African Journal of Biotechnology Vol. 7 (20), pp. 3753-3760, 20 October, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Isolation and characterization of synthetic detergent-degraders from wastewater

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Accepted 18 July, 2008

The biodegradability of the principal component of synthetic detergent products known as linear alkylbenzene sulphonate (LAS) has been contentious, hence the need to evaluate its primary biodegradation by indigenous microorganisms in wastewater ecosystem. The native microbial consortium of a wastewater ecosystem found to utilize detergent components were characterized using standard and conventional methods. The organisms identified 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.* Alkaline pH and mesophilic temperature range (33.9 – 34.3°C) was found to be supportive of the metabolic activities of the detergent-degraders in the tropical wastewater ecosystem. The bacterial detergent-degraders were more of gram-negative than gram-positive. Fungal detergent-degrader activities were abruptly terminated as the pH shifted to the alkaline range probably due to production of alkaline intermediates. The biodegradation of the synthetic detergent components that occurs in wastewaters, sewage treatment plants and in the ultimate open-water receiving ecosystems is primarily the result of microbial activities.

Key words: Biodegradation, detergents, linear alkylbenzene sulphonate, microorganisms.

INTRODUCTION

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 (Okpokwasili and Nwabuzor, 1988). Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the surfactant component is between 10 and 20%. 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 surfactant (Swisher, 1975; Okpokwasili and Nwabuzor, 1988).

Linear alkylbenzene sulphonates (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 (Gledhill, 1975; Nomura et al., 1998).

Surfactants constitute a major ingredient of detergent components and they are usually 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 sludge are discharged into the environment. These chemicals through several transport mechanisms enter the hydro-geological cycle. Assessment of the environmental contamination levels of

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surfactants and related compounds is achieved through a wide range of laboratory biodegradation tests and eco-toxicological studies (Di Corcia et al., 1999b). 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).

The primary biodegradability of LAS has been established by the Methylene blue – active substance (MBAS) method (Leidner et al., 1980; Pitter and Fuka, 1979). However, there have been conflicting reports about the ultimate biodegradability (mineralization) of the LAS carbon skeleton, particularly the benzene ring (Swisher, 1963; Pitter and Fuka, 1979; Thomas et al., 1997).

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 samples generated were untreated according to the personnel of the companies. The experimental design was a randomized complete block design.

Detergents used

Domestic detergents used were; powdered 'Omo' 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 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 (Larson and Payne, 1981; Okpokwasili and Nwabuzor, 1988).

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 as described by 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 changes and total aerobic viable counts (Okpokwasili and Olisa, 1991).

RESULTS AND DISCUSSION

The mean physico-chemical properties of composite effluent showed that the anions for microbial growth were in optimum concentration except for the PO_4^{3-} and NO_3^- ions that were relatively low. The composite wastewater was at mesophilic temperature range which favored the proliferation of the mesophiles (Table 1). The effluent was aerated since the STP had fixed aerators and this enhanced the mineralization process that was subsequently observed. The mean heterotrophic bacterial count from the 76 randomly collected effluent samples from industrial concerns and Agbara STP was 42.9×10^6 cfu/ml. The mean bacterial detergent-degrader count was 20.94×10^6 cfu/ml while the mean fungal population from the composite effluent was 4.5×10^6 cfu/ml. This result suggested that more bacterial populations were involved in the biodegradation of detergent products than fungi.

The results of this study were predicated on the fact that microorganisms are ubiquitous. Hence, isolation of microorganisms in wastewater is crucial since both domestic and industrial wastewater ultimately get discharged in open rivers. Although, 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 FEPA, EU and WHO standards (Table 1). 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 (Table 1) of the composite wastewater used exceeds the WHO and EU limits which is suggestive of high organic chemical pollution and this may slow down

Table 1. 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

Source: FEPA (1991), Degremont (1991) and WWI (2005).
NS = Not specified.

the rate of mineralization by microorganisms, since high concentration of N and P may be toxic to micro-organisms. Although, the dissolved O_2 was relatively adequate, it was due to presence of aerators in the Agbara STP. In temperate climate, mineralization of synthetic detergent products in wastewater has been achieved under 25 days (WWI, 2005, 2004), whereas the present study carried out under tropical climatic conditions 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 treatment plant and the archaic technology being used in sub-Sahara African countries.

Compliance with EU regulations on discharged effluent (WWI, 2005) is yet to be met by any country in sub-Sahara 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). Whereas, under natural conditions or during treatment processes, the degradation of pollutants is controlled most 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 were subjected to detergent degradation under similar physico-chemical conditions and they were able to utilize the detergent but for longer acclimatization time.

