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Biodeterioration Abilities of Microorganisms in Brake Fluids

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Authors' contributions

This work was carried out in collaboration between both authors. Author CMM performed the statistical analysis, wrote the first draft of the manuscript and managed literature searches. Author GCO designed and supervised the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To identify the parameters that get affected when brake fluids biodeteriorate and also to make known that brake fluids biodeteriorates into novel compounds. To also identify the organisms that deteriorates brake fluids most.

Place and Duration of Study: Department of Microbiology, University of Port Harcourt, between November 2013 and August 2014.

Methodology: Two brands of brake fluids were used (Ate and Allied). Used and Unused samples of these brake fluids were included. Microbiological analysis and physico-chemical analysis were carried out. Deterioration testing was also carried out after which gas chromatography was done to know the level of deterioration.

Results: Ate brake fluids deteriorated more than the Allied brake fluid. Biodeterioration of brake fluid is more pronounced when a mixed culture of bacteria and fungi is involved. The more the biodeterioration of the brake fluid, the lesser the pH and the higher the total viable count or the optical density. Gas chromatograms revealed that the carbon numbers shown on the peaks indicated the novel compounds the brake fluids deteriorated to. Statistical analysis using ANOVA

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(excel) showed that there were significant differences in the total viable counts, pH and optical densities between the mixed microbial cultures of bacteria and fungi in used Ate and used Allied brake fluids and between the mixed cultures in the used brake fluids and the unused brake fluids at $P < 0.05$. Significant difference was also noticed in the ANOVA readings of the viscosities of the used and unused brake fluids.

Conclusion: Brake fluid which is one of the fluids used in vehicles should be changed from time to time following the directives of the manufacturer and the idea of mixing different types of brake fluids for use in a particular vehicle should not be practiced by the owners of these automobiles.

Keywords: Brake fluids; biodeterioration; novel compounds; used and unused brake fluids.

1. INTRODUCTION

Brake fluids are hydraulic fluids. They transmit power, lubricate all moving parts, act as a heat transfer medium, as a sealing medium, too and maintain the system in good working order. Desirable properties of brake fluids are proper viscosity, good lubricity, high viscosity index, compatibility with system, stable or non-degrading, non-toxic or poses few or no known health concerns, low compressibility, low foaming, filterable, low specific gravity, high heat transfer capabilities, low vapor pressure, non-flammable (high flash point), low pour point, good demulsibility, high boiling point, non-corrosive and rust inhibitory, good pH value and good sealing compatibility. Biodeterioration as defined by H.J. Hueck is “any undesirable change in the properties of a material caused by the vital activities of organisms” [1]. Biodeterioration may be defined as “The deterioration of materials of economic importance by microorganisms [2].

Used oil is any oil that has been refined from crude oil or any synthetic oil that has been used and as a result of such use, is contaminated by physical and chemical impurities [3]. Used oil can be as a result of the use of the following refined products such as used lubricants, synthetic oil, transmission and brake fluid, refrigerator oil, compressor oil, hydraulic fluids, heat transfer fluids and used motor engine oil. Used oil is typically contaminated or mixed with dirt, fine particles, water or chemicals, all of which affect the performance of the oil and eventually render it unstable [4].

Negligence by drivers on the importance of changing the brake fluid of their vehicles after the specified period of time given by their manufacturers has become a great problem. As this have resulted to a very low performance of vehicles and at last, the death of individuals (driver, passengers and also pedestrians). For this reason, drivers must avoid mixing different brands and types of brake fluids for use in a vehicle and should also know which type of brake fluid best suits their vehicle and so adhere to its use for their automobiles. Also, the Federal Motor Safety Standard 116 of changing brake fluid after 1-2 years of usage must be the guide of every vehicle owner.

The usual hydraulic brake fluid comprises a lubricating component and a viscosity-reducing solvent. Brake fluid is known as a hygroscopic fluid. A wet brake fluid has a boiling point lower than pure brake fluids. The boiling point is the most important parameter of brake fluid [5] which is for performance driving, the higher the boiling point the better but it is also worth noting that if any brake caliper itself gets above 250 degrees in temperature, there will be a problem. This means that brake fluid is easily made wet by contacting moist air during driving and mainly as a result of a lack of tightness in the system and the brake tubes [6]. The moisture lowers the boiling point of the brake fluid and causes potentially an “airlock” as well as brake malfunction. A number of limiting factors have been recognized to affect the biodegradation of petroleum hydrocarbons, many of which have been discussed by Brusseau. 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. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants especially nitrogen, phosphorus, and in some cases iron [7]. Some of these nutrients could become limiting factor thus affecting the biodegradation processes. The most rapid and complete degradation of the majority of organic pollutants occurs under aerobic conditions [8].

Many studies have indicated the importance of microbial contamination of stored hydrocarbon fuels, which can lead to blocking of pipelines and filters, reduced fuel quality and corrosion of storage tanks [9]. According to Geneviève et al. [10], total bacterial and cultivable Gram-negative bacteria were analyzed for each metalworking fluid. Genus *Citrobacter*, *Staphylococcus*, *Alcaligenes*, *Acinetobacter*, *Bacillus*, *Shewenella* and *Brevundimonas* were identified in more than one machine. Brake fluid was brought into focus because of some experiences automobiles face such as brake pad failure, clog filters, etc.

