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# Biodegradation of Synthetic Detergents

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Additional information is available at the end of the chapter

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## 1. Introduction

The synthetic detergent industry is a lucrative industry considering the world population and the need for washing and cleaning by every individual in the world. In USA detergent is often described as surfactant or syndet while in Europe the term 'tenside' (for tensio-active material) is fashionable. *The Comité International de Dérivés Tension Actifs* has agreed after extensive deliberation on the following definitions: A detergent is a product, with the formulation comprising essential constituents (surface-active agents) and subsidiary constituents (builders, boosters, fillers and auxiliaries) (that is, surface-active agents are chemicals made of hydrophilic and hydrophobic molecules, that is, amphiphilic products). The first detergent to be utilized by man was soap, an innovation traditionally attributed to ancient Egyptian culture. However, when the word detergent is used today, it is assumed to refer to the synthetic detergents (tenside, syndets or surfactants), which have assumed increasing chemical and economic importance in the post-world war periods. The first synthetic detergents were developed by the Germans during the first world war period; they were generally referred to as Nekal. In the late nineteen-twenties and early thirties long-chained sulphonate-alcohols were sold as neutralized sodium salts. This metamorphosed to the long-chain alkyl aryl sulphonates sodium salts with benzene as the aromatic nucleus. The alkyl portion was from the kerosene fraction produced from petroleum industries. In the United Kingdom, Teepol a secondary Olefine sulphate from petrochemical sources was produced and still being produced in England and Western Europe to this day [1]. The worldwide manufacture of synthetic detergents has increased from the 1949 level of 30,000 tons to an estimated 1.5 to 2 million tons per year [2]. A significant increase in the material expectations of industrialized societies and the concomitant rise in popularity of the automatic washing machine has been the primary influence on the increased production levels demanded of the synthetic detergent industry. The failure of this increase in synthetic detergent manufacture and usage to cause a parallel increase in the detectable levels of waste detergent accumulating in various aquatic ecosystems

is indirect evidence that the biodegradation of synthetic detergents occurs in nature. Moreover, due to its ease of manufacture and versatility, the alkyl benzene sulphonate (ABS) very quickly gained a foothold on the synthetic detergent market shortly after the Second World War more than half the detergents used throughout the world were based on the formulation.

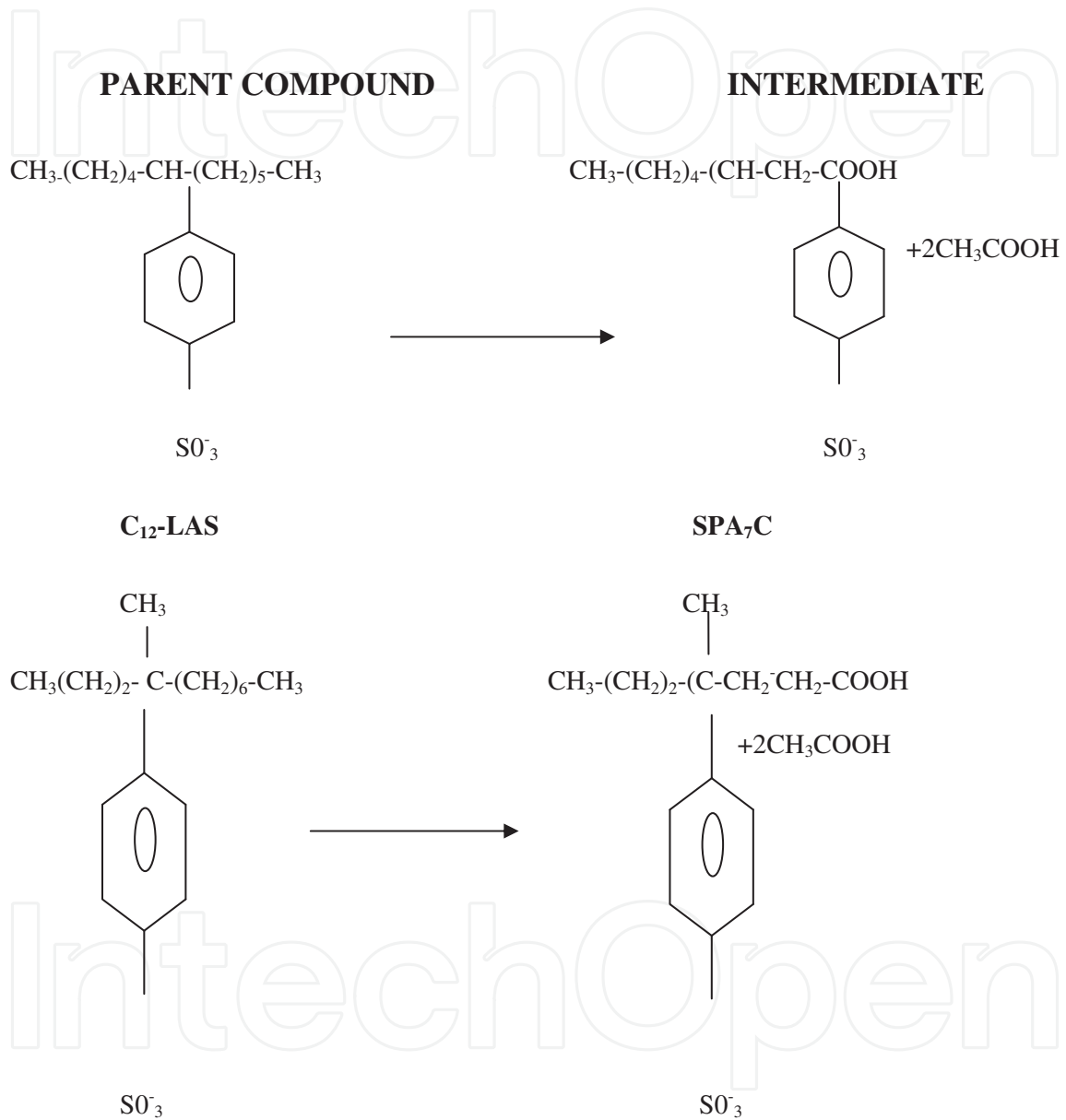
Detergents are cleaning products derived from synthetic organic chemicals. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it an advantage over soaps. Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactant component is between 10 and 20% while the other components include bleach, filler, foam stabilizer, builders, perfume, soil-suspending agents, enzymes, dyes, optical brighteners and other materials designed to enhance the cleaning action of the surfactants[3].

A surfactant is formed when a strongly lipophilic, hydrophobic group is bonded together with a strongly hydrophilic group in the same molecule. Usually surfactants are disposed after use to sewage treatment plants (STPs). Biodegradation processes and adsorption on sludge particles remove these chemicals from wastewater to a greater or lesser extent, depending on the chemical structure of the surfactant molecule and on the operating conditions of the STP. After treatment, residual surfactants, refractory co-products and biodegradation products dissolved in STPs effluents or adsorbed on sludge are discharged into the environment. These chemicals through several transport mechanisms enter the hydro-geological cycle. Assessment of the environmental contamination levels of surfactants and related compounds is achieved through a wide range of laboratory biodegradation tests and eco-toxicological studies.

