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Biodegradability of Unused Lubricating Engine Oils in Fresh Aquatic System

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ABSTRACT

The biodegradability of four unused lubricating engine oils (Total engine oil, Tonimas engine oil, Oando engine and Lubical engine oil) was investigated in fresh aquatic system obtained from Isokpo stream of Rivers State in the Niger Delta, Nigeria. Biodegradability (mineralization) of the lubricating oil samples were monitored for a 56 – day period using the percentage ratio of Biological Oxygen Demand (BOD) to Chemical Oxygen Demand (COD). Olive oil served as a positive control while sodium azide served as a negative control. The results obtained indicated the following rate of mineralization, Total engine oil 5.3 percent, Oando engine oil 7%, Tonimas engine oil 16% and Lubicol engines oil 73%. Statistical analysis using ANOVA, showed that there was no significant difference (P=0.05) in the percentage mineralization of the engine oils. Result obtained from the viable bacterial and fungi counts, indicated higher total heterotrophic bacteria (THB) counts than total fungi (TF) counts, and higher hydrocarbon utilizing bacteria (HUB) counts than hydrocarbon utilizing, fungi (HUF) counts. Characterization and identification test reveal that a microbial consortium comprising of the following genera; Bacillus, *Pseudomonas*, *Microcosus*, *Proteus*, *Escherichia*, Enterobacter, *Arthrobacter* were implicated in the biodegradation process in the fresh water source. Similarly the molds encountered in the degradation process were *Aspergillus*, Geotricum, *Cladosporium*, *Penicillium*, *Fusarium* and *Candida* species. Changes in the physicochemical parameters during the biodegradability monitoring period included, pH, temperature, alkalinity, conductivity, biological oxygen demand, chemical oxygen demand, total organic carbon, dissolved oxygen, nitrate, sulphate, phosphate and inorganic carbon. Biodegradability result obtained indicated that the degradation of any petroleum product (engine oil) in the environment depends on interplay of many factors which includes; type of lubricating oil, microbial load/species present in the environment and physicochemical characteristics of the natural habitat. The results of this study have provided an insight into the biodegradability of some lubricating engine oils in fresh water ecosystem. Three out of the four petroleum lubricating engine oils investigated were not readily biodegradable, hence research into the production of biobased lubricating oils that are environmentally friendly, cost effective and efficient in performance like the petroleum base oils is recommended and as well as enhancing the biodegradation of these petroleum base lubricating oils that may spill into the environment.

Key Words: Biodegradability, Biodegradation, Lubricating Oil, Mineralization

INTRODUCTION

The major proportion of lubricants enters the environment in one way or another, mainly due to use of lubricants. As far as automobile engine oil is concerned, the result of this usage can be seen on motorways (roadside), car parks, and roadsides mechanic workshops as black coating of oil on the road as well as pollution of aquatic environment due to activities of marine engine boat operators in our water ways. Lubricatants both unused and used can cause considerable damage due to their high potential of serious water pollution (Betton, 1992). Lubricating oils contain variable amounts of chemical substance toxic to humans and/or other organisms (Clyton and Clyton, 1981). Lubricating oils are formulated from a range of base fluids and chemical additives. The base fluid which is essentially petroleum base has several functions, but primarily to reduce friction between moving surfaces in machines (Prince, 1992). The additives contained in lubricating oils, can be toxic

to flora and fauna. Lubricating oils persistence in the environment depends largely upon the base fluid (Mortier and Orszulik, 1992). Lubricants are among the most widespread containments in soil and ground water due to their common use in industries and automobiles for lubricating purpose (Norton, 1995).

The menace posed by these environmental pollutants and their possible elimination and control have been the preoccupation of various National and International Environmental Agencies. One widely employed measure for removal of spilled oil in the aquatic environment is the application of chemical dispersants. The most efficient and cost effective means of eliminating these contaminants from the environment is through microbial degradation. Microbial degradation contributes significantly to the elimination or ultimate removal of oil and detergent chemicals from the environment (Okpokwasile and Odokuma, 1990). Many researchers have utilized the degradative potentials of microorganisms in the remediation of petroleum product polluted water and soil environments (Atlas, 1984, Okpokwasili and Amachukwu, 1988, Okpokwasili and Okorie, 1988; Wokoma, 2002; Atim and Antai, 2002, Odokuma, and Ibor, 2003; Odokuma and Otokunefor 2003; Odokuma and Okara, 2005; Adekunle and Oluyode, 2005). Biodegradability, a measure of the extent by which organic compound is biodegraded to be finally converted into inorganic compound – carbon dioxide and water has provided a standard guide for assessing the degree of biodegradation of pollutants in a given ecosystem (ASTM, 2003). The objective of this study therefore was to evaluate the biodegradability of four lubricating engine oils in fresh water ecosystem.