2. MATERIALS AND METHODS

2.1 Source of Samples

Used brake fluids were withdrawn using a syringe into a sterile bottle from the brake fluid cylinders of cars which had been driven with it for about eight months. Unused oil was bought directly from the store. The trade names of the fluids are Ate and Allied brake fluids. The isolation, characterization and identification of cultures were adopted from Okpokwasili and Okorie [11]. Mixed culture of bacteria used were single isolates of *Pseudomonas*, *Micrococcus*, *Serratia* while for mixed culture of fungi, single isolates of *Aspergillus*, *Penicillium* and *Mucor* were used.

2.2 Viscosity-temperature Characteristics

The flow rate of cold brake fluid at 22ºC and hot brake fluid after it has been heated up to 40ºC in a beaker of water over a gas flame for the used and unused oil samples was determined by using a Canon-Fenske 150/601B viscometer[12].

2.3 Growth of the Mixed Culture of the Bacterial and / or Fungal Isolates in the Brake Fluids

This was used to examine the differences in characteristics of used and unused brake fluid in relation to their decomposition by a consortium of microorganisms. A mineral salt broth composed of 10 g of NaCl, 0.42 g of $MgSO_4.7H_2O$, 0.29 g of KCl, 0.83 g of KH_2PO_4, 1.25 g of $NaHPO_4$, 0.42 g of $NaNO_3$ [13] in [14], 1 litre of deionized water with the pH of 7.2 were dispensed in 99ml amounts into four 250 ml Erlenmeyer flasks. One millilitre (1 ml) of used fluid was added into two of the flasks respectively and to the remaining two flasks was added 1 ml of unused fluid [15]. The flasks were autoclaved at 121ºC for 15minutes. Upon cooling, two of the flasks containing used and unused fluid were inoculated with the mixed culture while the other remaining two uninoculated flasks containing used and unused fluid served as control. The flasks were incubated at room temperature (30ºC) on a rotary shaker operated at 140 rpm for 21 days. The optical density (OD) at 560 nm, total viable count (TVC) and pH of the culture in each flask were monitored at intervals; at day0, day3,day6, day9, day12, day15, day18 and day 21. Total viable counts were obtained after serial dilution in physiological saline (deionized water containing 0.85% w/v NaCl); spread plating on nutrient agar and incubation of plates for 48 hours at room temperature. Gas chromatographic analysis was carried out to determine the extent of biodegradation of the used and unused brake fluid and the formation of other novel compounds as a result of microbial growth on the brake fluid.

2.4 Total Petroleum Hydrocarbons Analysis

The samples were extracted for total petroleum hydrocarbon with Analar grade dichloromethane (DCM), hexane and acetone (3:1:1, v/v/v) with 2 g of anhydrous sodium sulphate in a separatory funnel, equipped with teflon cover and shaken for 10 minutes and vented. This process was repeated three times. The two phases were separated by decantation. Extracted organic phase was cleaned using activated silica and concentrated to 2 ml using vacuum rotary evaporator. 1.0 µl of the final extract was injected and eluted in already calibrated HP 6890 gas chromatograph. The calibration was carried out by using commercially available total petroleum hydrocarbon primary standards (Accu standards, USA). The peak area was used in the quantifications. All quality control / quality assurance procedures were strictly followed [16].

2.5 Statistical Analysis

Statistical analysis was carried out using one-way Analysis of Variance (ANOVA). P-values which showed test of significance was carried out at 95% level of confidence. P-values were also used to determine the significance levels between the mixed microbial culture and the control during the experimental study.

3. RESULTS AND DISCUSSION

This study was carried out using Allied and Ate DOT3 brake fluids which met Federal Motor Safety Standard and Society of Automotive Engineers J1703 standard. There are various international, national and company standards which specify their physical-chemical properties, the following ones are most substantial SAE

J1703/1704, ISO 4925 and FMVSS (CRF 571.116) [5]. These brake fluids contain various additives to improve the viscosity. Additives are chemicals added to the base stocks to improve performance and to obtain optimum lubricating performance in today's equipment. The most striking feature of a survey of the microorganisms involved in biodegradation processes is their large numbers, ubiquitous presence and varied capabilities. The organisms involved (including the bacteria, fungi, actinomycetes, protozoa, etc.) [17].

Table 1 shows the results of viscosity test of used and unused brake fluids at different temperatures. From the table, it could be seen that as the temperature increased the viscosity reduced and *vice versa*. Temperature is perhaps one of the most important factors, affecting both biodegradation and the consistency of the oil spilled. Temperature is a crucial factor in the beginning stages of biodegradation [18]. Also, as temperature decreases, viscosity of oil increases, becoming thicker. Therefore, biodegradation is significantly more successful at warmer temperatures as found in the tropics [19]. Atlas [20] found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons was reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons [21]. Used brake fluid flowed faster whether hot or cold and it also gets heated up faster than the unused brake fluid. The fact that under both temperatures regimes, used brake fluid flowed faster than unused brake fluid is probably due to used brake fluid been subjected to several thermal stress during use in the vehicle results in the loss of viscosity and freer flow. Used oil heats up faster than unused oil perhaps due to the decreased viscosity of used oil. The metal particles produced by wear and collected by oil in service may contribute to greater heat conduction in used oil than in unused oil with fewer particles [15]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperature. According to Krisnangkura et al. [22] viscosity may be considered the integral of the interaction forces of molecules. When heat is applied to fluids, molecules can then slide over each other more easily making the liquid to become less viscous. Viscosity is a basic property of brake fluid. For in-service brake fluid, the viscosity should remain consistent over years of service, unless the fluid has become contaminated or severely oxidized. A decrease in viscosity is an indication of contamination or excessive deterioration.