Linear alkylbenzene sulphonate (LAS) is a commonly used anionic surfactant in detergents and it is easily biodegraded than non-linear alkylbenzene sulphonate (ABS) even though, total biodegradation still requires several days. Commercial LAS also contain co-products called dialkyltetralinsulphonates (DATS) and iso-LAS. Over seventy major isomers of DATS have been detected in Commercial LAS [4]. Under aerobic conditions rate and pathway of LAS biodegradation have been studied; the primary biodegradation (biotransformation) begins with oxidation of the external methyl group ( $\omega$ -oxidation) followed by stepwise shortening of the alkyl chain via oxidative cleavage of  $C_2$  units ( $\beta$ -oxidation). The process leads to formation of Sulpho-phenyl carboxylic acids (SPACs) [5]. The second cycle (ultimate biodegradation or mineralization) involves opening of the aromatic ring and/or desulphonation of SPACs leading ultimately to  $CO_2$ ,  $H_2O$ , inorganic salts and biomass.

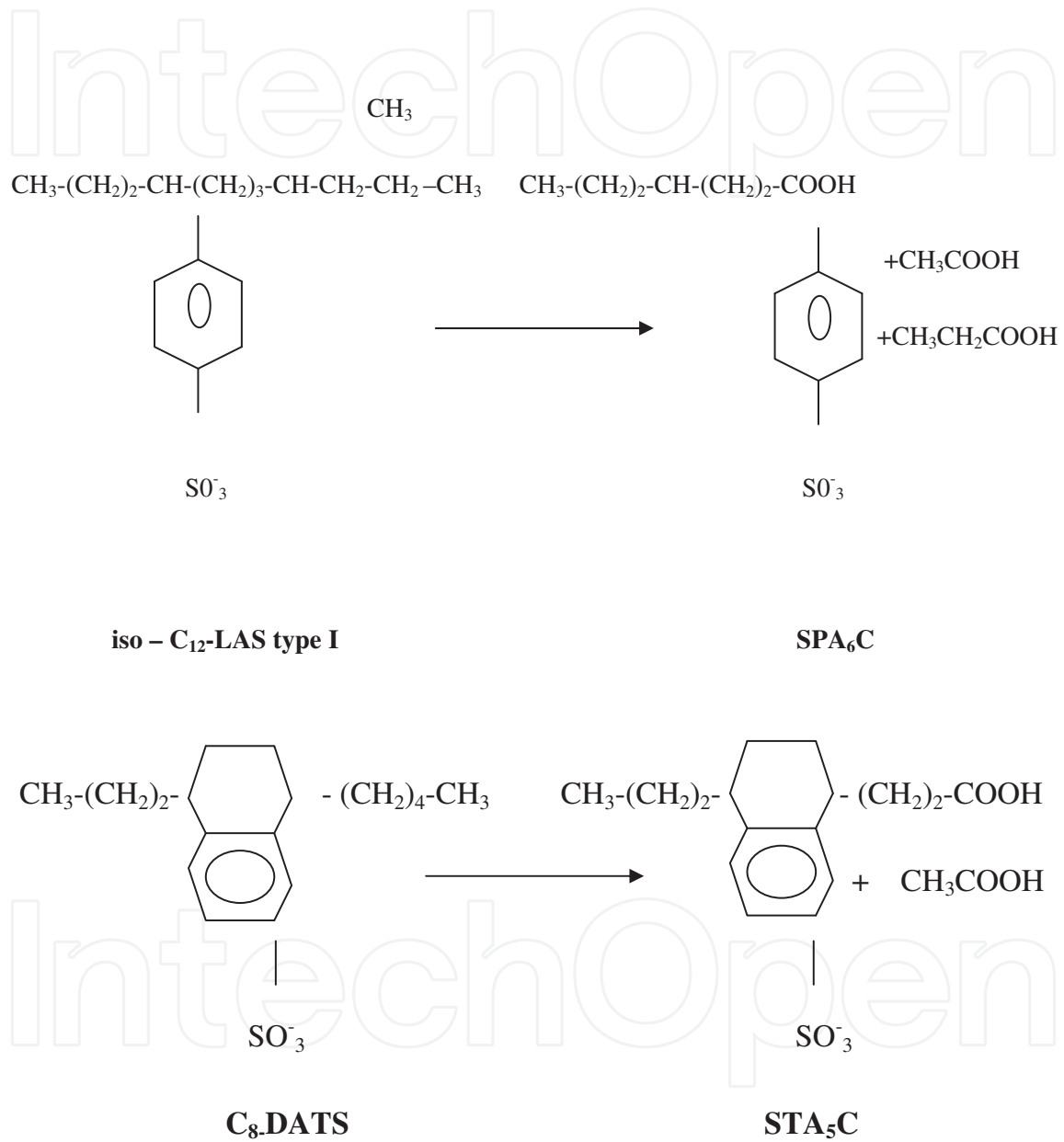
It has however, been recognized that the molecular architecture of a synthetic detergent can influence its biodegradability potential. Linear alkylbenzene sulphonate (LAS) biodegradation rate and acute toxicity on aquatic life are both very much related to the chain length and phenyl position of the alkyl chain [7, 8]. The primary biodegradability of LAS has been established by the Methylene blue – Active substance (MBAS) method. Sulphonated aromatic compounds are xenobiotics and these compounds are produced in large amounts as linear alkylbenzene sulphate (LAS). It was believed for a long time that desulphonation always occurs early in the aromatic sulphonate degradation pathway, however, it has been discovered that desulphonation occurs at later stages prior to the mineralization of the benzene ring. Commercial LAS is present in sewage at levels of 1- 20 mg/L and it is a mixture of many different isomers and

homologues. Toluene sulphonate is an additive in some detergent formulations and as such it's released into the environment in large amounts [9, 10, 11-15].

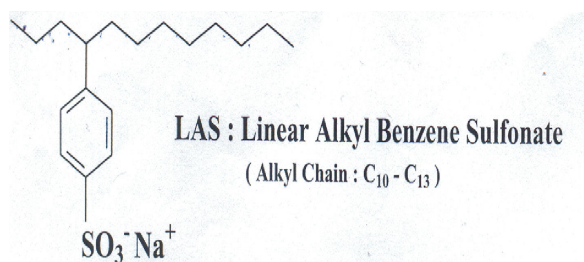


iso -C<sub>12</sub>-LAS type II

SPA<sub>7</sub>C



**Figure 1.** Examples of Structures and Acronyms of Linear Alkylbenzene Sulphonate (Surfactants), Co-Products and Related Catabolic Products



Source: [6]

**Figure 2.** Chemical Structure of LAS

## 2. Methods

The biodegradability of both domestic and industrial detergents were evaluated by collecting large numbers of representative untreated effluent samples (morning and evening) from many industrial and domestic sources, thereafter a composite wastewater sample can be generated. The determination of the physico-chemical properties of wastewater samples (morning and evening) is crucial to the outcome of the investigation and this can be done using the *Standard methods for the examination of water and wastewater* [17]. First, it is important to determine the heterotrophic microbial population in effluent samples using the Nutrient agar (bacterial) as well as Sabouraud dextrose agar (fungal). The Methylene-blue Active substance (MBAS) analysis is used to determine the percentage anionic matter (that is, surfactant component) in each of the commercial detergent product as well as in the wastewater samples. It is important to ascertain the presence of detergent components in the wastewater because this is the natural habitat of the 'detergent – degrader' microbial population. The strategic evaluation of the 'detergent – degrader' population would involve serial dilution technique, isolation of detergent-degraders on minimal salt medium supplemented with detergent at industry stipulated concentration(0.01% w/v) as well as the development of pure cultures of each microbial species that utilizes detergent components in wastewater environment.

