkanes with 1–3 ringed cyclic alkanes usually containing 5–6 member polycyclic aromatics with small amounts of PAHs while used oils may contain minute quantities of additives, heavy metals resulting from engine wear and a higher percentage of alkylbenzenes, naphthalene, methyl naphthalene and higher PAHs probably as a result of pyrosynthesis. Spent oils will normally contain heavy metals such as Lead, Iron, Tin and Silicon as well [5–8]. These components serve as a source of carbon and energy for several groups of microorganisms.

Microbial utilization of oils leads to changes in performance as well as foul odors, blocking of pipes by microbial slime and sludge, the release of toxic by-products, which raise health and safety concerns and sometimes even engine failure with its attendant economic implications. A lot of these changes are difficult to quantify [9–10]. Oils that are in contact with water tend to be more prone to microbial deterioration in the absence of adequate preservatives and biocides. The car engine combustion process and the microbial decomposition of the oil produce free water as well as carbon (IV) oxide and other degradation by-products and the microbes live in the free-water phase, thus can only live in straight cutting oils if they are contaminated with water. Microorganisms are capable of breaking down many complex molecules by adaptation of their degradative enzyme systems [11]. Crankcase oils are readily deteriorated by algae, fungi, and bacteria [12–15]. The accumulation of water within these products, at the bottom of storage tanks and similar containers and even within the engine while in use is credited with the attack of these products by microorganisms [10], [16]. During the manufacture of oil products, crude oil is exposed to thermal processing. The products generated are sterile but get contaminated during warehousing and storage [17]. A study showed that after oil refining, 3.2 x 10^4 cells/ml were found in oil products following pumping into a factory tank; this value increased to 7.0 x 10^4 cells/ml in a petroleum storage depot and tests carried out at the distribution oil depot revealed counts of approximately 2.8 x 10^5 cells/ml [10]. Bio-corrosion of pipelines and tanks play a role in introducing microbial contaminants into oils [18]. This microbial utilization of hydrocarbon products would normally result in the production of by-products that impact negatively on desirable chemical and physical qualities [9].

The deterioration of engine oils and similar petroleum derivatives by microorganisms is a serious economic and environmental problem all over the world [18–20]. While it is difficult to prevent the activities of microorganisms, in oil fields, storage tanks and blending plants, it is essential to identify likely culprits with a view to developing effective preventive processes, biocides and deterioration inhibitors. This study, therefore, looked to investigate the stability of synthetic crankcase oils to degradation by microbial isolates obtained from them.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation of Microorganisms

The oil samples employed in this study are Lenoil GTX synthetic motor oil 15W/ 40 and Lenoil GLX synthetic motor oil 20W/ 50. The used oils were collected as “clean catch” from the crankcases of cars which had driven with them for about 3 months while the fresh samples of the same oils were bought directly from the store. Isolation and characterization were done according to the method of Gerhardt et al. [21] while identification was as outlined by Holt [22].

2.2 Determination of Flow Rate

The flow rate of the various oil types was determined at both cold and hot temperatures. Ten milliliters (10 ml) of the cool oil (30°C) in a pipette was released in drops and the number of drops per time was counted. A stopwatch was used for timing. The same procedure was repeated with the oil samples after heating to a constant temperature of 60°C in a water bath. Counting was done at one-minute intervals.

2.3 Determination of Bacterial Growth Rate and Utilisation of the Oils

Nine milliliters (9 ml) of sterile Mineral Salt broth was dispensed into 28 test tubes which were then labeled 1 – 7 in groups of four for the seven bacterial isolates obtained per oil sample [23]. To the 9 ml of medium in each test tube, 1 ml of the relevant test oil was added before autoclaving at 121°C and 15 psi for 15 minutes. On cooling, each test tube was inoculated with the corresponding bacterial isolate (the bacteria initially isolated from the test oil in the test tube); the fifth set of test tubes containing only media and the test oil without any inoculum served as control. The tubes were incubated at room temperature for 14 days at the end of which each tube was examined for turbidity.