Table 1. Viscosities of used and unused brake fluids

Brake fluid	Temperature	Viscosity (cSt)	
		Unused	**Used**
Allied	22ºC	11.99	9.04
	40ºC	11.87	4.34
Ate	22ºC	12.97	9.24
	40ºC	12.90	6.31

Fungi was not isolated from unused brake fluid (Figs. 3 and 4), it could be said that fungi are secondary invaders of brake fluid (Figs. 7 and 11). Also, that effective deterioration is carried out by a mixture of bacteria and fungi in used brake fluid (Figs. 1, 2, 6 and 12). It also confirms an earlier work [11] which employed single cultures of bacteria rather than a mixture (Figs. 8, 10, 14 and 16). *Pseudomonads* are the best known bacteria capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants [23-25]. Biosurfactants increase the oil surface area and that amount of oil is actually available for bacteria to utilize it [26]. Okerentugba and Ezeronye [27] demonstrated the ability of *Penicillium* spp, *Aspergillus* spp and *Rhizopus* spp to degrade petroleum hydrocarbons especially when used as single cultures. The fungi *Aspergillus fumigatus* and *Candida silvicola* were isolated from the interfacial biomass of diesel storage systems in Brazil [9]. The pH of the used Ate brake fluid dropped from 6.4 - 4.3 (Fig. 1) when the mixed culture (bacteria and fungi) was utilizing it, while the drop for unused Ate brake fluid was from 7.6–6.5 (Fig. 3) when mixed culture of bacteria utilized it. For the used Allied brake fluid, the drop in pH was 6.7-5.1 (Fig. 2) and for the unused brake fluid, it was 8.4-7.4 (Fig. 4). The optical density due to the increase in cell numbers rose. For the used Ate brake fluid, it rose from 0.30 - 0.76 (Fig. 1) while for its unused it rose from 0.19-0.36 (Fig. 3). The optical density for used Allied brake fluid rose from 0.21-0.57 (Fig. 2) while for the unused Allied brake fluid; it rose from 0.07-0.27 (Fig. 4). All these occurred over the same period of 21 days. These results were also compared to the control values (Figs. 5,9,13 and 15). *A. fumigatus* is a known fuel-deteriogenic mould that reduces the pH of the aqueous phase [9,28,29]. In a previous investigation, Bento et al. [29] have reported that when *A. fumigatus* was grown in Bushnell Hass mineral medium (2 g l^{-1} phosphate) containing diesel oil as carbon source, the pH of the aqueous phase was reduced from 7.0 to 4.8 after 60 days. Propionic acid was identified, among other metabolites (including alcohols and ketones), in the aqueous phase. In addition to the metabolites produced during growth on hydrocarbons, the reduction of pH of the aqueous phase may also be caused by cell lysis or by organic acids generated during abiotic degradation of diesel or biodiesel. The total viable count was also higher for the used brake fluid (Figs. 1 and 2) than the unused brake fluid (Figs. 3 and 4). It can also be said that microorganisms used up the Ate brake fluid (Figs. 5, 6, 7, 8, 13 and 14) more than the Allied brake fluid (Figs. 9, 10, 11, 12, 15 and 16); this could be as a result of constituents of its make-up. It is possible that physico-chemical stress on the brake fluid changed the brake fluid's properties during use and this altered the components of the brake fluid making it prone to deterioration. This resulted in a build-up of organisms making the fluids cloudy, that is, a high optical density. This is consistent with the reports of [11,30]. There is always an increase in biomass when biodegradation takes place [31].

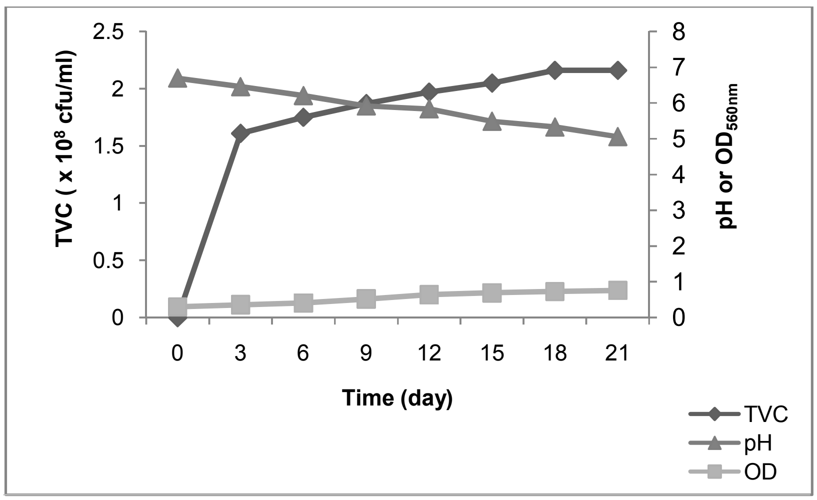

Fig. 1. Growth profile of total viable count (TVC), pH and optical density (OD) of mixed microbial culture of bacterial (*Pseudomonas* sp., *Micrococcus* sp. and *Serratia* sp.) and fungal (*Mucor* sp., *Aspergillus* sp. and *Penicillium* sp.) colonies from used Ate brake fluid