MATERIALS AND METHODS

Fresh water sample was obtained from Isiokpo stream in Ikwerre Local Government Area of Rivers State, Nigeria. 4 – litre plastic jerican was employed. Sample was capped and transported in Ice Park to the laboratory. Analyses were carried out and stored in the refrigerator at 4°C. The lubricating engine oils used in thin study were obtained from the company's headquarters, and major distributors located in Port Harcourt, Nigeria. All reagents employed in this study were of analytical grade and were obtained from BPH chemical Ltd., Poole, England. Nutrient Agar and Potato Dextrose Agar were obtained from International Diagnostic Groups, Lancashire, England. Filter papers (Whatman No. 1) WER Bauston Ltd., London were also used. The Bonny light crude oil used was obtained from Shell Petroleum Development Company (SPDC) Port Harcourt

Preliminary Toxicity Test

This was carried out to determine the non-toxic concentration of the various lubricating oils to the indigenous microflora of the fresh water sample. It involved plating out in duplicates 0.1ml of 1:100 dilution of water sample on mineral salt agar (MSA) using spread plate method (APHA, 1998) containing different concentration of lubricating oils and incubating at room temperature (28±2°C) for 48h. Concentrations of the

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test lubricating oils employed were 100mg/l, 10mg/l, 1mg/l, and 0.1mg/l, 0.01. This was performed by a ten fold serial dilution of each lubricating oil. Enumeration of colonies was performed after incubation at room temperature (28 ± 2°c) for 48h. (Table 1).

Enumeration of Microbial Population

The total heterotrophic bacteria (THB) count of water sample and total viable bacteria count during the preliminary toxicity test were performed on nutrient agar (oxoid) using the spread plate method (APHA, 1998). Plates were properly labeled and incubated at 37°c for 24h after which they were examined for colony formation and enumeration. The hydrocarbon utilizing bacterial (HUB) count of water sample was performed in duplicates on mineral salt agar (MSA) of Mills *et al;* (1978) as modified by Okpokwasili and Odokuma (1990). Sterile filter paper (Whatman No.1) saturated with Bonny light crude oil were aseptically placed on the inside cover of each petri dish kept in an inverted position and incubated at 30°c for 48h.The total fungal (TF) count of water sample was estimated by plating 1ml of serial dilution onto the surface of Potato Dextrose Agar (PDA) plates in duplicates. Approximately 10ml of 10 percent lactic acid was added to 500ml of PDA media before pouring of the plates in order to suppress bacterial growth. Plates were examined and enumerated after incubation at 30°c for 5 days. The same technique was employed for hydrocarbon utilizing fungal (HUF) counts by using PDA and incorporating with sterile filter paper saturated with Bonny light crude oil incubating at 30°c for 5 – 7 days.

The initial (day O) THB count of biodegradation test set up was enumerated by plating 0.1ml of serial dilution on MSA plates in duplicates and subsequent THB counts at day (14, 28, 42 and 56), dilution of each set up were plated out in duplicates as earlier described. On the other hand, the initial (day O) TFC density of test set up as well as day 14, 28, 42 and 56 were estimated by spread plate method on PDA plates in duplicates and incubated at 30°C for 48h. The HUB and HUF counts for (day O, 14, 28, 42 and 56) the use of sterile filter paper saturated with crude oil as earlier described for enumeration of HUB and HUF for the natural water source was employed. Biodegradation tests were carried out in six 2L Erlenmeyer flasks. To each flask was added 900ml mineral salt broth (MSB) and sterilized by autoclaving at 121°C for 15mins. After cooling appropriate concentration of test lubricating oil was aseptically added with sterile pipette based on the preliminary toxicity tests carried out. To each set up, 100ml of water sample was added as the inoculums. To the positive control test set up, 0.1ml of olive oil was added while 2g of sodium azide as to inhibit growth of microorganisms. The entire tests set up were labeled as shown in Table 2. Repeated sampling for microbiological and physicochemical analysis were carried out at day O and subsequently at 2 weeks intervals for a 56 day monitoring.

Biodegradability Monitoring

Biodegradability (mineralization) for a 56 day incubation period was monitored using the percentage ratio of BOD to

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COD. The BOD for each biodegradation test system was monitored using method adopted from Stewart *et al;* (1974) at day O, 14, 28, 42 and 56. The COD for each set up was determined at day O (initial day). The ultimate biodegradability also referred to as the percentage of carbon in the material that is potentially mineralizable was calculated from the percentage of ratio of BOD for day O, 14, 28, 42 and 56 to COD at day O.

$$\text{Mineralization} = \frac{BOD}{COD\ at\ day\ 0} x\ 100\%$$

Physiochemical parameters of water sample and biodegradation experimental set up analyzed were pH, temperature, salinity, conductivity, alkalinity, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Organic Carbon (TOC), Dissolved Oxygen (DO) nitrate, phosphate, sulphate and inorganic carbon. They were determined using methods adopted from Stewart *et al;* (1974). Isolation and identification of bacterial and fungal hydrocarbon utilizes were accomplished on basis of their cultural, morphological characteristics and by use of Gram staining. The isolates were further subjected to series of biochemical test for identification and characterization using the determination schemes of Holt *et al;* (1994). Similarly, moulds were identified through their cultural as well as microscopic analysis.

