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Full Length Research Paper

Biodegradation of synthetic detergents in wastewater

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A total of 76 wastewater samples were randomly collected from pharmaceutical, textile, and detergent-manufacturing industries as well as the Agbara Sewage Treatment Plant (STP). Thirty-eight samples each in 2-L plastic containers were collected for morning and evening effluent used for this study. Composite samples were later developed and the physico-chemical properties of these samples determined. The physico-chemical properties of the composite wastewater influenced the selected microbial population adapted to utilization of detergent components. The optimum temperature range of the composite wastewater was 33.9 – 34.3°C while the mean optimum pH ranged from 6.9 – 8.8 for the laboratory simulated biodegradation of test detergents. Although, the fungal consortium was eliminated as the medium approached the alkaline pH, this is as a result of the metabolites produced. The macro-elements, the BOD and the hydrocarbon concentration of the composite effluent were above the EU and FEPA limits for discharged effluent. The composite effluent was thereafter spiked with test detergents (Elephant, Omo, Klin, Ariel Persil, Teepol, and SDS) at 0.01% (w/v) and its progressive degradation monitored for 30 days. The microbial detergent-degraders population changed between Day 0 and 15, thereafter it stabilized. The heterotrophic bacterial count from the seventy-six randomly collected effluent samples was 42.9 x 10^6 cfu/ml, while the mean bacterial detergent-degrader population was 20.94 x 10^6 cfu/ml. The mean fungal population from the randomly collected effluent sample was 4.5 x 10^6 cfu/ml. The bacterial detergent-degraders characterized and identified include *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus majodoratus*, *Klebsiella liquefasciens*, *Enterobacter liquefasciens*, *Klebsiella aerogenes*, *Enterobacter agglomerans*, *Staphylococcus albus*, *Proteus* sp., *Klebsiella oxytoca* and *Brevibacterium* sp., while the fungal detergent-degrader included; *Myceliophthora thermophila*, *Geomyces* sp., *Alternaria alternata*, *Fusarium* sp., *Aspergillus flavus* and *Asperigillus oryzae.* The primary biodegradability of synthetic detergent was confirmed by the Methylene Blue–Active Substance (MBAS) method. Gas chromatography (GC) provided the convincing evidence of synthetic detergent mineralization within the 30 day period in a sewage treatment plant. The detection of unusual peaks in the GC profiles provided the scientific evidence of inclusion of certain hydrocarbons in detergent formulation outside that of industry specifications. The unusual peaks are attributable to inclusion of certain chemical optical brighteners (C_{17}–C_{24}). Linear alkyl benzene sulphonates (LAS) which is the principal synthetic detergent component are thus biodegradable and its use in detergent formulation is environment - friendly.

Key words: Biodegradation, detergents, linear alkylbenzene sulphonate, sustainable development.

INTRODUCTION

The increasing releases of organic pollutants by industries cause many health–related problems. However, increased awareness of the harmful effects of environmental pollution has led to a dramatic increase in research on various strategies that may be employed to clean up the environment. It is now realized that microbial metabolism provides a safer, more efficient, and less expensive alternative to physico-chemical methods for pollution abatement (Hebes and Schwall, 1987).

Linear alkyl benzene sulphonates (LAS) is a commonly used anionic surfactant in detergents and it is easily bio-

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degraded than non-linear alkylbenzene sulphonate (ABS) even though, total biodegradation still requires several days (Gledhill, 1975; Nomura et al., 1998). After soaps linear alkylbenzene sulphonates (LAS) are the most widely used surfactants in domestic and industrial detergents. In 1995, the global production of LAS was ca 2.8×10^6 ton (Ainsworth, 1996).

Surfactants constitute a major ingredient of detergent components. Usually surfactants are disposed after use to sewage treatment plants (STPs). Here, biodegradation processes and adsorption on sludge particles remove these chemicals from wastewaters 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 sludges 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. There are many evidences showing that the primary biodegradation begins with oxidation of the external methyl group (ω-oxidation) followed by stepwise shortening of the alkyl chain via oxidative cleavage of C_2 units (β-oxidation). This process leads to the formation of sulpho-phenyl carboxylic acids (SPACs) (Cook, 1998).

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 is generally accepted that dialkyltetralin sulphonates (DATS) and iso-LAS which are co-products of commercial LAS, form carboxylated intermediates upon biodegradation; this detection has been facilitated through mass spectrometric study of sewage contaminated groundwater (Field et al., 1992).

Many researchers have studied dialkyltetralin sulphonates (DATS) and iso-LAS mineralization in the laboratory and high levels of these chemicals in ultimate biodegradation has been detected and that many refractory organic carbons associated with impurities characterized LAS mineralization (Cavalli et al., 1976; Kolbener et al., 1995a,b). Recently, laboratory simulations have confirmed that the microbial populations of domestic and industrial activated sludge were effective in primary biodegradation of DATS and iso-LAS but were not capable of mineralizing most of the related metabolites (Nielsen et al., 1997). However, these metabolites cannot be considered as refractory species, under appropriate conditions, they can be utilized as a sulphur source for bacterial growth (Cook, 1998). Liquid chromatography/Mass Spectrometry (MS) with an electrospray (ES) ion source and a single quadrupole is a powerful technique (Di Corcia et al., 1999a) for characterizing the structures of break-down product originated from biotransformation of alkyl branched alcohol ethoxylate (Di Corcia et al., 1998) and nonylphenol ethoxylate (Di Corcia et al., 1993) surfactants.

Principally, co-products of commercial mixtures of LAS surfactants are DATS and iso-LAS, they make-up to 15% of LAS. A previous method based on solid – phase extraction (SPE) and liquid Chromatography / MS has been modified for monitoring the above analyses in aqueous samples of STPs. The metabolites as well as iso-LAS metabolites discharged from a STP into river water continued to degrade in the aquatic environment (Di Corcia et al., 1999a).

MATERIALS AND METHODS

Sources of wastewater samples

Wastewater samples were obtained from sewage treatment plant (STP), detergent-manufacturers and industries that utilize detergents as cleaning agent after production in Lagos and Ogun states, Nigeria.

Sample collection

Sampling was done with sterile plastic container (2 L) and collection of effluent was randomly done at all points of discharge of effluent along the production line and stored in the refrigerator at 4°C. All the effluent generated was untreated according to the personnel of the companies. The experimental design was a randomized complete block design.

Detergents used

Domestic detergents used included powdered 'Omo' which was purchased from Unilever Nigeria Plc., 'Elephant Extra' from PZ, Ariel from PT. Sayap Mas Utama, Jakarta Timur 13910, Indonesia. 'Persil' from Lever Brothers Ltd., Ireland. Teepol' was obtained from National Oil and Chemical Marketing Plc., (NOLCHEM) Lagos. Sodium Dodecyl Sulfate (SDS) was obtained from Fischer Scientific coy, New Jersey, USA.

Determination of anionic matter in test detergent products

The Research and Development Department (R&D) of PZ factory, Nigeria developed a modified Methylene Blue-Active Substance (MBAS) analysis method for detergent powders and the protocols of that methodology was used to determine % anionic matter in each of the test detergents (PZ R&D, 1991).

Determination of the physico-chemical properties of wastewater samples

The physico-chemical properties of the composite (morning and evening) wastewater samples were determined using the standard methods for the examination of water and wastewater (APHA, 1985; 1992).

Aerobic heterotrophic microbial counts

The effluent samples collected from each sampling point at 0 – 30 cm depths were serially diluted and inoculated onto Nutrient agar

plates in duplicates. The plates were then incubated at room temperature, (28 ± 2°C) for 24 – 48 h after which colony counts were taken (Okpokwasili and Nwabuzor, 1988; Larson and Payne, 1981).

Viable counts of detergent – utilizing microorganisms

The number of bacterial detergent-utilizers in each of the effluent sample collected was determined by inoculating minimal salt agar medium supplemented with test detergent at 0.01% (w/v) with 0.1 ml of the serially diluted effluent sample using spread plate technique. The inoculations were done in duplicates. The control plates were not inoculated. Incubation was at 28 ± 2°C for 48 - 72 h (Thysse and Wanders, 1972; Okpokwasili and Nwabuzor, 1988).

Bacterial isolates were characterized using standard and conventional methods. These tests were according to the methods of Gerhardt et al. (1981) and *Bergey's manual of systematic bacteriology* (1984). The fungal isolates were characterized using standard and conventional methods (Smith, 1981).

Microbial growth in wastewater spiked with detergents

Composite effluent sample (1 L) was dispensed into 2 L Erlenmeyer flask. A total of 16 flasks were filled with the composite effluent. The flasks were in duplicates. Then, 5 mg/L of test detergent was spiked into each wastewater flask with perforated plug for aeration. These were kept at ambient temperature (28 ± 2°C) for 30 days.

Samples were taken at Day 0, 5, 10, 20 and 30 from the Erlenmeyer flasks containing composite effluent samples spiked with 5 mg/L of detergent; this was to determine the pH, LAS concentration and total aerobic viable counts (Okpokwasili and Olisa, 1991).

Determination of LAS concentration using the Methylene Blue-Active Substance (MBAS) method

The method for determining the concentration of MBAS in the detergents was that adapted from *Standard Methods for the Examination of water and wastewater* (APHA, 1985; 1992). This involved the preparation of a series of ten separatory funnels for each of the test detergents. Each series of funnels contained different volumes, 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5 7.5 and 10 ml of solutions of the test detergents each made up to 50 ml with de-ionized water such that with the exception of sodium dodecyl sulphate (SDS), the concentration of the detergents in the above solution were 0.51, 1.60, 2.60, 3.76, 4.95, 6.18, 7.50, 8.8 12.5 µg/ml respectively. In the case of SDS, dilutions were prepared such that the concentration of detergents in the resultant solution after making up to 50 ml with de-ionized water corresponded to 0.1, 0.31, 0.53, 0.75, 0.99, 1.24, 1.49, 1.77, 2.50 µg/ml respectively. The lower SDS concentration was because it contained more surfactant than the other detergents under test. The tenth funnel in each series contained no detergent and served as control since a total of eight detergents were under test and needed determination, each test detergent sample was diluted taking a series of ten different volumes, a total of eighty samples were analyzed for MBAS determination to generate standard curve prior to the ultimate biodegradation studies.

The solutions of detergents in each series of separatory funnels were made alkaline by adding 1 N NaOH using 1 drop of 1% phenolphthalein solution as indicator to obtain a change in colour from colorless to pink. Then, 1 N H_2S0_4 was added in droplets to make the solution acidic thereby reverting the colour from pink to colorless. Thereafter, 5 ml of chloroform and 13 ml of methylene-blue reagent were added respectively to the funnel after which each funnel was shaken vigorously for 30 s for the contents to mix. The flasks were then kept still for 30 min for the phases to separate.

The chloroform layer was drawn off into a 100 ml Erlenmeyer flask. Extraction was performed three times employing 5 ml of chloroform each time. All extracts were pooled in the 100 ml Erlenmeyer flask. Extracts collected were later transferred back to the separatory funnel and 25 ml wash solution (6.7 mM Phosphate buffer, pH 7.1) was added to each funnel. The funnels were vigorously shaken for 30 s after which they were allowed 30 min to settle before the chloroform layer was drawn off through glass wool into 50 ml volumetric flasks. The chloroform extracts were finally shaken to ensure uniform mixing; Absorbance measurements of the extracts were done using Ultra Violet - visible Spectrophotometer (PHOTOMECH – 301 D^+ Model 100 – 20 U–V Spectrophotometer, OPTMA Co., Japan) set at 652 nm wavelength against blank chloroform. The concentration of the residual surfactant present in each test detergent in terms of methylene blue - active substance (MBAS) were then plotted against the time (Days) for the 30 day biodegradation period. The result obtained with the SDS served as the standard.