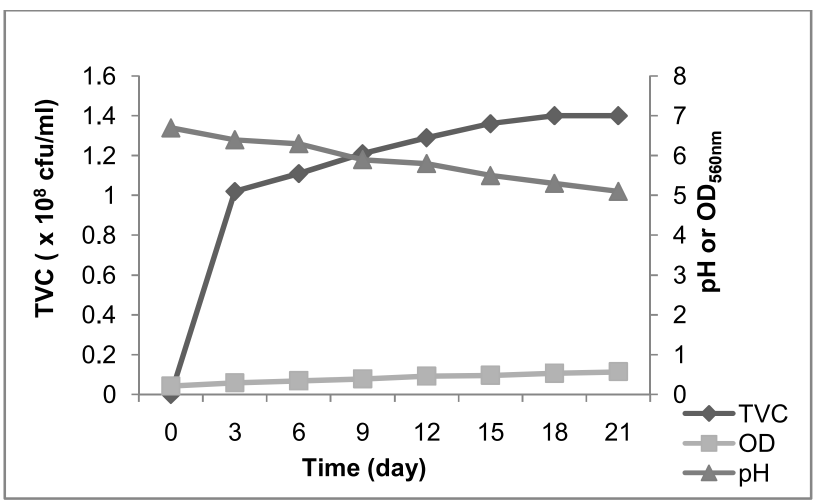

Fig. 2. Growth profile of total viable count (TVC), pH and optical density (OD) of mixed microbial culture of bacterial (*Pseudomonas* sp., *Micrococcus* sp. and *Serratia* sp.) and fungal (*Mucor* sp., *Aspergillus* sp. and *Penicillium* sp.) colonies from used allied brake fluid

Nour et al. [32] illustrated a mathematical correlation between viable cell count and biomass dry weight of four different bacterial strains. Their work aimed to predict mathematical correlations expressing relationship between optical density, total viable count and dry cell weight of some bacterial strains used in different biotreatment applications in petroleum industry.

The biodeterioration of fluids as analyzed using gas chromatography is represented in Figs. 5-16. Unused brake fluids showed higher number of peaks (Figs. 13-16) than the used brake fluid (Figs. 5-12) in both inoculated (degraded) and uninoculated (undegraded) control cultures. This can explain the fact that, the higher number of peaks (represented by a carbon number) was as a result of undegraded compounds in the brake fluid. It could be said that physico-chemical stress on the brake fluid cylinder has caused cracking of brake fluid into fractions which are used up by the organisms. The appearance of new chromatographic peaks which is concomitant with synthesis of novel organic compounds during degradation of petroleum has been reported by Amund [33]. Biodeterioration of Ate brake fluid was higher than that of Allied

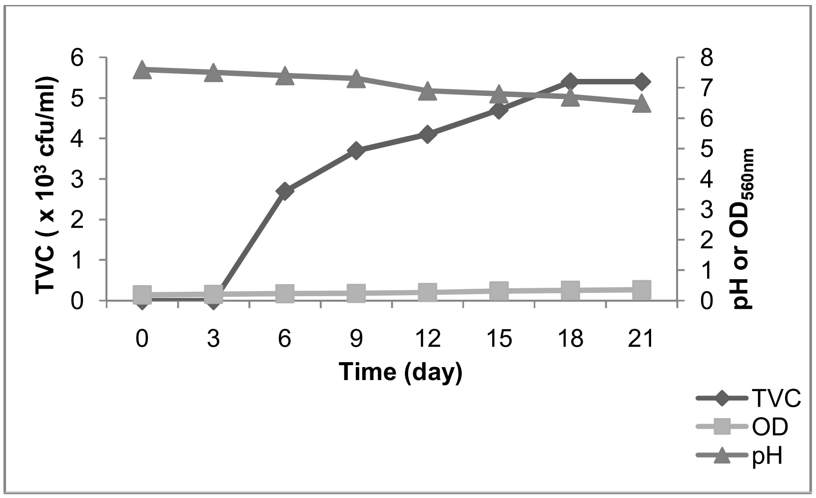

Fig. 3. Growth profile of total viable count (TVC), pH and optical density (OD) of mixed bacterial (*Pseudomonas* sp., *Micrococcus* sp. and *Serratia* sp.) colonies from unused Ate brake fluid

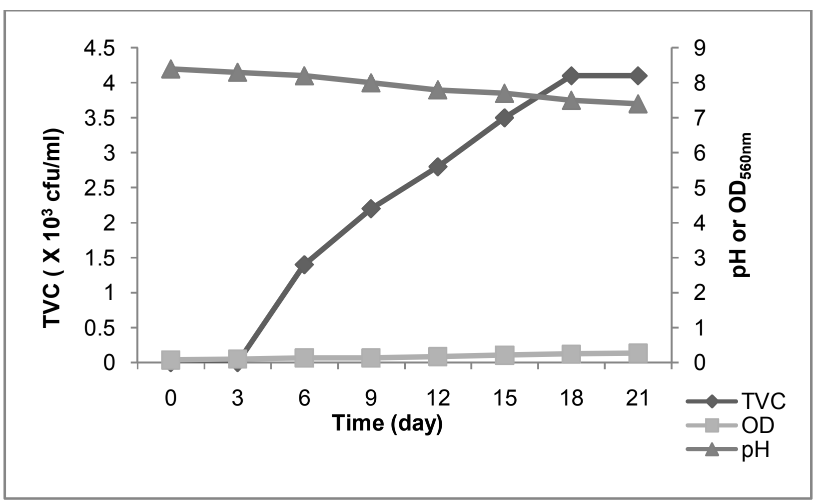

Fig. 4. Growth profile of total viable count (TVC), pH and optical density (OD) of mixed bacterial (*Pseudomonas* sp., *Micrococcus* sp. and *Serratia* sp.) colonies from unused allied brake fluid

brake fluid. Mineral oil is used in the preparation of Ate brake fluid while Allied brake fluid is made from synthetics. This was seen in the total viable counts and also in the gas chromatograms. Fermentation studies are currently being conducted to determine to what use this novel microbial synthesis can be put into bioconversion of used lubricating oils in a bid to abate pollution

