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BIODEGRADABILITY OF UNUSED LUBRICATING BRAKE FLUIDS IN FRESH AND MARINE ECOSYSTEM.

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ABSTRACT

The biodegradability of four unused lubricating brake fluids (Total brake fluid, Allied brake fluid, Oando brake fluid and Ate brake fluid) was carried out in fresh and marine water obtained from Isiokpo stream and Bonny river of the Niger Delta, South South Nigeria. Biodegradability, of the brake fluids were obtained after a 56 day period of incubation monitored at 2 weeks intervals using the percentage ratio of Biological Oxygen Demand (BOD) to Chemical Oxygen Demand (COD). Olive oil was used as the positive control while sodium azide served as the negative control. The results obtained showed the following rate of biodegradability in fresh water and marine water; Total brake fluid (20, 2.3 percent), Allied brake fluid (40%, 1%), Oando brake fluid (44%, 2.5%), and Ate brake fluid (13.3%, 2.1%). Statistical analysis using ANOVA, showed that there was significant difference ($P<0.05$) in the percentage mineralization of Allied brake fluid in both fresh and marine water sources. Biodegradability of the brake fluids was higher in fresh water than in the marine water. Results obtained from the viable bacterial and fungal counts (TVC) indicated higher total heterotrophic bacterial (THB) count than total fungal (TF) counts and higher hydrocarbon utilizing bacteria (HUB) counts than Hydrocarbon Utilizing Fungi (HUF) counts. Characterization and identification tests revealed that a microbial consortium comprising of the following genera; Bacillus, Pseudomonas, Proteus, Escherichia, Micrococcus, Arthrobacter, Enterobacter and Citrobacter was implicated in the biodegradation process in fresh water, while Bacillus, Pseudomonas, Staphylococcus, Enterobacter and Citrobacter was implicated in the marine water source. Similarly, the moulds encountered from the fresh water were, Aspergillus, Fusarium, Penicillium, Geotricum and Cladosporuim. The yeast was candida species. In marine water, the moulds were Aspergillus and Fusarium. Physicochemical parameters monitored were pH, salinity, BOD, COD, SO_4^{2-} and PO_4^{3-}. The study indicates that the lubricating brake fluids which are petroleum based were not readily biodegradable in fresh and marine aquatic ecosystems, hence research into production of biobased lubricating oils that are environmentally friendly, cost effective and efficient in performance is highly recommended.

Key words: Biodegradation, Mineralization, Lubricating Oils, Biodegradability, Brake fluids.

INTRODUCTION

Hydraulic fluids (brake fluids) are fluids that are used in machines that perform work through transfer of power from one location to another. In addition to transferring power, hydraulic fluids much lubricate, transfer heat and be compatible with other materials such as seals, gaskets and metal components in the system. Most brake glycol fluids used today are glycol-either based, but mineral oil and silicone based fluids are available. They contain a base fluid, metal corrosion inhibitor, and an antioxidant (Givens and Michael, 2003; Placek, 2006). Hydraulic fluids are petroleum – based (Mortier and Orszuliki, 1992). Hydraulic fluids are essentially hydrocarbons and their release to the environment are referred to as petroleum contamination which results from leaking above ground and underground storage tanks, high –pressure hydraulic lines that fractures, including carelessness of automobile technicians. All these contribute to the environmental burden (Betton, 1992). Hydraulic fluids contain variable amounts of chemical substances toxic to humans and/or other organisms in terrestrial and aquatic environments. It is estimated that, approximately 50% of all lubricating oils sold worldwide end up in the environment via total loss applications, volatility, spills or accident. More than 95% of these materials are currently mineral oil based. In view of their high ecotoxicity and low biodegradability, mineral oil based lubricating oils constitute a considerable threat to the environment (Leth and Gregesen, 2005; Manfred, 2006; Takahashi *et al*., 2007; APHA, 2012). An immediate effect of the presence of hydraulic fluid in the soil is a decrease in the population of soil microorganisms. The aquatic environment is the most severely threatened by oil pollution (Anka *et al;* 1998). When various products gets into the water bodies, they may be biodegraded by the indigenous microorganisms (Odokuma and Otakunefor 2003; Adesodu and Mbagwu, 2008; Agarry *et al.,* 2010). They may pose toxicity problems to the indigenous microflora (Okpokwasili and Odokuma, 1996; Barron *et al.,* 2003). Biodegradability, a measure of the extent by which organic compound is biodegraded or totally utilized by microorganisms resulting in the production of carbon dioxide, water, mineral salts and new biological cellular constituents (biomas). This is also known as mineralization (Atlas, 1984). It has provided a standard guide for assessing the degree of biodegradation of pollutants in a given ecosystem (ASTM, 2003). The biodegradation process relies on microorganisms, to break down chemical substances. Certain chemical structures are more susceptible to microbial break down than others, for example vegetable oils will biodegrade more rapidly than petroleum oils (Madsen, 1996; Manfred, 2006). The objective of this study therefore was to evaluate the biodegradability of four lubricating hydraulic brake fluids (Total brake fluid; Allied brake fluid, Oando brake fluid and Ate brake fluid) in fresh and marine aquatic ecosystems of the Niger Delta.

MATERIALS AND METHODS

Fresh water sample was obtained from Isiokpo stream in Ikwerre Local Government Area of Rivers State, while marine water sample was collected from Bonny river estuary of Rivers State. 4litre plastic containers were employed. Samples were capped and transported in Ice Park to the laboratory. Analyses were carried out and samples were stored in refrigerator at 4^{o}C. The lubricating hydraulic brake fluids used in this study were obtained from the company's headquarters, and major

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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 paper (Whatman No. 1) WER Bauston Ltd, London were also used. The Bonny Light crude used was obtained from Shell Petroleum Development Company (SPDC) Port Harcourt.

Preliminary Toxicity Test (Range Finding)

This was carried out to determine the non toxic concentrations of the various hydraulic brake fluids to the indegenious microflora of the fresh and marine water samples. It involved plating out in duplicates 0.lml of serial dilutions of water sample on Mineral Salt Agar (MSA) using spread plate method (APHA, 1998) containing different concentrations of brake fluids and incubating at room temperature (28 ± 2^0C) for 48h. Concentrations of the test hydraulic brake fluids employed were 100mg/l, 10mg/l, 1.0mg/l, 0.lmg/l and 0.0lmg/l respectively. This was performed by a ten fold serial dilution of each brake fluid. Aseptically, 1.0ml of brake fluid was transferred into the 10^{-1} flask containing 100ml of water source. This was thoroughly mixed and 1.0ml of the mixture was transferred to 10^{-2} flask containing 99ml of the water sample. The procedure was repeated up to 10^{-5} flask containing 99ml of water source. Enumeration of colonies was carried out after incubation at room temperature (28 ± 2^0C) for 48h (Table 1).

Enumeration of Microbial Populations

The Total Heterotrophic Bacterial (THB) count of water samples and total viable bacterial count (TVC) during the preliminary toxicity test were carried out on nutrient agar (oxoid) using the spread plate method (APHA, 1998). Plates were properly labeled and incubated at 37^0C for 24h after which the plates were examined for colony formation and enumeration. The Hydrocarbon Utilizing Bacterial (HUB) count of water samples was performed in duplicates on MSA of Mills *et al.,* (1978) as modified by Okpokwasili and Odokuma (1990). Sterile filter papers (Whatman No. 1) saturated with Bonny light crude oil were aseptically placed on the inside cover of each plate and kept in an inverted position and incubated at 30^0C for 48h. The same method was employed for Hydrocarbon Utilizing Fungi (HUF) counts, using PDA and incorporating with sterile filter paper saturated with Bonny light crude oil incubating at 30^0C for 5–7 days.

The initial day THB count of biodegradation test set up were enumerated by spread plate method on MSA plates. Plating was carried out by plating 0.lml of serial dilution in duplicates and subsequent THB counts at day (14, 28, 42 and 56), were carried out as earlier described. The initial day (day 0) TF count population of test set up as well as day 14, 28 42 and 56 were estimated by spread plate method on PDA plates in duplicates as earlier described for enumeration of HUB and HUF for the natural water sources were employed.

Biodegradation test were carried out in 12 2L Erlenmeyer flasks. To each flask was added 900ml MSB and sterilized by autoclaving at 121^0C for 15mins. After cooling appropriate concentration of test hydraulic brake fluid was aseptically added based on the preliminary toxicity tests (range finding) carried out (Table 3). (TTBF – 1.0mg/l, ALBF – 1.0mg/l, OABF – 0.01mg/L, ATBF – 1.0mg/L, and 0.1mg/L

of olive oil for positive control). To the negative control 2g of sodium azide was added. To each set up, 100ml of water sample was aseptically added as the inoculum.

The entire tests set up were labeled as shown in Table 2. Repeated samplings for microbiological and physicochemical analyses were carried out at initial day and subsequently at 2 weeks intervals for a 56 day monitoring.

Biodegradability for a 56 day incubation period was monitored using the percentage ratio of BOD to COD. The BOD for each test set up was monitored using method adopted from Stewart *et al.,* (1974) at day 0, 14, 28 42 and day 56. The COD for each set up were determined by method adopted from Stewart *et al.,* (1974) at initial day, 14, 28, 42 and day 56.

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 0, 14, 28, 42 and 56 to COD at day 0.

$$\text{Mineralization} = \frac{BOD}{COD \quad at \quad day \quad 0} x100\%$$

Physicochemical parameters of water samples and biodegradation experimental set up analyzed were pH, salinity, BOD, COD, TOC, DO, NO_3^{2-} SO_4^{2-} and PO_4^{3-}. 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 Gram staining. The isolates were further subjected to series of biochemical tests for identification and characterization using the determination schemes Holt *et al;* (1994). Similarly, moulds were identified through their cultural as well as microscopic features.