To determine the primary and ultimate biodegradation of test detergent samples using the *river die–away method*, 16 (2 L) Erlenmeyer flasks each holding 1000 ml of freshly collected 24 h composite effluent samples from both domestic and industrial sources, southwest Nigeria were obtained. The composite effluent samples in each flask were spiked with 5 mg/L test detergents coded as: AK17 (Klin), AK 27 (Omo), AK37 (Elephant), AK47 (Persil), AK57 (Ariel), AK77 (Teepol) respectively, while AK 67 (SDS) served as the standard containing 1.0 µg/ml of (SDS) detergent. The control flask was spiked with no detergent. The flasks were then left still under room temperature for 30 days.

One milliliter (1 ml) samples were drawn from each of the sixteen flasks and diluted with de-ionized water twenty times (x20) at day 0, 5, 10, 20 and 30 in order to determine the residual surfactant concentration in terms of MBAS for each test detergent (Larson and Payne, 1981; Okpokwasili and Olisa, 1991).

Ultimate biodegradation of the linear alkyl benzene sulphonates (LAS) the active matter in detergents was monitored using a Gas Chromatogram (GC). Samples from MBAS analyses on days 0, 5, 10, 20 and 30 were used. Calibration of the GC was done with Acenaphthelene, an aromatic compound. The Gas Chromatogram (SRI 8610 instrument, Model USA) was fed with samples by syringe injection. The residual surfactant–chloroform extracts were desulfonated by boiling in 5 ml concentrated phosphoric acid. The evolved volatile materials were trapped in 3 ml n-Hexane using soxhlet apparatus and a condenser heated electrically. The evolved volatile materials were brought to 25 λ with n-Hexane; they were cooled for 30 min before they were decanted in glass bottles and then taken to the GC laboratory for analysis.

The content of the glass bottles were allowed to evaporate for 24 h, leaving behind concentrates (that is, LAS biodegradation residues). The volume of sample injected into GC was 1- to –2 λ, and this was in a split ratio 10 – to– 1 for the GC; SRI 8610 instrument, 200ft x 0.01 in. (60m x 0.25 mm) FID channel 1 packed capillary column, 3% OV – 17 carrier gas of Nitrogen at 30ml/min. Components: STD – Mix CPT, Temp. 80°C (Sullivan and Swisher, 1969).

Gas chromatographic analysis

The gas chromatographic analyses for days 0, 10 and 20 were determined as reported by Sullivan and Swisher (1969). The GC (Perkins Elmer Auto–System Gas Chromatography, USA) analysis of the total hydrocarbon was carried out using a GC equipped with flame ionization detector (FID). A 30 m fused capillary column with internal diameter 0.25 mm and 0.25 m film thickness was used and the peak areas were analyzed with a *SRI Model 203 Peak Simple Chromatography Data System*. The column temperature was 60°C for 2 min to 300°C programmed at 12°C/min. Nitrogen was used as

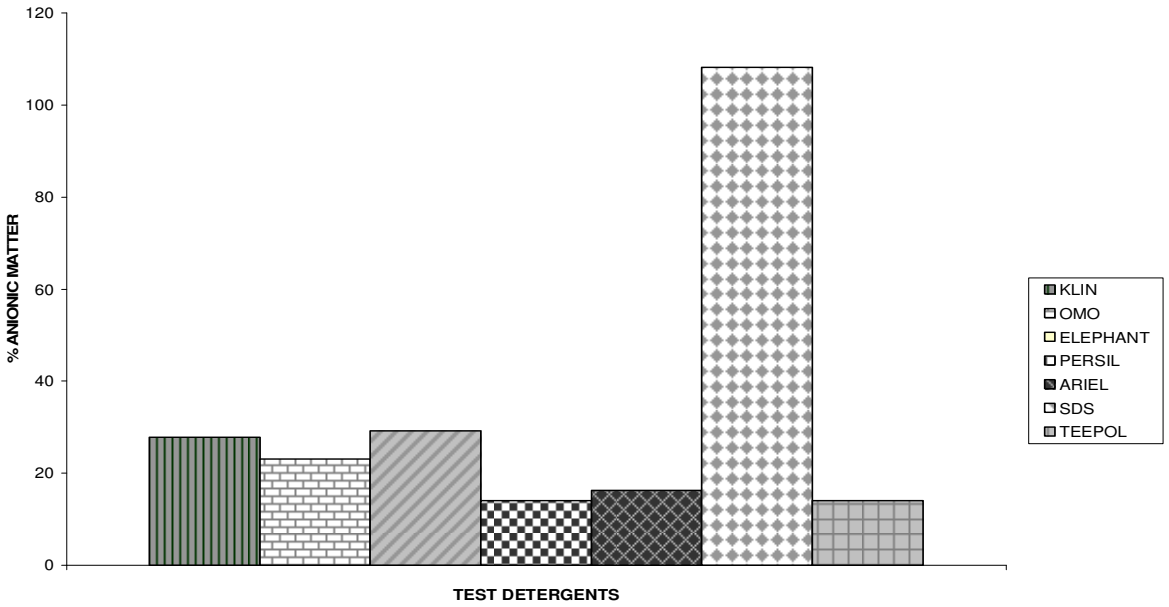


Figure 1. Determination of anion.

carrier gas at 37 psi. Hydrogen and air flow rates were 9 and 13 psi respectively. The injector port and detector temperatures were 250 and 320°C respectively as well as 1 – 2 µl of sample was injected.

Effect of detergent on the growth of four fungal isolates

Four fungal detergent-degraders were transferred independently and aseptically from Sabouraud dextrose-detergent agar slant and inoculated onto sterile yeast-extract peptone dextrose (YEPD) medium supplemented with detergent at 0.01% (w/v). The quadruplicate test tubes containing the 100 ml of medium were incubated at room temperature for 5 days. These isolates were B_1 *(Aspergillus oryzae*) B2 (*Myceliophthora thermophila*), B_3 (*Geomyces* sp.), and B_4 *(Alternaria alternata).* After 5 days of incubation, day 6, 7, 8 and 9 harvested mycelia's weight were determined intermittently after 24 h, by filtering the content of each set of test tubes using sterile filter paper while the mycelia were Oven-dried (LTE G150 Oven, UK) at 85°C for 24 h. Thereafter, the mycelia were cooled and weighed. This process was repeated for days 6, 7, 8 and 9. The control was YEPD-detergent medium without inoculants (Gerhardt et al., 1981).

Detergent degradation by microbial isolates (microcosm experiment)

The microbial detergent- utilizers obtained from *river die-away* methods were tested in a laboratory simulated biodegradation study. Nutrient broth (1 L) supplemented with test detergent product (Elephant) at 0.1 mg/ml was dispensed into eight conical flasks (250 ml) prior to sterilization at 121°C for 15 min using the autoclave, such that each flask had 250 ml of nutrient-detergent broth. This was inoculated with five bacterial isolates and four fungal isolates in this pattern; X1 (fungal consortium), X2, X3, X4, X5, X6 (bacterial isolates) and X50 (microbial consortium) all duplicated. The control flasks were uninoculated. This experiment was monitored over a period of 30 days with samples taken on days 0, 10, 20, and 30 for microbial population count, pH measurement (Metrohm 780 pH Meter, UK) and *Absorbance readings at 652 nm on U-V spectrophotometer* (Heλios Gamma & Delta Spectrophotometer Model 9423 UVG, Spectronic Unicam Ltd., Mercers Row, UK.) (Okpokwasili and Olisa, 1991).

RESULTS AND DISCUSSION

The results of this study were predicated on the fact that microorganisms are ubiquitous. Hence, the detection of the microbial consortium involved in detergent degradation. The anionic matter (LAS) content of SDS (sodium dodecyl sulphate) was the highest of the seven detergent products used, while the least was found with Persil. The LAS concentration in both Persil and Teepol which are foreign products were relatively low compared with those of other detergent products analyzed (Figure 1). The microbial detergent-utilizers were characterized using standard and conventional methods (Table 1).

The physico-chemical properties of the composite wastewater used for this study showed that it was heavily polluted with organic matter, hence, the relatively high BOD value. Comparatively, the COD falls short of Federal Environmental Protection Agency (FEPA), European Union (EU) and World Health Organization (WHO) standards (Table 2). This might be the reason for the slow rate of mineralization of xenobiotic compounds in this ecosystem. The NO_3 – N, SO_4^{2-}, PO_4^{3-}, NH_4 – 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 this is the reason for the longer time required for mineralization to be effected, since high concentration of N and P may be toxic to microorganisms. Although, the dissolv-

Table 1. Micromorphology and biochemical characterization of bacterial detergent–degraders.

Isolate code	Gram reaction	Cellular – morphology	Catalase	Oxidase	Indole test	Motility test	MR	VP	Citrate utilization	Urease activity	Starch hydrolusis	Gelatin hydrolysis	Growth on MacConkey	NO_3 reduction	Coagulase test	Spore test	Glucose	Xylose	Lactose	Suscrose	Aratinose	Galactose	Maltose	Mannitol	Sulicin	Raffimose	Probable identity
X^1	+	O	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	*E. majodoratus*
X_2	-	R	+	-	-	-	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	*K. liquefasciens*
X_3	-	R	+	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	*E. liquefasciens*
X_4	-	R	+	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	-	+	*K. aerogenes*
X_5	-	R	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	-	-	*E. coli*
X_6	-	R	+	-	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	*E. agglomerans*
A	+	C	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	-	-	-	*S. albus*
B	-	R	+	-	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	*E. agglomerans*
C	-	R	+	-	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	+	+	-	-	*Proteus sp.*
X_{55}	-	R	+	-	+	-	-	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	*K. oxytoca*
U	+	R	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	*Brevibacterium sp.*
X8	-	R	+	+	-	+	+	-	+	-	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	*P. aeruginosa*

R = Rods, O = oval, C = cocci, + = positive, and − = negative.

ed O_2 was relatively adequate, it was due to presence of aerators in the Agbara STP. This is suggestive of the fact that optimal presence of a single physico-chemical factor does not determine the rate of mineralization of xenobiotics in the environment. In temperate climate, mineralization of synthetic detergent products in wastewater has been achieved under 25 days (WWI, 2005, 2004), whereas under tropical climatic conditions this study showed that for some of the commercial detergents it would take more than 30 days for some of them to be mineralized by microorganisms which might be due to absence of optimal physico–chemical conditions in wastewater and the archaic technology being used in sub-Sahara African countries STP.

Compliance with EU regulations on discharged effluent (WWI, 2005) is yet to be met by any country in sub-Saharan Africa due to problem of system design as regards STPs and heavy discharge of synthetic organic materials in both domestic and industrial sewers.

Centralized wastewater treatment plants can achieve total nitrogen concentrations of 3 mg/L for discharged effluent from STP which is the currently set limit of technology in the United State of America as at 2004 (US EPA, 2000), under natural conditions or during treatment processes, the degradation of pollutants is controlled often by a variety of physical and chemical parameters such as temperature, pH and availability of the substrate, and not by the presence or absence of the appropriate population of microorganisms. The presence of optimal physical and chemical conditions will allow eventual evolution and growth of the best-adapted microbial population (WWI, 2005, 2004). This fact was corroborated when similar strains of detergent-degraders from Central Medical Laboratory, Nigeria were subjected to detergent degradation under similar physico-chemical conditions and they were able to utilize the detergent but for longer acclimatization time.

Table 2. Mean physico-chemical properties of composite wastewater

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_20)	10.54	11.08	6 – 9	7.5 – 8.5
Conductivity @ 25^0C	204 Usm^{-1}	185 Usm^{-1}	NS	340
Temperature	34.3^0C	33.9^0C	40^0C	$20 – 25^0C$
PO_4^{3-}	99.9 mg/L	90.3 mg/L	5 mg/L	10 – 25 mg/L
SO_4^{2-}	92.7 mg/L	88.6 mg/L	500 mg/L	NS
NO_3^{-1}	26.29 mg/L	22.86 mg/L	20 mg/L	20 mg/L
Total suspended solid (TSS)	170 mg/L	200 mg/L	30 mg/L	35 mg/L
COD	57.51 mg/L	52.01 mg/L	200 mg/L	<125 mg/L
Specific gravity	1.009	1.022	NS	NS
$NH_4 – N$	193.5 mg/L	178.7 mg/L	NS	15 mg/L
Cl^{-1}	36.18 mg/L	37.95 mg/L	600 mg/L	600 mg/L
Dissolved oxygen (DO)	9.05 mg/L	9.45 mg/L	>2 mg/L	2 mg/L
BOD	38.08 mg/L	34.41 mg/L	30 mg/L	<25 mg/L
Total hydrocarbon (THC)	15.0 mg/L	13.6 mg/L	10 mg/L	<10 mg/L
DO_5	36.04 mg/L	32.67 mg/L	>2 mg/L	NS
Total dissolved solid (TDS)	NS	NS	NS	NS

NS = Not Specified
(Source: FEPA, 1991; Degremont, 1991; WWI, 2005).