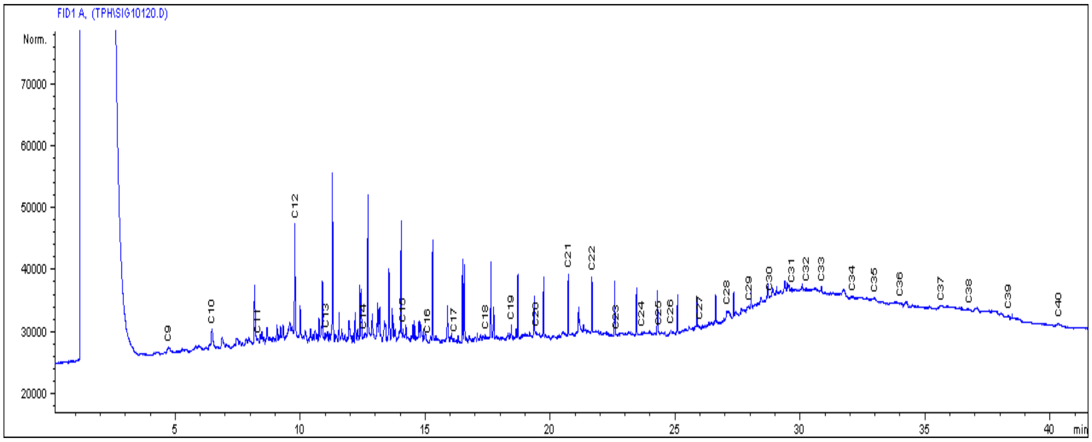

Fig. 5. Gas chromatogram for control sample of used Ate brake fluid after day 21

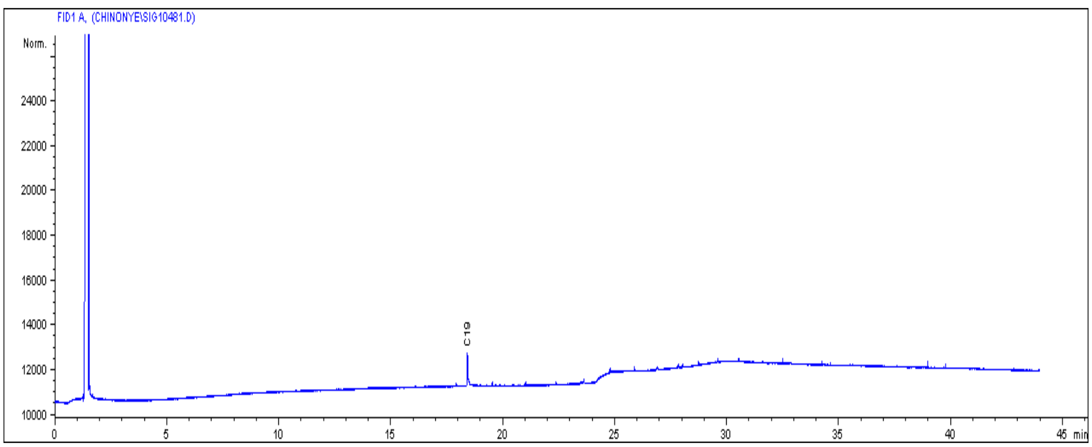

Fig. 6. Gas chromatogram of mixed microbial culture of bacterial and fungal isolates from used Ate brake fluid after day 21

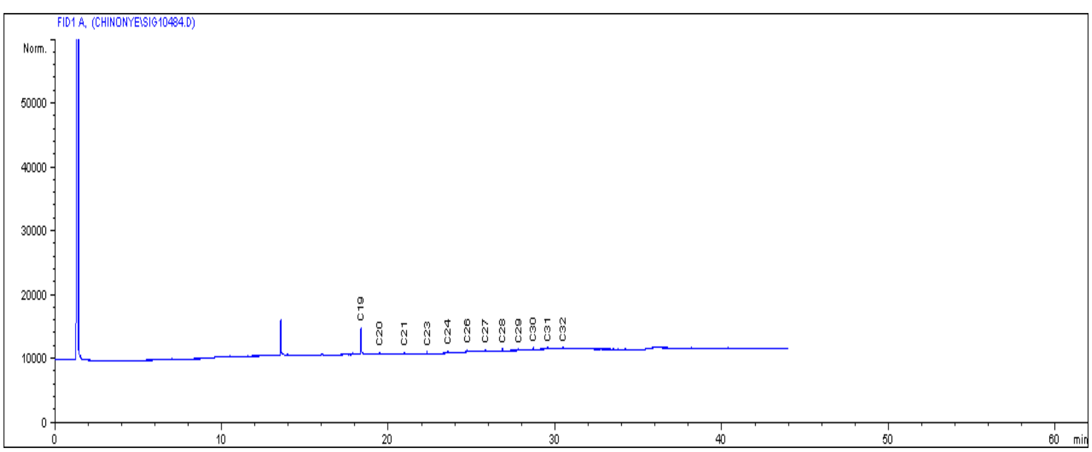

Fig. 7. Gas chromatogram of mixed fungal culture for used Ate brake fluid after day 21

by them [15]. Among the different contaminants of brake fluid, the most common is probably water. It reduces the boiling point of the fluid, creates vapor at the "hot spots" of the circuit, and prevents proper compression. Another frequent contaminant is petroleum distillates such as engine oil, automatic transmission fluid or power steering fluid [34].