Statistical Analysis

Analysis of variance (ANOVA) method (Finey, 1978) was employed to analyze data obtained

RESULTS

The result of physiochemical characteristics of the fresh water aquatic system used in the study before biodegradation test is presented in Table 3. The THB and TF, counts of the fresh water source as well as HUB and HUF are presented in Table 4. Forty Nine percent of the THB densities enumerated were HUB, while 25% of the count represented HUF. The THB, THF, HUB and HUF counts of biodegradation test lubricating oil samples during the monitoring period are illustrated in figures (1-4). Total engine oil (TTEC) supported the highest growth with microbial load of 2.33×10^6 cfu/ml on day 28. Tonimas engine oil (TMEO) and Oando engine oil (OAEO) followed with 2.05×10^5 cfu/ml, and 1.99×10^5 cfu/ml respectively while lubicol engine oil (LUEO) supported the least microbial growth of 2.88×10^4 cfu/ml. Generally, the total viable counts (TVC) of bacteria and fungi (growth profile) during the biodegradation period followed the same pattern. They increased exponentially from initial day to day 28, and gradually increased thereafter to day 28, and declined sharply from day 42 to day 56.

DISCUSSION

In Nigeria, like in most developing nations, environmental problems are severe and very challenging, the survival of many species both plants and animals are threatened by improper disposal of industrial chemicals and hydrocarbon wastes into the environment. However, microorganisms in natural environments make major contribution to the natural attenuation of pollutants. This study

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was therefore designed to basically determine the biodegradability of four unused lubricating engine oil samples in fresh water. Studies have shown that biodegradation experiment with environmental samples incubated under controlled laboratory conditions are probably, the best routine test for biodegradability and are indeed, superior to experiments with environmental samples with pure cultures. The use of microcosms as test system for biodegradation is of great value because it gives not only qualitative but also reasonably good quantitative result about the behaviours of the pollutants in the sampled environment (Hus and Bartha, 1979).

The results of microbial counts during the biodegradation period showed that the unused engine oil samples were utilizable sources of carbon and energy for heterotrophic microorganisms. Moreover, the exponential growth of both bacterial and fungi from day O to day 14 (Fig. 1-4) indicates that the lubricating oils were being metabolized as sole sources of carbon and energy within the period. The decline in population of THB and TF counts from day 42 to day 56 may be due to nutrient exhaustion with possible accumulation of toxic metabolites in the media which mark the on set of stationary and death phases. Table 6 shows the result obtained from the characterization and identification of bacterial genera isolated from the fresh water source, which include; *Bacillus, Pseudomonas, Proteus, Escherichia, Micrococcus, Arthrobacter, Enterobacter* and *Citrobacter*. Comparison of bacterial and fungal counts in Table 4 suggests that bacteria played a greater role during the degradation of the lubricating oil samples than fungi; hence the highest THB counts than TF counts through out the test period. Benneth and Faison (1977) attributed the dominance of bacteria degraders to the fact that fungi are more proficient at co-metabolism and bioaccumulation than at using of pollutants as sole carbon source. The proportion of the microbial population capable of hydrocarbon degradation in an aquatic habitat is influenced by a number of factors one of which is environmental condition (Fought and Westlake, 1987).

The molds isolated from the fresh water source were *Aspergillus, Fasarium, Penicillium, Geotricum* and *Cladosporium*. Yeast was *Candida* sp. Some of the organisms isolated in this study, were also implicated as being capable to degrade car engine lubricating oil by Okpokwasili and Okerie (1988) and Ekwenye and Ike (2007). They included *Bacillus, Pseudonones, Micrococcus* and *Citrobacter* while fungi included *Aspergillus, Cladosporium* and *Penicillium.*

Changes in PH during the biodegradation period showed pH near neutrality. This favours most heterotrophic bacteria (Atlas, 1984). Generally, the pH of the various test system could be a function of chemical composition of microbial activities. There were slight decreases in pH initially from day O to day 14. According to Benneth and Faison (1977), the mechanism of fungal degradation involves secretion of protons into the medium, which ultimately leads to lowering of pH. On the other hand, the slight increase observed at day 28 and 42 may be due to