Statistical Analysis

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

RESULTS

The result of physicochemical characteristics of the fresh and marine aquatic systems used in the study before biodegradation test is presented in Table 1. The THB and TF counts of fresh and marine water sources as well as HUB and HUF are presented in Table 2. Forty eight percent of the THB populations enumerated from fresh water sample were HUB, while 25% of the THF count represented HUF. On the other hand, in the marine water sample 9% of the THB, were HUB, and 7% of the THF enumerated were HUF the result showed that the fresh water sample had higher density of HUB than the marine water sample.

The THB, TF, HUB and HUF counts during the monitoring period are illustrated in Figs. (1 – 8). Generally, the growth profile of brake fluid samples in fresh and marine

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water followed the same pattern. They increased exponentially from day 0 to day 14, gradually increased thereafter to day 28, and declined sharply from day 42 to day 56 (Figs.1 – 8).

Table 1: Physicochemical parameters of habitat water samples.

Parameters	**Values**	
	Freshwater	Marine water
pH	6.39	4.55
Salinity (mg/L)	32.5	35,262.5
BOD (mg/L)	8	16.64
COD mg/L	7.2	20.0
SO_4^{2-}(mg/L)	68.80	688.89
PO_4^{3-} (mg/L)	0.0825	0.00824

Table 2: Bacterial and fungal counts of habitat water samples

Type of count	Fresh water		Marine water	
	(cfu/ml)	(%) count	Cfu/ml	(%) count
THB	4.7×10^3	52	5.6×10^3	91
HUB	2.3×10^3	48	5.0×10^2	9
TFC	8.0×10^2	75	3.0×10^2	93
HUF	2.0×10^2	25	2.0×10	7

Table 3: TVC (cfu/ml) during preliminary toxicity testing of the various brake fluids samples in fresh and marine water samples after 48h incubation at room temperature (28 ± 2^0C).

	Test hydraulic brake fluid									
Conc. (mg/l)	TTBF		ALBF		OABF		ATBF		Olive oil	
	FW	MW	FW	MW	FW	MW	FW	MW	FW	MW
100	$3.0x10^4$	$1.50x10^4$	$9.0x10^3$	$1.0x10^4$	$4.0x10^3$	$8.0x10^3$	$1.2x10^3$	$3.2x10^3$	$4.3x10^4$	$6.0x10^3$
10	$4.0x10^4$	$7.0x10^3$	$3.6x10^4$	$5.6x10^4$	$6.0x10^3$	$9.0x10^3$	$6.0x10^3$	$8.0x10^3$	$9.0x10^4$	$6.4x10^4$
1.0	$8.9x10^5$	$1.15x10^5$	$1.35x10^5$	$2.3x10^5$	$1.2x10^4$	$3.2x10^4$	$1.25x10^5$	$2.25x10^5$	$3.2x10^5$	$7.3x10^4$
0.1	$5.0x10^4$	$4.0x10^4$	$4.0x10^3$	$6.0x10^3$	$6.3x10^4$	$6.3x10^4$	$3.0x10^3$	$5.0x10^3$	$7.8x10^5$	$8.5x10^5$
0.01	$4.0x10^3$	$6.0x10^3$	$4.0x10^3$	$6.0x10^3$	$7.5x10^4$	$9.5x10^3$	$5.0x10^4$	$8.0x10^3$	$8.6x10^5$	$9.7x10^5$

Table 4: Biodegradation test set up

Brake fluid Test code	Fresh Water	Marine Water	Description
TTBF	TTBF FW	TTBF MW	MSB+ Water sample + Total brake fluid
OABF	OABF FW	OABF MW	MSB+ Water sample + Oando brake fluid
ALBF	ALBF FW	ALBF MW	MSB+ Water sample + Allied brake fluid
ATBF	ATBF FW	ATBF MW	MSB+ Water sample + Ate brake fluid
O.Oil	O.Oil FW	O.Oil MW	MSB+ Water sample + Olive oil (+ve control)
Naz	Naz FW	Naz MW	MSB+ Water sample + Sodium azide (-ve control)

MSB = Mineral salt broth, FW = fresh water, MW = Marine water.

Table 5: Percentage mineralization (Biodegradability) of hydraulic brake fluid samples at day 56 in fresh and marine water samples.

Brake fluid Code	Biodegradability	
	Fresh Water (%)	Marine Water (%)
TTBF = Total brake fluid	20	2.3
ALBF = Allied brake fluid	40	1.6
OABF = Oando brake fluid	44	2.5
ATBF = Ate brake fluid control	13.3	2.1
O.Oil – Olive Oil (+ve control)	80	5.3
Naz – Sodium azide (-ve control)	5.3	1.2

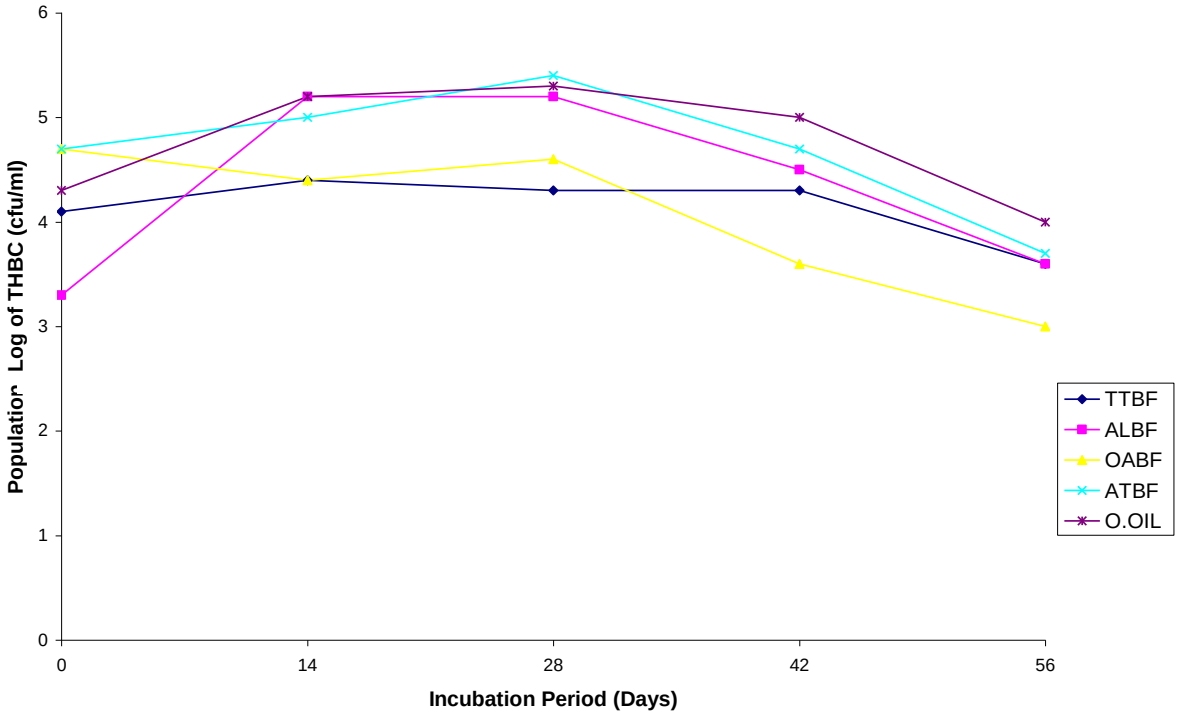

Fig 1 Growth Profile of THB in Fresh water inoculum during Biodegradation of Test Brake Fluids

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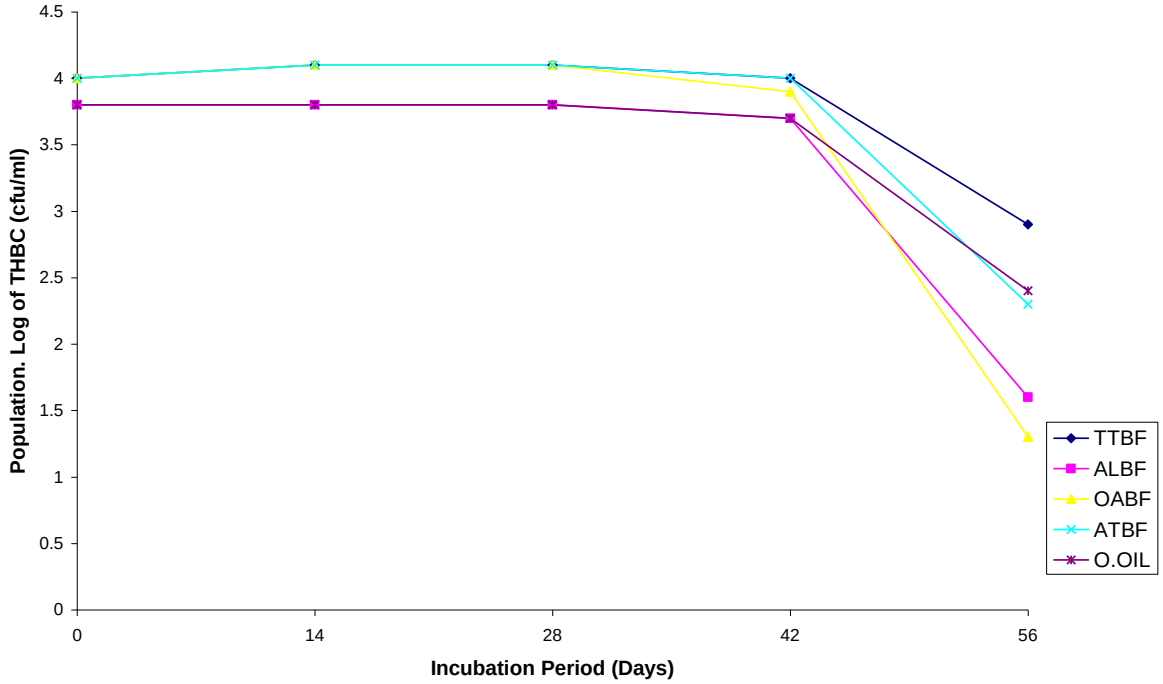

Fig 2 Growth Profile of THB in Marine Water inoculm during Biodegradation of Test Brake Fluid