The MBAS method is principally a titrimetric method similar to the common titration technique that identifies chemicals by neutralization process that involves Acid-Base reactions. When the end-point is reached a color change is observed. Thereafter, the concentration of the surfactant can be determined after the anionic substance is neutralized [3, 21, 22].

### 2.1. Aerobic heterotrophic microbial counts

The effluent samples collected from each sampling point at 0 -30cm depth were serially diluted and inoculated simultaneously onto Nutrient agar plates and Sabouraud dextrose agar in duplicates aseptically. The plates were incubated at ambient temperature for 24 - 48hrs and 7 days for fungi respectively. Thereafter, the colonies were counted [4,18,19]. The Micro-morphology of both the fungal and bacterial isolates was then used as well as their biochemical reactions for their characterization and identification. This was done following standard and

conventional methods [20]. *Thereafter, the schemes of Bergey's manual of systematic bacteriology and Smith's introduction to industrial mycology can be followed for the identification of bacterial and fungal isolates respectively.*

## 2.2. Microbial growth in wastewater spiked with detergents

The ability of native microorganisms to use detergent hydrocarbon components as sole source of carbon and energy for growth under laboratory simulated conditions suggested the biodegradation pattern in natural environment. Therefore, the composite effluent samples allowed to stand for minimum of 48hrs can be used to determine the microbial growth rate as well as the residual detergent components using Degradation – Time course graphical illustration. Wastewater samples were thereafter taken aseptically at intervals (Days; 0, 5, 10, 20, 25 and 30) to monitor the pH, total aerobic viable counts and spectrophotometry readings for growth changes. Serial dilution and spread plate methods were used to inoculate simultaneously Nutrient agar, Minimal medium and Sabouraud dextrose agar supplemented with test detergent at 0.01% (w/v), incubated at ambient temperature for 48hrs and 7 days respectively [21, 22]. This process is imperative to detect the actual utilization of detergent components for growth with time by the indigenous microbial population. Consequently, the pH of the simulated growth environment ought to change as biodegradation process proceeds.

## 2.3. Determination of LAS concentration using MBAS method

The titrimetric method (MBAS) is done in such a way that wastewater samples obtained from the biodegradation assay at intervals (Day 0, 5, 10, 20 and 30) were subjected to extraction procedures using organic solvent, that is, Chloroform ( $\text{CCl}_4$ ). The chloroform- surfactant complex formed when the end-point is reached is indicated due to the presence of an indicator in the reaction medium. This process is repeated several times in order to extract all the detergent residues before its transfer to the Gas chromatogram(GC). The Gas chromatographic(GC)analysis detects the background LAS in wastewater as well as the residual detergent components from the biodegradation assay. Although, some other equipment such as High pressure liquefied chromatography (HPLC), spectrophotometer e.t.c can be used to monitor the ultimate biodegradation of LAS once these are calibrated with relevant standard aromatic hydrocarbon [19, 22, 23].

## 2.4. Gas chromatographic analysis

The gas chromatographic analyses of samples for selected time intervals Days 0, 10 and 20 were determined. The GC (Perkin Elmer Auto – System Gas Chromatography, USA) analysis of the total hydrocarbon was carried out using a GC equipped with flame ionization detector (FID). A 30m fused capillary column with internal diameter 0.25mm and 0.25m film thickness was used and the peak areas were analyzed with a SRI Model 203 Peak Simple Chromatography Data System. The column temperature was 60C for 2 minutes to 300°C programmed at 12°C/min. Nitrogen was used as carrier gas at 37 psi. Hydrogen and air flow rates were 9 psi and 13 psi respectively.



## 2.5. Molecular biology of detergent-degraders

### 2.5.1. Plasmid profile analysis

The plasmid profiles of the bacterial consortium metabolizing detergent components were evaluated purposely to use it as the basis of 'typing' for the detergent-degraders. The 'Mini-prep' plasmid isolation method was found to be inadequate because the plasmid DNA of isolated detergent-degrader consortium was very small hence they were not detectable with the 'Mini prep' method. However, the large-scale (Maxi-prep) plasmid DNA extraction method proved to be adequate for the size of plasmids encoded in this detergent-degrader microbial population (24, 25).

### 2.6. Curing of plasmid

This process becomes unavoidable since it is mandatory that plasmid encoded features would not be metabolically functional after curing process. The detergent-degrader bacterial population were thereafter inoculated on minimal salt medium supplemented with detergent, this is to evaluate the functionality of detergent utilization capacity of these isolates after going through curing process (25, 26).

### 2.7. Results

The findings of this study conducted in a tropical climate environment becomes very significant because previously this type of study were conducted in temperate climate environment. Although, similar results were obtained except for the fact that microbial detergent-degrader were observed to degrade and mineralize detergents at a slower rate than it occurred in temperate climate. This is suggestive of the consequential effect of the prevailing environmental factors in the tropics, sub-Saharan Africa. The mean physico-chemical parameter of the receiving wastewater body for the study was pre - determined following conventional and standard methods. The physico-chemical parameters (Table 1) measured revealed that the wastewater used for the study was within the mean alkaline pH range (10.54 -11.08), mesophilic temperature range (33.9 – 34.3°C), total hydrocarbon (THC) (13.6 – 15.0 mg/L)  $\text{NH}_4\text{-N}$  content (178.7 – 193.5mg/L) and BOD (34.41 – 38.08mg/L). The physico – chemical properties of the wastewater is typical of that of a tropical environment. The organic matter component is evidently high and this is the reason for the high  $\text{NH}_4\text{-N}$  content. The oxidation of organic matter and hydrocarbon component being responsible for the high BOD.

The anionic matter (LAS) content of SDS (sodium dodecyl sulphate) was the highest of detergents used. Hence, it was used as test detergent in all GC analysis while Persil had the least LAS content (Fig. 3). Although, it should be noted that physico-chemical analysis of the composite wastewater used revealed that it was heavily polluted with organic matter; high BOD value, the COD falls short of Federal Environmental Protection Agency (FEPA), European Union (EU) and World Health Organization (WHO) standards. This was observed as one of the strong reasons for the slow rate of mineralization of xenobiotic components in the tropics.

