GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 13 NO. 1 , JANUARY, 2007 : 1 - 5
 ISSN 1118 - 0579

# STABILITY TO MICROBIAL DETERIORATION OF LUBRICATION OIL



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(Received 20 October, 2005; Revision Accepted 21 February, 2006)

**ABSTRACT**

A study was carried out on the stability to microbial deterioration of car engine lubricating oil Six samples which were classified into used and unused oil were employed The used oil samples were collected from five kinds of vehicles namely Peugeot (504), Japanese (Toyota), Luxurious bus (Marcopolo), Mitsubishi bus and Mercedes Benz (flat boot) car The predominant bacteria species were the *Bacillus, Actinomyces* and *Corgnebacterium*, while the fungal species in order of predominance in all samples included *Aspergillus, Cladosporium, Cephalosporium* and *Penicillium* species the growth of fungal and bacterial organisms at different temperatures and their utilization of the oil indicated the ability of those organisms to proliferate in the oil as well as to utilize the carbon content of the oil as their sole energy source. Also, the results showed that lubricating oil in service is more prone to biodeterioration than unused oil. The biodegradative potentials of these organisms apparently caused changes of various sorts in the quality and hence performance of the oil. Keywords: deterioration, lubricating oil, vehicles, bacterial species, fungal species

## INTRODUCTION

Microorganism play an important role in causing changes in the quality of lubricating oils. The agents which cause the undesirable changes do this as part of their normal activities. The microorganisms of importance in biodeteriorative processes are the bacteria, fungi, actinomycetes and the algae. These groups have been reported widely as agents of biodeterioration in all parts of the world and under a large number of environmental conditions (Richmond and Norris, 1990).

Biodeterioration is not a unique process, as long as the environmental conditions of the organisms are not considered. As a result, studies of biodeterioration problems draw on basic information regarding the organism such as nutritional, ecological and physiological preferences (Sidney, 1994).

Lubricants are among the most widespread contaminants in soil and ground water due to their common use in industry and automobiles for lubricating purposes (Norton, 1995). Lubricating oil has the function besides that of friction reduction to keeping the engine clean by sweeping away metal wear particles from between all moving metal surfaces. It also cools the pistons. The performance of these functions may be adversely affected by microbial growth in the oil (okpokwasili and Okorie, 1988).

Amund and Adebiyi (1991) report that biodeterioration potential of lubricating oil appears to be closely related to their viscosities. Ogiri *et al.* (2001) studied the degradation of lube oil in polluted soil of an abandoned motor mechanic village in Nigeria after ten (10) years and found out that the population of heterotrophs was distribution of oil degraders varied. The present study was in deterioration of car engine lubricating oil and to suggest the potential application and importance of these organisms in the environment and the discard oils after months by car owners.

## MATERIALS AND METHODS

### Lubricating oil samples

The used lubricating oil samples of AGIP SAE 20W – 50 were obtained from the engines of five different cars which had been used to drive them for about two months. Used oil was obtained as a "clean catch" into a sterile bottle from their crankcase of the cars after running continuously for one hour. The unused oil (AGIP SAE) sample was purchased from the store.

### Microbial Isolation

Viable aerobic bacteria present in both used and unused oil were isolated on nutrient agar plates into which sterile fungizone (50 *Mgme* $-^{1}$) had been incorporated to inhibit fungal growth Five drops of aech type of oil were added to 10me of nutrient broth and incubated at room temperature ($30^{0}$c – $31^{0}$c) for 48hours.

Following this incubation, the culture was serially diluted in tubes of physiological saline. One mililitre of each dilution was aseptically pipetted and delivered into sterile petri dishes. To this was added 20ml of molten nutrient agar which had been previously autoclaved and left to cool to 450c the plate was gently rotated for adequate mixing to occur. Upon solidification of the agar, the plates were inverted and incubated at room temperature (300c – 310c) for 4-8 hours The bacterial colonies which developed on the plates were randomly picked and purified by subculturing onto fresh nutrient agar using the streak-plate technique Isolated colonies which appeared on the plates were then transferred onto nutrient agar slants and stored as stock cultures for further tests.