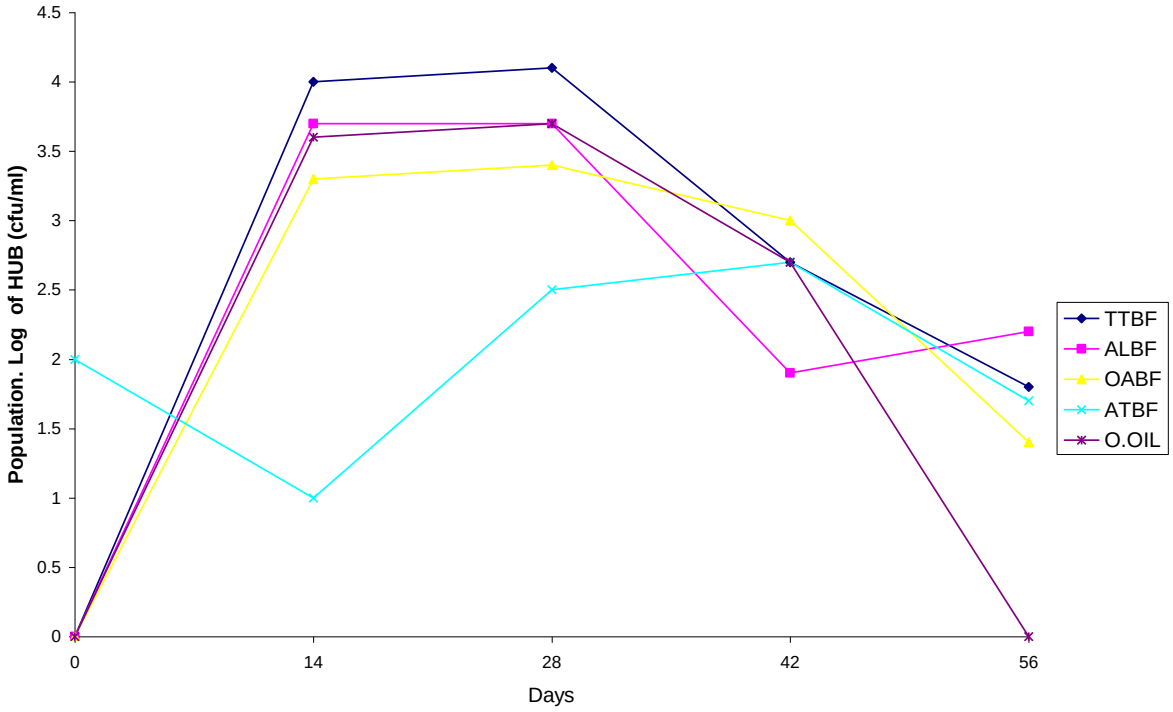

Fig 3 Growth Profile of HUB in Fresh water inoculum during biodegradation of test brake fluids.

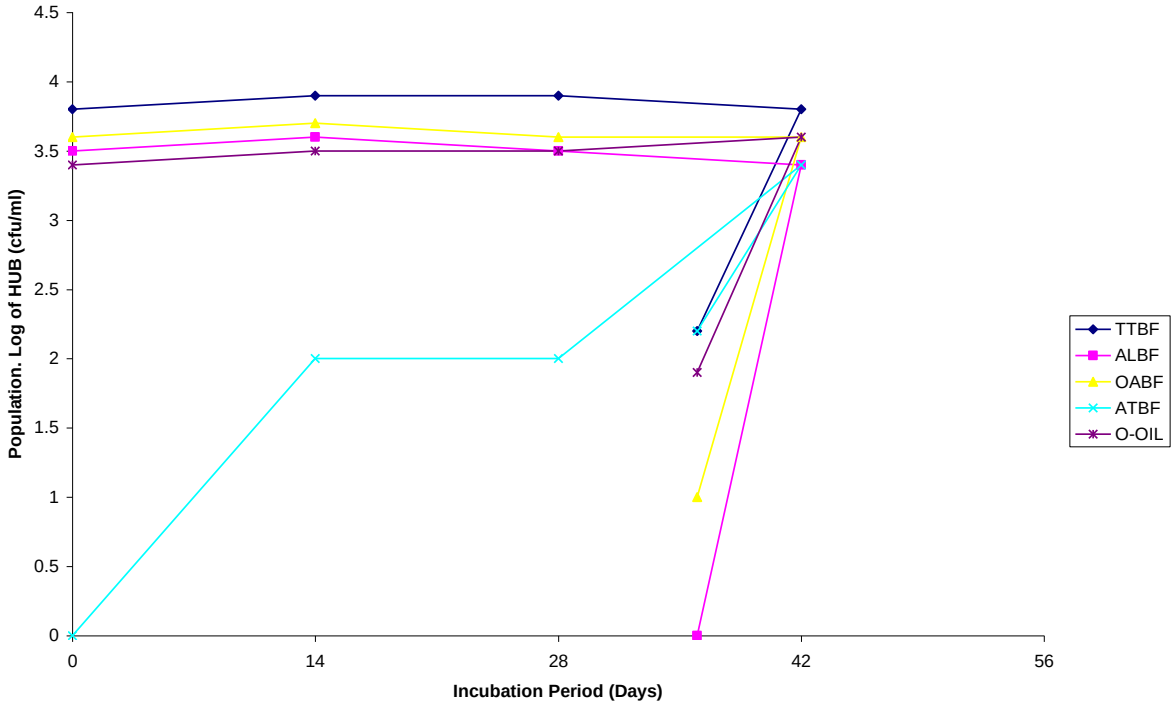

Fig 4 Growth Profile of HUB in marine water inoculum during biodegradation of test Brake fluids.

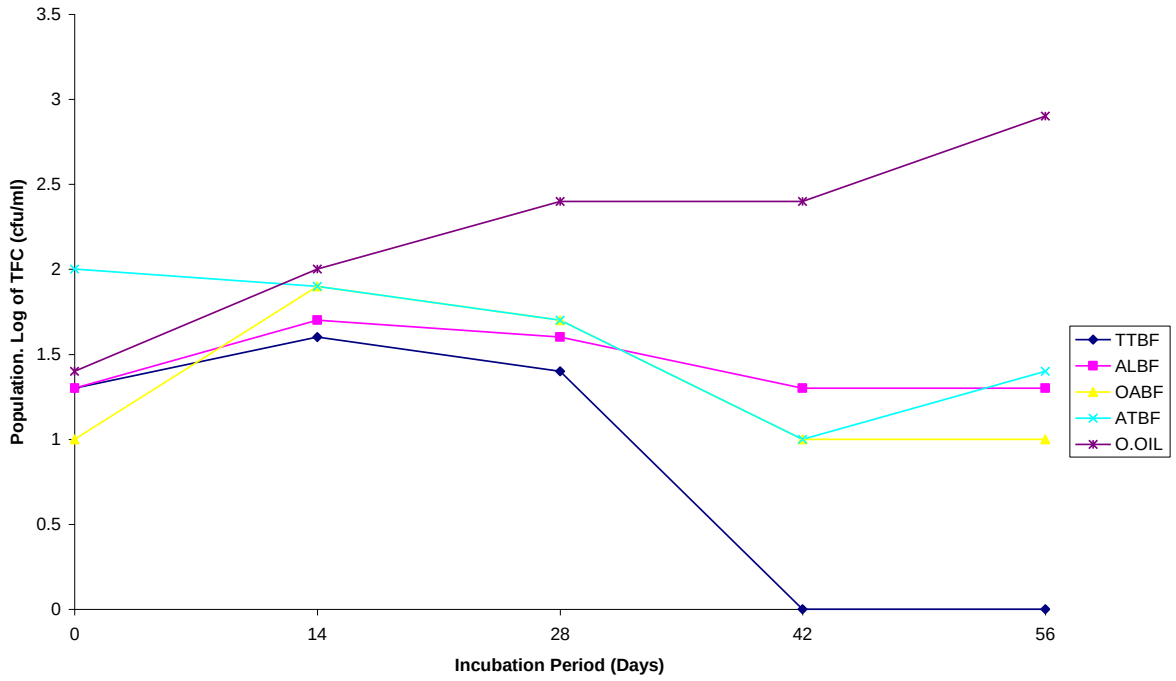

Fig 5 Growth Profile of TFC in test sytem containing Fresh Water inoculum during biodegradation of Test Brake Fluids

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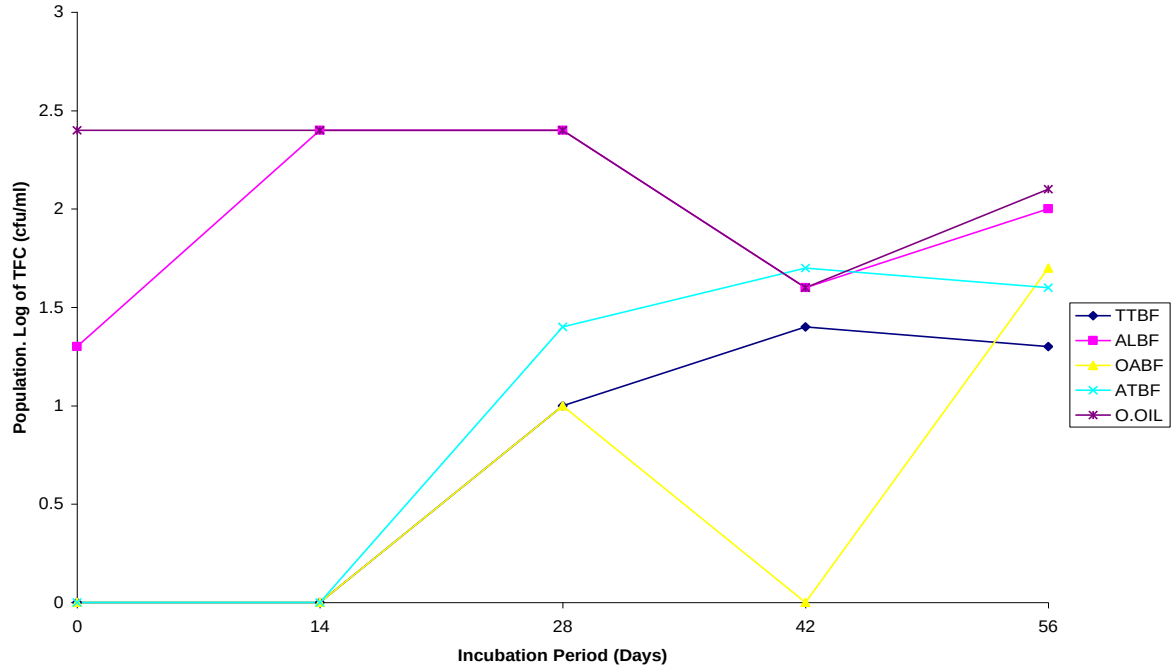

Fig 6 Growth Profile of TFC in test system containing Marine water inoculum during biodegradation of test Brake fluids.