The mean aerobic heterotrophic bacterial count from effluent was 42.9 x 10^6 cfu/ml, while the mean aerobic heterotrophic fungal population count was 4.5x10^6 cfu/ml. The total viable count (TVC) for Detergent–utilizing bacterial population was 209.4 x 10^5 cfu/ml. These were determined with composite wastewater samples from all the sampling points including the Agbara STP. Acclimatization of this microbial population to detergent components enhances the biodegradation efficiency of the microorganisms. Although, bacterial population was more than fungal detergent-degrader population in tropical wastewater, this agrees with the previous findings of researchers like Okpokwasili and Olisa, (1991); Amund et al. (1997). The adaptability of native microbial population in wastewater to detergent component would be the reason for their success at mineralizing LAS component in effluent where the physico-chemical properties of the wastewater ecosystem were supportive of the survival of these microorganisms (Spain and van Veld, 1983).

Alkaline pH range as well as mesophilic temperature range was observed to favor the acclimatization process for the native detergent–utilizing microbial population as soon as the optimum conditions became prevalent within the wastewater ecosystem (Figure 2). These physico-chemical factors were particularly important for the survival of detergent–utilizing microbial consortium in the wastewater. These findings in connection with the pH and temperature range corroborated the findings of Okpokwasili and Olisa (1991). Responding to changes in the environment is a fundamental property of a living cell and chemo taxis is the best studied bacterial behavioral response that navigates the bacteria to niches that are optimum for their growth and survival (Bren and Eisenbach, 2000). Bacterial chemo taxis (Bacterial heterotrophic population) was in this order KLIN > PERSIL > OMO > ELEPHANT > ARIEL > SDS and least with TEEPOL, while PERSIL attracted the highest fungal heterotrophic population (Figures 3 and 4). TEEPOL attracted the least fungal heterotrophic population from the field experiment while SDS has the highest anionic matter (LAS) content of all the test detergent products and it's the most easily mineralized because of its chemical structure (Figure 3). This corroborated the submission of Willets (1973a). SDS is being used as the standard in this study. In the course of the field study, the composite wastewater was spiked with each of the different test detergents, the chemical changes were monitored via the pH changes. At Day 0, pH changes was in this order KLIN>ARIEL >OMO >ELEPHANT >TEEPOL >PERSIL > SDS while at Day 30, OMO had the highest value with ELEPHANT having the least value (Figure 2) whereas during the microcosm study the pH range was adjusted by microbial metabolism to the range 6.9 – 8.8 (Figure 6). Thus, alkaline pH range supported the microbial consortium that mineralizes synthetic detergents. This explains the absence and reducing population of some detergent–utilizing fungal species after day 10 during the laboratory simulated biodegradation of test detergents (Figures 5 and 7), the pH shifted

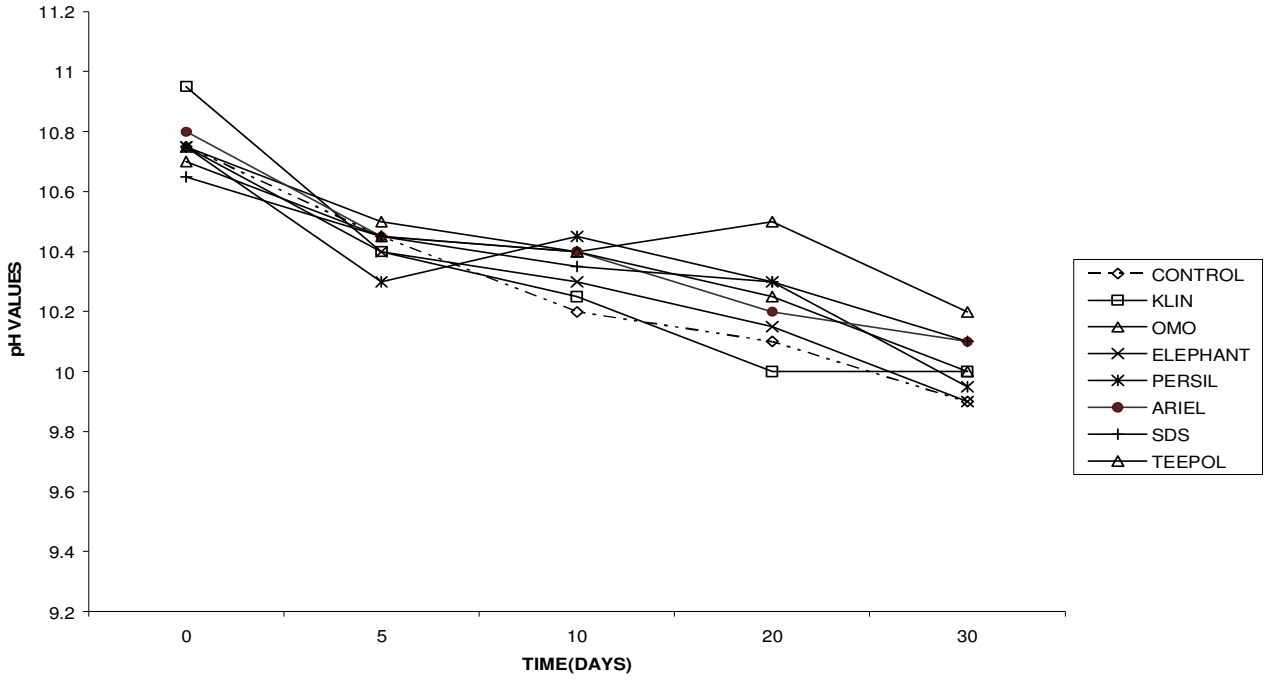


Figure 2. pH readings of primary biodegradation from field experiment (shake-flask).

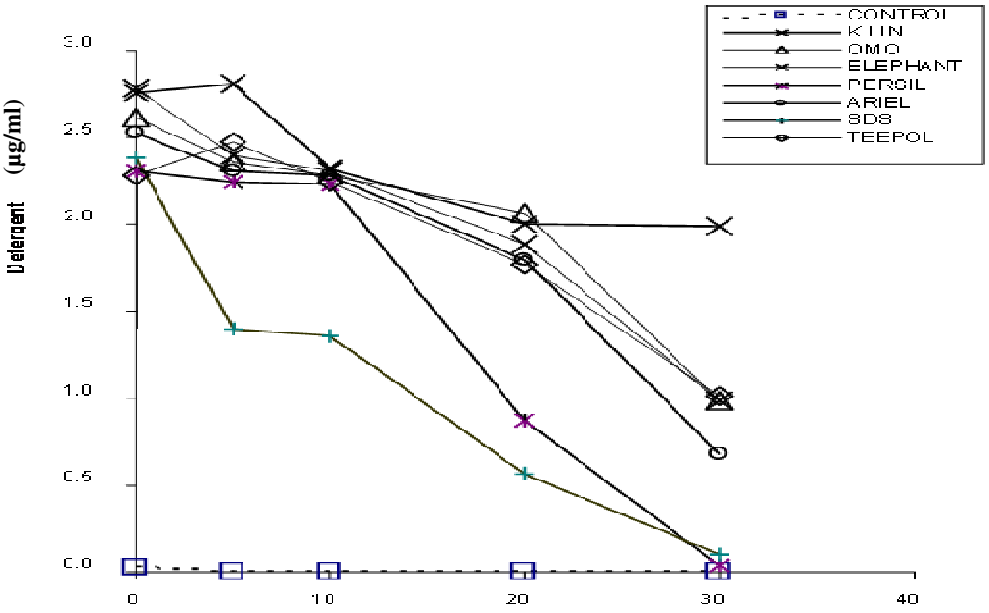


Figure 3. Biodegradation residues from (shake flask) field experiment.

to the alkaline range as a result of generation of alkaline intermediates which accounted for the initial pH increases. Although, the pH falls as the number of days increased further probably as a result of production of some acidic metabolites (SO_4^{2-}), this has been reported by other researchers (Hales et al., 1986; Okpokwasili and Olisa, 1991). Macro nutrients such as P and S are fundamentally essential in microbial cell physiology and biochemistry, being a part of such important bio-molecules as phospholipids, nucleic acids, proteins as well as nucleotides, cofactors involved in energy transport and catalysis of many cell processes (Hales et

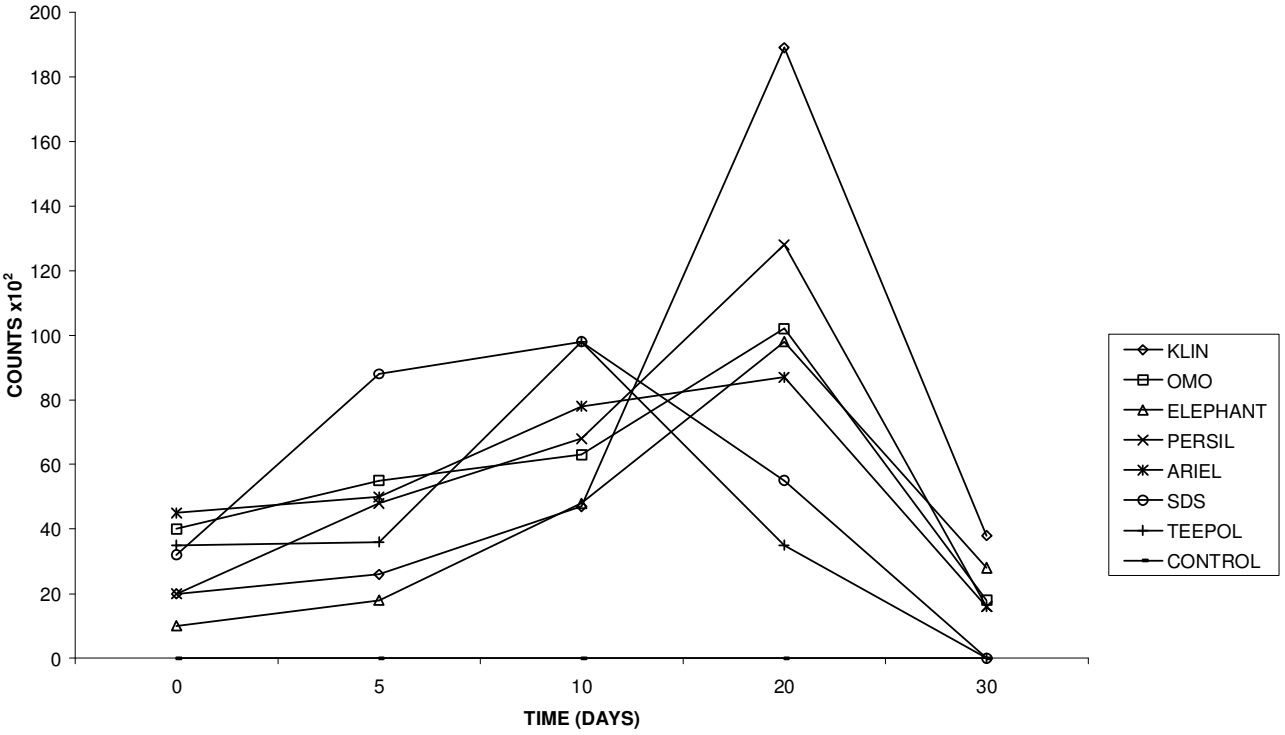


Figure 4. Mean aerobic bacterial detergent-degrader count (shake-flask experiment).