The fungi present in the oil were isolated using the same procedure as for bacteria isolation above but employing sabouraud Dextrose agar (SDA) plates into which sterile streptomycin (50 *mgme* $-^{1}$) had been incorporated to suppress bacterial growth. A portion of each fungal colony which developed was picked using a sterile inoculating needle and aseptically subcultured onto fresh sabouraud Dextrose Agar plates. The plates were kept as cultures for identification tests (Okpokwasili and Okorie, 1988). Heterotrophic Bacterial Count.

Ten-fold serial dilution of the culture sample was carried out to obtain various dilutions. An aliquot of lme from 3 dilutions: $10^{-8}$, $10^{-9}$, $10^{-10}$ was aseptically pipetted and delivered into sterile petri dishes after which molten nutrient agar was added for each dilution The plates were labeled and incubated at 370c for 48hours. At the end of incubation, the total heterotrophic count was determined by counting the number of colonies on the agar and multiplying by the reciprocal of the dilution factor, and answers given in colony forming units perme (cfulme)

### Characterization and Identification of the isolates

The bacterial isolates were examined for colonial morphology and microscopy and biochemical characteristics Gram reaction, spore staining, motility test, oxidase test, catalase test were carried out according to the methods

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described by Tasie, 1999. Indole test, $H_{28}$ production from kliger iron agar (KIA), fermentation of sugars, methyl red test, Voges- Proskauer test, nitrate reduction test, KCN test, gelatin hydrolysis test, urease test and citrate utilization test were carried out according to the methods described by Cowan *et al*, 1974.

Identification of the bacteria to the generic level followed the scheme of Holt (1994) Fungal isolates were examined macroscopically and then microscopically using the needle mount method (Hunter and Bennett, 1973).

**Growth Of The Bacteria Isolates At Different Temperatures**

The ability of the bacterial isolates from the test oil samples to grow under different temperature regions was examined To do this, the ten isolates were inoculated singly into tubes of nutrient broth. The tubes were then incubated at 300c, 450c, 550c, 650c, 750c, and 850c for 48 hours

Test of bacterial utilization of oil samples

Bacterial isolates from the oil samples were tested on their ability to utilize the oil samples as sole carbon source The modified mineral salt medium (Mills et al, 1978) was used The method described by Okpokwasili and Okorie (1988) was employed. The medium was contained in 9.9ml amounts in test tubes. To half of these tubes were added 0.1ml each of used lubricating oil, and to the remaining tubes were added 0.1ml each of used oil. After capping, all the tubes were sterilized by autoclaving at 121$^0$c for 15 minutes and allowed to cool Upon cooling, each set of lubes was inoculated with corresponding isolates Two control tubes (one containing used oil and the other unused oil) remained uninoculated all the tubes were incubated at room temperature for 14 days Each tube was checked for turbidity after the incubation period, using HACH spectrophotometer 3000 at 400nm

## RESULTS AND DISCUSSION

Different types of bacteria and fungi were found in lubricating oils but in larger numbers in the used oil than in the unused oil (Table 1) this means that the components of used oil have been affected during use which made it conducive for microbes to utilize it This report has drawn attention to the fact

Table 1 Viable (Heterotrophic) Colony Count

| Sample | Identify | Bacteria x $10^6$ | Fungi $10^4$ |
|---|---|---|---|
| | | Cfu/ml | Cfu/ml |
| 1 | Peugeot | 58 0 | 8.0 |
| 2 | Japanese | 49 0 | 16.0 |
| 3 | Luxury | 52 0 | 9.0 |
| 4 | Mistsubishi | 51 0 | 11 0 |
| 5 | Mercedes | 44 0 | 9.0 |
| 6 | Unused | 18 0 | 4 0 |