The isolates that showed some level of turbidity on the selected test oil were used for further studies. These isolates were inoculated into mineral salt broth containing the test crankcase oil as the sole carbon in order to test the organism's ability to utilize the test oil. A known quantity (1 ml) of the test oil was added to 99 ml of mineral salt broth, sterilized at 12°C and 15 psi for 15 minutes and then inoculated with the test organism(s) from the turbid test tubes. Incubation was at room temperature for 17 days with regular shaking. The optical density at 600 nm (OD), pH and total viable counts (TVC) were monitored throughout the period of incubation.

3. RESULTS AND DISCUSSION

The microbial strains isolated from the used and fresh test oils are shown in Table 1. Among the bacterial isolates, the implicated genera were *Bacillus, Edwardsiella, Pseudomonas, Micrococcus, Actinomyces* and *Citrobacter* with *Aspergillus, Cladosporium, Cephalosporium, Penicillium,* and *Mucor* amongst the fungi. These results are corroborated by earlier reports [3], [14,24,25]. Gram-negative bacteria such as *Alcaligens*, *Pseudomonas* and *Achromobacter* are the initial contaminants of cutting fluids while their Gram-positive spore-forming counterparts and some fungal species have been shown to dominate in lubricating oils [26]. Commonly reported bacterial degraders of lubricating oils are *Achromobacter, Bacillus, Enterobacter, Escherichia, Pseudomonas, Rhodococcus, Serratia* and *Staphylococcus* [27–35]. *Aspergillus*, *Rhizopus* and *Penicillium* spp. dominate amongst the fungi [13,36]. Basuki et al. [33] reported the removal of 35 of the 47 components of used engine oil by *Acinetobacter junii* while Harikrishna et al. [37] found that *Bacillus subtilis* degraded fresh engine oil by 71% in 10 days.

Table 1. Microbial isolates from the oil samples

Isolate designation	Identity
A. Bacteria	
C/N 1	*Edwardsiella* sp.
C/N 2	*Bacillu*s sp.
C/N 3	*Pseudomonas* sp.
C/N 4	*Bacillus* sp.
C/N 5	*Micrococcus* sp.
C/N 6	*Actinomyces* sp.
C/N 7	*Citrobacter* sp.
B. Fungi	
K1	*Cephalosporium* sp.
K2	*Aspergillus* sp.
K3	*Mucor* sp.
K4	*Cladosporium* sp.
K5	*Penicillium* sp.
K6	*Aspergillus* sp.

Microbial loads were noticeably higher in the used oil samples when compared to fresh samples as shown in Fig. 1 and fungi had higher counts than their bacterial counterparts. The organisms seemed to utilize the GTX 15W/40 better than GLX 20W/50. Commonly such differences will be chiefly due to the presence of water within the engine from the engine combustion process and then changes in the chemical components of used oils following subjection to wear and tear from the engine; the hydrocarbon content will normally be degraded to the more easily utilisable groups with fewer aromatic rings and less complex chain structure. The additives such as biocides used in manufacture may also be broken down leaving the used oil more susceptible to microbial contamination. The additives make the oil a

somewhat more complex substrate and could alter its biostability especially as they sometimes impact on the efficiency of biocides included in the oil formulation [3,38]. Studies have shown that microorganisms were able to utilize used oils better because while in service, the soluble organic components of the oil come in contact with water providing nutrients for microbial colonization [25].

The degrees of turbidity recorded for the different bacterial isolates following a 14 day incubation period are outlined in Table 2. *Pseudomonas, Edwardsiella, Bacillus* and *Actinomyces* isolates grew on both used and fresh GLX and GTX synthetic oil types while the *Citrobacter* isolate grew on only used oil samples of both test oils. Only the *Bacillus* isolates showed growth on both used and fresh samples of GTX 15W/40 so this oil was not used for further tests. The *Micrococcus* isolate showed no growth on either test oil type. It should be noted that the isolates that did not grow were still viable as they grew when streaked onto sterile nutrient agar.

