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Degradation of Commercial Detergent Products by Microbial Populations of the Lagos Lagoon

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ABSTRACT. The biodegradability potentials of three detergent products with the trade names Omo, Teepol and sodium dodecyl sulfate (SDS) by the native bacteria of the Lagos lagoon was carried out using the lagoon die-away method. Physico-chemical parameters of the water samples showed that the lagoon in Apapa was more polluted than at the *University of Lagos*. In 12 days, approximately 30, 60 and 97 % of Omo, Teepol and SDS respectively were degraded. SDS with an alkyl sulfate moiety as surfactant supported the highest growth of the detergent-utilizing organisms, indicating that the components of Omo and Teepol are more resistant to microbial attack. The detergent-utilizing bacteria identified were mainly Gram-negative and of the following genera: *Vibrio*, *Klebsiella*, *Flavobacterium*, *Pseudomonas*, *Escherichia*, *Enterobacter*, *Proteus*, *Shigella* and *Citrobacter*. *Vibrio* was the most frequently encountered organism while *Proteus* was the rarest. Results of this investigation had shown that detergents made in Nigeria may still contain components that are recalcitrant to biodegradation.

The presence of man-made hazardous compounds in the environment is a subject of intense concern to many countries in the world. Soap, an alkaline salt of fatty acid, is one of man's earliest synthetic products. Its extensive use as products for washing and cleaning has now been replaced by more effective synthetic detergents. The cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it advantages over soaps (Okpokwasili and Nwabuzor 1988). Surfactants are the components mainly responsible for the cleaning action of detergents. In commercial detergents, the content of the active surfactant is between 10 and 20 %. The remainder consists of bleach, filler, foam stabilizer, builders, perfume, soil-suspending agents and other materials designed to enhance the cleaning action of the surfactant (Swisher 1975; Okpokwasili and Nwabuzor 1988).

A surfactant generally results when a strongly lipophilic, hydrophobic group is bound together with a strongly hydrophilic group in the same molecule. The resulting molecule is simultaneously attracted and repelled by water. Consequently, it tends to congregate at the surface and interface and this leads to its cleaning and foaming action. The hydrophobes in general use for detergent formulation are aliphatic or alkylaromatic hydrocarbon radicals containing from 12 to 20 carbon atoms. The hydrophilic groups are generally anionic, such as sulfonates, sulfates, esters, carboxylates or nonionic polyethers or polyalcohols (Swisher 1975).

The major surfactant component employed in the 1950s were alkylbenzene sulfonates (ABS). The surfactant was later discovered to be resistant to microbial attack and retained its foaming property upon discharge from sewage plants into natural waters. This led to the development and subsequent adoption of a more readily biodegradable surfactant called linear alkylbenzene sulfonate or LAS (Willets 1973).

Microorganisms implicated in LAS degradation include *Nocardia* sp., *Fusarium* sp., *Aspergillus* sp., *Pseudomonas* sp., *Micrococcus* sp. and *Acinetobacter* sp. (Kobayashi and Rittmann 1982). Degradation of the LAS molecule begins with carboxylation of the terminal methyl group which brings about loss of toxicity and surfactancy (Swisher 1975).

The interactions among environmental factors, such as dissolved oxygen, oxidation–reduction potential, temperature, pH, availability of other compounds, salinity, particulate matter, competing organisms and concentration of compounds often control the feasibility of biodegradation (Kobayashi and Rittmann 1982).

The Lagos Lagoon is a wide expanse of shallow waters covering an area of about 208 km^2 (Hill and Webb 1958). Increased industrialization and urbanization in Lagos, Nigeria, has led to the generation of large amounts of industrial and domestic sewage containing large quantities of synthetic detergents which eventually find their way into the lagoon. In this work, the lagoon die-away test method was used to assess primary biodegradation of an industrial detergent (Teepol) and a common domestic

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detergent (Omo). Microorganisms associated with detergent degradation in the Lagos lagoon are also defined.

MATERIALS AND METHODS

Water samples. Lagoon water samples were collected from the *University of Lagos* jetty and the Marine beach jetty Apapa. Tap water was collected from the laboratory.

Detergents and reagents. Domestic detergent powder 'Omo' was purchased from *Lever Brothers Nigeria PLC*, Lagos. Teepol was obtained from *National Oil and Chemical Marketing Company*, Lagos. Sodium dodecyl sulfate (SDS) was purchased from *Sigma Chemical Company* (St. Louis, Mo, USA). All chemical reagents used in this study were of analytical grade and were purchased from *Sigma*.

Media. The medium used for the isolation of detergent-utilizing organisms was the minimal salts–detergent agar. It contained (in g/L distilled water): NaCl 5.0, KCl 0.6, $MgSO_4 \cdot 7H_2O$ 7.0, NH_4NO_3 1.0, detergent 1 % (*W/V*), and agar 20. The pH was adjusted to 8.0 with the aid of a pH-meter (*Siebold*). Sterilization was carried out by autoclaving at 121 °C for 15 min.

Isolation and identification of detergent utilizing bacteria. Aliquots of diluted water samples were plated on sterile minimal salts–detergent agar. The inocula were spread evenly with the aid of a sterile glass rod. Incubation was carried out at room temperature (27 ± 3 °C) for 3 d. Identification was carried out as described by Buchanan and Gibbons (1974).

Physico-chemical characteristics of Lagoon water. Temperature, pH, salinity, dissolved oxygen, nitrate content, conductivity and total hydrocarbon content of the lagoon water samples were determined using the standard methods for the examination of water and waste waters (*APHA* 1985).

Aerobic heterotrophic bacterial counts. Samples of the lagoon water and tap water were serially diluted and plated on nutrient agar in triplicates. The plates were incubated at room temperature for 2 d.