**Table 2: characterization and identification of bacteria isolates**

| Identification | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Gram stain | + | + | - | - | + | - | - | - | + | + |
| Flagella | - | - | + | - | - | + | + | + | + | - |
| Spire | - | - | - | - | - | - | - | - | + | - |
| Motility | - | - | + | - | - | + | + | + | + | - |
| Cell Arrangement | Short rods | Shorts Rods | Short rods | Short circular rods | Rods | Rods | | Rods | Sporing rods | Cluster of small uniform cocci |
| Catalase | + | - | + | + | + | + | + | + | + | + |
| Oxidase | - | + | + | - | - | - | - | - | - | - |
| Gelatin | + | - | + | + | + | + | - | - | - | + |
| Citrate | + | | + | + | + | + | + | | | |
| Indole | - | - | - | - | - | - | + | + | - | - |
| Methyl red | + | | + | + | + | - | + | + | + | |
| Voges | - | - | | - | + | + | - | - | + | + |
| Proskauer | | | | | | | | | | |
| $H_2s$ | - | - | - | - | - | - | - | + | + | - |
| Urease | - | | + | | + | - | + | + | - | - |
| No | + | - | + | + | + | + | + | + | | + |
| (reduction) | | | | | | | | | | |
| KCN | | | + | - | + | + | + | + | - | - |
| Lactose | - | + | - | - | | - | - | + | - | - |
| Glicose | + | + | + | + | + | + | + | + | + | + |
| Xylose | - | - | + | - | + | | + | - | - | - |
| Sucrose | - | + | - | - | | - | + | - | - | - |
| Maltose | + | + | - | + | - | - | + | + | + | - |
| Mannitol | - | - | + | - | + | + | + | - | - | - |

Key:
+ = Positive reaction
- = Negative reaction.

**Bacterial Isolations**

1. Corynebacterium
2. Actinomyces
3. Pseudomonas
4. Acinetobacter
5. Norcardia
6. Serratia
7. Citrobacter
8. Edwardsiella
9. Bacillus
10. Micrococcus

that lubricating oils are susceptible to microbial attack. Several reports on petroleum biodegradation are available (Bartha and Atlas, 1992; Atlas, 1993). Also in virtually all samples was the prepon derance of members of the genus Bacillus (Table 3). This agrees with the report of Okpokwasili and Okorie (1988)

In the test for utilization of both unused and used oils as sole carbon sources by the bacterial isolates in mineral medium, it was found that the isolates utilized the oils to various extents. Generally, the used oil. It was also observed that the highest turbidity and occurrences in the six samples were Bacillus sp., Edwardsillea sp. And Serratia sp.

Figure I showed the results of the effects of temperature on the organisms indicating that all isolates grew at 300c and a few at temperatures of $45^0$c and $55^0$c although species exhibited grow that $65^0$c and $75^0$c. However, there was no growth at 850c. This may be attributed to the fact that as spore-formers, Bacillus species resist from engine parts (Ugwu, 1988).

Also, gelatin hydrolysis or liquefaction was carried out to detect whether or not a bacterium could produce proteases that hydrolyse gelatin and liqliefy solid gelatin medium. The ability of an organism to possess this property is attributable to the decrease in viscosity of oil when attacked by microbes.