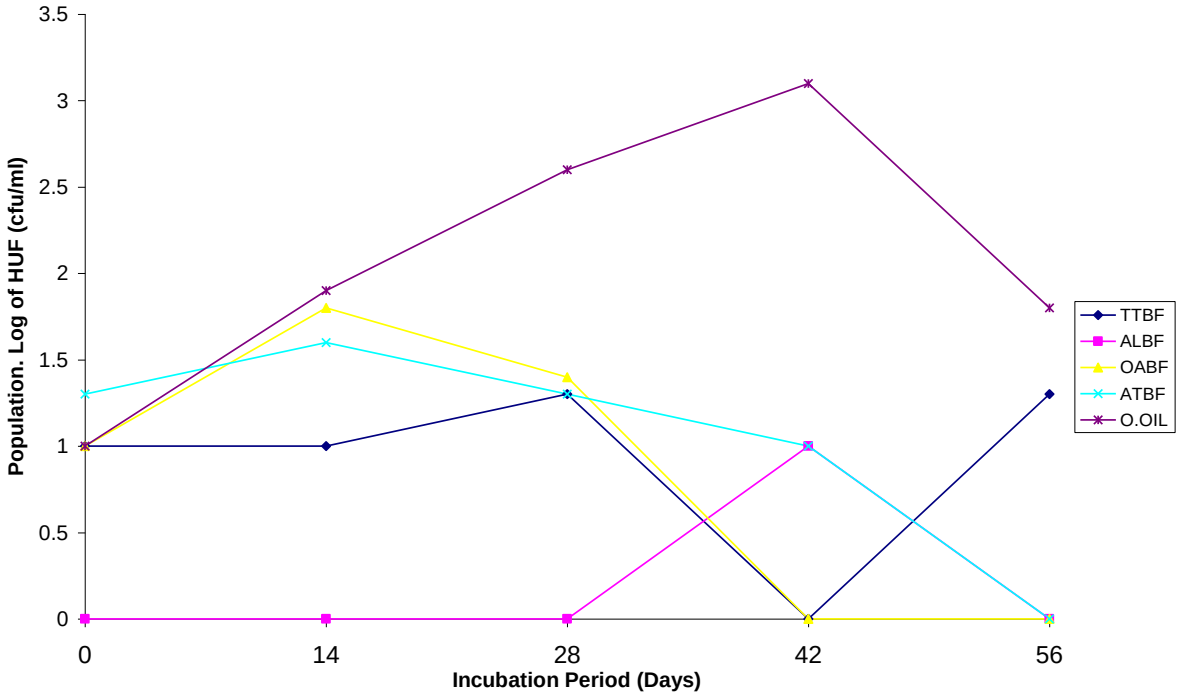

Fig 7: Growth Profile of HUF in test system containing fresh water inoculum during biodegradation of Test Brake Fluids.

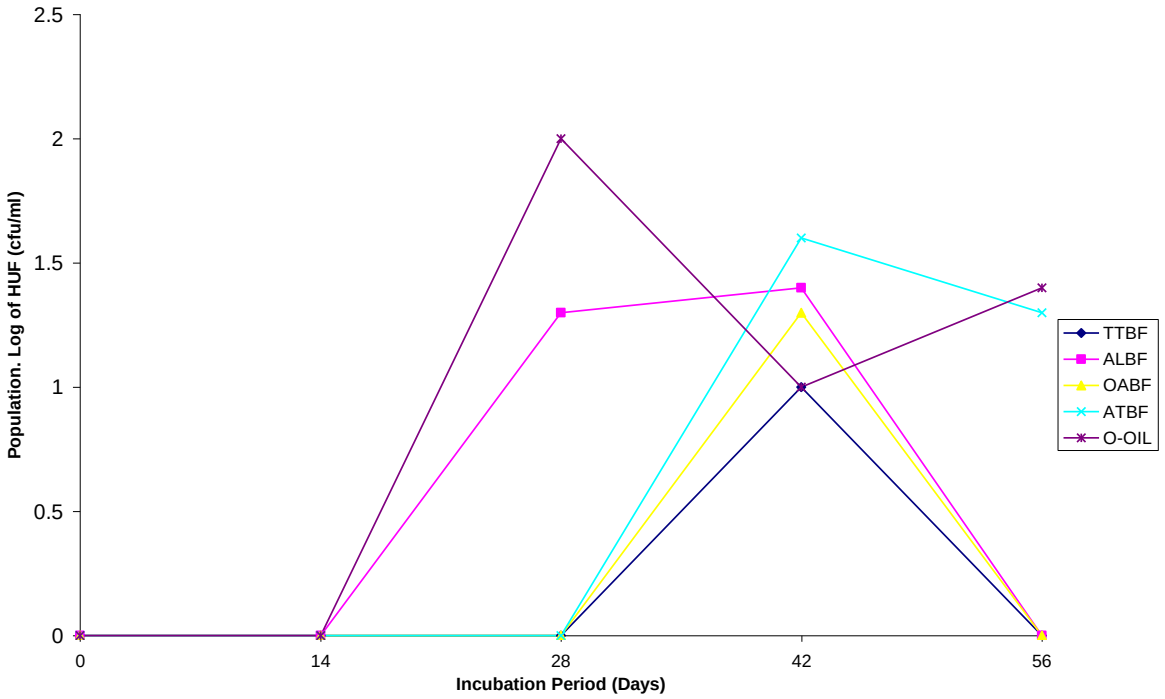

Fig 8 Growth Profile of HUF in test system containing fresh water inoculum during biodegradation of test Brake Fluids

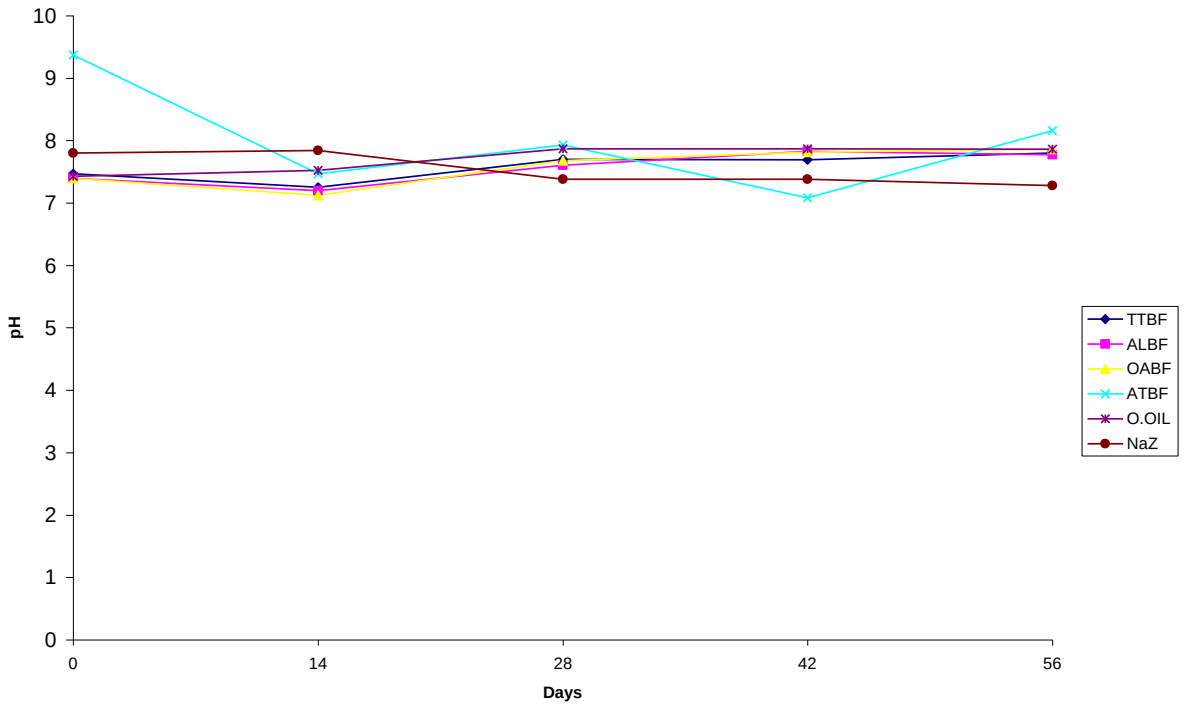

Fig. 9 Changes in pH level in test system containing Fresh water inoculum during biodegradation of various brake fluids