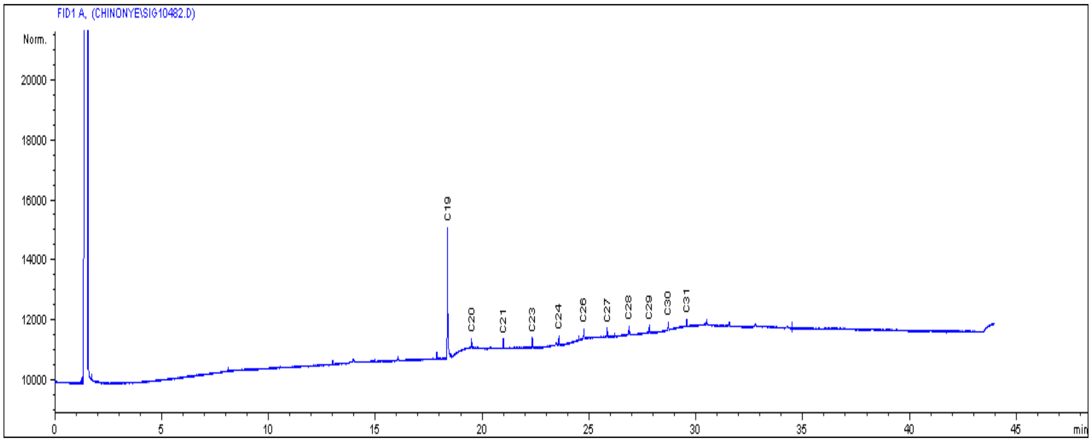

Fig. 8. Gas chromatogram of mixed bacterial culture for used Ate brake fluid after day 21

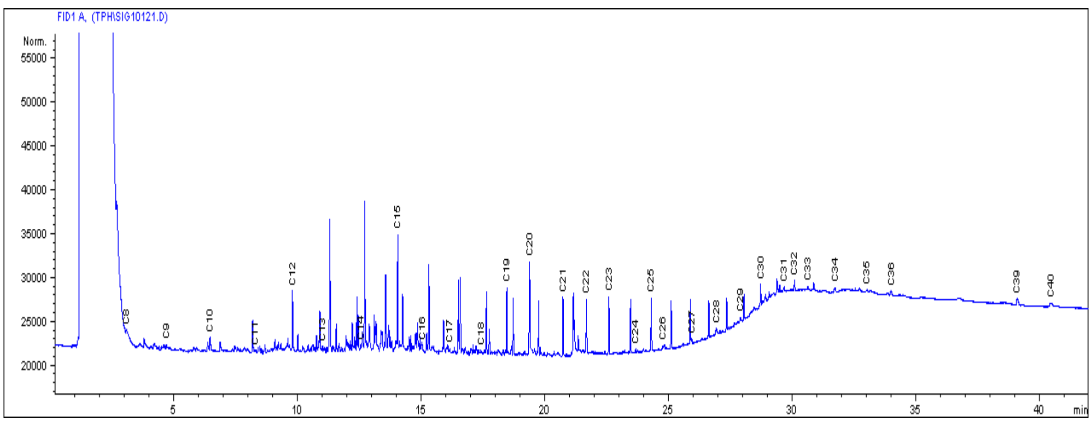

Fig. 9. Gas chromatogram for control sample of used allied brake fluid after day 21

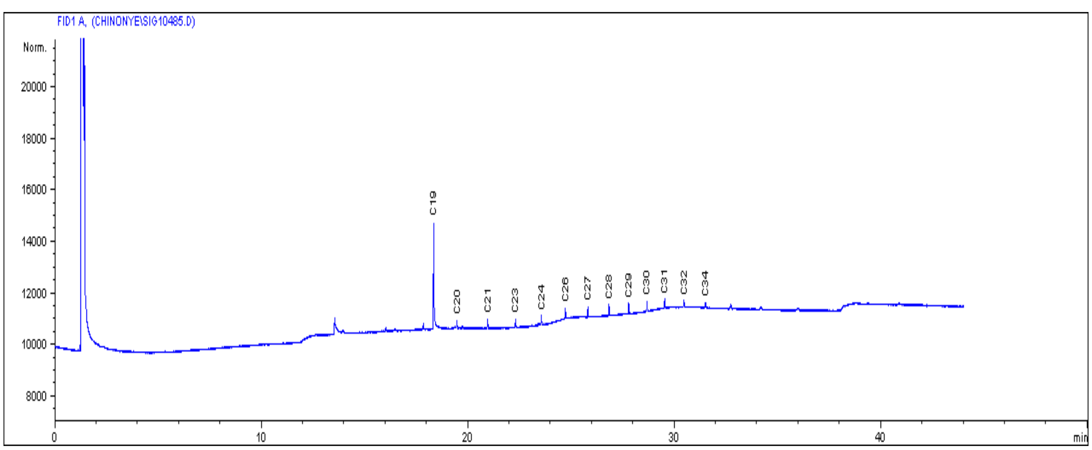

Fig. 10. Gas chromatogram of mixed bacterial culture from used allied brake fluid after day 21