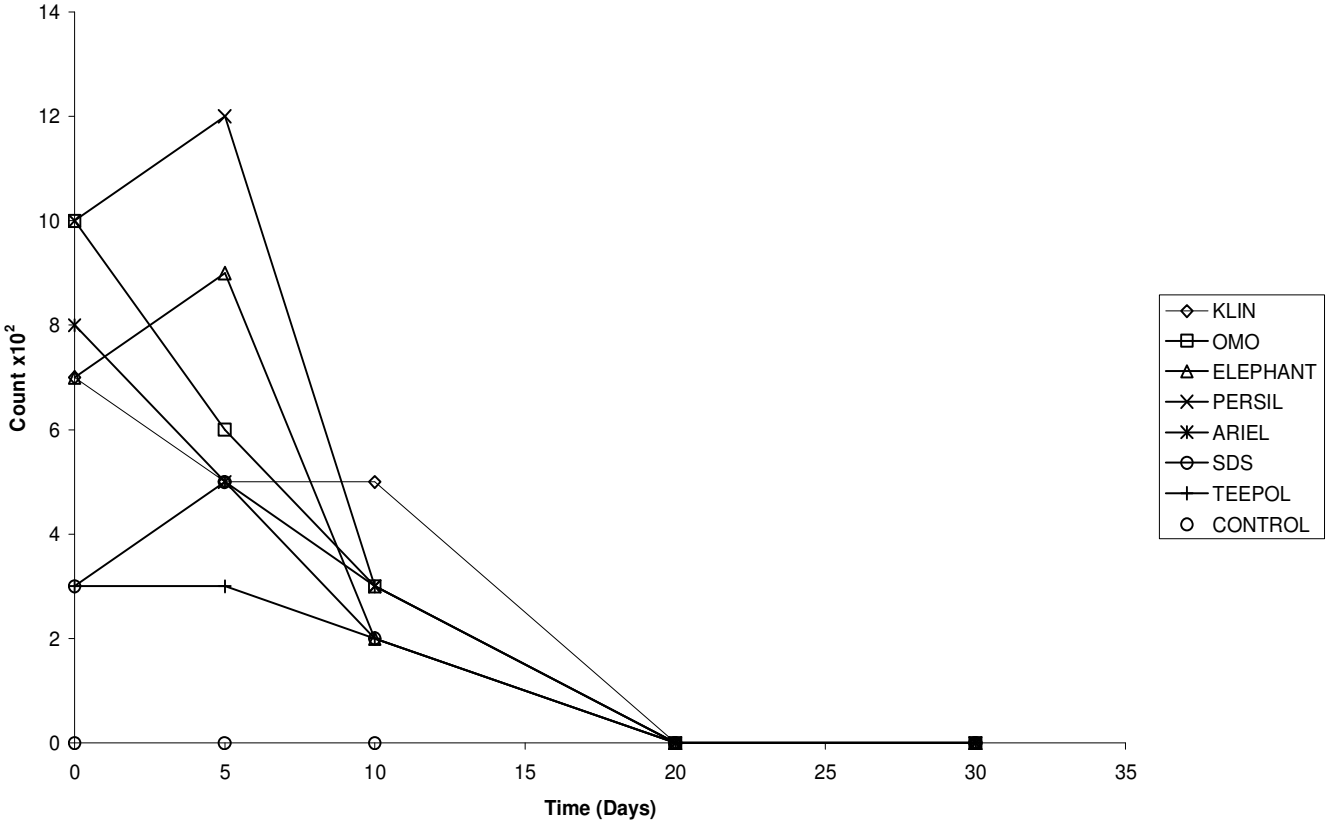


Figure 5. Mean fungal detergent-degrader count (shake-flask experiment).

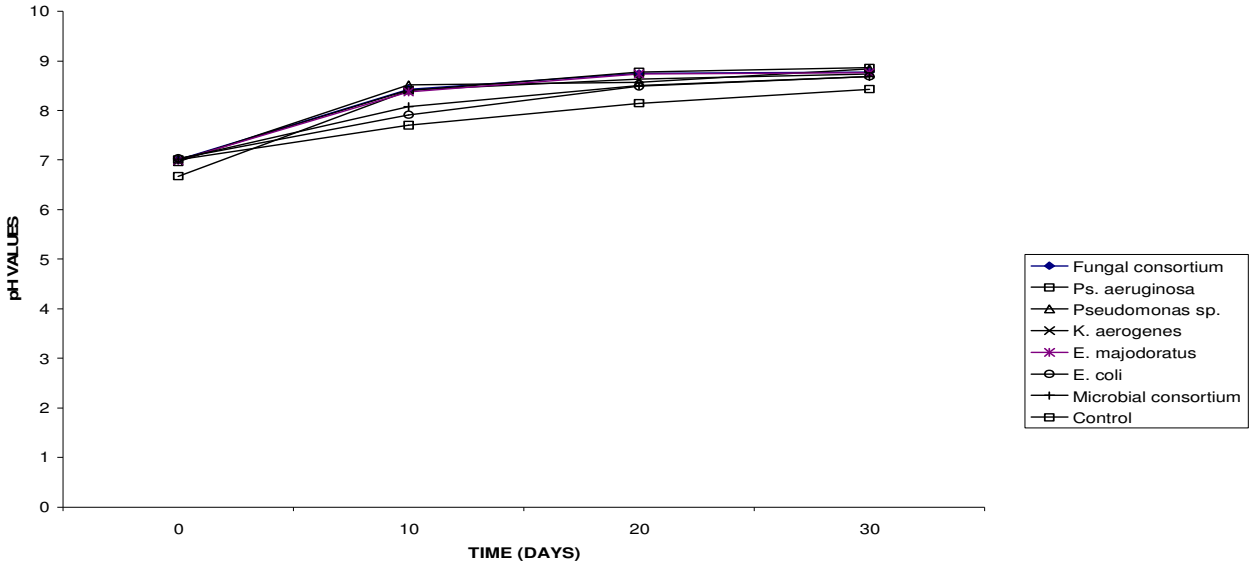


Figure 6. Mean pH readings of biodegradation of test detergents (Microcosm experiment).

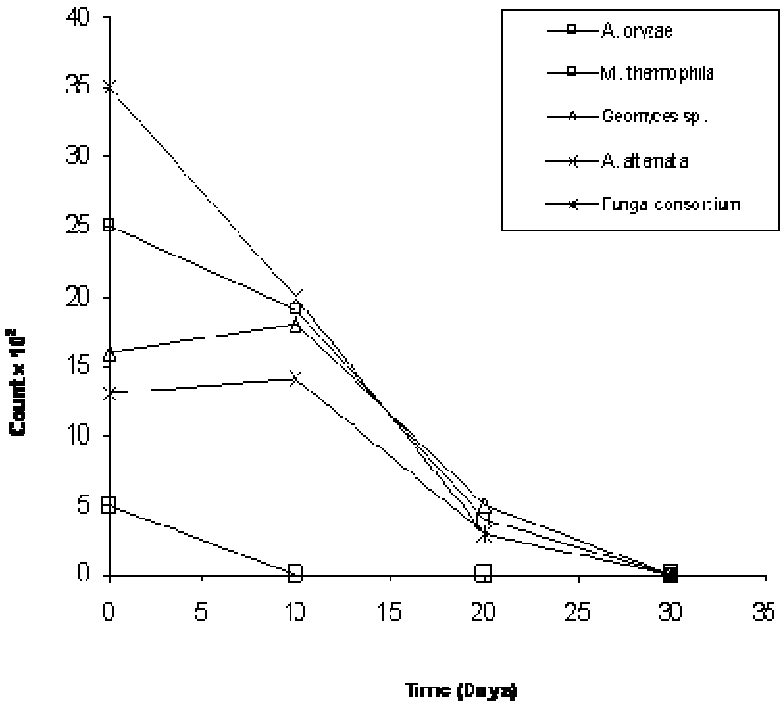


Figure 7. Mean fungal detergent-degrader count (microcosm experiment).

al., 1999). Thus, the overall increase in microbial numbers in the 30-day biodegradation period may be attributed to the availability of carbon source and sulphate in the detergent product for energy and growth (Figures 7 and 8) (Kertesz et al., 1994; Zurrer et al., 1987). The microbial culture media lacks C and sufficient SO_4^{2-} sources. Hence, commercial detergent products with relatively high SO_4^{2-} concentrations exhibit rapid degradation because this enhances both biomass accumulation and increase in cell number of the detergent-degraders (Konopka et al., 1996). This supports the observations of Higgins and Burns (1975) who stated that the relationship between surfactants and microbes is complex and involves factors other than biodegradation

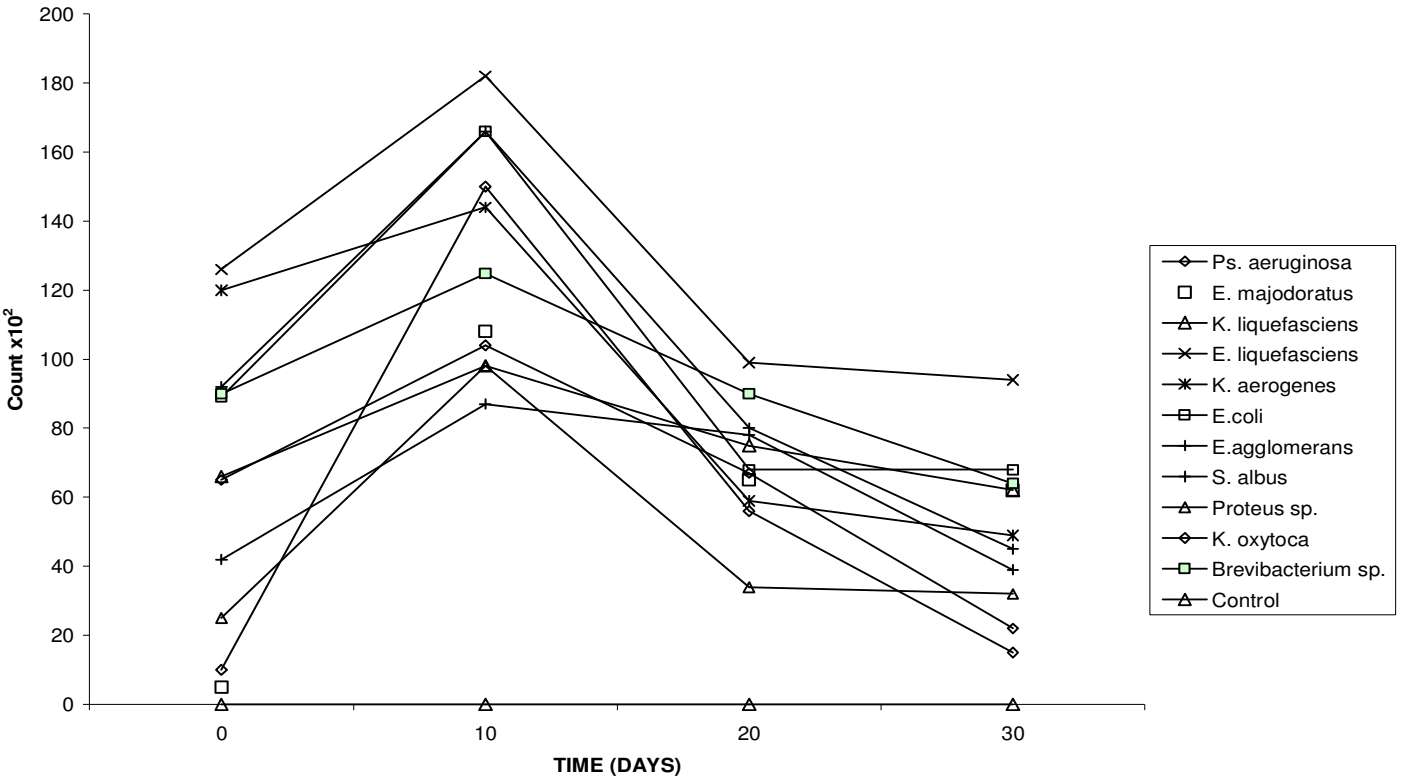


Figure 8. Bacterial colony count (microcosm experiment).