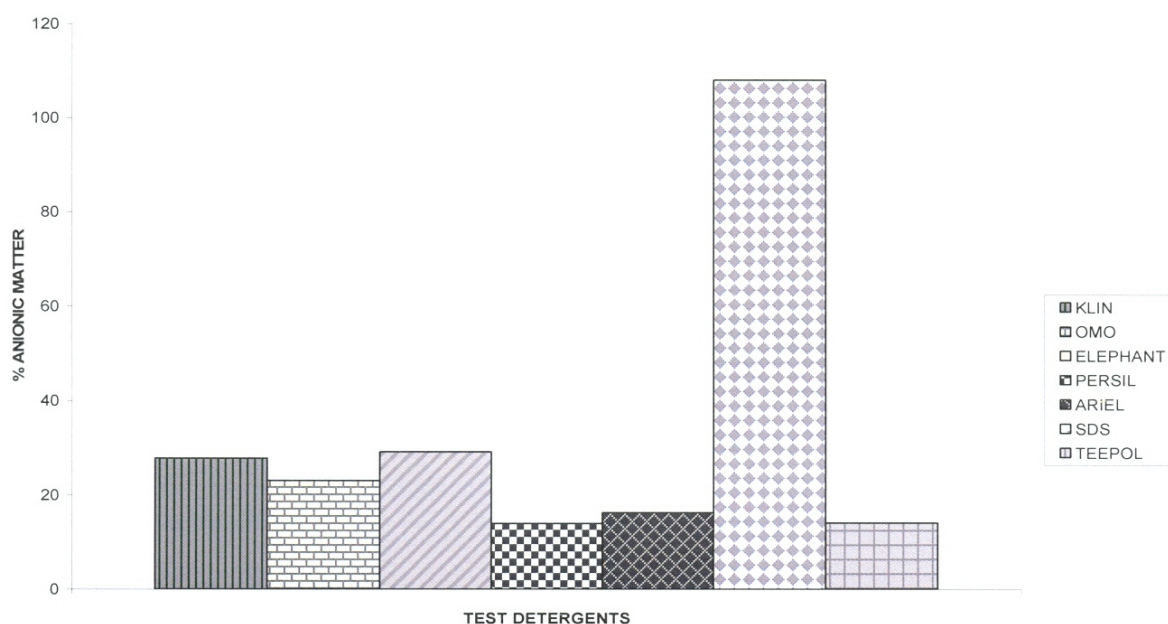


PARAMETER	MORNING	EVENING	FEPA/WHO STANDARDS	EU STANDARDS
General appearance	Cloudy foaming	Foaming	NS	NS
Colour	Blue	Light green	NS	NS
Odour	Soapy smell	Soapy smell	NS	NS
pH(H <sub>2</sub> O)	10.54	11.08	6 – 9	7.5 – 8.5
Conductivity @ 25 °C	204 Usm <sup>-1</sup>	185 Usm <sup>-1</sup>	NS	340
Temperature	34.3 °C	33.9 °C	40 °C	20 – 25 °C
PO <sub>4</sub> <sup>3-</sup>	99.9mg/L	90.3mg/L	5mg/L	10 – 25mg/L
SO <sub>4</sub> <sup>2-</sup>	92.7mg/L	88.6mg/L	500mg/L	NS
NO <sub>3</sub> <sup>-1</sup>	26.29mg/L	22.86mg/L	20mg/L	20mg/L
Total suspended solid (TSS)	170mg/L	200mg/L	30mg/L	35mg/L
COD	57.51mg/L	52.01mg/L	200mg/L	<125mg/L
Specific gravity	1.009	1.022	NS	NS
NH <sub>4</sub> – N	193.5mg/L	178.7mg/L	NS	15mg/L
Cl <sup>-1</sup>	36.18mg/L	37.95mg/L	600mg/L	600mg/L
Dissolved oxygen (DO)	9.05mg/L	9.45mg/L	"/>2mg/L	2mg/L
BOD	38.08mg/L	34.41mg/L	30mg/L	<25mg/L
Total hydrocarbon (THC)	15.0mg/L	13.6mg/L	10mg/L	<10mg/L
DO <sub>5</sub>	36.04mg/L	32.67mg/L	"/>2mg/L	NS
Total dissolved solid (TDS)	NS	NS	NS	NS

NS = Not Specified

**Table 1.** Mean Physico-Chemical Properties of Composite Wastewater Sample

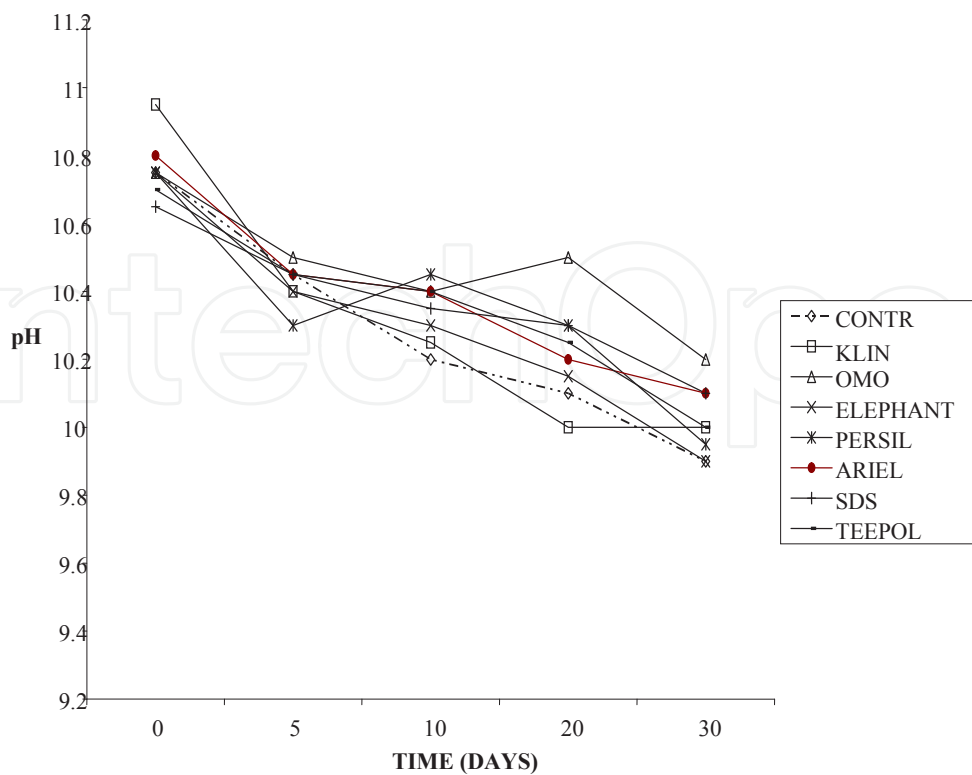
The NO<sub>3</sub> – N, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup>, NH<sub>4</sub> – N and total hydrocarbon (THC) content of the composite wastewater used exceeds the WHO and EU limits which is suggestive of high organic chemical pollution and longer time required for mineralization to be effected (Table 1). The heterotrophic bacterial population was more than those of the fungal because the wastewater mean pH was alkaline. Hence, the microbial consortium involved in detergent degradation process had more of bacterial species than fungal. Alkaliphilic microbial consortium succeeded the acidophilic population that started the biodegradation process in an autogenic succession fashion. The alkaline pH as well as the mesophilic temperature range favored the acclimation of the indigenous microbial population to the test detergents, as well as enhanced the biodegradation efficiency of the microorganisms. This fluctuation in pH values over a 30-day period was suggestive of production of acidic and alkaline metabolites during detergent degradation as well as serves as the evidence of occurrence of chemical changes in the original formulation



**Figure 3.** Determination of Anionic Matter in Test Detergents

of the test detergents (Fig. 4). The test detergents were coded as follows: AK 17 (Klin), AK 27 (OMO) AK 37 (Elephant), AK 47 (Peril), AK 57 (Ariel), AK 77 (Teepol) and AK 67 (SDS) which served as the standard detergent. *This is to facilitate unbiased analysis in the laboratory.*