The slow degradation of surfactants in the 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 accessibility of the substrates (solubility, dissociation from adsorbed materials etc.), or predation may dictate the rate of mineralization of xenobiotics in wastewater (Willets, 1973a, b; Kertesz et al., 1994).

Aromatic compounds are abundant in the biosphere due to natural and anthropogenic activities and some of them are pollutants (Ghisalba, 1993). Basic research with commercial surfactants is often made more complex by the nature of the commercial product which is due to the presence of LAS where the single structure represents some 26 major phenyl positional isomers. LAS is the principal surfactant component of all synthetic detergents and it is non-volatile (Willets, 1973a, b). The non-volatility gives it a measure of recalcitrance which is often up-turned by the presence of acclimatized microbial community which is capable of using it as energy source (Bren and Eisenbach, 2000).The heterotrophic microbial population isolated from both the field experiment and

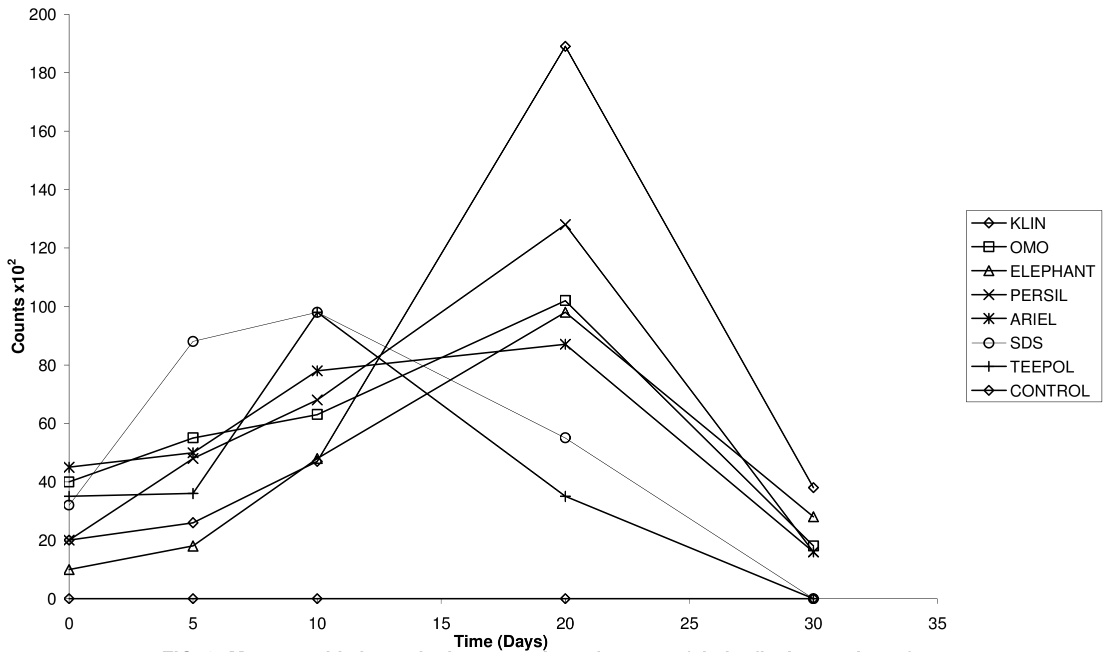

Figure 1. Mean aerobic bacteria detergent-degrader count (shake-flash experiment).

laboratory simulated biodegradation of synthetic detergent is indicative of either ease of biodegradation or the bioavailability of the surfactant incorporated in the detergent formulations.

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 including those from the Agbara STP. Acclimatization of this microbial population to detergent components enhances the biodegradation efficiency of the microorganisms. The adaptability of native microbial population in wastewater to detergent component would be the reason for their success at utilizing LAS components in effluent since the physico-chemical properties of the wastewater ecosystem was 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. 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). Genetic and biochemical evidence have showed that bacterial chemotaxis is independent of uptake or metabolism (Adler, 1969). 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 1 and 2). 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 is the most easily mineralized because of its chemical structure. This agrees with 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 in alkaline direction was in this order KLIN > ARIEL > OMO > ELEPHANT > TEEPOL > PERSIL > SDS while at Day 30, OMO had the highest alkaline value with ELEPHANT having the least value (Figure 3), the pH range for the field experiment was 10.0 - 11.0 whereas during the microcosm study the pH range was adjusted by microbial metabolism to the range 6.9 – 8.8. Thus, alkaline pH range supported the microbial consortium that mineralized the synthetic detergents. This explains the absence of detergent–utilizing fungal species after day 10 during the laboratory simulated biodegradation of test detergents; the pH shifted to the alkaline range as a result of generation of alkaline intermediates which accounted for the initial pH increases. Although, the pH