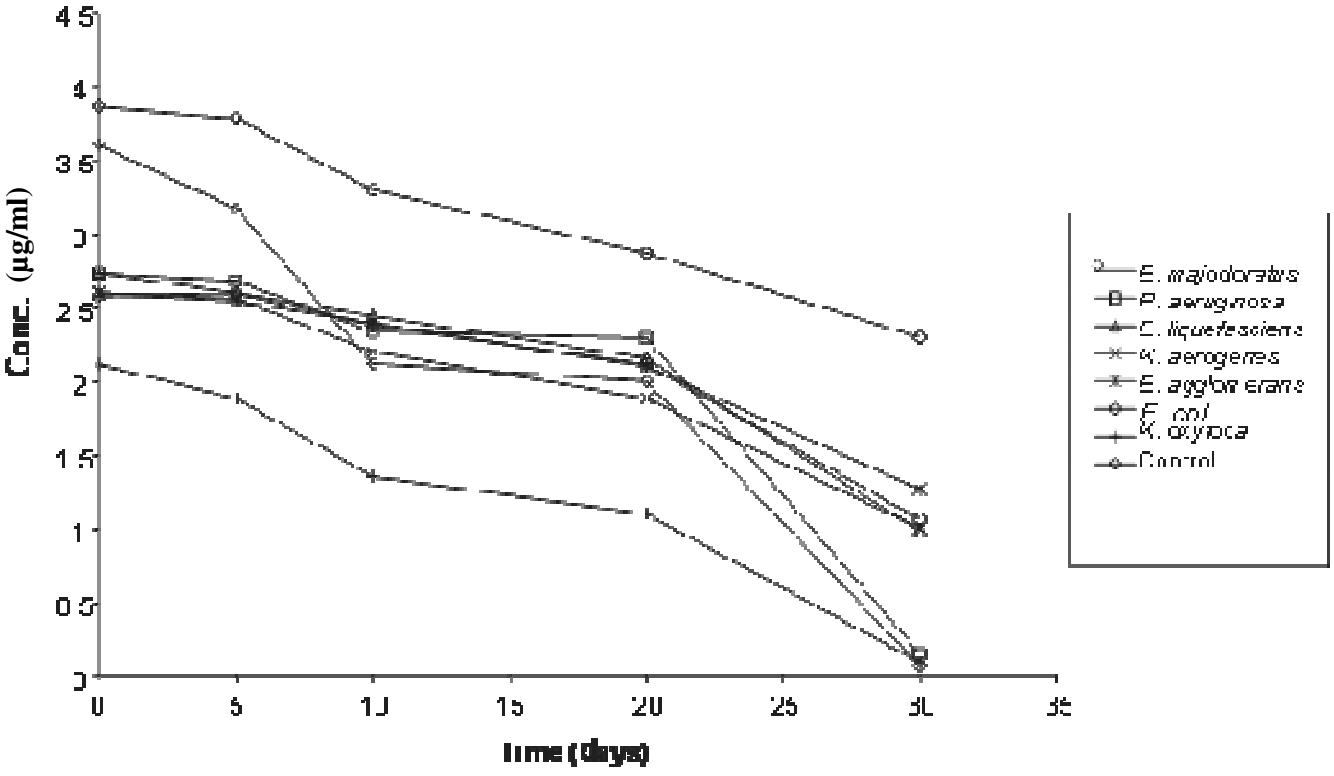


Figure 9. Biodegradation residues (microcosm experiment).

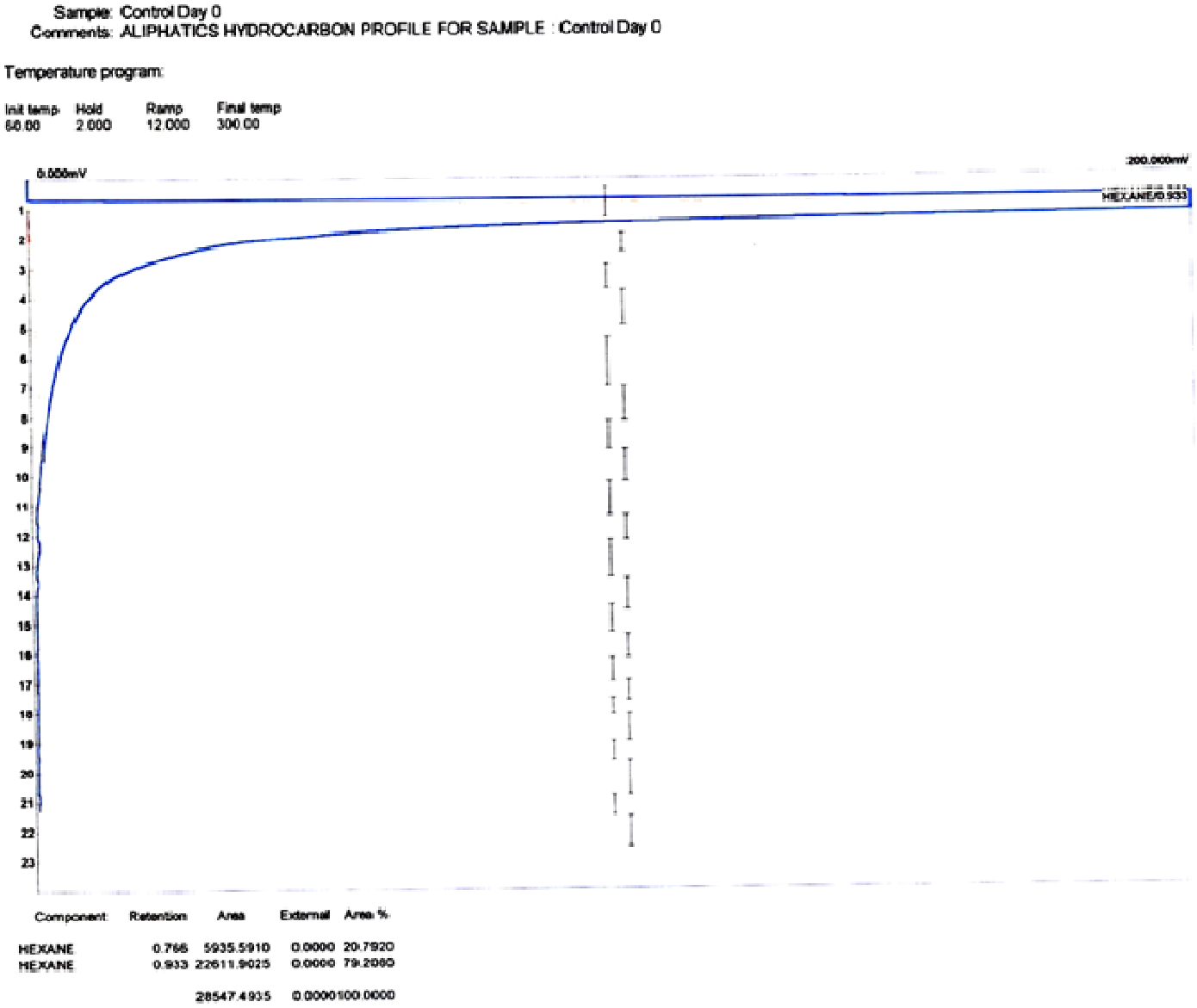


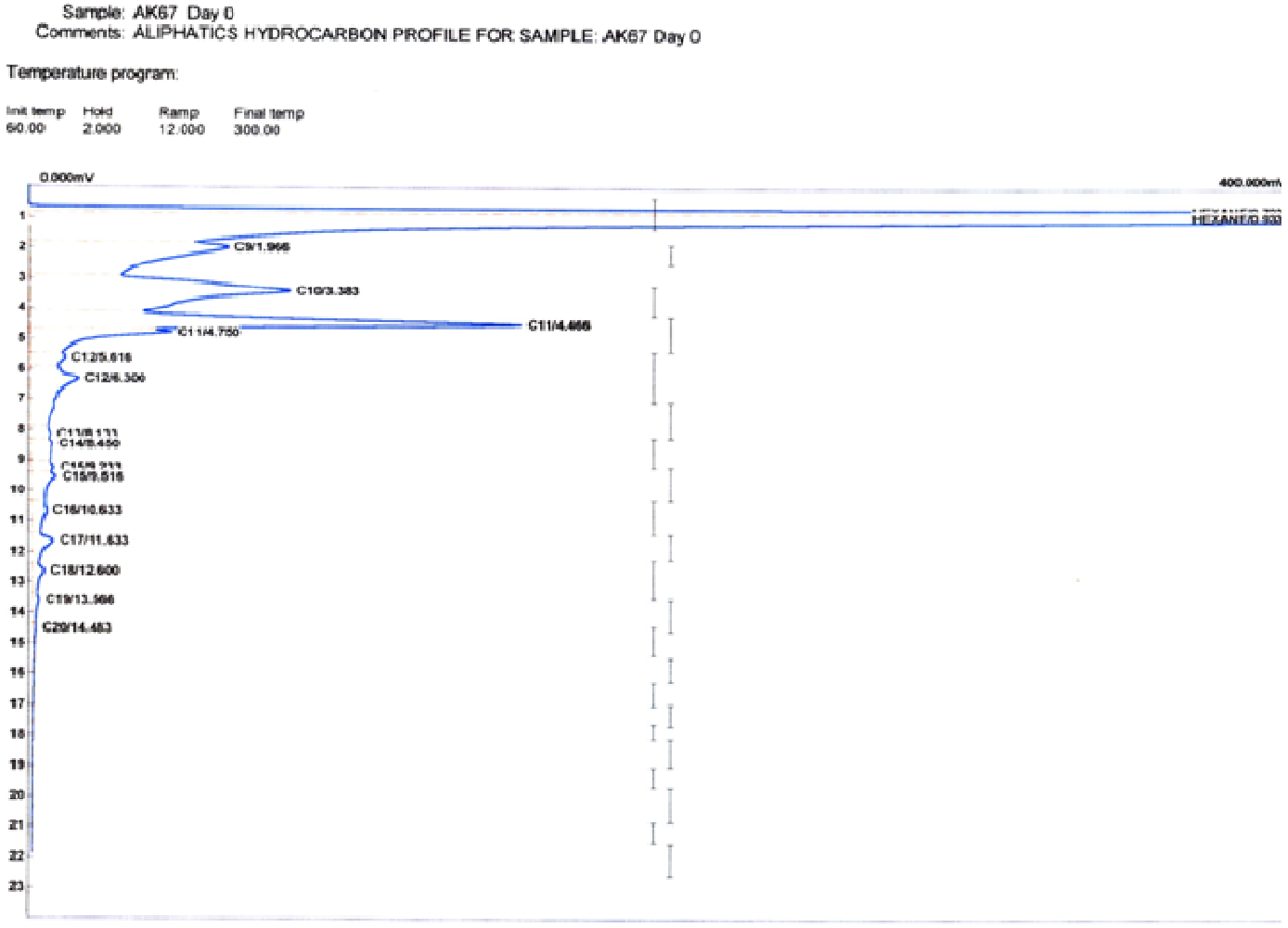
Figure 10. GC profile of detergent residues (shake flask experiment). Control: no detergent.

and that under appropriate conditions, surfactants can act as bactericides and bacteriostats. However, the ability of a surfactant to be bactericidal depends largely on the microbial species, size of the hydrophobic portion of the surfactant molecule, purity of the water sample in terms of organic matter such as sewage and the presence of divalent metal ions (Higgins and Burns, 1975).

The microbial isolates from the shake-flask experiment capable of utilizing the test detergents as C and energy sources were *Enterococcus majodoratus*, *Klebsiella liquefasciens*, *Enterobacter liquefasciens*, *Klebsiella aerogenes*, *Escherichia coli*, *Enterobacter agglomerans*, *Staphylococcus albus*, *Pseudomonas aeruginosa*, *Proteus* sp, *Klebsiella oxytoca*, *Brevibacterium* sp., *Myceliophthora thermophila*, *Geomyces* sp, *Alternaria alternata*, *Verticillium alboatrum*, *Aspergillus flavus*, *Trichoderma* sp, and *Aspergillus oryzae.*

Some of these isolates have been reported as capable of utilizing pure anionic surfactant molecule (Gledhill, 1974; Sigoillot and Nguyen, 1992; Schleheck et al., 2004) and surfactant components of detergents (Okpokwasili and Nwabuzor, 1988; Amund et al., 1997; Kertesz et al., 1994).