Furthermore, it seems that bacteria are the principal biodeteriogen of oil when pH is near neutral but they give way to the fungi as the pH becomes acidic (Okpokwasili and

Table 3: Incidence of Isolates on Oil Sample

| Isolates | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| | Unused | Peugeot | Mitubishi | Japanese | Mercedes | Luxury |
| Corynebacterium | - | + | + | + | + | + |
| Actinomyces | + | + | + | - | + | + |
| Pseudomonas | + | - | - | - | - | - |
| Acinetobacter | + | - | + | + | + | + |
| Norcardia | + | - | + | + | + | + |
| Serratia | - | + | - | + | + | - |
| Citrobacter | - | + | + | + | + | - |
| Edwardsiella | - | + | - | - | + | + |
| Bacilsus | + | + | + | + | + | = |
| Micrococcus | + | + | - | + | - | + |

**Key:**

+ = Presence = Absence

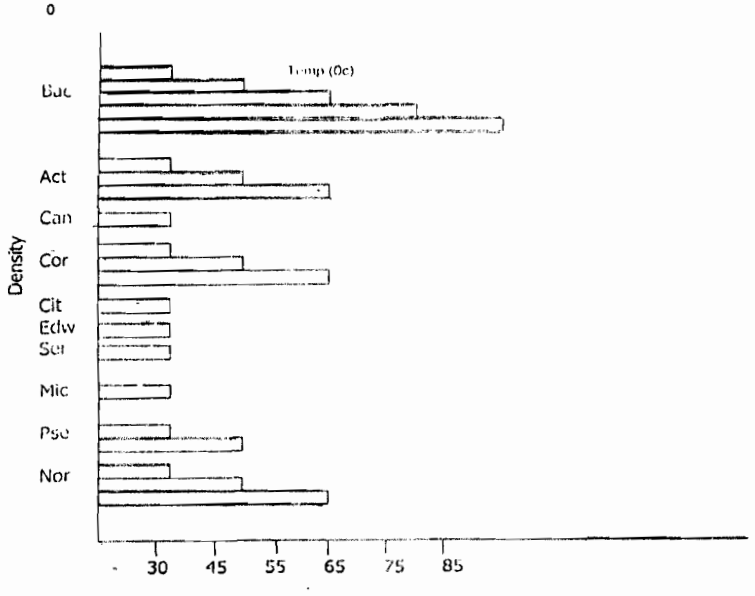


Fig 1 Growth of bacterial isolates at different temperatures

Key

Bac = Bacillus
Act = Actinomyces
Aco = Acinetobacter
Cor = Corynebacterium
Edw = Edwardsiella
Cit = Citrobacter
Ser = Serratia
Mic = Micrococcus
Pse = Pseudomonas
Nor = Norcadia

Table 4: Bacteria Utilization Of Oil Sample

| Isolates | Peugeot | Japanese | Luxury | Mitsubishi | Mercedes | Unused |
|---|---|---|---|---|---|---|
| Corynebacterium | - | + | - | - | - | - |
| Actinomyces | +++ | + | ++ | + | ++ | ++ |
| Peseeudomones | ++ | + | + | ++ | + | + |
| Acine; tobacter | - | - | + | + | + | - |
| Norcarddia | - | + | - | + | + | - |
| Seriatia | - | - | + | + | + | + |
| Citrobacter | +++ | ++ | + | ++ | ++ | + |
| Edwardsilla | - | + | - | + | - | + |
| Bacillus | +++ | +++ | + | +++ | + | + |
| Micrcoccus | + | ++ | + | - | - | + |

Key.
+++ = Highly turbid.
++ = Turbid
+ = Slightly turbid
- = Not turbid

Table 5: Identification of Fungil Isolated from the different Lubricating Oil

| Colony morphology on SDA | Microscopic morphology | Identification | Occurrence in sample |
|---|---|---|---|
| Dark grey surface, dark reverse side velvety or cottony | Non-septate conidiophore terminating in a globus or clarate swelling bearing phialides at the apex There are primary and secondary sterigmata | Aspergillus sp | All sample |
| Green powdering surface, while reverse side | The conidiophore arise from mycelium singly, branched near the apex end in phialides hyaline or brightly coloured in mass | Penicillium sp | Unused only |
| Greenish dark grey surface. Dark on reverse side | Tall dark upright conidiophore branched variously near the apex. Have clustered conidia, ovoid and irregular, simple or branched acropetalous chains | Cladosporium sp | All sample |
| Pink to violet surface, fellow reverse side. | Have branched slender conidiophores. Slightly enlarged at the apex. Conidia appear to rise one at a time conidiophore is short, straight from the ium sp mycelium. | Cephalosporium sp | Except unused only |