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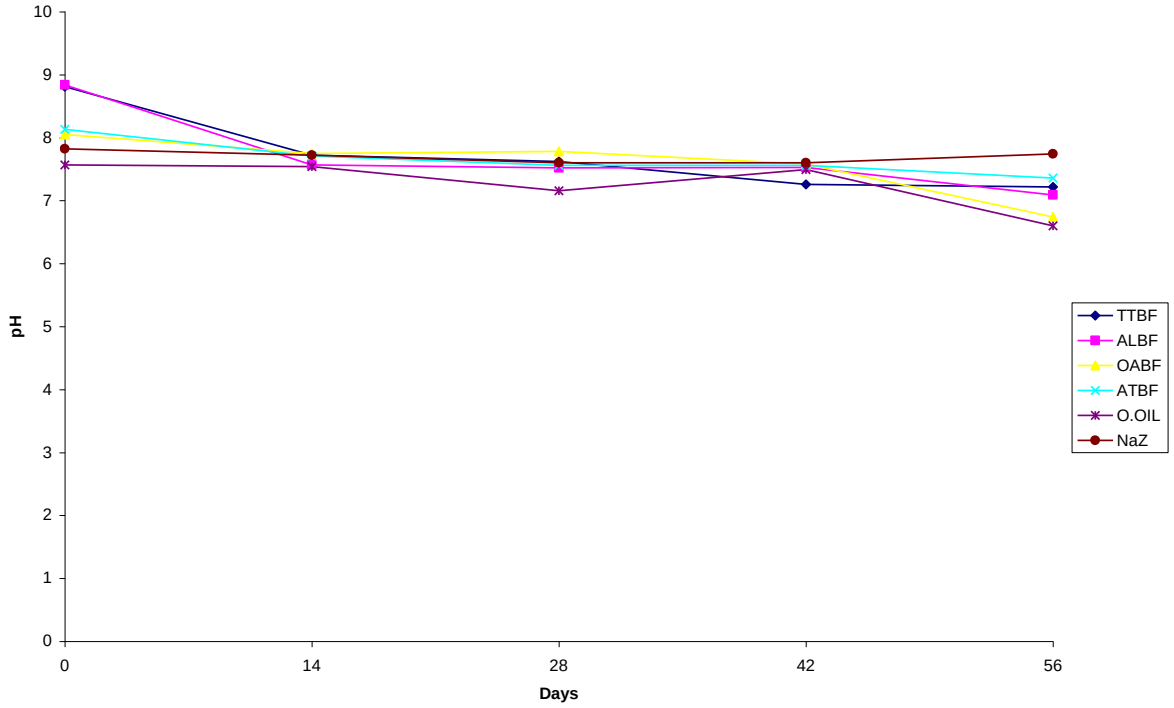

Fig. 10 Changes in pH level in test system containing marine water inoculum during biodegradation of various brake fluids

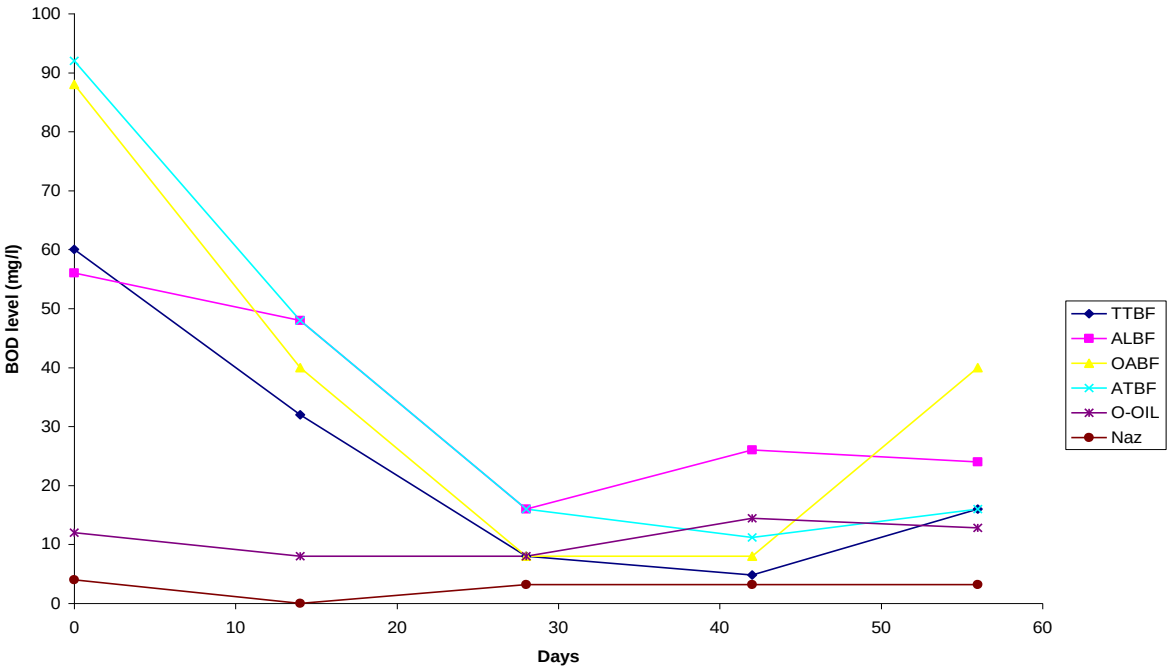

Fig 11 Changes in BOD level during Biodegradation of test Brake Fluids in Fres Water inoculum

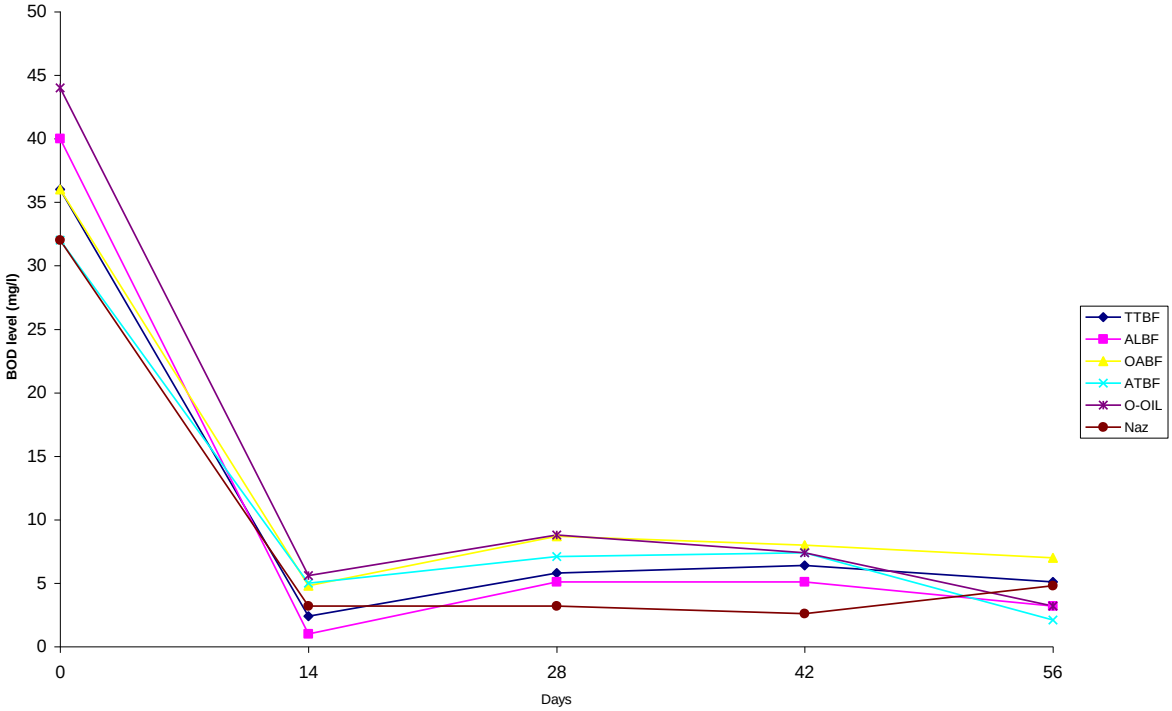

Fig 12 ıanges in BOD level during biodegradation of test Brake Fluids in Marine Water inoculum

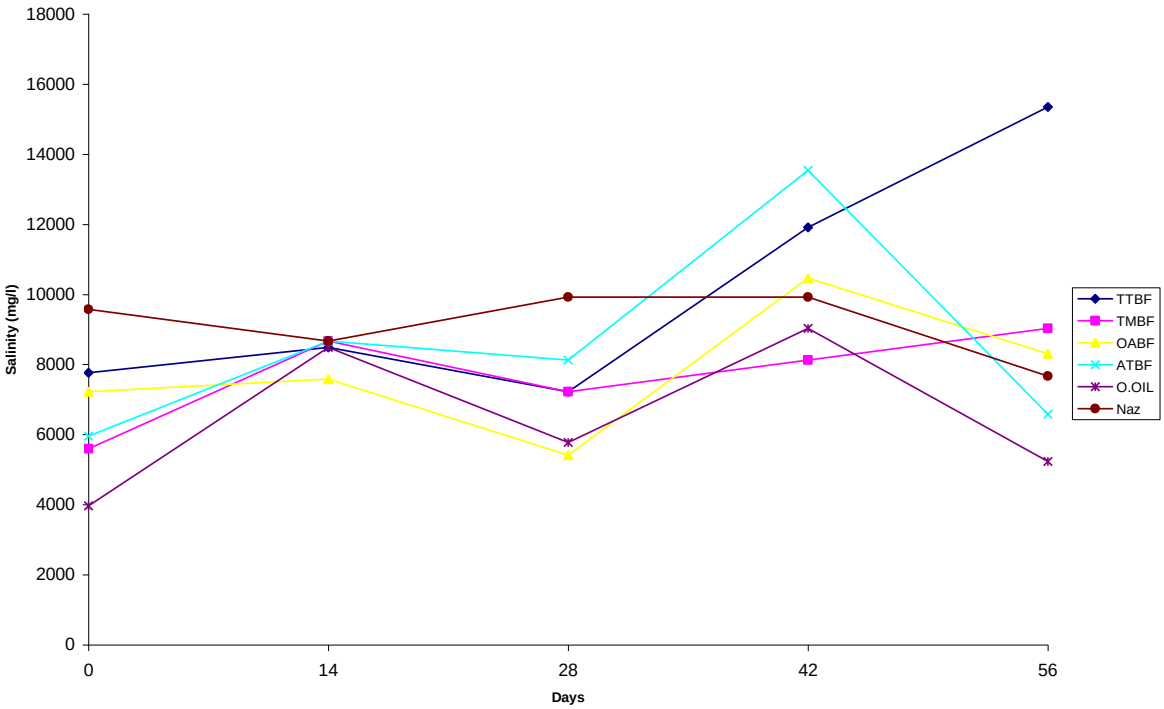

Fig. 13 hanges in Salinity level during biodegradation of test Brake Fluid in Fresh Water inoculum