When the test synthetic detergents were subjected to



Component	R-etention	Area	External	Area %
HEXANE	0.733	5356.1485	0.0000	15.1356
HEXANE	0.933	15692.1460	0.0000	44.2609
C9	1.966	3078.3500	0.0000	8.6827
C10	3.383	4054.6230	0.0000	11.4364
C11	4.466	2044.4885	0.0000	5.5872
C11	4.750	1105.8760	0.0000	3.1192
C12	5.616	277.4800	0.0000	0.7827
C12	6.300	1120.3270	0.0000	3.1600
C13	8.133	155.5335	0.0000	0.4387
C14	8.450	308.9290	0.0000	0.8714
C15	9.233	132.7250	0.0000	0.3744
C15	9.516	334.2895	0.0000	0.9429
C16	10.633	257.7525	0.0000	0.7270
C17	11.633	250.2370	0.0000	0.7058
C18	12.600	148.2380	0.0000	0.4181
C19	13.566	84.0630	0.0000	0.2371
C20	14.483	42.5150	0.0000	0.1199
		35453.7235	0.0000	100.0000

Figure 11. GC Profile of detergent residues (shake flask experiment). AK 67 = Sodium dodecyl sulphate (SDS).

ultimate biodegradation in both the shake – flask and laboratory simulated experiments, the native micro-organisms metabolized the detergent components for growth and biomass accumulation (Figure 9), as a result the gas chromatography was used at intervals to analyze the samples within a 30 day period 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 (Larson and Payne,1981; Swisher, 1987; Di Corcia et al., 1999a,b; Konopka et al., 1996). Although, unusual peaks in GC profiles were detected by other researchers but it was

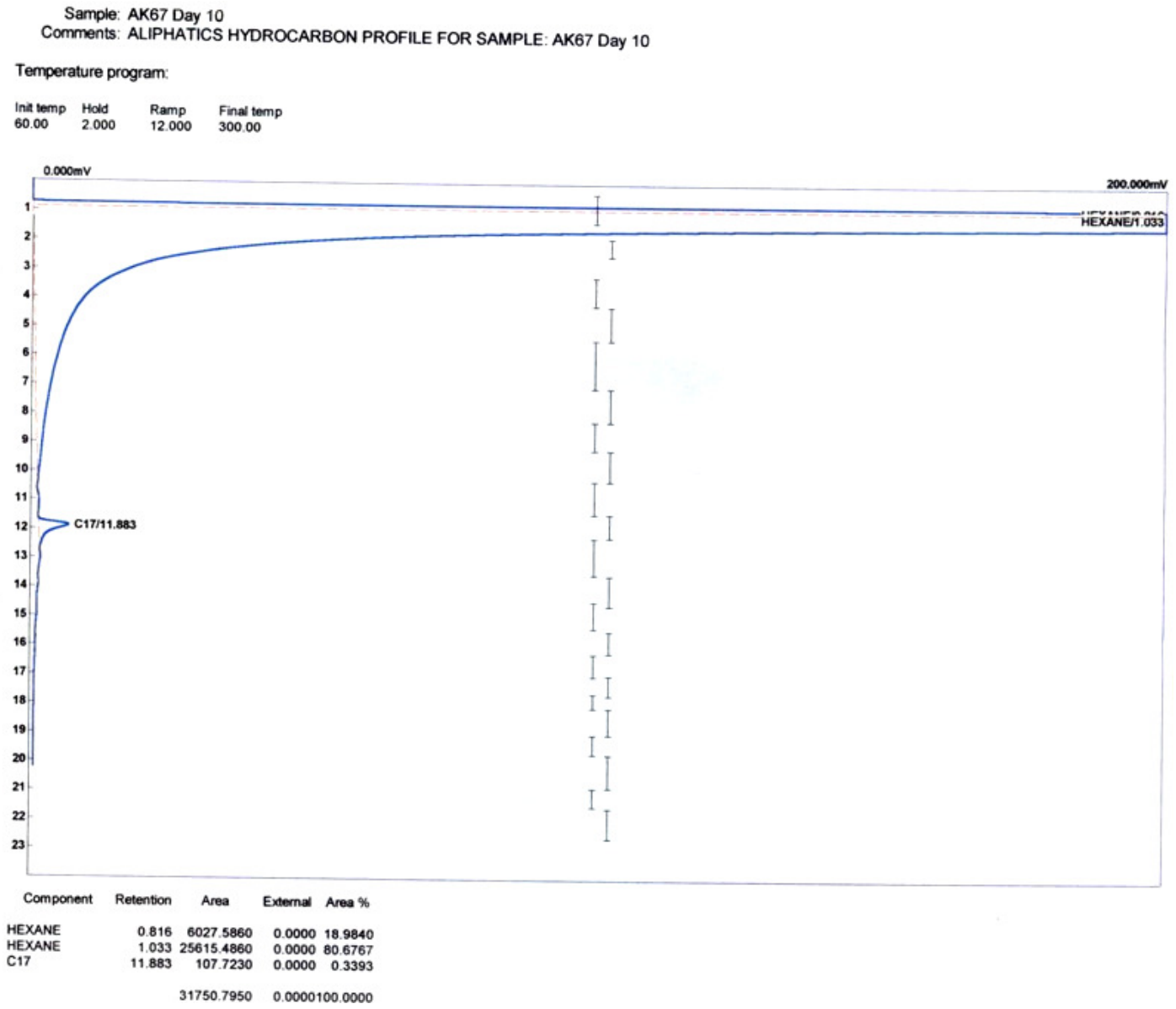


Figure 12. GC Profile of detergent residues (shake flask experiment). AK 67 = Sodium dodecyl sulphate (SDS).

sparsely reported, these unusual peaks were not strange because previous researchers had also observed it (Kertesz et al., 1994).

It has long been recognized that susceptibility to primary biodegradation is insufficient in itself to prove the environmental acceptability of a compound. Information on the intermediates formed in the course of biodegradation is needed as well. Hence, the desire for a conclusive evidence for the ultimate biodegradation of synthetic detergent component in open-rivers, this was provided by the GC analysis (Swisher, 1987). Although, few high peaks were detected in the chromatograms suggesting inclusion of certain hydrocarbons in detergent formulations outside that of industry prescriptions (Figures 11, 13, 18 and 19). It has been legislated by the international committee on synthetic detergents that commercial synthetic detergents should be manufactured with C_{10} – C_{14} atoms (CLER, 1999) but this study discovered some other C atoms up to C_{20} from the GC profiles of analyzed samples from the shake – flask experiment while in the microcosm study, the GC profile revealed presence of C_{21} atoms (Figure 17, 18 and 19). It is either other chemical substances were included in detergent formulation which are undisclosed to con-

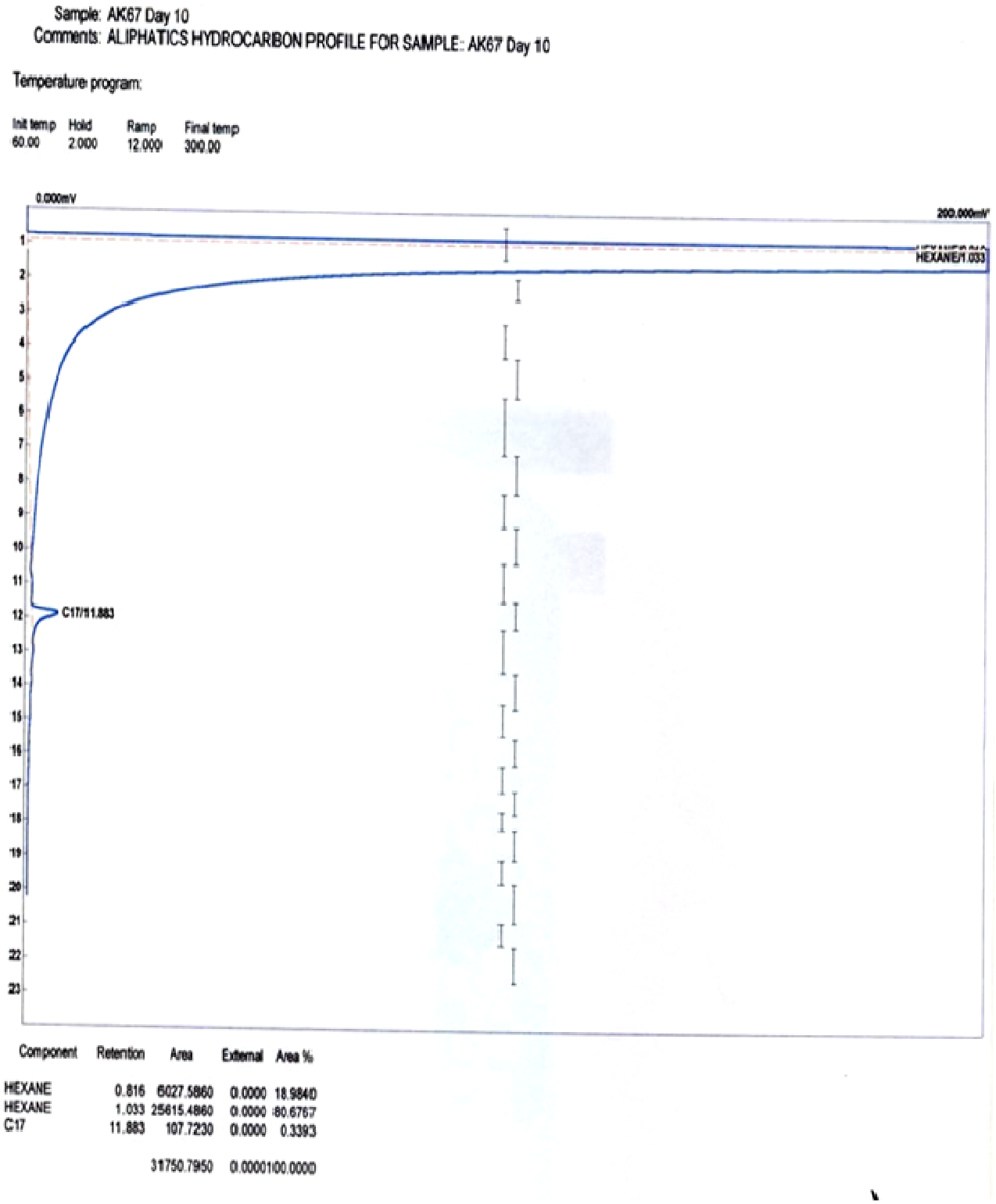
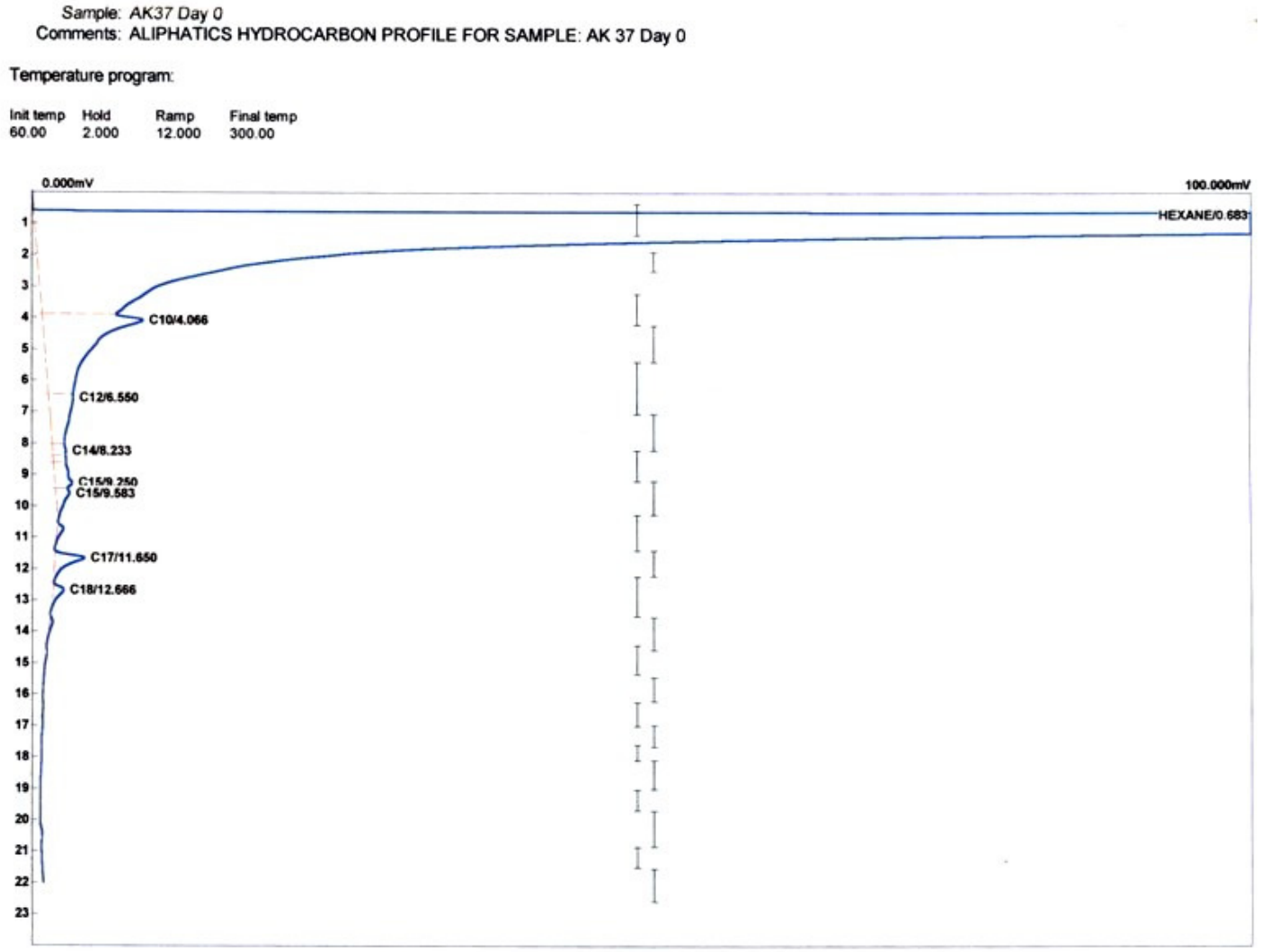