Used oil samples of GLX 20W/50 showed greater flow rates than fresh samples at both 30°C and 60°C and hot samples flowed better than cool samples (Fig. 2). Temperature exerts a strong influence on both biodegradation and the consistency of the test oil [39]. Oils will normally be less viscous at higher temperatures such as found in the car engine making it more susceptible to microbial attack. Rowland et al. [40] therefore opine that biodegradation is more successful at warmer temperatures associated with the tropics. The observed drop in viscosity may play a role in the ready invasion of used oils by microbial agents.

The results for the utilization of the oils are presented in Figs. 3–6. The differences in turbidity (Table 2) observed are indicative of the varying abilities of isolates C/N 1 – 7 to utilize the oils as an energy source. The poor and differential growth pattern observed could be attributed to the differences in the oil formulations especially as regards the presence of biocides and preservatives which are inhibitory to

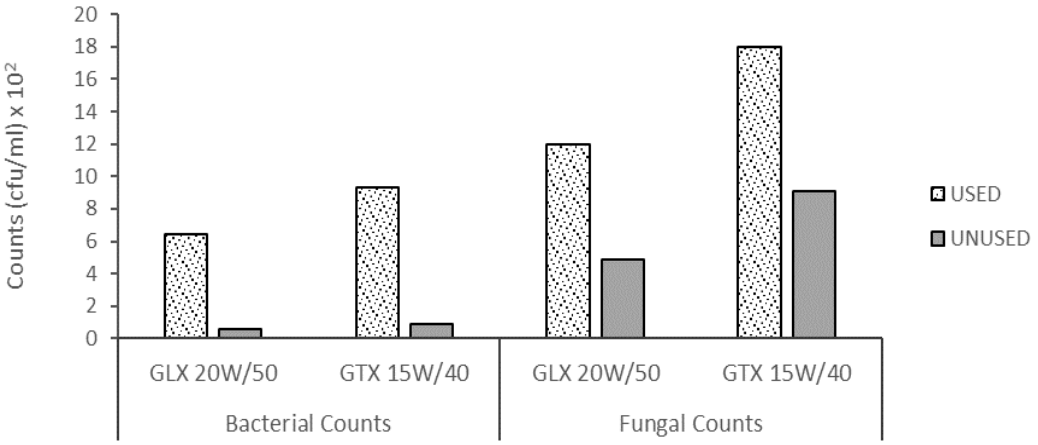

Fig. 1. Mean microbial counts in used and fresh oil samples

Table 2. Bacterial utilisation of the oil samples

Isolate	Isolate identity	Degree of turbidity			
		GLX 20W/50		GTX 15W/40	
		Used oil	Fresh oil	Used oil	Fresh oil
C/N 1	*Edwardsiella* sp.	+	+	-	-
C/N 2	*Bacillus* sp.	-	-	+	+
C/N 3	*Pseudomonas* sp.	+	+	-	+
C/N 4	*Bacillus* sp.	-	-	++	+
C/N 5	*Micrococcus* sp.	-	-	-	-
C/N 6	*Actinomyces* sp.	++	+	-	-
C/N 7	*Citrobacter* sp.	-	-	+	-