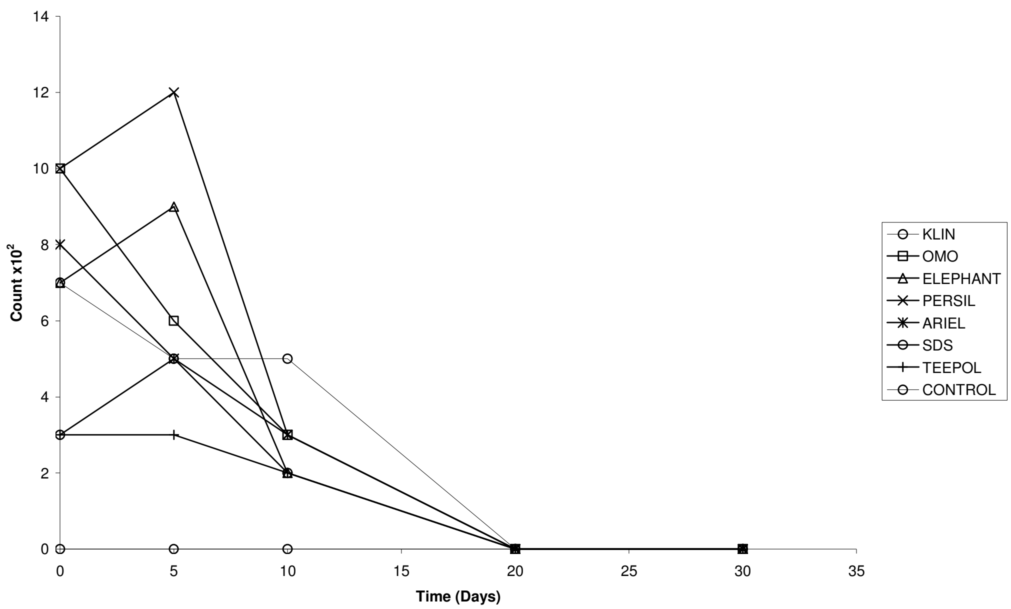

Figure 2. Mean fungal detergent-degrader count (shake-flask experiment).