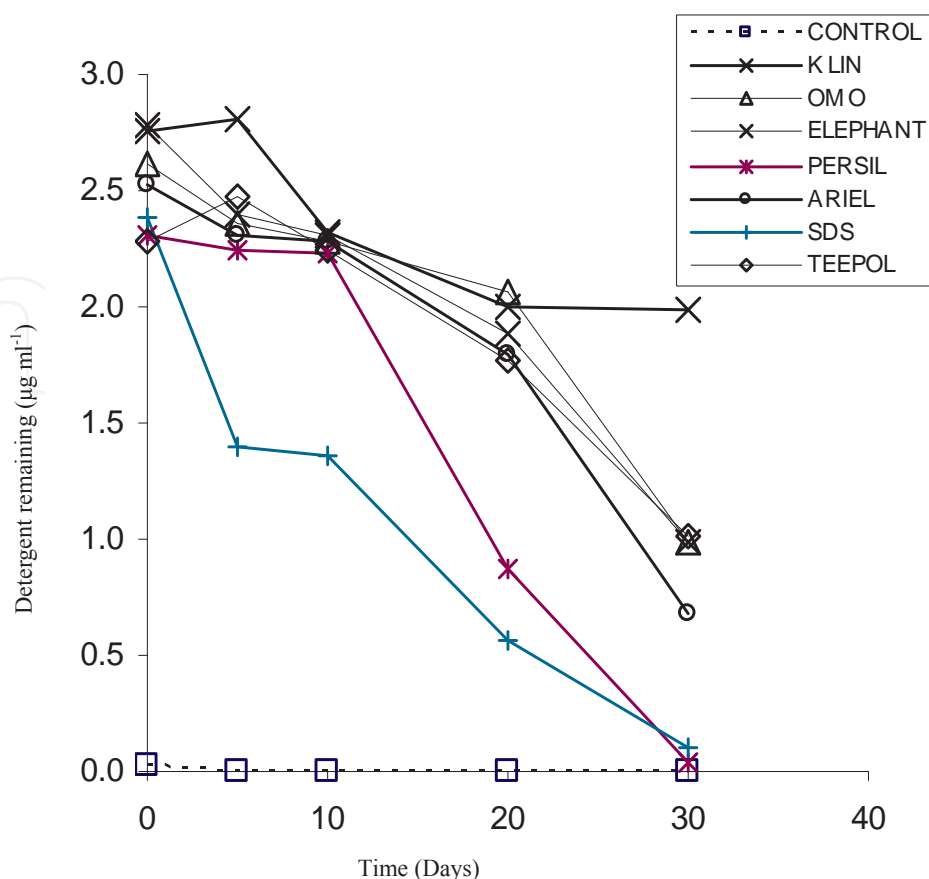
This study revealed that though the test detergents contained similar formulation but the duration for the mineralization process was different from what has been previously reported, that is, 17-20 days in temperate climate. It has been reported that commercial LAS mixtures usually contain about 15% of co-products which may be responsible for differences in their rate of mineralization [28]. Major co-products of commercial mixtures of linear alkylbenzene sulphonate (LAS) surfactants are dialkyltetralinsulphonate (DATS) and methyl-branched isomers of LAS (iso-LAS). Unlike LAS, little and contrasting information on the fate of DATS and iso-LAS is available. The use of liquid chromatography/mass spectrometry (LC/ms) has confirmed that DATS were more resistant than iso-LAS to primary biodegradation. Biotransformation of both LAS-type compounds and DATS produced besides expected sulphophenyl alkyl monocarboxylated (SPAC) LAS and sulphotetralin alkyl carboxylated (STAC) DATS metabolites, significant amounts of sulphophenyl alkyl dicarboxylated and sulphotetralin alkyl dicarboxylated (SPADC and STADC) compounds which may be responsible for the measure of recalcitrancy found in synthetic detergents[28]. The mesophilic temperature range (25- 35°C) favored the activities of microbial detergent-degrader in tropical wastewater. The slow degradation of surfactants in natural environment may be as a result of unfavorable physico-chemical conditions (such as temperature, pH, redox potential, salinity, oxygen concentration) or the availability of other nutrients. The presence of optimal physico- chemical conditions will allow eventual evolution and growth of best-adapted microbial population to the detergent component. Although, industry specifications states that C<sub>10</sub> – C<sub>14</sub> gives the best



**Figure 4.** pH readings of primary biodegradation from field experiment (Shake-flask).

biodegradable detergent product. SDS was found to be the most rapidly biodegraded of all the test detergent products utilized for this study followed by Elephant. This is due to the fact that straight chain LAS are rapidly biodegraded than branched chain LAS. Although, SDS is a purer detergent of analytical grade often used in the laboratory with over 95% purity level while Elephant's purity level cannot be guaranteed up to 90% this was true for other commercial test detergents too. In comparisons, SDS relatively degraded faster than all the test detergents in the presence of the microbial consortium apart from the fact that it contains  $C_9 - C_{12}$ . Under 10 days, SDS (AK 67) was almost completely mineralized (except  $C_{17}$ ), as at Day 20, ELEPHANT (AK 37) had components of  $C_{11}$ ,  $C_{12}$  and  $C_{17}$  unmineralized (Fig. 5). Persil (AK 47) was quickly mineralized almost at the same time as SDS because of the level of purity and their molecular architecture (Fig 5).

The scientific evidence of microbial utilization of the synthetic detergent components for growth as well as source of energy is as shown in Fig.6. In less than 10 days of running the biodegradation assay, the bacterial detergent-degrader had reached the stationary phase on SDS whereas those growing on Elephant detergent got to stationary phase about 20<sup>th</sup> day. This was due to the differences in molecular architecture of the detergent being metabolized. The fungal population utilizing detergent as source of carbon and energy for growth dominated the culture vessel within the first 20 days prior to pH alteration from acidic to alkaline in an autogenic succession fashion (Fig.7). The pH of the intermediates formed also selects the microbial colonizers for detergents components, however, it should be noted that the test

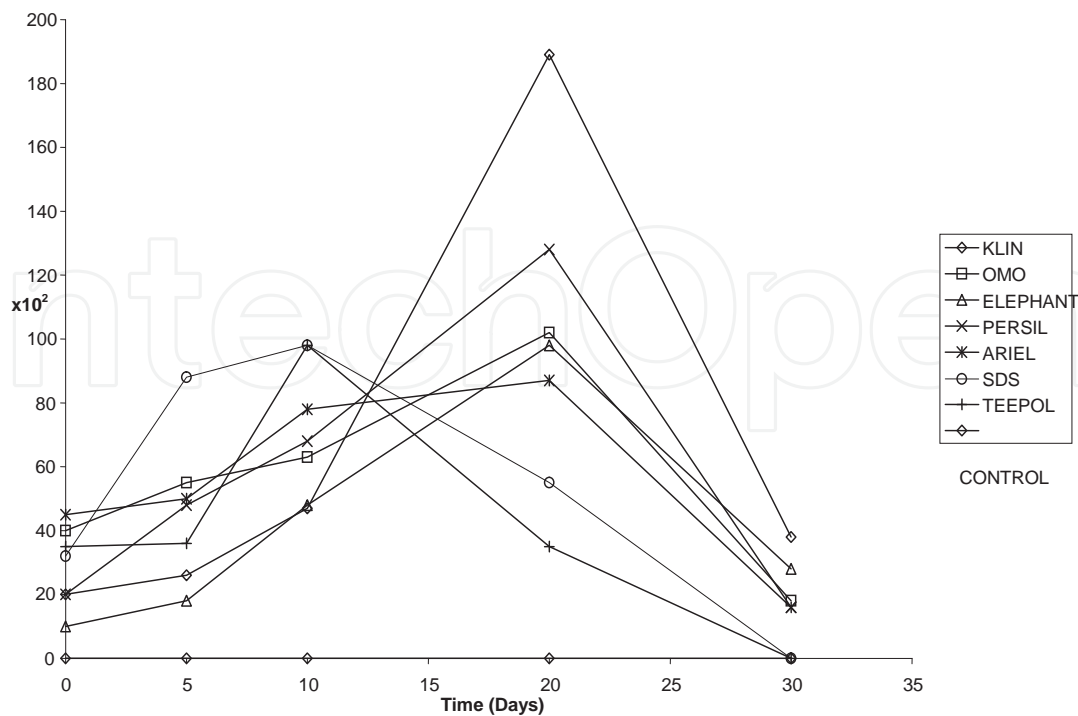


**Figure 5.** Biodegradation residues (shake-flask experiment).

detergents were from different manufacturers and that sometimes apart from industry stipulated additives for synthetic detergent products some manufacturers incorporates other chemicals as optical brighteners which sometimes are recalcitrant.