Legend: **-** *No Growth, + Little Growth, ++ Moderate Growth*

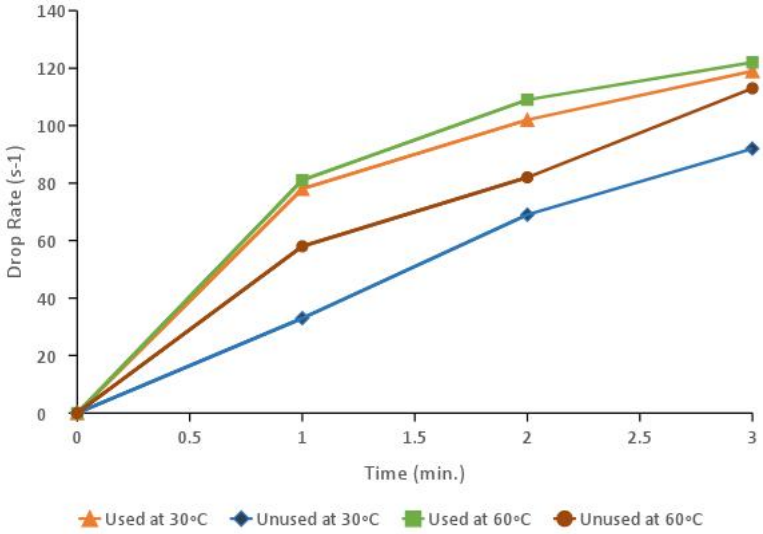

Fig. 2. Variation in flow rates of used and fresh GLX 20W/50 oil samples with time at different temperatures

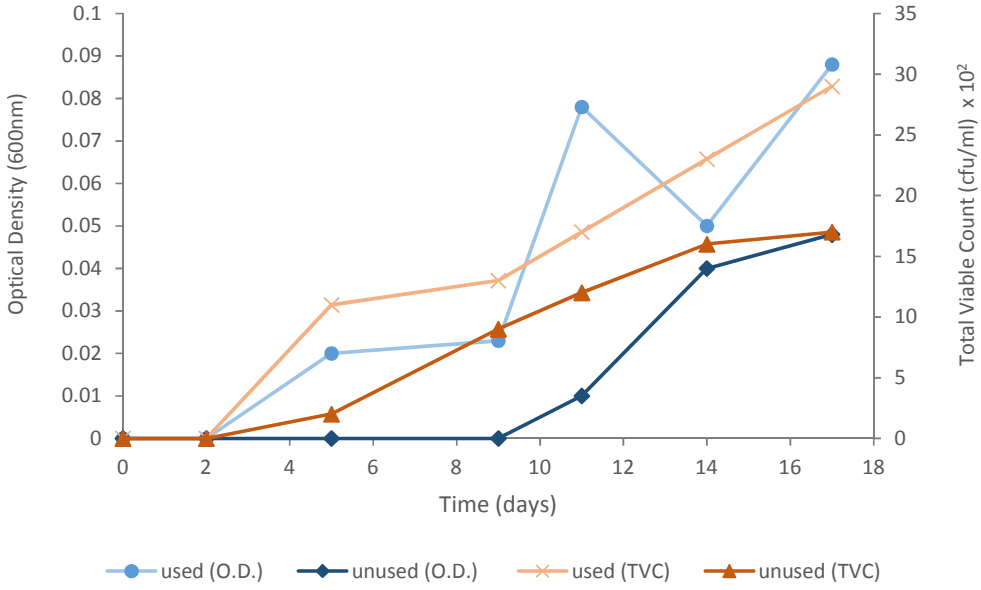

Fig. 3. Growth profile of *Edwardsiella* sp. on used and fresh GLX oil

microbial growth [41]. The physical and chemical changes that occur during degradation of the oils will exert pressures that are selective for different microbial groups. Most of the organisms did not show growth until the fifth day of incubation indicating a period of adaptation (lag phase). It is inferred that during this period, the organisms synthesize requisite enzymes for the breakdown. The mixed cultures, as expected, utilized the samples more readily than single cultures very likely because of the synergistic action of the microbial combination.

With the mixed culture, the lag growth phase was not as distinct as seen in single cultures (Figs. 7 – 8). The lag phase was absent in the growth of the mixed culture on the used oil samples but with the fresh oils, there was a distinct lag phase.

The organisms seemed to enter the stationary growth phase between Day 4 and Day 8.