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reduction in acidic compounds, production and/or proton secretion as a result of reduction in biodegradation rates. It is also possible that co-metabolic reactions much later resulted in production of slightly alkaline compounds by day 28 (Odokuma and Ibe, 2003). The decline in pH and alkalinity for most of the test systems with time may be due to production of acidic metabolites (Delyan *et al;* 1990). The TOC showed substantial percentage decrease from day 0 to day 28 during the monitoring period (fig. 7). Other chemical parameters that showed substantial decreases at one period or the other in course of the biodegradability monitoring were; No^{-}_{3}, PQ^{3-}_{4}, and inorganic carbon (IC) (figs. 8, 9, 18). Generally, these reductions indicated that the degraders were actively utilizing the metallic salts of these anions as sources of nitrogen, phosphorus and sulphur respectively. Similar observations have been made by Odokuma and Akpokodje (2004), and Okoduma and Okara (2005). The increase in conductivity for the test systems (fig. 15) with time may be due to increase in ionic strength of the test samples as a result of microbial and metabolic substrate thereby converting them to higher conductivity (Stewart *et al;* 1974). The decline in BOD in the various test systems (fig. 15) indicates that the amount of degradable organic material present in the sample were being degraded by the microorganisms. BOD represents the amount of oxygen required for the microbial decomposition of organic matter in waste water sample; it is roughly proportional to the amount of degradable organic material present in the water sample (Pelczer *et al;* 1982). The changes in dissolved oxygen (DO) showed relative decrease in some of the test systems (fig. 10). These results indicate that the microorganisms present in the water sample were utilizing oxygen for metabolic activities. This followed the result of BOD. As BOD was decreasing, DO was also decreasing in some of the test systems during the period of monitoring.

The initial decrease of temperature from day 0 to day 14 and subsequent rise in temperature from day 14 to day 28 may have favoured microbial growth (fig. 14). The changes in COD (fig. 12) may have been supported by the microbial growth pattern since the highest THB counts during the period of monitoring were recorded at day 28. The highest values of COD were recorded on day 28 for all the lubricating test engine oils during the incubation period. COD provides a measure of the oxygen equivalent of that portion of the organic matter in a water sample that is susceptible to oxidation (Stewart *et al;* 1974). The high value of COD in the negative control on day 28 may be due to chemical reactions in the test system. Comparing the percentage mineralization at day 56 for the various test engine oils, it was observed that mineralization was highest in lubicol engine oil with 73%. The olive oil had 80% mineralization being a vegetable oil was more degradable than the petroleum based lubricating engine oils used in this study. The percentage mineralization of 5.3% observed in the negative control test set up could be attributed to natural attenuation process other than biodegradation, since microorganisms were eliminated by addition of sodium azide (biocide). The minor decreases observed in some of physiochemical

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parameters in the negative control suggest the involvement of non-biological factors, possibly photo-oxidation. The differences in the rate of mineralization of the lubricating engine oil samples in the fresh water sample could be attributed to different factors such as; the microbial population of the aquatic system, the interplay of the physicochemical parameters, available nutrients as well as chemical composition of each of the petroleum lubricating engine oil samples. These factors determine lubricating engine oil's biodegradability or its recalcitrance. In the light of the findings of this study, it is recommended that more research on the manufacture and use of renewable bio-based lubricating oils should be enhanced and reduced dependence on petroleum base lubricating products. These bio-based products, if spilled into the environment, will degrade and disappear with little or no harm to the environment.

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Table 1: Total viable bacteria count (cfu/ml) during toxicity testing of the various lubricating engine oil samples in fresh water sample after 48h incubation at room temperature (28 ± 2°C)

	Test Engine Oils				
Conc. (mg/l)	TTEO (cfu/ml)	TMEO (cfu/ml)	OAEO (cfu/ml)	LUEO (cfu/ml)	Control Olive Oil (cfu/ml)
100	1.0×10^3	1.0×10^3	2.0×10^3	1.0×10^4	4.3×10^4
10	2.0×10^3	7.0×10^3	3.0×10^3	1.1×10^5	9.0×10^4
1.0	4.0×10^3	80×10^3	5.0×10^3	7.8×10^7	3.2×10^5
0.1	6.0×10^3	1.4×10^4	7.0×10^3	2.0×10^4	7.8×10^5
0.01	9.4×10^4	1.15×10^5	3.0×10^4	3.0×10^4	8.6×10^5

Table 2: Biodegradation test set up

Lubricating oil Test code	Fresh water sample	Mineral salt broth	Description
TTEO	FW	MSB	Total engine oil +FW+MSB
TMEO	FW	MSB	Tonimas engine oil+FW+MSB
OAEO	FW	MSB	Oando engine oil +FW+MSB
LUEO	FW	MSB	Lubicol engine oil +FW+MSB
O.Oil	FW	MSB	Olive oil +FW+MSB (+v control)
Na.Z	FW	MBS	sodium azide +FW+MSB(-ve control)

Table 3: physicochemical parameters of habitat water sample

Parameters	Values (freshwater)
PH	6.39
Temperature capacity ^{O}C	28.6
Salanity (mg/l)	32.49
Conductivity (Ns/cm)	18
Alkalinity	8
BOD (mg/l)	8
COD (mg/l)	7.2
TOC (%)	0.0258
DO (mg/l)	6.56
SO_4^{2-} (mg/l)	68.80
NO_3 (mg/l)	3.088
PO_4^{3-} (mg/l)	0.0825
Inorganic carbon (%)	0.8796