The GC profiles (Fig 8 and 9) represented the monitoring process for SDS degradation from the commencement (day 0) of the biodegradation assay as well as the detergent residues detected using the GC result for Day 10 (Fig. 9). Although, at Day 0, 9 peaks were detectable on sampling for SDS residues in the biodegradation assay (Fig. 8) but as at Day 10 only C17 residues peak was detectable. This showed that biodegradation was both progressive and successful. On further studies the SDS (AK 67) was completely mineralized by Day 20. This study was done for all the test detergents and none of these test detergents were recalcitrant (19, 22). The present observation differs from the reported 17 to 20 days requirements for synthetic detergent mineralization in wastewater from temperate regions of the world. This present finding is peculiar to sub-Saharan Africa which has tropical climate. Moreover, at the end of the 30-day biodegradation assay, all test detergents were mineralized.

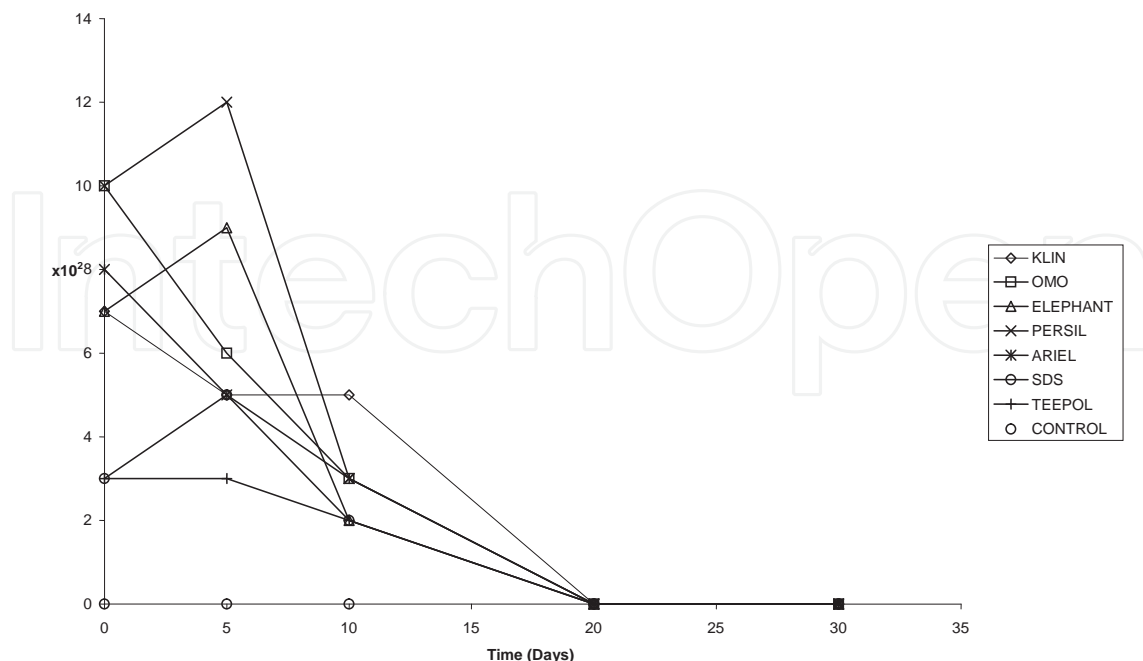
The second cycle (ultimate biodegradation or mineralization) involves opening of the aromatic ring and / or desulphonation of SPACs leading ultimately to CO<sub>2</sub>, H<sub>2</sub>O, inorganic salts and biomass formation [9, 28, 31]. It has been reported that the primary biodegradation of DATS



**Figure 6.** Mean aerobic bacteria detergent-degrader count (shake-flask experiment).

was completed by Day 17 [28]. Hence, both DATS and iso-LAS are not issues of concerns as regards their mineralization, both undergo primary biodegradation. The microbial populations of domestic and industrial activated sludge are very effective in the primary biodegradation of DATS and iso-LAS, but are not capable of mineralization of the related metabolites. However, these metabolites cannot be considered as refractory since under appropriate conditions they can be utilized as a sulphur source for bacterial growth and biomass accumulation [5, 15, 16].

These discoveries of co-products and LAS-type compounds explain the presence of some unusual peaks in the Gas chromatogram obtained in this study (Fig. 9). When the test synthetic detergents were subjected to ultimate biodegradation in laboratory simulated experiments, the native microorganisms metabolized the detergent components for growth and biomass accumulation as a result the gas chromatography was used at intervals to analyze the samples within a 30-day period. This is to monitor the transitory intermediates formed as well as to provide the convincing evidence for the mineralization of the detergent spiked into wastewater and nutrient broth respectively [18, 28]. The Control was sterile water with no detergent addition as revealed by the GC analysis of the control flask (Fig. 10). Although, unusual peaks in GC profiles were detected by other researchers but it was sparsely reported [9]. Under aerobic conditions, rate and pathway of detergent biodegradation have been the objectives of many studies. Although, the biodegradation pathway of LAS has not been fully explained, there are many evidences showing that the first cycle of biotransformation (primary biodegradation) begins with



**Figure 7.** Mean fungal detergent-degrader count (shake-flask experiment).

oxidation of the external methyl groups ( $\psi$ - oxidation) followed by stepwise shortening of the alkyl chain via oxidative cleavage of  $C_2$  units ( $\beta$  - oxidation) [28, 31].

The microbial isolates both from the shake-flask studies and sewage treatment plant (STP) utilizing the test detergents as C and energy sources include; *Enterococcus majodoratus*, *Klebsiella liquefasciens*, *Enterobacter liquefasciens*, *Klebsiella aerogenes*, *Escherichia coli*, *Enterobacter agglomerans*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Myceliophthora thermophila*, *Geomyces sp.*, *Alternaria alternata*, *Aspergillus flavus*, *A. oryzae* and *Trichoderma sp.* The other bacterial species for which their identity could not be ascertained were those that would require high throughput DNA technology to confirm their identity. The rate of multiplication of bacterial cells was highest for *Pseudomonas aeruginosa* followed by *Klebsiella aerogenes*, and *Enterobacter agglomerans*, then *E. coli* in this descending order. The bacterial consortium stabilized and reached the stationary growth phase at Day 10 for the microcosm experiment (Fig. 6). The fungal detergent utilizers were characterized following the methods of *Smith's Introduction to mycology* (Smith, 1981). Comparative study of the mycelia features as well as microscopy was used to identify the fungal species. The fungal population could not tolerate the rise in pH value towards the alkaline pH range, hence their elimination. The fungal detergent-degrader population started decreasing after Day 10 (Fig.7). When *E. coli*, *Ps. aeruginosa*, *K. aerogenes*, *S. albus* and *K. liquefasciens* strains isolated from diseased-patients were inoculated onto detergent medium they utilized detergent component as C and energy source for growth. The production of detergent catabolic enzymes thus seems to be inductive by this observation.