The enhanced degradation observed in the presence of a consortium corroborated other studies [27,42–44]. Commonly, the individual strains are only able to utilize a few components of the oil; complete degradation often requires the involvement of multiple organisms [29]. For the single cultures, *Pseudomonas* showed the greatest degrading capacity for the used crankcase oils while *Edwardsiella* excelled with

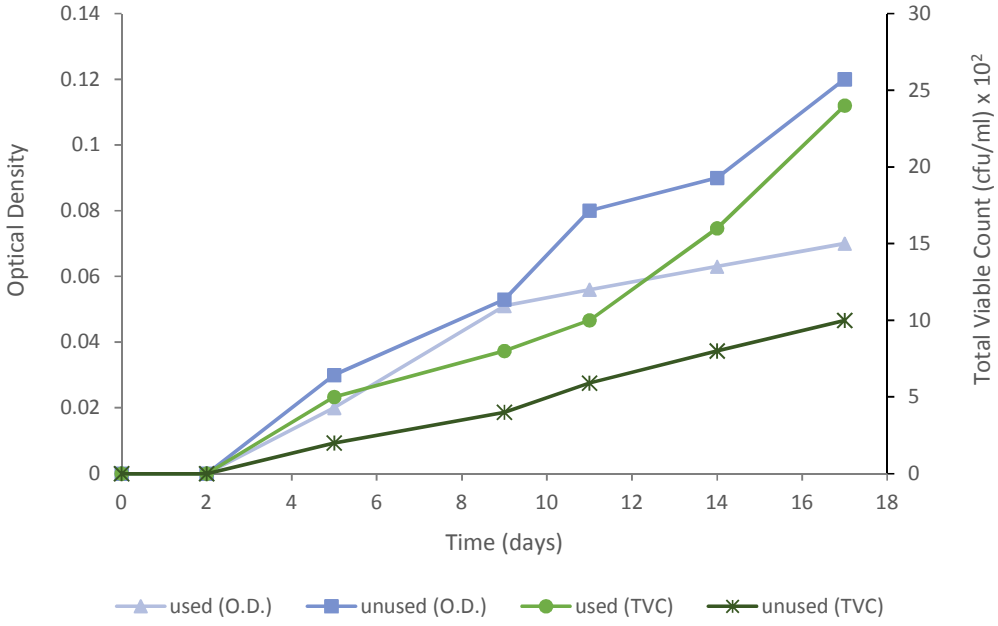

Fig. 4. Growth profile of *Pseudomonas* sp. on used and fresh GLX oil

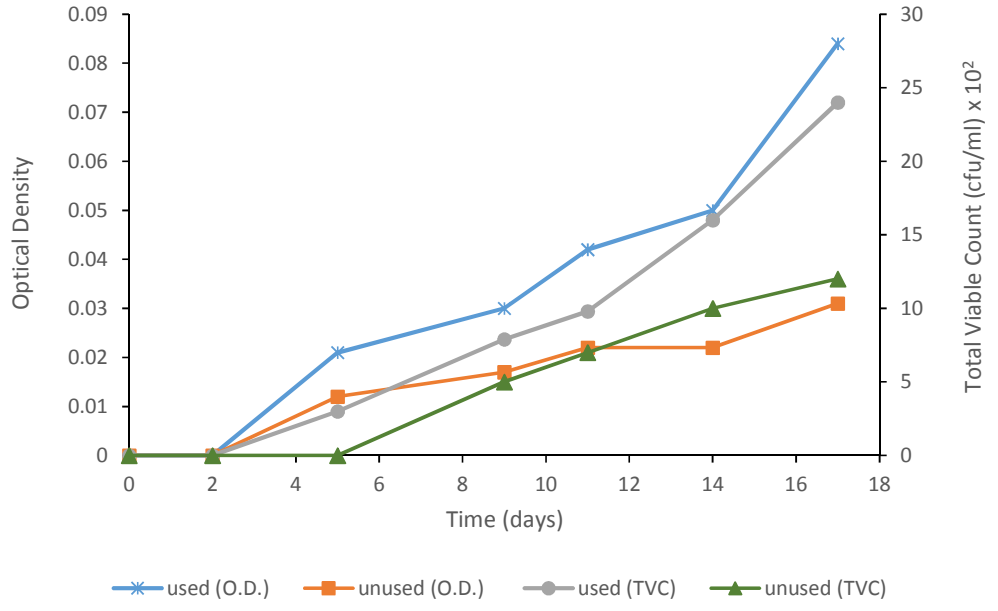

Fig. 5. Growth profile of *Actinomyces* sp. on used and fresh GLX oil

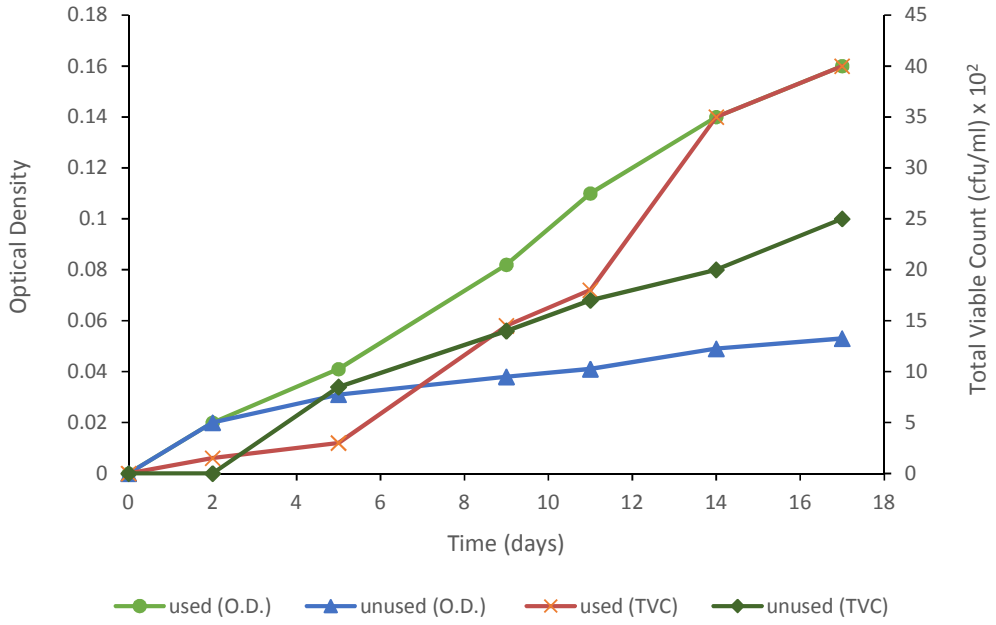

Fig. 6. Growth profile of a mixed culture of *Edwardsiella*, *Pseudomonas* and *Actinomyces* on used and fresh GLX oil