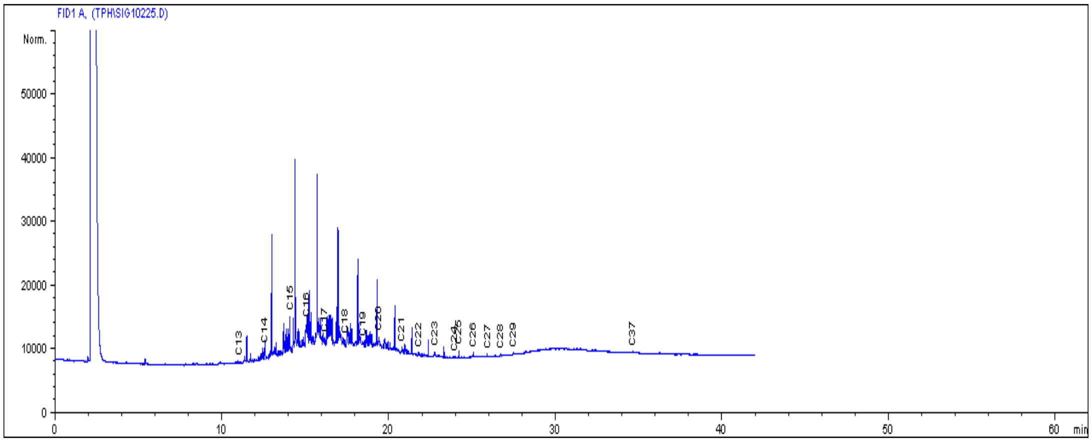

Fig. 11. Gas chromatogram of mixed fungal culture for used Allied brake fluid after day 21

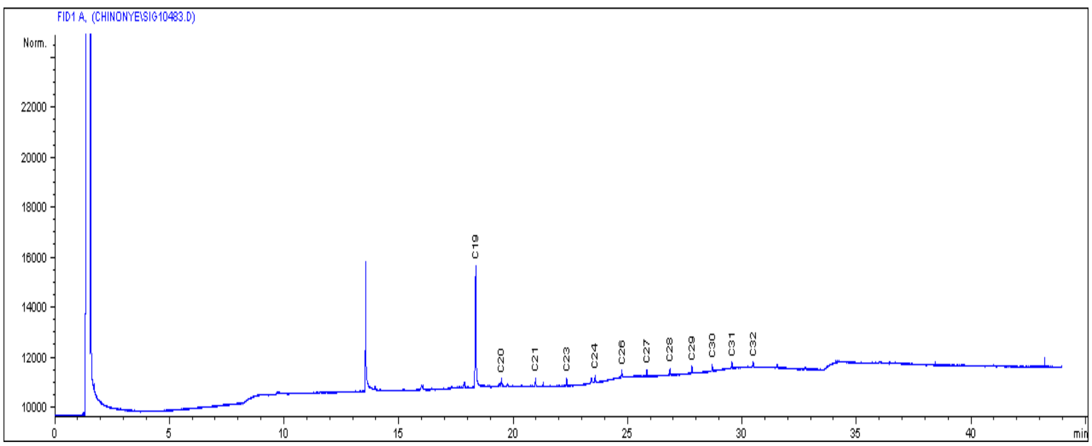

Fig. 12. Gas chromatogram of mixed microbial culture of bacterial and fungal isolates from used allied brake fluid after day 21

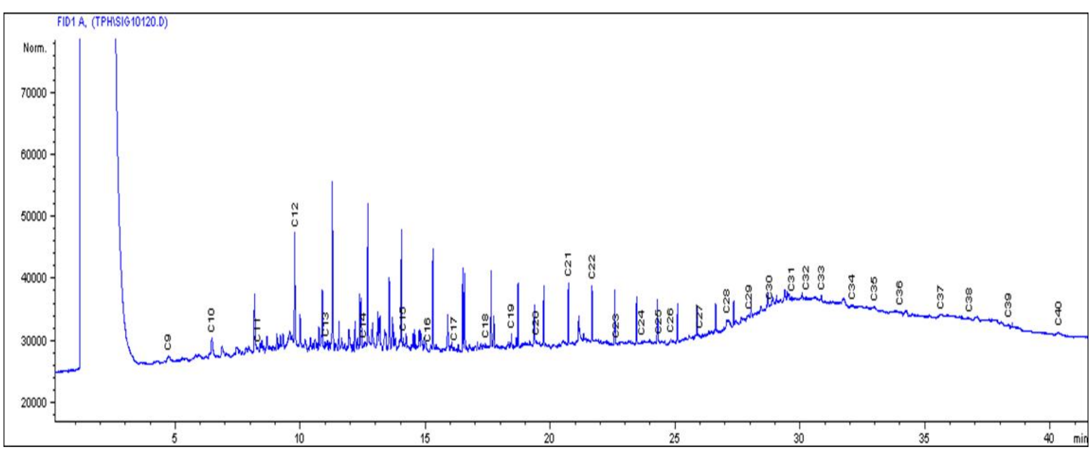

Fig. 13. Gas chromatogram for control sample of unused Ate brake fluid after day 21