Figure 13. GC profile of detergent residues (shake flask experiment). AK 67 = Sodium dodecyl sulphate (SDS).



Component	Retention	Area	External	Area %
HEXANE	0.683	20648.5590	0.0000	95.4798
C10	4.066	635.5460	0.0000	2.9388
C12	6.550	149.2730	0.0000	0.6902
C14	8.233	22.6565	0.0000	0.1048
C15	9.250	59.1290	0.0000	0.2734
C15	9.583	35.6750	0.0000	0.1650
C17	11.650	54.3140	0.0000	0.2512
C18	12.666	20.9570	0.0000	0.0969
		21626.1095	0.0000	100.0000

Figure 14. GC profile of detergent residues (shake flask experiment). AK 37 = ELEPHANT detergent.

sumers which is certainly the case because substances such as toluene sulphonate has been reported in some detergent formulations (Schoberl and Huber, 1988). Shake–flask experiment with wastewater samples when subjected to GC analysis after 0, 5, 10, 20 days biodegradation process showed that C_{14} LAS homologues were mineralized faster than C_{12} homologues while during the laboratory simulated (microcosm study) biodegradation process the result was the same, thus corroborating the fact that increased distance between sulphonate group (phenyl position and chain length) and the far end of the hydrophobic group increases the speed of primary biodegradation (Huddleston and Allred, 1963; Swisher, 1970; Swisher, 1975). The residual total hydrocarbon content (THC) from extracted samples for the laboratory simulated biodegradation was from 0.13×10^{-6} – 1.82×10^{-6} mg/ml for the 30 day biodegradation process. The more sophisticated desulphonation and gas

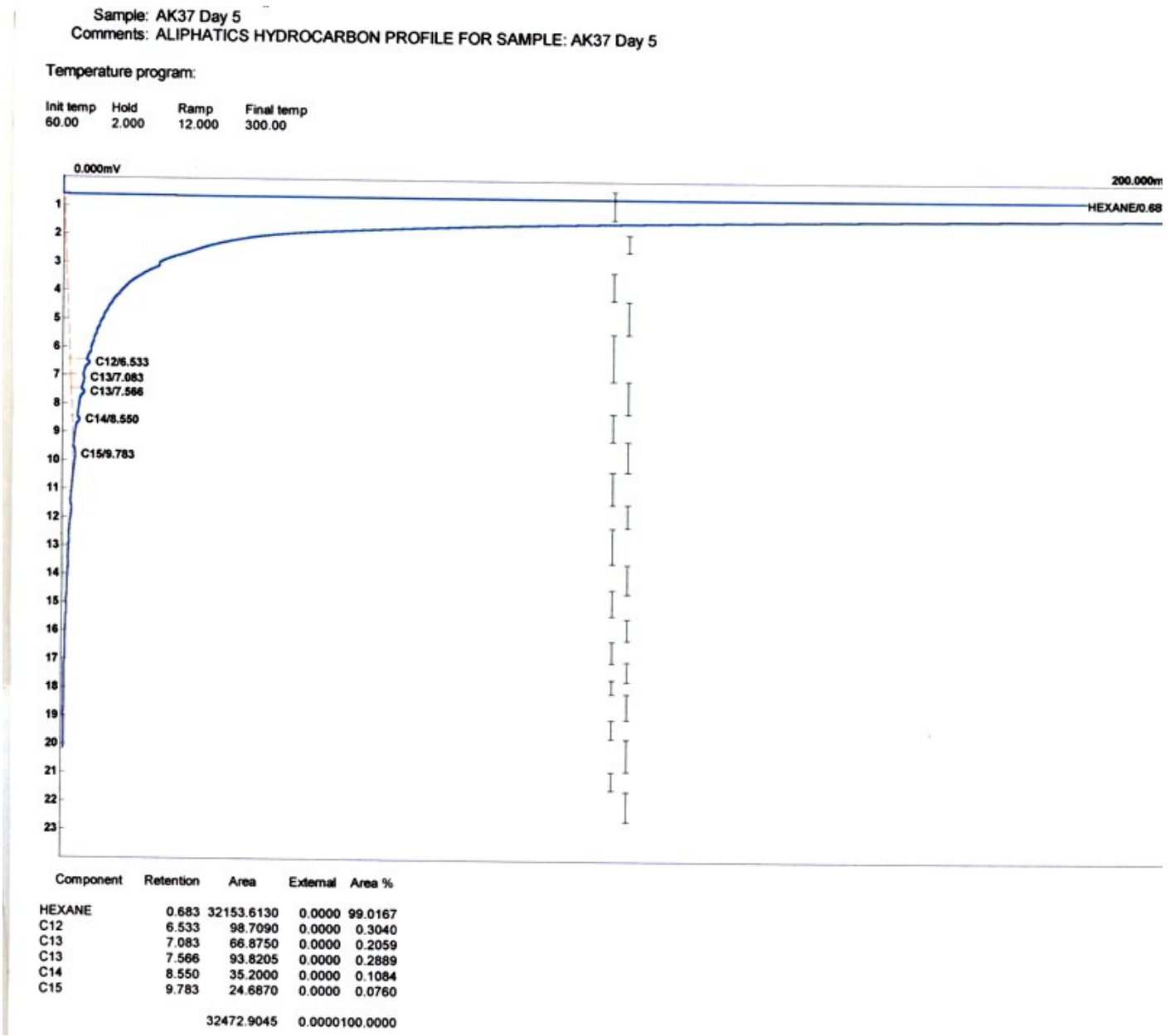


Figure 15. GC profile of detergent residues (shake flask experiment). AK 37 = ELEPHANT detergent.

chromatographic method enables quantization of the LAS present as well as the relative concentration of each of the chain length. The performance level for the microbial consortium was assessed with fungal consortium (X_1) and microbial consortium (X_{50}). The microbial consortium (X_{50}) (Figure 20) was second to the best in performance because bacterial isolate *Ps. aeruginosa* was able to metabolize detergent product with only 1.86 $x10^{-6}$ mg /ml remaining after 20-day incubation period in this study.

The best culture of detergent–utilizing bacterial strains were *Ps.* a*eruginosa* and *K. oxytoca* while bacterial isolate *E. coli* was the slowest in terms of rate of detergent – utilization as shown by the GC profile (Figures 18 and 19). SDS was found to be the most rapidly biodegraded of all the test detergent products utilized for this study followed by Elephant (Figures 11 – 16). This is due to the fact that straight chain LAS are rapidly biodegraded than branched chain LAS, also SDS is a purer detergent of analytical grade often used in the laboratory with over 95% purity level while Elephant's

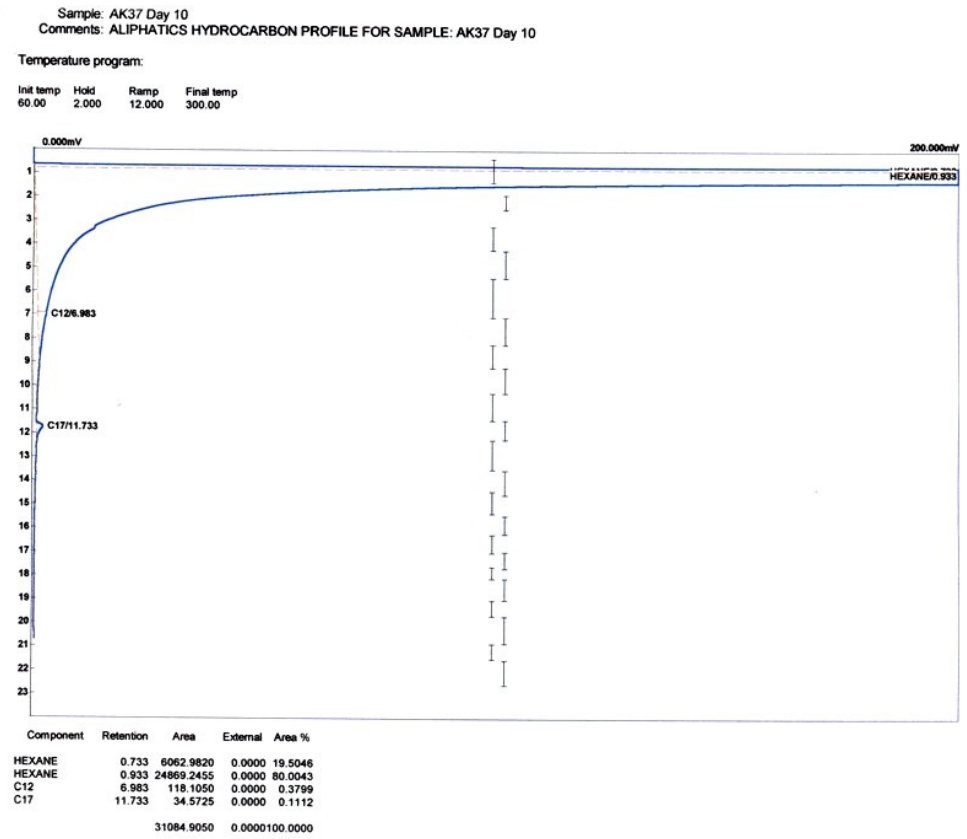


Figure 16. GC Profile of detergent residues (shake flask experiment). AK 37 = ELEPHANT detergent.