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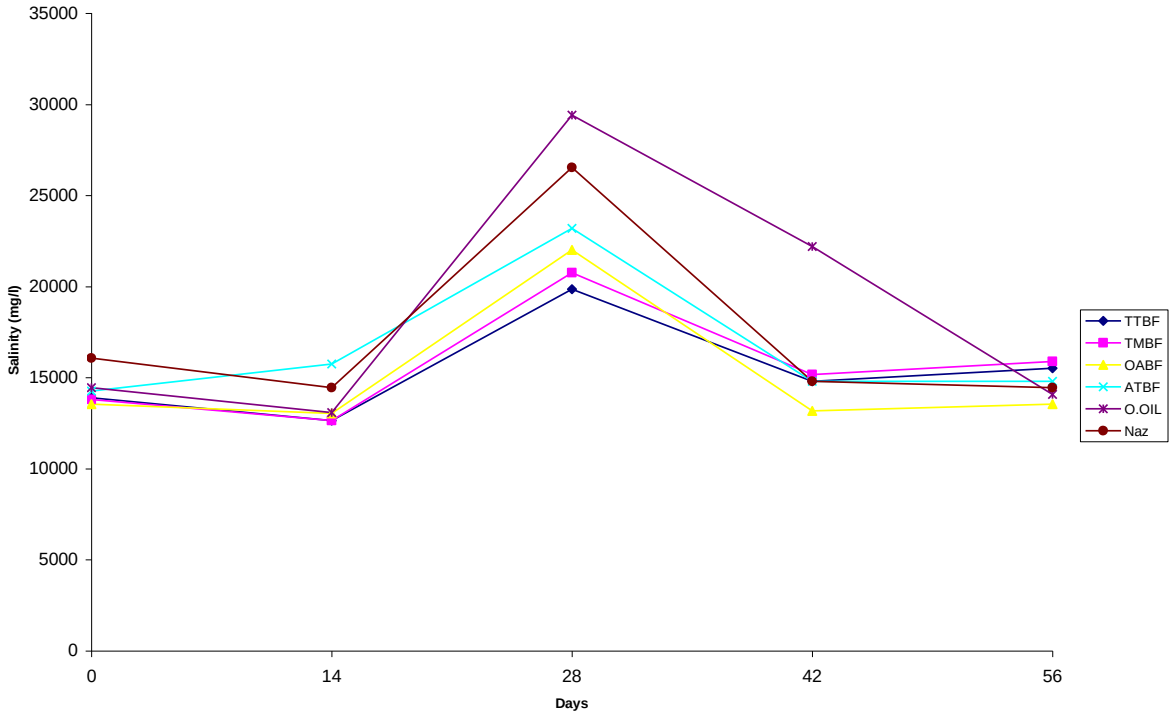

Fig. 14 Changes in Salinity level during biodegradation of test Brake Fluids in Marine Water inoculum

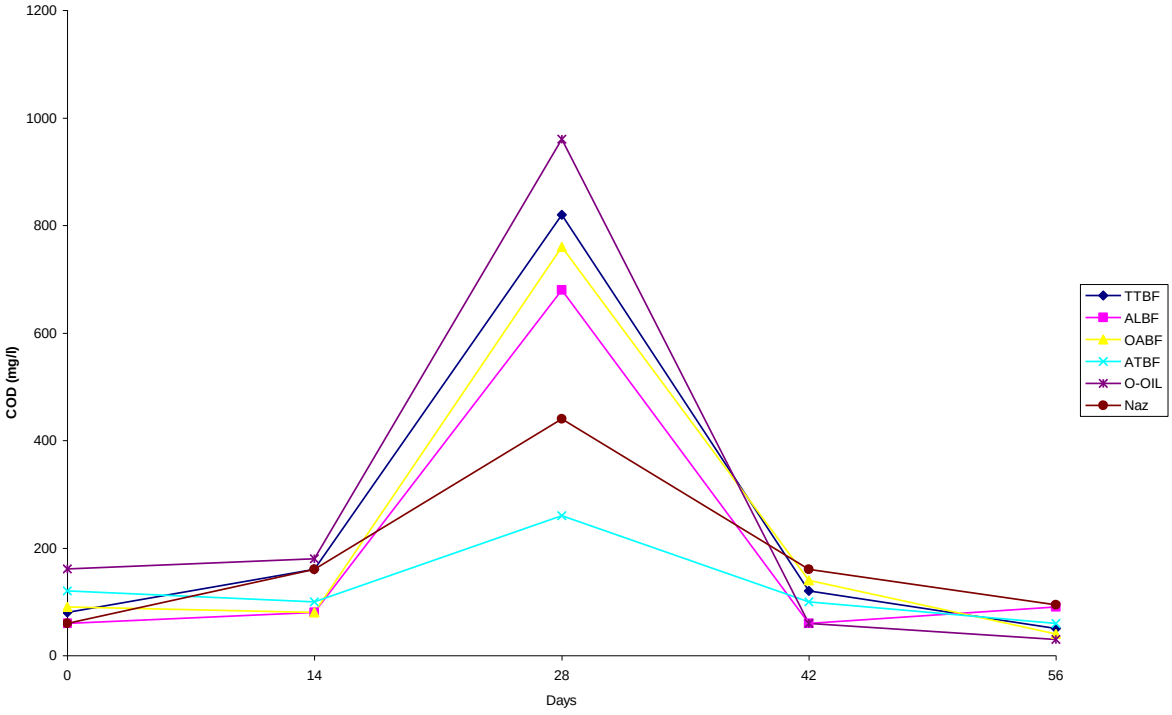

Fig. 15 Changes in COD level during biodegradation of test Brake Fluids in Fresh Water inoculum

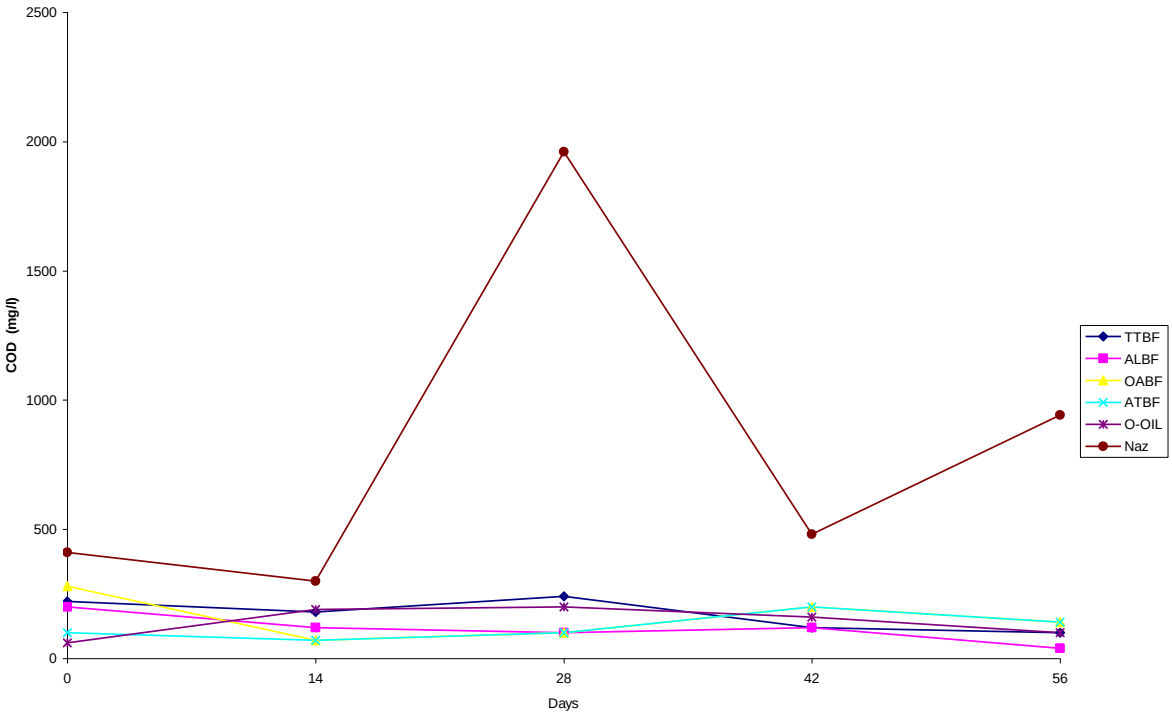

Fig. 16 hanges in COD level during biodegradation of test Brake Fluids in Marine Water inoculum