Table 4: Bacterial and fungal counts of fresh water source

Type of count	value(cfu/ml)	%count
THB	$4.7 X10^3$	52
HUB	$2.3 X10^3$	48
TFC	$8.0 X10^2$	75
HUF	$2.0 X10^2$	25

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Table 5: Percentage mineralization of unused lubricating engine oils at day 56 in fresh water sample

Engine oil code/name			Percentage mineralization (%)
TTEO	-	Total engine oil	5.3
TMEO	-	Tonimas engine oil	16
OAEO	-	Oando engine oil	7
LUEO	-	Lubicol engine oil	73
O.Oil	-	Olive oil (positive control)	80
Na.Z	-	Sodium azide (negative control)	5.3

Changes in physicochemical parameters during the biodegradation of unused lubricating engine oil samples are illustrated in figures 5 – 18. Percentage mineralization of the various tests lubricating engine oil samples including positive and negative controls at day 56 are shown in table 5. The result showed that lubicol engine oil had the highest mineralization of 73%, the least was total engine oil with 5.3%. Statistical analysis results showed that generally there was no significant difference (at 95% probability level) between the bacterial populations during the biodegradation of the lubricating engine oil samples. Although there was significant difference in population of HUF during biodegradation of the engine oil samples Fcal.=3.160, while Ftab. = 2.86. For physicochemical parameters statistical analysis showed that there was no significant difference (p=0.05) in changes of the parameters during the biodegradation of the oil samples in the fresh water sample.

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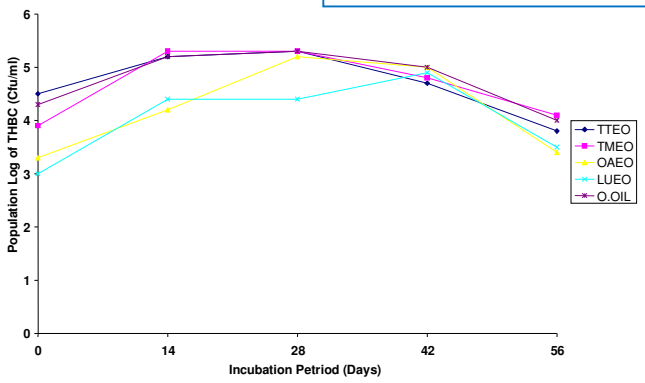

Fig.1: Growth profile of THB in freshwater inoculum during biodegradation of test engine oils.

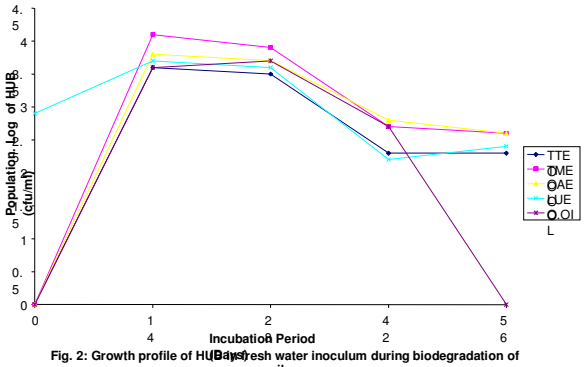

Fig. 2: Growth profile of HUB in fresh water inoculum during biodegradation of test engine oils

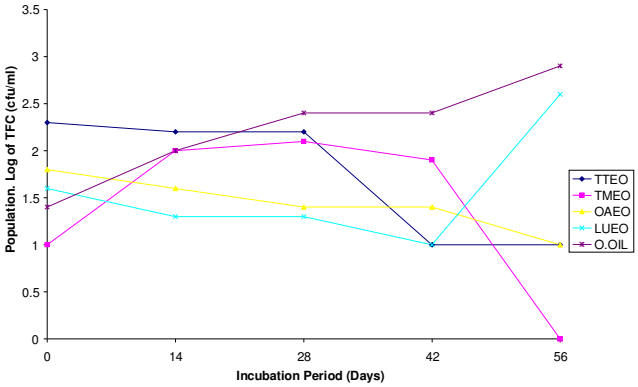

Fig 3: Growth profile of TFC in test system containing freshwater inoculum during biodegradation of test engine oils

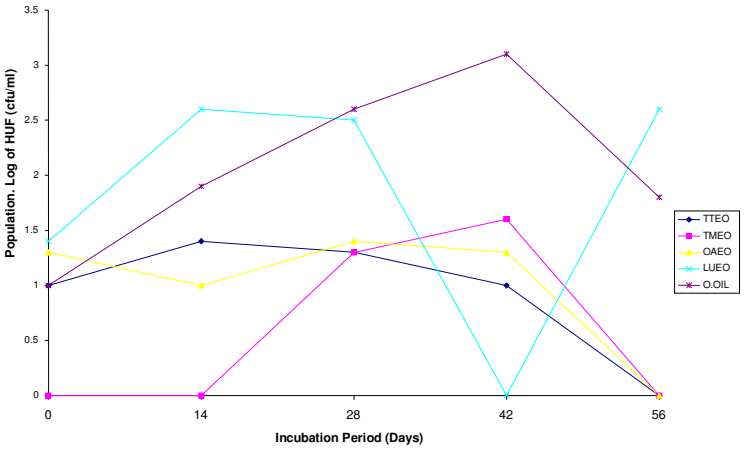

Fig 4: Growth Profile of HUF in test system containing freshwater inoculum during biodegradation of test engine oils