Figure 8. GC profile showing peaks of unmineralized detergent component (Day 0)

The GC profiles thus reveal that the actual process of detergent degradation truly occurs in natural environment (Fig. 8, 9 and 10). The presence of residues represented by the unusual peaks was identifiable. This is due to activities of some detergent manufacturers who incorporated unwholesome chemicals in synthetic detergents to enhance detergency as well as aid optical brighteners in detergents. This unwholesome practice is common in sub-Saharan Africa as revealed in research works (19, 22) these inclusions are contrary to regulatory body specifications.

This observation lays to rest the issue of recalcitrancy of domestic and industrial detergents in natural environment. Desulphonation process is the rate limiting process and this has been shown to have taken place by microbial activities in natural environment.

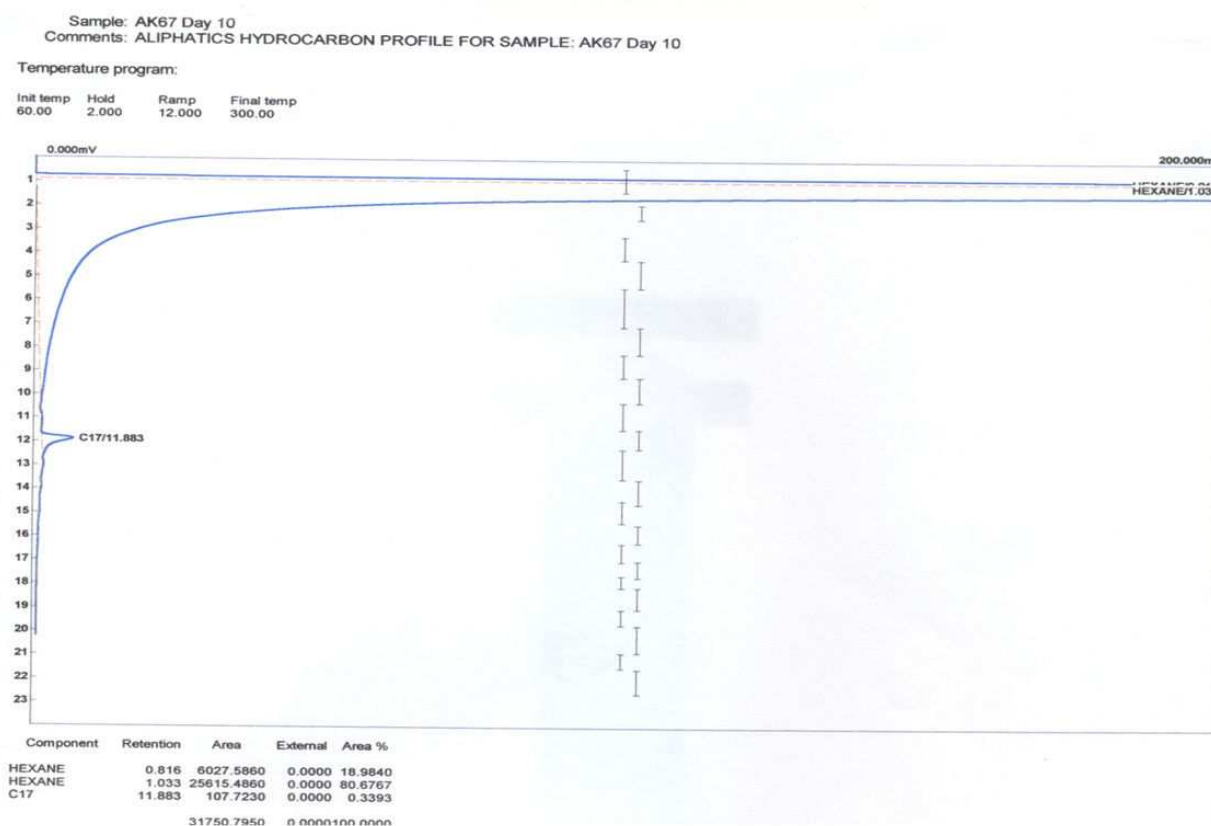
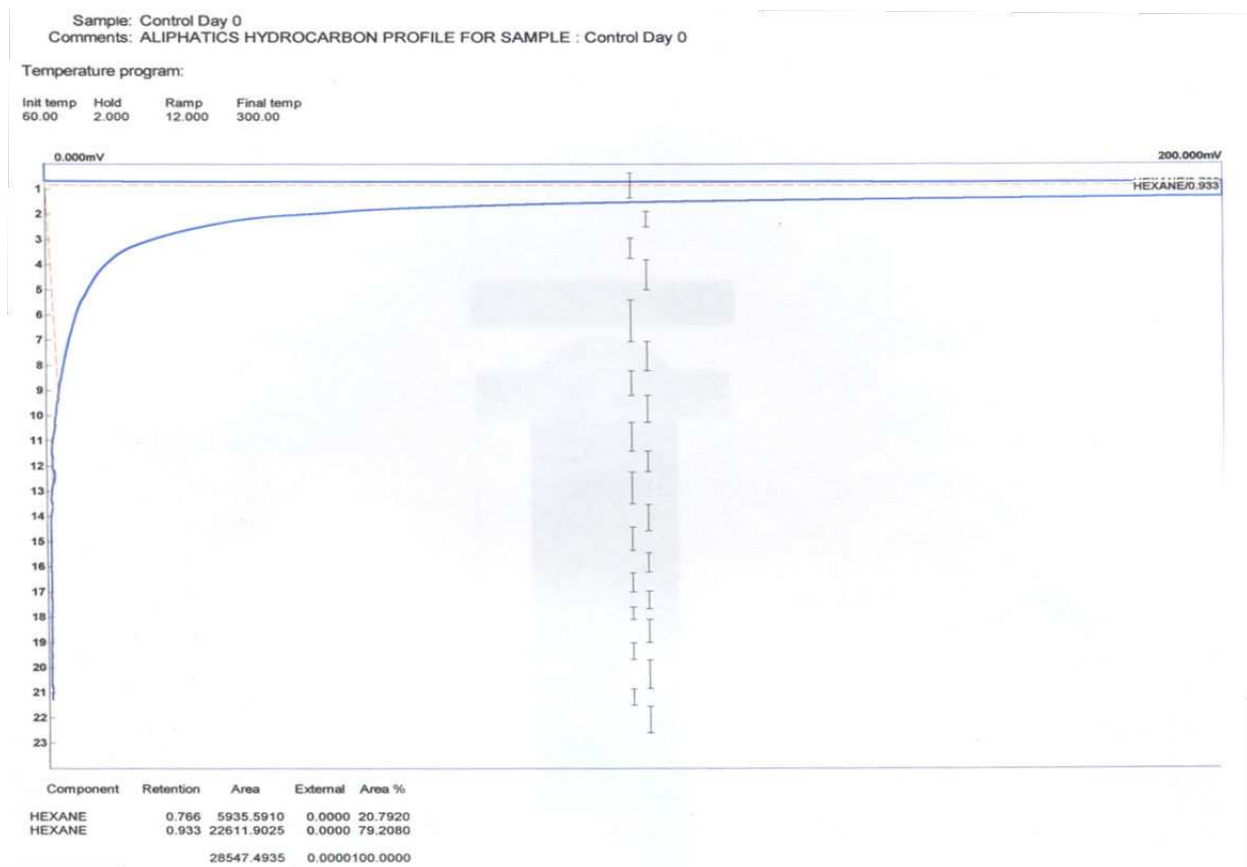