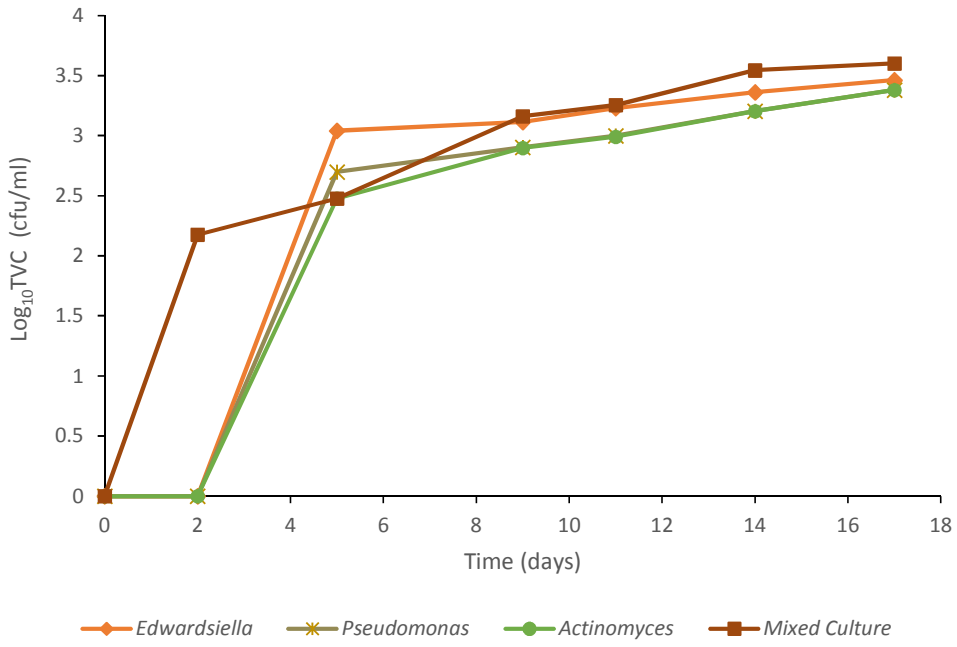

Fig. 7. Growth curve of isolates on used GLX 20W/50 oil

regards to the fresh oil. The growth rates were generally observed to be slower in the fresh oils while utilization rates were higher in used oil samples than in the fresh ones. There was a steady increase in total viable counts and optical densities as time progressed; this was accompanied by a decrease in pH. Observed pH dropped from 7.2–7.0 to 6.88–6.0 on average

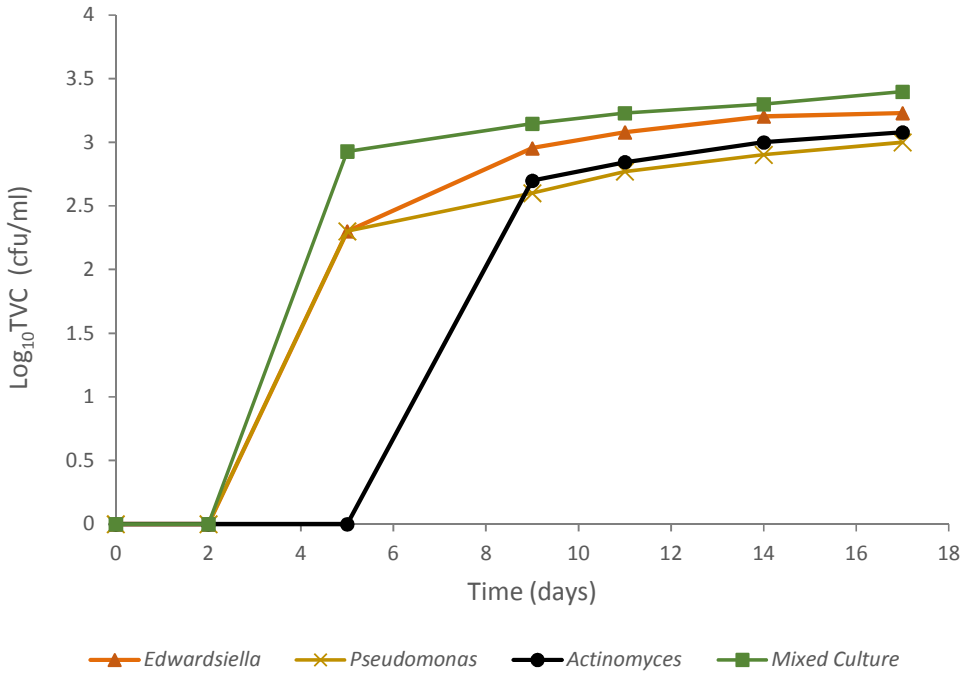

Fig. 8. Growth curve of isolates on fresh GLX 20W/50 oil

across all the samples. The drop in pH has been identified as being due to the production of acidic metabolites [25]. This fall in pH is pertinent to the secondary invasion of the crankcase oils by fungi. Normally, the presence of an alternative carbon source would further enhance the biodeterioration process. The physical wear and tear of the engine on the oil have been known to produce products that constitute alternative carbon sources enhancing biodeterioration activities on used oils. The presence of an alternative carbon source while undesired when the oil is in use would be useful in bioremediation of soils impacted with these oils.

4. CONCLUSION

The exposure to thermal and physicochemical stresses together with the presence of water in the motor engine make used synthetic crankcase oils more susceptible to microbial deterioration than fresh oils. With synthetic crankcase oils that contain biocides and preservatives, bio-deterioration may not occur as readily. The alteration of its components would be required before any significant microbial problem can be noticed. The presence of microorganisms that have the requisite enzyme systems to catalyze the initial breakdown of the original components of the oil is essential here. It is advisable to change the oil regularly to safeguard the engine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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