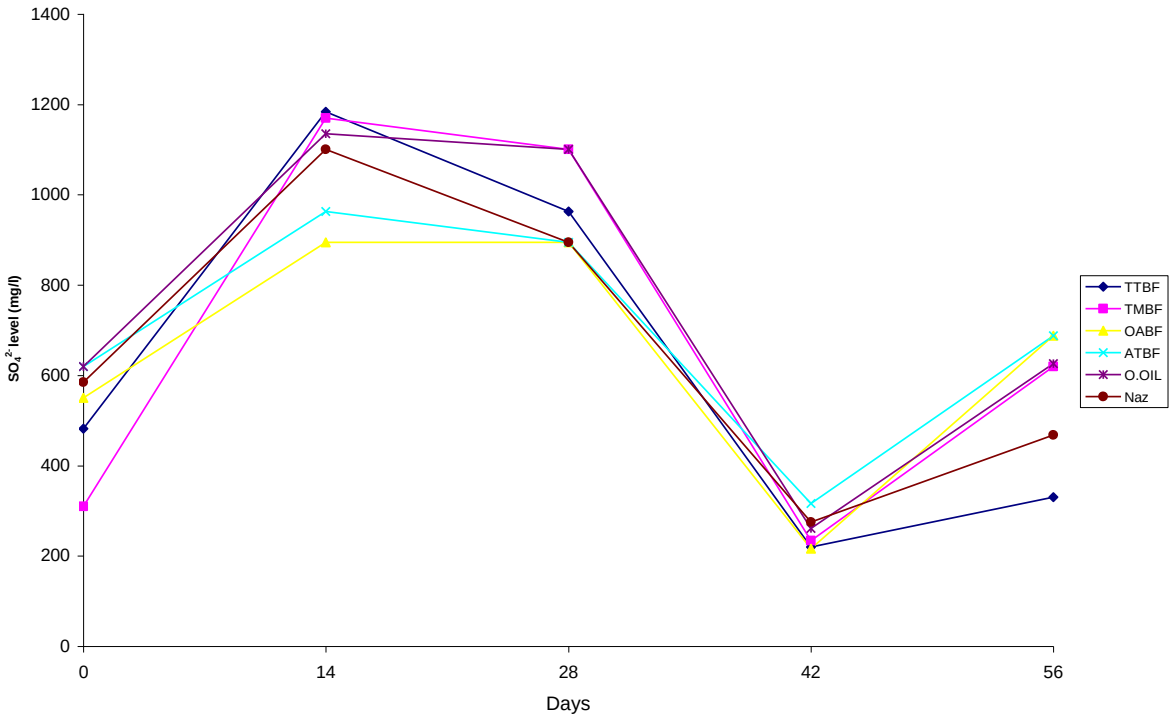

Fig. 17 hanges in SO_4^{2-} during biodegradation of test Brake Fluids in Fresh Water inoculum

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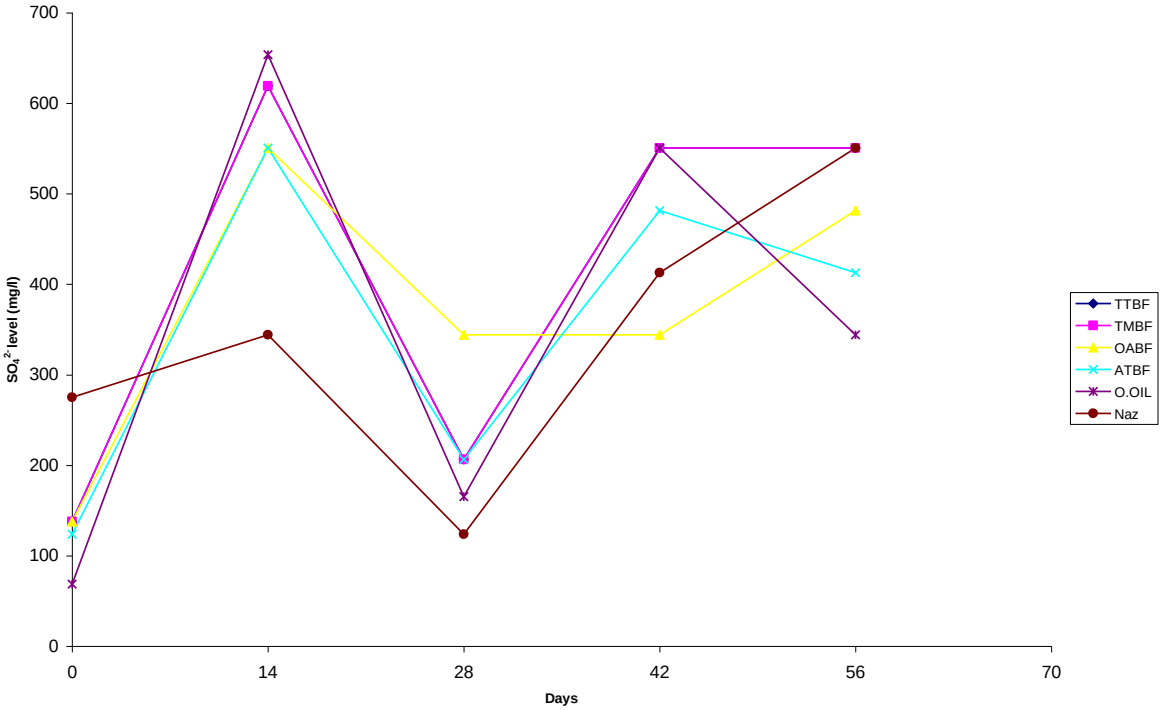

Fi 18 :hanges in SO_4^{2-} during biodegradation of test Brake Fluids in Marine Water inoculum

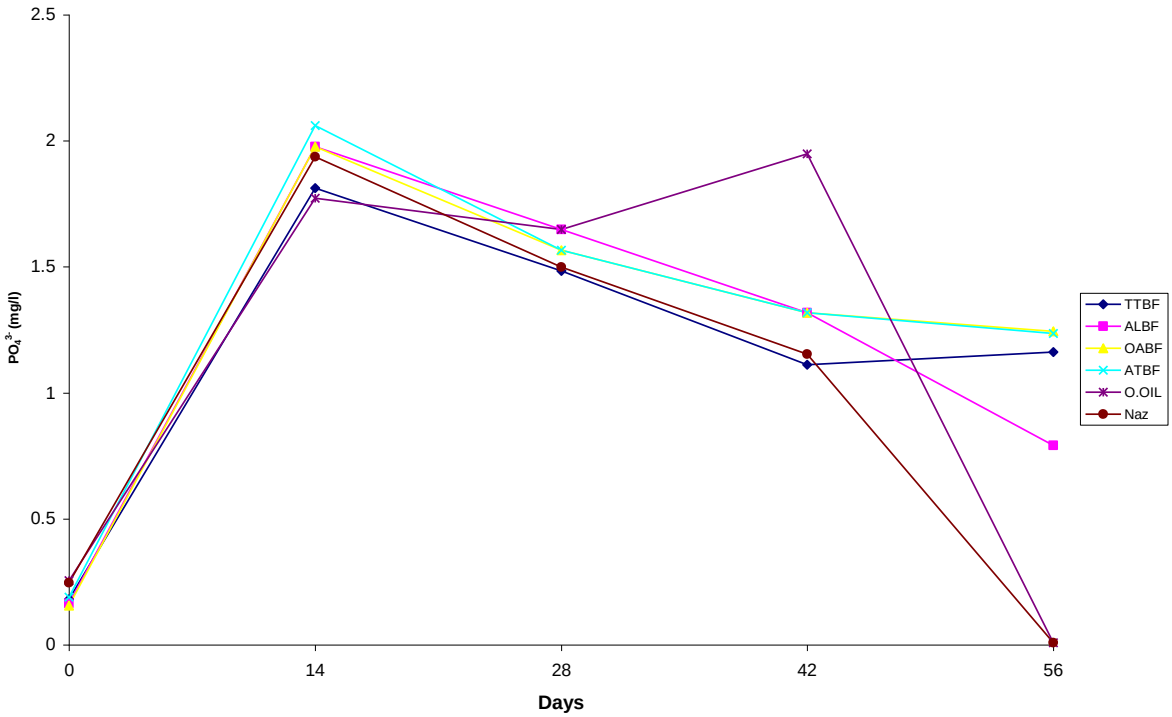

Fig. 19 :hanges in PO_4^{3-} during biodegradation of test Brake Fluids in Fresh Water inoculum

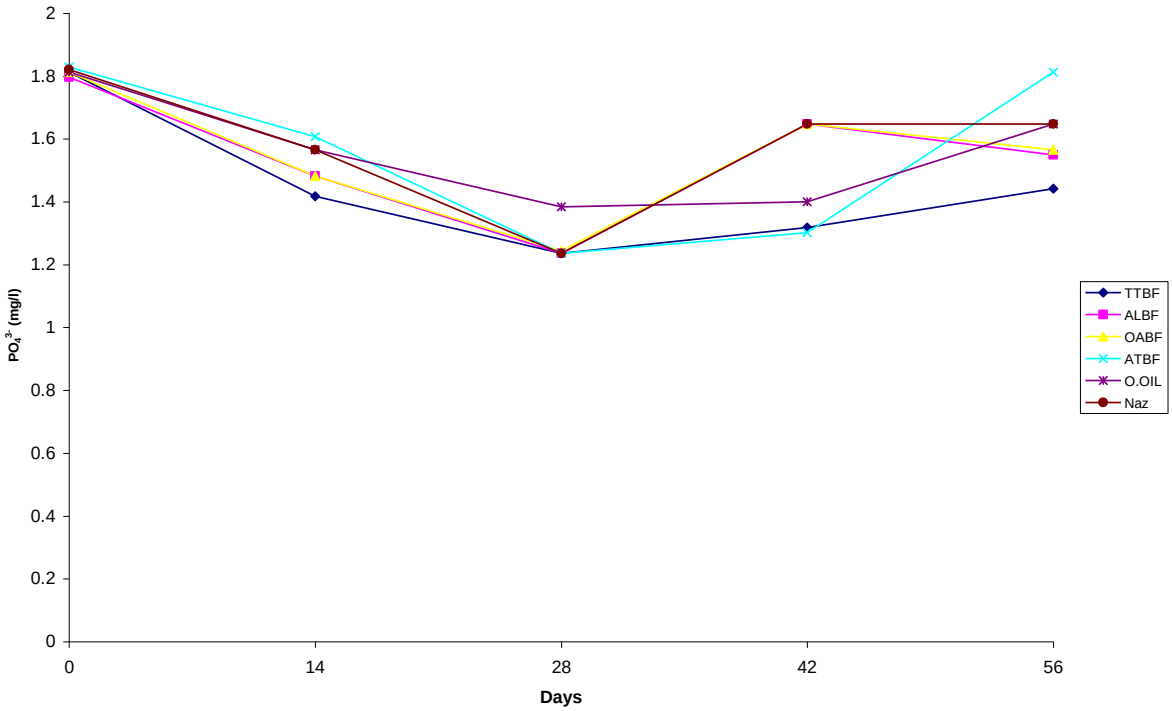

Fig. 20 Changes in PO_4^{3-} during biodegradation of test Brake Fluids in Fresh Water inoculum

Changes in physicochemical parameters during the biodegradation monitoring of hydraulic brake fluid samples are illustrated in Figs. (9 – 20). Percentage mineralizations (Biodegradability) of the various hydraulic brake fluid samples including positive and negative controls at day 56 are shown in Table 5. The result showed that Oando brake fluid had the highest value of 44% biodegradability in fresh water sample and Ate brake fluid had the least value of 20% while biodegradability of the brake fluids were generally very low in marine water sample with 2.5% for Oando brake fluid being the highest and 1.6% for Allied brake fluid being the least.