Figure 9. GC profile showing a single peak of detergent residues (C<sub>17</sub>) Day 10

### 3. Molecular biology of detergent-degraders

The plasmid-profile of selected bacteria detergent-degraders was successfully detected with the Maxi-prep method. It was evident that the genetic information for detergent-hydrocarbon utilization was not plasmid - mediated since the cured isolates grew on detergent supplemented medium after plasmid was removed. Hence, the genes for detergent hydrocarbon utilization can be said to be resident within the genome of the selected bacterial population [19].

The ability to utilize detergent components as carbon and energy source for growth and biomass accumulation suggested some peculiarity in the genome of microorganisms with this trait. Since not all microorganisms have the ability, particularly acclimation to xenobiotic compounds. Plasmid DNA-coded character often plays significant role in bacteria adaptation to xenobiotics in the environment. However, the convincing evidence on the genetic linkage of the catabolic trait would be found using high through-put techniques such as the PCR and DNA sequencing to trace the nucleotide sequence encoding for detergent – catabolism which may be resident in the plasmid – DNA or the genomic DNA. The genetic diversity among xenobiotic-degrader has been confirmed by several researchers [32]. The recently developed high through-put technologies such as construction of metagenomic libraries and DNA array technology would authenticate as well as enhance the knowledge of the identity of detergent-



**Figure 10.** GC profile of control experiment with no peak due to absence of detergent component ( Day 0)

degrader genes with greater precision [32, 33]. These new technologies involve DNA hybridization via construction of DNA probes which has been described as being a relatively rapid method of analysis compared to PCR [34]. Recent advances in molecular techniques, including high through-put approaches such as DNA microarrays and metagenomic libraries have opened up new perspectives towards new opportunities in pollution abatement and environment management. [34]. Compared with traditional molecular techniques which are dependent on the isolation of pure cultures in the laboratory. DNA microarrays and metagenomic libraries allows the detection of many uncultivable and uncharacterized xenobiotic-degraders, thus enhancing the knowledge of the genetic diversity of environmentally relevant microorganisms.

Most countries are upgrading their effluent treatment plant to Membrane Bioreactor Technology (MBR) which improves the quality of domestic sewage and wastewater discharged without increasing the plant foot-print [29]. This MBR has a single line designed to handle effluent flow of 1000 fold/day more than that of conventional STPs. The up-graded process design increases the quality of discharged effluent to satisfy consent levels and achieve effluent of unrestricted irrigation re-use standard [27, 29]. The compact size and efficient operation of this advanced process allowed countries such as Japan, Brescia to achieve a higher quality discharged- effluent without expanding the plant's footprint [27].

The above strategic and holistic approach would enhance sustainable development as well as effluent quality when strictly adhered to. When a sub-lethal environment change is imposed on a mixed microbial population, two possible mechanisms of shift in the population may occur; (i) the predominance of the population may shift to those species whose activities are enhanced by the change; and (ii) those species of the population having the metabolic capability may acclimatize to the change.

The presently available information from this study suggested that microorganisms may eventually be able to deal with any kind of organic compound, particularly synthetic detergent provided that the compounds are intrinsically degradable. The surfactant component (LAS) supposedly recalcitrant is biodegradable under optimum environmental conditions. The following deductions can be made from this study;

- The biodegradation process was favored at the mesophilic temperature range 25 - 30°C
- Alkaline pH was predominant in the field (STP) as well as in the microcosm studies.
- The pH of the wastewater ecosystem dictated the type of microbial heterotrophs that metabolized the detergent components.
- Although acidic pH selected the fungal detergent-degraders they were only able to tolerate the pH in the culture media spiked with detergent up to pH 7.0. This was responsible for the disappearance of fungal isolates after Day 10 while conducting the laboratory simulated detergent degradation.
- The NO<sub>3</sub>-N in the composite wastewater was too high compared to newly legislated European Union environmental standard limits of 3mg/L for treated wastewater. The problem of eutrophication is inevitable and this would reduce the volume of oxygen (BOD) required for biodegradation process which may be responsible for the observed length of time taken to mineralize the test detergents in tropical environment as opposed to the 17 – 20-day often reported in temperate climates.
- Mineralization of LAS is true; hence its inclusion in synthetic detergent formulation poses no public health or aquatic life threats.
- Gas chromatographic analysis of biodegradation residues provided convincing evidence of the mineralization of the surfactant component of detergent products which was said to be recalcitrant.
- The result of this study showed the presence of C<sub>17</sub> – C<sub>21</sub> hydrocarbon component in detergent formulation, signifying some measures of inconsistency on the part of detergent manufacturers who might have incorporated other organic chemicals in detergent products to enhance their performance (that is, fluorescent optical brighteners) at the expense of public and aquatic life safety.
- Although aquatic toxicity of detergent products have been reported, this might be due to unwholesome additives incorporated (C<sub>17</sub> – C<sub>21</sub>) in the product which takes longer time than expected for it to be mineralized in STPs and open-river. These unwholesome additives

include 4, 4'-bis (2-sulphostyryl) biphenyl and toluene sulphonate which are typical representative of the stilbene class of fluorescent optical brighteners.

- This study has shown that microorganisms possess a range of genetic mechanisms allowing evolutionary changes in existing metabolic pathways, specialized enzyme systems and pathways for the microorganisms for degradation of xenobiotics such as LAS or synthetic detergents which have been found in geographically separated areas of the biosphere.
- Detergent – degraders were principally bacteria of Gram-negative group suggesting their ability to tolerate the surfactant than the Gram-positive bacteria.
- Plasmid profile analysis also revealed the presence of single plasmid in all the bacterial isolates but plasmid – DNA may not be absolutely responsible for the detergent–hydrocarbon utilization trait.
- Future studies with PCR and DNA sequence analysis would reveal the DNA 'fingerprint' of each of the 'detergent-degraders' species. This would enhance the processes of surveillance for these organisms in similar ecosystems and the detection of new serotypes as well as assist in environmental impact assessment (EIA) study for sustainable development.

The question whether enzymes specialized for the degradation of xenobiotics in these bacteria evolved from more common isozymes only after the large-scale introduction of xenobiotic chemicals into the environment can now be answered with the recent advances in molecular techniques, including approaches such as DNA microarrays and metagenomic libraries.

This study has given insight into the molecular events and regulatory mechanisms responsible for evolution of detergent catabolic trait, and that the presence of a selective pressure of a specific substance or a toxic chemical (LAS) can spur microbes to metabolize such xenobiotics.

The current study summarized clearly evidences of the potential of microorganisms to evolve new and desired biochemical pathways themselves after surviving natural selection. The introduction of LAS (C<sub>10</sub>-C<sub>14</sub>) into detergent formulation as the principal surfactant component is thus environment friendly and would enhance sustainable development processes.

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