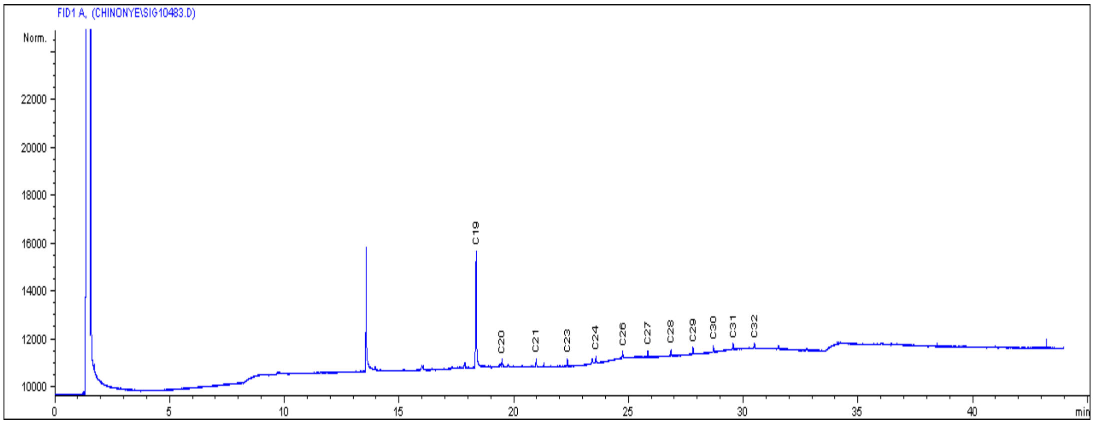

Fig. 14. Gas chromatogram of mixed bacterial isolates in unused Ate brake fluid after day 21

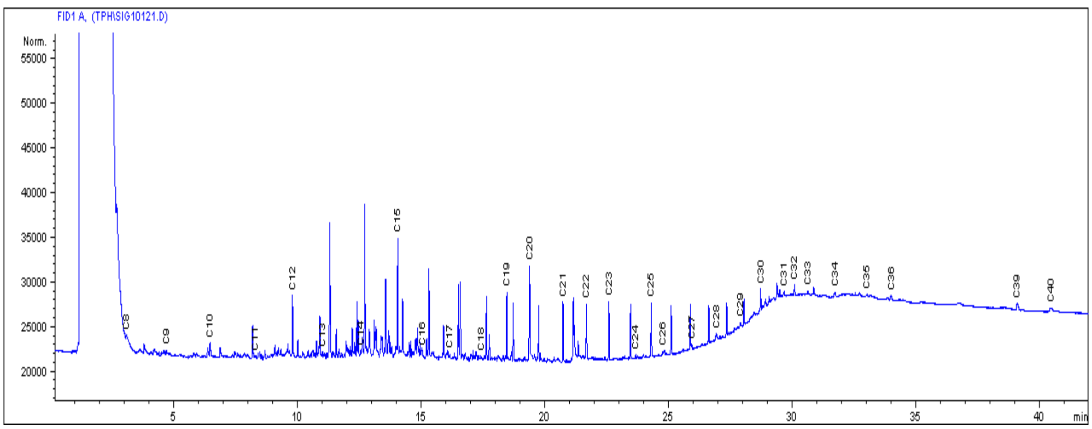

Fig. 15. Gas chromatogram for control sample of unused Allied brake fluid after day 21

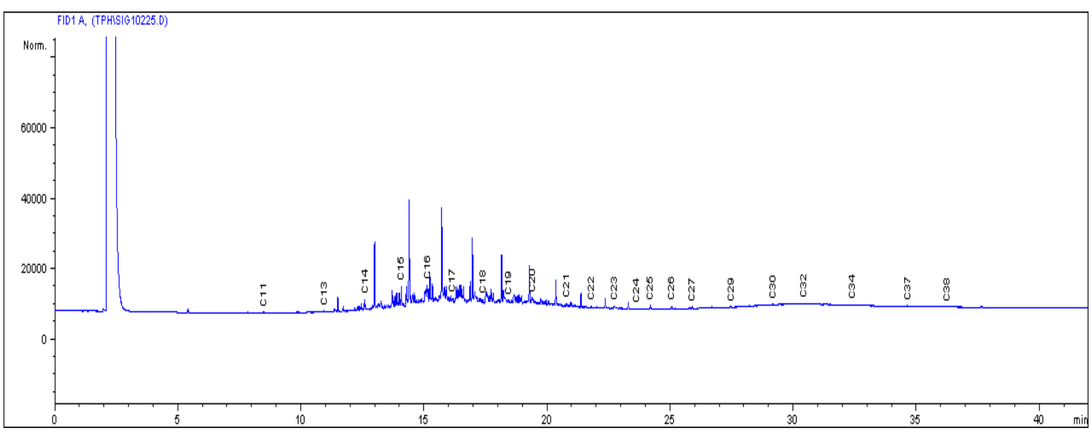

Fig. 16. Gas chromatogram of mixed bacterial culture in unused allied brake fluid after day 21

4. CONCLUSION

Microorganisms truly live in brake fluids and could possibly be the cause of clogged filters, brake lines, pads and brake failures. It has been confirmed that in fuels, microorganisms are present both in diesel fuel, as well as in biodiesel. Microorganisms are sensitive to fluctuations or changes in their environment. Whenever their chemical or physical environment is suddenly altered, there is a lag period during which the microbial community adapts to the new conditions of the brake fluid [35].

The presence of a hydrocarbon mixture in the brake system leads to disastrous consequences. In all GC analyses, compounds are identified by the retention time of the analyte. Total petroleum hydrocarbon fraction methods can be used to measure both volatile and extractable hydrocarbons. Gas chromatography has already been shown as a valuable tool in analyzing high molecular weight petroleum products [36]. Incontaminated cases, the glycol content of the bottom layer is a sufficient characteristic to conclude that it is brake fluid [37]. The material safety data sheet of the STP brake fluid analyzed as a reference sample describes the content of the fluid as a "mixture of glycol ethers, polyglycols, oxidation inhibitors and corrosive inhibitors". Water is an essential factor for microbial activity [1]. Consequently, the most commonly recommended measure for mitigating against microbial activity in fuel systems is water control.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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