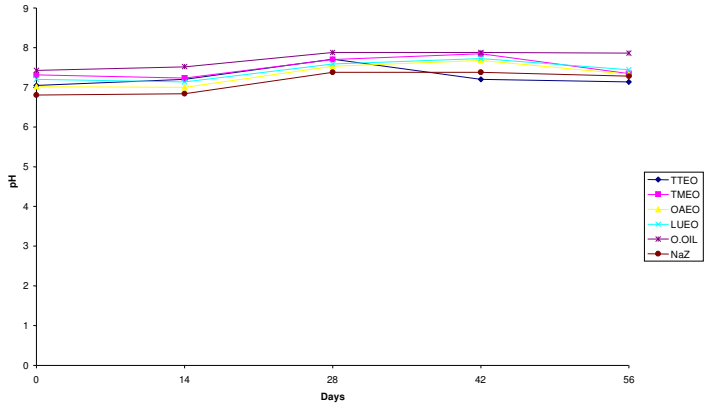

Fig. 5: Changes in pH level in test system containing fresh water inoculum during biodegradation of various engine oil samples

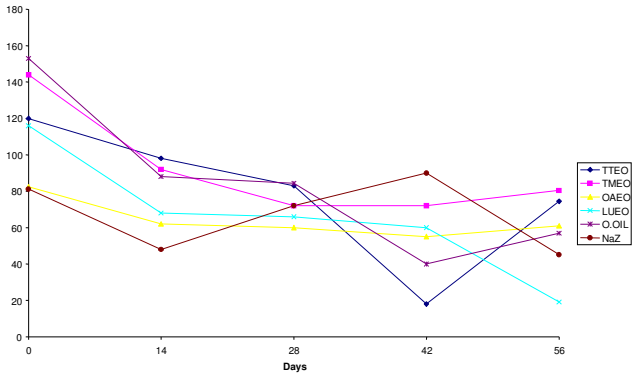

Fig. 6: Changes in Alkalinity level in test system containing fresh water inoculum during biodegradation of various engine oils

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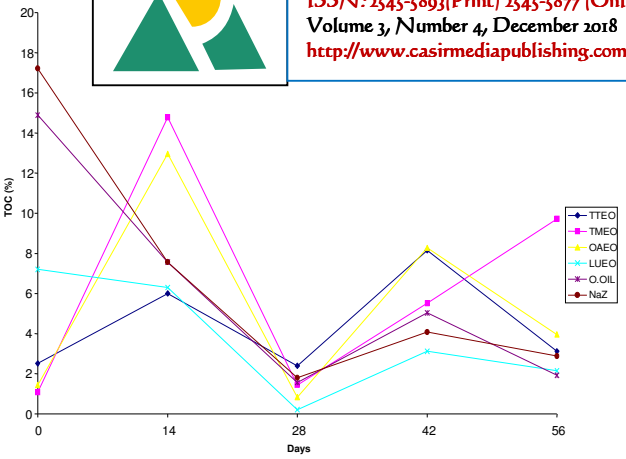

Fig. 7: Changes in TOC level in test system containing fresh water inoculum during biodegradation of various engine oils

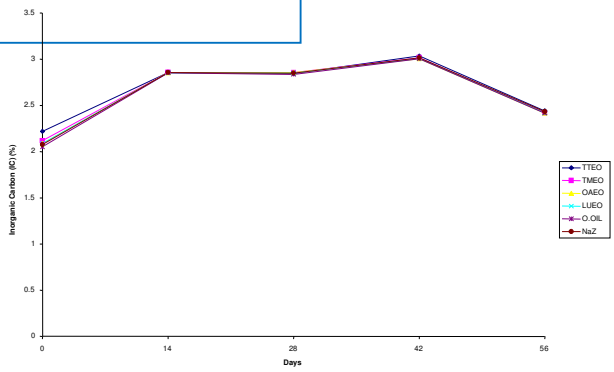

Fig. 8: Changes in (IC) level in test system containing fresh water inoculum during biodegradation of various engine oils

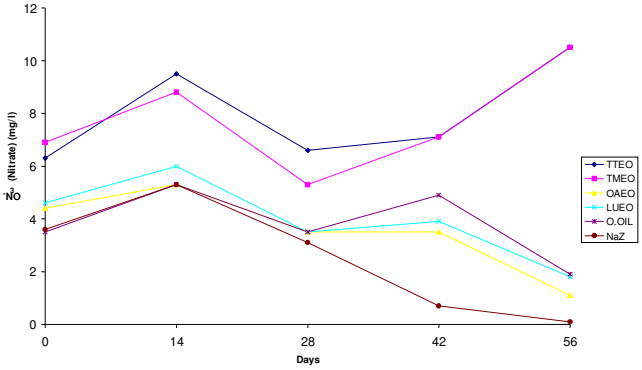

Fig 9: Changes in NO_3^- (Nitrate) in test system con taining Fresh water inoculum during biodegradation of various engine oils