Okorie, 1988). Fungi isolates from the oils were identified as Cladosporium sp., Aspergillus sp., Cephalosporium sp. And Penicillium sp. (Table 5) the above fungal genera have already been incriminated as hydrocarbon degraders by previous workers (Hill, 1984) with Cladosporium species and Aspergillus fumigatus (Ogiri et al, 2001) being the most significant. Both organisms have been found to persistently occur in hydrocarbon products such as diesal (hill 1984 and Sidney, 1994).

Identification of the oil utilizing bacteria showed that they belong to the genera: *Bacillus, Serratia, Cornebacterium, Citrobacter, Actinomyces, Edwardsiella, Micrococcus, Pseudomonas, Norcardia and Acinetobacter* (Table 2)

Microbial deterioration of lubricating oil is harzardous both to the engine parts (loss of function) to man and animals by infection. There has been growing awareness that potential adverse temperature conditions by going into the spore stage. In this endospore state, 850c is not hot enough to penetrate the spore in the organism and kill the organism. This explains the situation in car engines. When the car runs, the engine becomes hot and the temperature of the oil rises Consequently, the viable microbial population in the oils falls Some thermotolerant and thermophilic microbial contaminants in the oil are not killed.

In the biochemical tests carried out (Table 2) oxidase test was used to detect the presence, in bacteria, of cytochrome c oxidase that is able to reduce oxygen and artificial electron acceptors This property confers on the loacteria the ability to carry out respiratory mode of metabolism. The carbohydrate utilization test was carried out to detect if acid or gas were produced during fermentation growth with various sugars (oxidation-fermentation test) It is known that biodegradation of hydrocarbon is an oxidative process and that it takes place in an aerobic environment only (Sidney, 1994) So the presence of cytochrome oxidase helps the organism to carrt out respiratory metabolism in an aerobic environment

The growth on kliger Irn Agar (KIA) showed that some of the organisms produced hydrogen sulphide ($H_2S$) it is expected that the release of this H2S into the oil changes the colour of the oil from yellowship – green to black. though darkening of the oil may also be caused by non-biological processes, such as deposition of metal particles and carbon health problems could exist when oils and oil emulsions particularly those used in machine shops, become infected (Hill and Alzubaidy, 1979) Most of the fungal and bacteria genera isolated from the lubricating oils are known to be pathogenic to man and animals. Some species of such genera as *bacillus, Corynebacterium, pseudomonas, Nocardia* and *Aspergillus* are known to cause infections in man. Others may be involved in plant inflections which eventually affect man. These organisms get to man through careless disposal of contaminated oils. When contaminated or spoilt oil is removed from the engine by mechanics, it is poured into the surrounding soil. This oil seeps into the soil and many find its

way into ground-water or by run-off into surface water for drinking and recreational purposes These waters get contaminated with these microorganisms originally present in the contaminated oil.

Man then becomes infected when this water is used or consumed. Alternatively, the inhalation of infected aerosol from oil emulsion may also be hazardous Hydrogen sulphide and fatty acid by-products of microbial infection are well known irritants of both lungs and skin. Inhalation of living or dead bacteria could invoke serological responses which could lead to respiratory ailments hence infected oils may pose a threat to life (Atlas, 1995).

In conclusion, most bacteria and fungi present in lubricating oil can utilize it as their sole source of carbon and energy. However, these organisms utilize the oil at different rates. On exposure of new (unused) oil to the environment, microbial attack on the oil occurs and its components become degraded resulting in the loss of function of the oil It is therefore evident that lubricating oils used in car engines are susceptible on the kind of vehicle which is using the oil Therefore further work will be done on the different brands of motor oil.

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