Statistical analysis results showed that there was significant difference (at 95% probability level) between the bacterial and fungal populations in fresh and marine water samples during the biodegradation process. There was significant difference in populations of THB, $F_{cal.}$ = 46.190 while = $F_{tab.}$ = 2.620. HUF was significantly greater in fresh water than in marine during the biodegradation of the hydraulic brake fluids, $F_{cal.}$= 7.411 while $F_{tab.}$ = 2.620. For physicochemical parameters, statistical analysis showed that there were significant differences ($P < 0.05$) in changes of the following parameters during the biodegradability monitoring of all the four brake fluids in fresh and marine water samples; salinity, sulphate, and dissolved oxygen. Only ATBF(Ate brake fluid) was significantly different in the changes when compared in fresh and marine water samples ($F_{cal.}$ = 5.912 while $F_{tab.}$ = 5.317). There were no significant differences in the changes of the following parameters; pH, BOD, COD, TOC and PO_4^{3-}.

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DISCUSSION

The results of the microbial counts during the biodegradation process (growth profile) showed the exponential growth of both bacterial and fungi from day O to day 14 (Figs.1 – 8) indicates that the hydraulic brake fluids were being metabolized as sole sources of carbon and energy. The decline in population of THB, HUB and TF counts from day 42 to 56 may be due to nutrient exhaustion with possible accumulation of toxic metabolites in the media, which marked the on set of stationary and death phases. The bacterial loads of fresh and marine water samples (Table 1) indicated that the fresh water had higher HUB and HUF counts than the marine water sample. This could have accounted for the higher biodegradability rates of the brake fluids in the fresh water system. The relative few or no growth recorded in the negative control test system was due to the application of sodium azide as a biocide, which eliminated microorganisms in both water samples. The results obtained from the characterization and identification of brake fluid utilizing bacterial and fungal isolates reveal the following genera from fresh water source; *Bacillus, Pseudomonas, Proteus, Escherichia, Microscoccus, Enterobacter,* and *Citrobacter,* while *Bacillus, Pseudomonas, Staphylococcus, Enterobacter,* and *Citrobacter,* were implicated from the marine water source. The fungal genera implicated from the fresh water source were *Aspergillus, Fusarium, Penicillium, Geotrichum and Cladosporium.* Yeast was *Candida* sp. In the marine water source, the moulds encountered were *Aspergillus, and Fusarium.* Some of the organisms isolated in this study were implicated in earlier studies as being able to degrade car engine lubricating oil by Ekwenye and Ike (2007). They included *Bacillus, Pseudomonas, Micrococcus* and *Citrobacter* while the fungi included *Aspergillus, Cladosporium and Penicillium.* A comparison of data in Table 2 (microbial counts) and Figs. 1 – 8 (growth profile), suggests that bacteria played a greater role in the biodegradation of hydraulic brake fluids than fungi; hence the higher THB and HUB counts than THF and FUB counts throughout the test period in both fresh and marine water samples. Benneth and Faison (1997) attributed the dominance of bacterial degraders to the fact that fungi are more proficient at co-metabolism and bioaccumulation than using pollutants as sole carbon source. The proportion of the microbial population capable of hydrocarbon degradation in aquatic habitat is influenced by a number of factors which include; physical, environmental and chemical factors (Leahy and Cowel, 1990; Ward *et al.,* 2003; Van Hamme *el al.,* 2003) and biological factors such as microbial consortium (Mishra *et al.,* 2001; Adekunle and Oluyode, 2005; adaptation, Maleszak *et al.,* 2004) and genetic enrichment and seeding (Onwura and Nwuke, 2004; Hamamura *et al.,* 2001).

The values of the physicochemical parameters of the habitat water sources (Table 1) showed differences in their characteristics. The values of salinity of 32.5mg/l for fresh water and 36,262.5 mg/L for marine water gave a mark difference between the two water sources. Since the NaCl concentration, salinity and sulphate are a function of chloride ion concentration, it is not surprising that the values of salinity and sulphate are higher in marine water than

in fresh water sample. These results indicate that dominant microflora in the fresh water source would be non-halophytic and halophytic organisms in the marine water source which had low pH value of 4.55. Phosphate is scarce in seawater (Nester *et al.,* 2001). Sea water usually contains fewer organisms than fresh water, but higher halophytic organisms, those that prefer or require high salt concentration thrive in it (Nester *et al.,* 2001). The value of phosphate of the marine water source showed low value of 0.00824 and 0.8824 mg/l, while that of fresh water was 0.0824. These mark differences in the physicochemical results of the two water samples may have influenced to large extent the biodegradability rates in both aquatic environments (Nester *et al.,* 2001). Changes in pH (Figs. 9 and 10) during the biodegradation period showed pH of nearly neutrality in most of the test systems in both fresh and marine water samples. Most important hydrocarbon degrading heterotrophic bacteria and fungi perform best when pH is near neutral. However, fungi are known to be more tolerant of acidic conditions (Delyan *et al.,* 1990). Changes in salinity showed higher values in marine water than in fresh water, with gradual increase and decline from day 42 to day 56 (Figs. 13 – 14). The result of percentage mineralization (Table 5) showed that biodegradability was higher in fresh water than marine water. Hyper salinity will result in the decrease in microbial metabolic rates. Okpokwasili and Odokuma (1990) have observed that biodegradation of oil spill dispersants decreased with increase in salinity in artificial media.

The BOD and COD values of the two water bodies were 8mg/L and 7.2mg /L for fresh water while 16.64mg/L and 20.0mg/L in marine water, indicating higher BOD and COD in the marine system; this implies that the marine water sample was potentially polluted than the fresh water sample (Osubanjo, 1992). The decrease in BOD in the various test systems in both fresh and marine water samples (except in the negative control tests) showed that the amount of degradable organic materials present in the water samples were being utilized by the microorganisms (Figs. 11 – 12). BOD represents the amount of oxygen required for the microbial decomposition of organic matter in water sample. It is roughly proportional to the amount of degradable organic matter present in the water sample (Pelczer *et al.,* 1982; Nester *et al*., 2001).

The changes in COD in fresh and marine water samples showed that the highest values were recorded on day 28 during the degradation 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 values of COD recorded on day 28 in the negative control in marine and fresh water during the incubation period may be due to chemical reactions in the systems.

Other chemical parameters that showed substantial decreases at one period or the other in course of the biodegradability monitoring were; SO_4^{2-} and PO_4^{3-} (Figs. 17 – 20). Generally, these reductions indicated that the degraders were utilizing some of the metallic salts of these anions as sources of nitrogen, sulphur and phosphorus respectively. Similar observations have been

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reported by Odokuma and Akpokodje (2004); Odokuma and Okara (2005).

The results of percentage mineralization (biodegradability) Table 5, showed that biodegradability of the hydraulic brake fluids were higher in fresh water than in marine water sample. The high salinity of the marine water source may have affected degradation of the brake fluids by microorganism in the marine water sample. Similar observations had been made by Okpokwasili and Odokuma (1990). It was observed that Oando brake fluid had the highest degradability with 44% followed by Allied brake fluid (40%), Total brake fluid (20%) and least Ate brake fluid (13.3%) in the fresh water sample while in the marine water sample, degradability was very low with 2.5% for Oando brake fluid being the highest while Allied brake fluid with 1.6% was the least. The olive Oil (positive control) had 80% degradability in fresh water sample. The Olive Oil being a vegetable Oil was more degradable than the petroleum based hydraulic fluids used in this study. Lubricants and hydraulic fluids based on plant oils are rapidly and completely biodegradable, and are of low ecotoxicity, display excellent tribiological properties and generally have very high viscosity indices and flashpoints (Manfred, 2006). The percentage mineralization values observed in negative controls of 5.3% in fresh water sample and 1.2% in marine water sample could be attributed to natural attenuation processes other than biodegradation since microorganisms were eliminated by application of biocide. The minor decreases observed in some of the physicochemical parameters in the negative controls (SO_4^{2-} and PO^{3-}_4) suggested the involvement of non-biological factors, possibly photo-oxidation. The differences in the rate of biodegradability of the hydraulic brake fluids used in this study, can be attributed to the following factors; the total viable counts of microbial populations of the two aquatic systems, the physicochemical parameters of the aquatic habitats, available nutrients and chemical composition of the different hydraulic brake fluids.

The findings of this study strongly suggest that the hydraulic brake fluids samples used in this study were not readily biodegradable having biodegradability less than 50 %(ASTM, 2003). More importantly, this study indicated that different factors come into play to determine the biodegradability or recalcitrance of a hydraulic brake fluid. The results of controlled experiments with environmental samples closely resemble what is obtainable *in situ*. However, it might be erroneous to extrapolate the rate of biodegradability observed in this study to what can be obtained in field situation.

In the light of the findings of this study, it is recommended that appropriate government agencies should regulate and monitor the type of additives used in formulating petroleum based hydraulic brake fluids, and encourage more research into the formulation/manufacture of bio-based lubricating hydraulic brake fluids/oils that are environmentally friendly, cost effective and are efficient in performance like the petroleum – based brake fluids. These bio-based products if spilled into the environment will readily degrade and disappear with little or no harm to the ecosystem. Petroleum products, when

spilled, remain for years and cause a lot of harm to the environment.

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