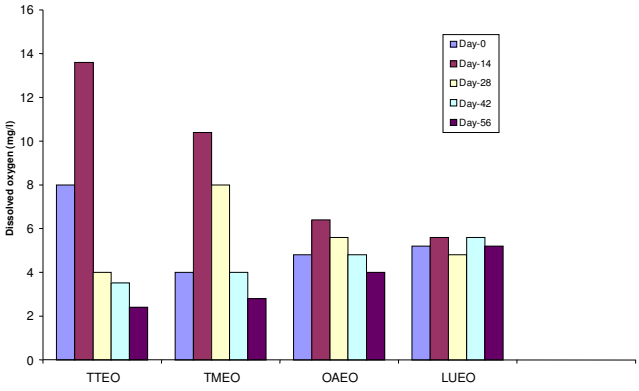

Fig . 10: Dissolved oxygen levels during biodegradation of test engine oils in fresh water inoculum

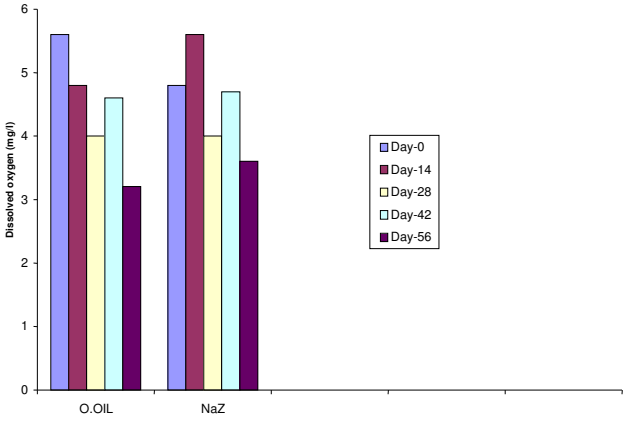

Fig. 11: Dissolved oxygen levels during biodegradation of olive oil and sodium azide in fresh water inoculum

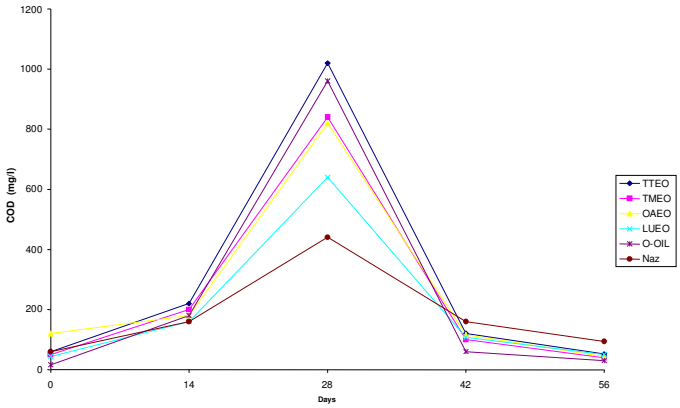

Fig. 12: Changes in COD level during biodegradation of test Engine Oils in Fresh Water inoculum

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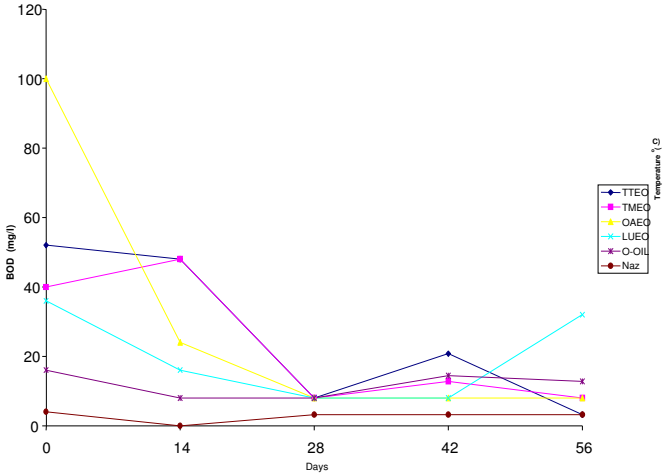

Fig 13: Changes in BOD level during biodegradation of test engine oils in fresh water inoculum