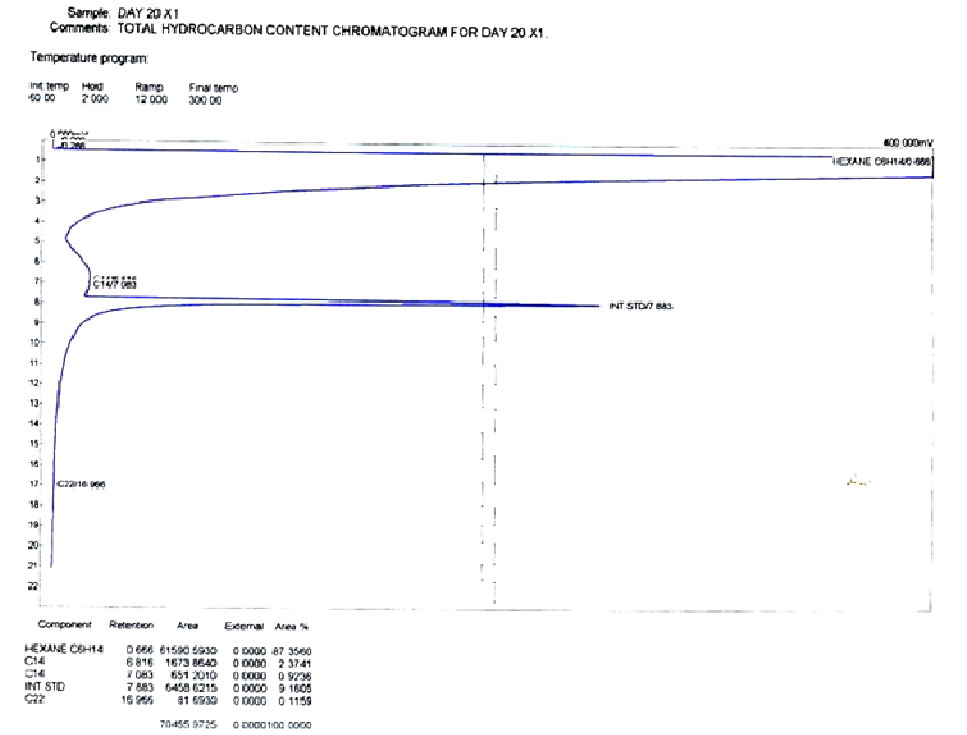


Figure 17. GC profile of detergent residues (microcosm experiment). X1 = Fungal consortium.

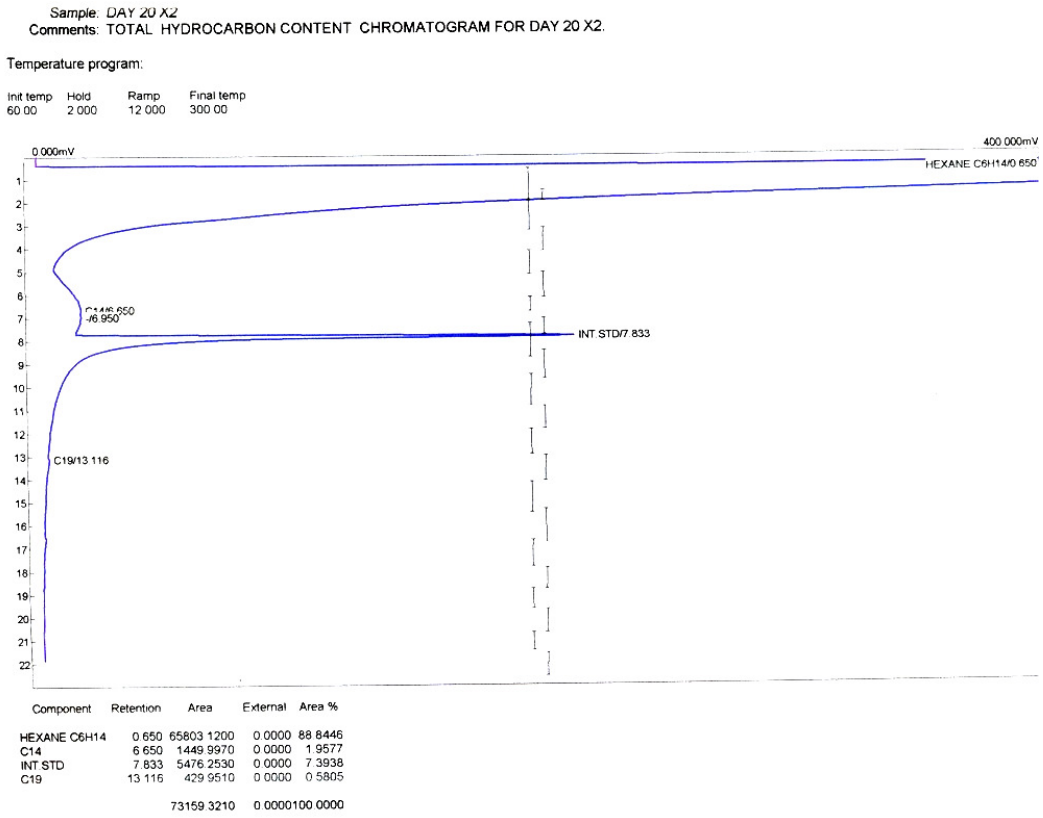


Figure 18. GC profile of detergent residues (microcosm experiment). X2 = *Pseudomonas aeruginosa*

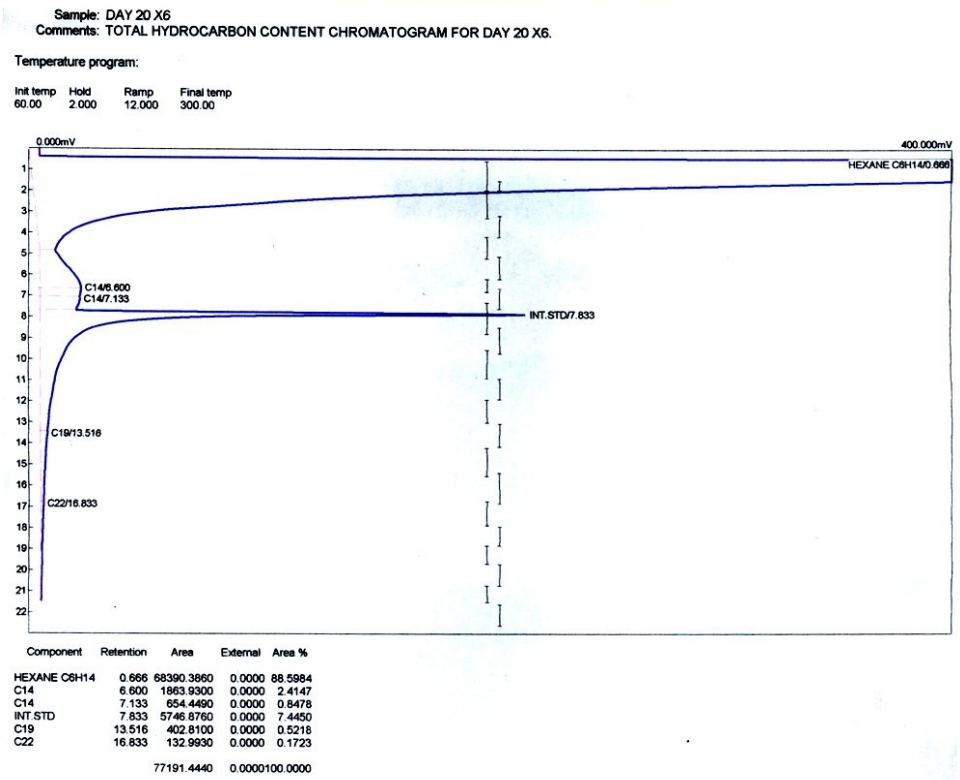


Figure 19. GC Profile of detergent residues (microcosm experiment). X6 = *Escherichia coli.*

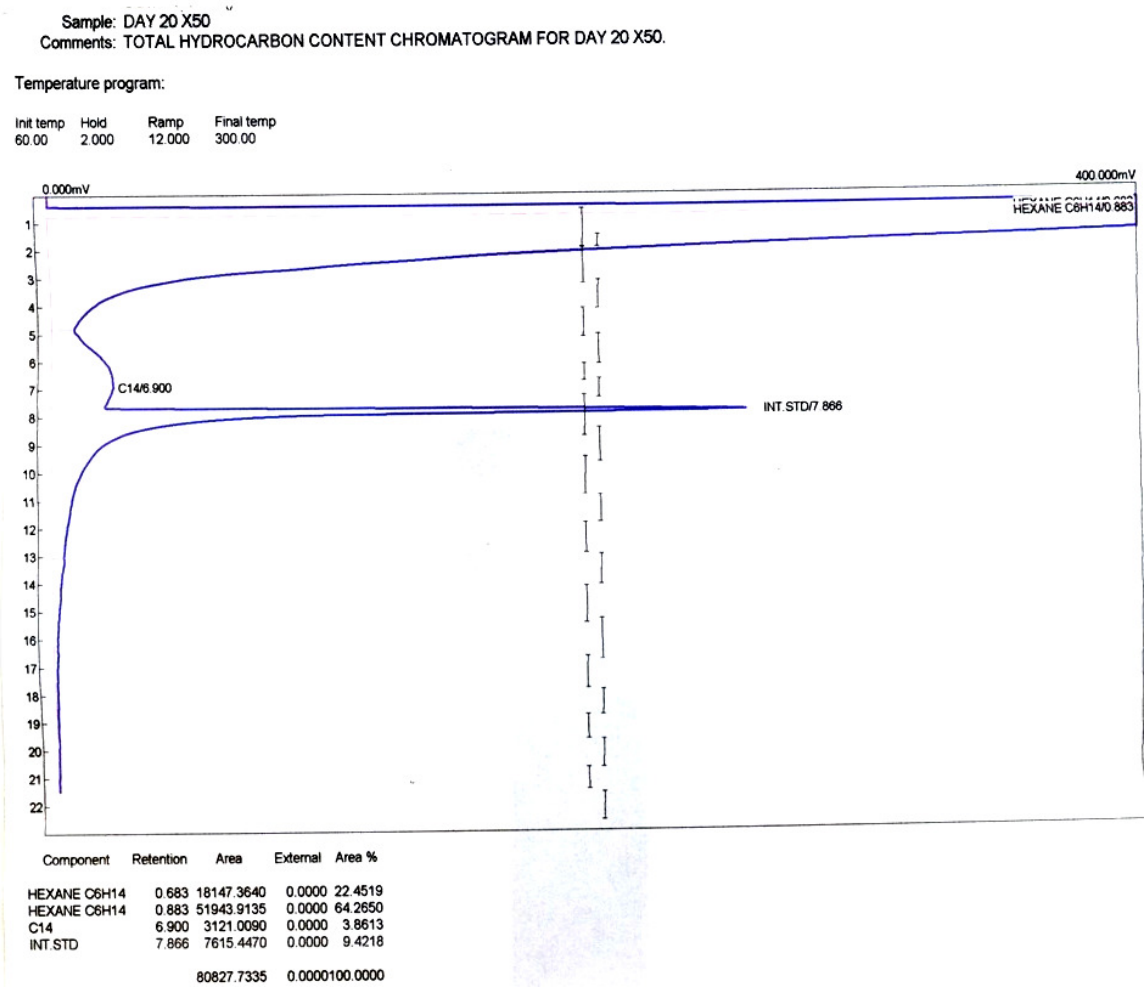


Figure 20. GC profile of detergent residues (microcosm experiment). X50 = Microbial consortium.

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 was almost completely mineralized (except C_{17}) (Figure 12), while as at Day 10, ELEPHANT had components of C_{11}, C_{12} and C_{17} unmineralised (Figure 16).

The inefficiency associated with the local technology used in STPs has made the change to membrane bioreactor technology inevitable. WWI (2006) reported that 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. 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 (WWI, 2005, 2006).

The introduction of LAS (C_{10}-C_{14}) into detergent formulation as the principal surfactant component is thus environment-friendly, since it is biodegradable and it would enhance sustainable development processes.

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