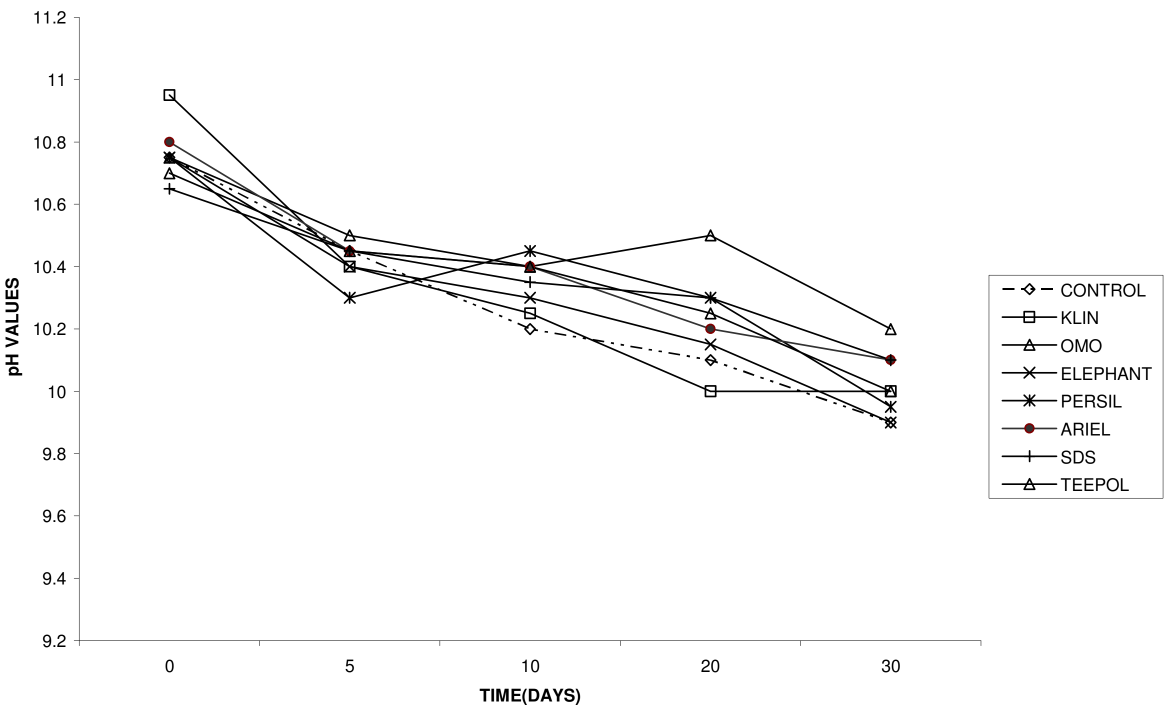

Figure 3. pH reading of primary biodegradation from experiment (shake-flash).

falls as the number of days increased further, which is suggestive of the 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 al., 1999). The building materials in detergent formulation complement the nutrient supply from the environment to facilitate the detergent-degrader activity

Table 2. 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 hydrolysis	Gelatin hydrolysis	Growth on MacConkey	NO_3 reduction	Coagulase test	Spore test	Glucose	Xylose	Lactose	Suscrose	Arabinose	Galactose	Maltose	Mannitol	Salicin	Raffimose	Probable Identity
X^1	+	O	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	*Enterococcus majodoratus*
X_2	-	R	+	-	-	-	+	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	+	*Klebsiella liquefasciens*
X_3	-	R	+	-	-	+	-	+	+	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	+	-	*Enterobacter liquefasciens*
X_4	-	R	+	-	-	-	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	+	-	+	*Klebsiella aerogenes*
X_5	-	R	+	-	+	+	-	-	-	-	-	-	+	+	-	-	+	+	+	+	+	-	+	+	-	-	*Escherichia coli*
X_6	-	R	+	-	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	*Enterobacter agglomerans*
A	+	C	+	+	-	-	-	+	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	-	-	-	*Staphylococcus albus*
B	-	R	+	-	-	+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	-	+	+	-	-	*Enterobacter agglomerans*
C	-	R	+	-	-	+	-	-	-	-	-	+	+	+	-	-	+	+	-	+	-	-	+	+	-	-	*Proteus* sp.
X_{55}	-	R	+	-	+	-	-	+	+	+	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	*Klebsiella oxytoca*
U	+	R	+	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	*Brevibacterium* sp.
X8	-	R	+	+	-	+	+	-	+	-	-	+	-	-	-	-	+	+	-	-	-	+	-	-	-	-	*Pseudomonas aeruginosa*

R = Rods; O = oval; C = cocci; + = positive; - = negative.

(Gledhill, 1974). Microbiological growth monitoring in bulk water may be useful; however it can also be deceptive, since it does not take into account bio-films growth, a critical phenomenon that can have direct effects on heat transfer. Usually, maintenance of low biocide residual, which is monitored carefully, will ensure process waters free of major health problems (Hales et al., 1999). The increase in cell numbers as the microbial consortium in composite wastewater sample utilized the detergents may be due to the utilization of the surfactants as carbon and energy sources for growth (Konopka et al., 1996; Schleheck et al., 2004). The use of builders such as sodium tripolyphosphate in the detergent products may also serve as sources of nutrients for microbial growth which corroborated the report of Imazu et al. (1998). It was observed that determination of physico-chemical properties of the composite wastewater revealed high PO_4^{3-} ions hence the addition of the 'Builders' PO_4^{3-} ions would supplement as well as may complicate phosphate requirement of the microbial communities, since high PO_4^{3-} concentration could lead to toxicity. Builders act as water softeners and are an aid to detergent action (Okpokwasili and Olisa, 1991).

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 (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 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).

It has been reported by Higgins and Burns (1975) that many Gram positive bacteria are noticeably affected by surfactant concentrations of 10 - 20 ppm while several thousand ppm may be without effect on Gram negative organisms. The microorganisms found growing on the test detergents were mainly Gram negative rods or cocci suggesting that they were more tolerant to the surfactant concentrations present in the isolation media than the Gram-positive organisms; *Enterococcus* sp. *Staphylococcus* sp. and *Brevibacterium* sp (Table 2). These findings corroborated the findings of Sigoillot and Nguyen (1992).

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 molecules (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). The widespread usage of LAS as principal anionic matter in detergent formulations contributed significantly to the presence of large quantities of refractory organics in the environment. The information presently available 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 surfacetant component (LAS) supposedly said to be recalcitrant is biodegradable under optimum environmental conditions.

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