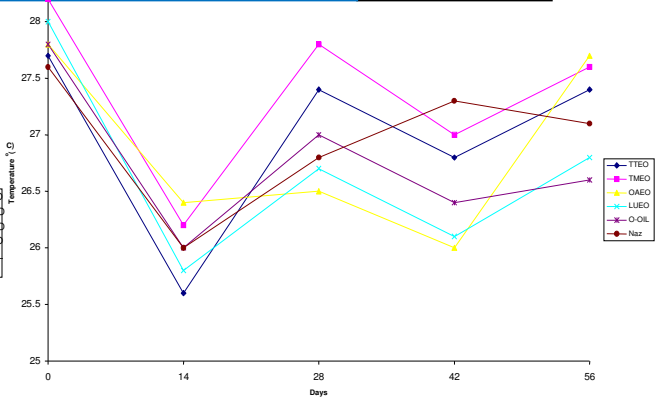

Fig.14: Changes in Temperature during biodegradation of test engine oil in fresh water inoculum

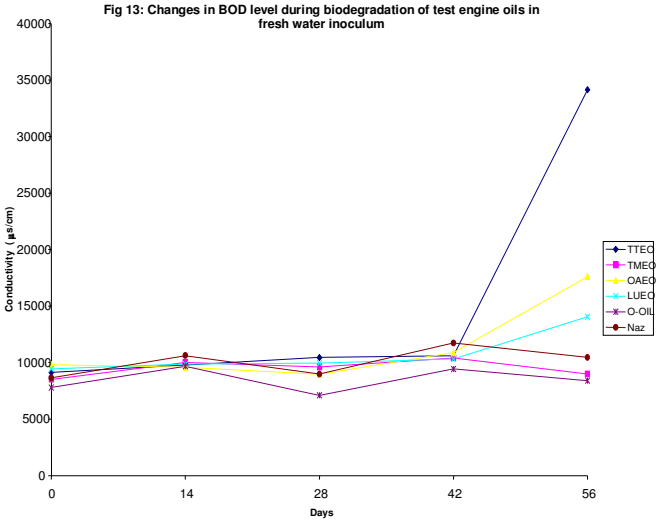

Fig. 15: Changes in conductivity level during biodegradation of test engine oils in fresh water inoculum

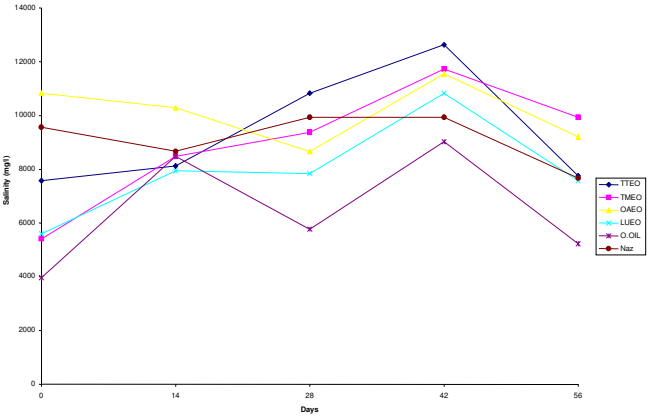

Fig.16: Changes in Salinity level during biodegradation of test engine oils in freshwater inoculum

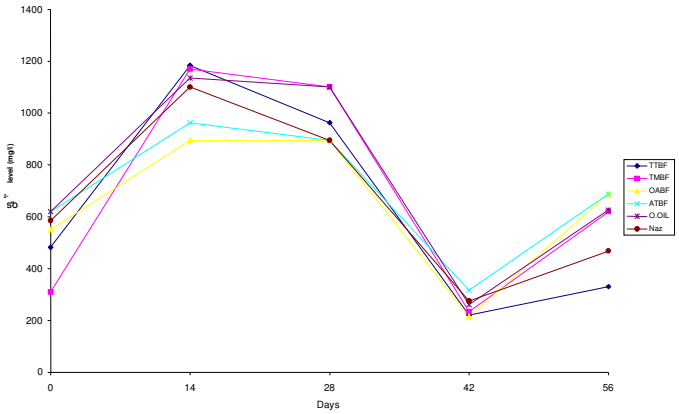

Fig. 17: Changes in SO_4^{2-} during biodegradation of test brake fluids in fresh water inoculum

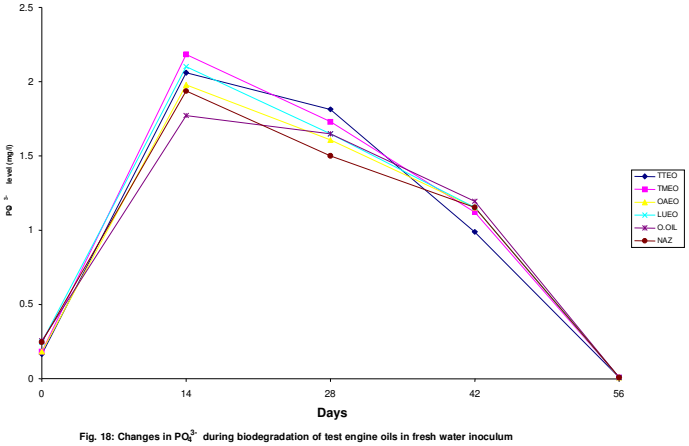

Fig. 18: Changes in PO_4^{3-} during biodegradation of test engine oils in fresh water inoculum