Viable counts of detergent-utilizing bacteria. The total number of bacteria that could grow on the agar medium containing the test detergent as the sole carbon source were determined by plating in duplicates 0.1 mL of the serially diluted water samples. After the incubation period, the bacteria growing on the plates were counted, isolated and characterized. Control plates with no added detergents were set up for comparison.

Determination of surfactant concentration in detergent samples. The methylene-blue-active substances (MBAS) method described by Okpokwasili and Nwabuzor (1988) was used. It involves the preparation of a series of ten separatory funnels for each of the test detergents. The flasks in series contained different volumes (mL) 1, 2, 5, 7, 9, 11, 13, 15 and 20 of a particular detergent solution. The tenth flask was with no detergent and it served as the control. The concentration of the detergent in solution was 30 mg/L and 10 mg/L for SDS.

The solutions of detergent in each series of separatory funnels were made alkaline by adding NaOH using a drop of phenolphthalein as indicator. Chloroform (10 mL) and methylene blue were added to the funnels after which each funnel was shaken vigorously for 30 s for the phases to separate. The chloroform layer was drawn off through glass wool into a 50-mL volumetric flask. The extracts were mixed and the absorbance measured at 652 nm in a spectrophotometer (*Beckman*) against a blank of chloroform. A calibration curve of surfactant concentration against absorbance reading for each detergent was then plotted.

Primary biodegradation in lagoon water die-away test. The method employed was as described by Okpokwasili and Olisa (1991). Three sterile Erlenmeyer flasks (2 L) were used in collecting fresh water samples. The flask each containing a different detergent at a concentration of 15.2, 15.2 and 1.0 mg/L for Omo, Teepol, and SDS, respectively, were set up. The flasks were left at room temperature with the organisms in the water samples serving as inoculum. Samples (100 mL) were withdrawn from each flask on the starting day of the experiment and subsequently at 3-d intervals for the analysis of methylene-blue-active substances. The residual surfactant concentration in terms of MBAS for each test detergent was read off the calibration curve and used to construct the degradation time charts.

RESULTS

The physico-chemical parameters of the lagoon water used for this study is as presented in Table I. The detergent concentration in the Marine beach sample was higher than in the sample from

the *University of Lagos*. The nitrate, phosphate, and total hydrocarbon content, and dissolved oxygen of the Marine beach sample indicated a higher pollution status at this point than in the *University of Lagos* area.

Table I. Some physico-chemical characteristics of the Lagos lagoon water[a]

Parameter	*University of Lagos*	Marine beach
Temperature, °C	24	27
Salinity, %	22.48	20.15
Dissolved oxygen, mg/L	9	8
Total hydrocarbon, mg/L	200	268
Nitrate content, ppm	1.0	2.2
pH	8.5	9.3
Phosphate content, μg/L	7.5	13.5
Conductivity, μS/cm	1.65	3.00
Detergent concentration as MBAS, mg/L	128	22.4

[a]The values are means of three replicates.

The detergent-utilizing bacteria isolated from the lagoon water belong to the following genera: *Vibrio*, *Klebsiella*, *Flavobacterium*, *Shigella*, *Proteus*, *Pseudomonas*, *Enterobacter*, *Bacillus*, *Escherichia* and *Citrobacter*. *Vibrio* species predominated, followed by *Flavobacterium* sp. while *Proteus* sp. was the least frequently encountered organism. Total aerobic plate counts of 8.41×10^6, 3.42×10^8 and 2.4×10^3 CFU/mL were obtained from the water samples from *University of Lagos*, Marine beach and tap water, respectively. Results of plate counts of detergent utilizers in lagoon water are shown in Table II.

Table II. Numbers of detergent-utilizing bacteria in the water samples (in CFU/mL)

Detergent	*University of Lagos*	Marine beach	Tap water
Omo	2.89×10^2	3.40×10^2	6.0×10
Teepol	1.10×10^5	2.20×10^4	3.6×10^2
SDS	7.80×10^5	8.99×10^4	2.2×10^2
Control (no detergent)	<10	<10	<10

Sodium dodecyl sulfate supported the highest growth of the detergent-utilizing organisms in the lagoon water. Smallest growth was observed in the tap water samples. The results of the biodegradation of the detergents in lagoon waters are presented as die-away curves (Fig. 1) which showed that the degradation of the detergents increased with increasing incubation period. Of the three detergents, SDS was the most readily attacked by the lagoon microorganisms while Omo was the least.

DISCUSSION

The lagoon water analysis showed that the Marine beach area was more polluted than that at the *University of Lagos*. This is not surprising since effluents from many industries situated in the Apapa area and discharges from ships at the Apapa harbor would contribute to the pollutant load in the Marine Beach area. Various genera of bacteria were isolated from lagoon waters as detergent utilizers. Similar organisms have also been reported by Okpokwasili and Olisa (1991). The ability to utilize the three types of detergents studied as sole carbon and energy sources is an indication of the possession of the necessary enzymes by the bacteria. The organisms also may have been previously exposed to the detergents in their natural environments and this might facilitate the rate of degradation.

Detergent-degrading bacterial isolates were mainly Gram-negative organisms, except for *Bacillus* sp. It is likely that the Gram-negative organisms are more tolerant to surfactants than the Gram-positive ones. The results of this work are in agreement with those of Higgins and Burns (1975) that many Gram-positive bacteria are notably affected by surfactant concentrations of 10–20 ppm while several thousand ppm may be without effect on Gram-negative organisms. The *Vibrio* sp. was the most frequently isolated organism and might probably possess the highest potential of degrading the

surfactant molecule relative to other species. Some of the organisms isolated have similarly been reported as capable of assimilating portions of pure anionic surfactant molecules (Gledhill 1974).

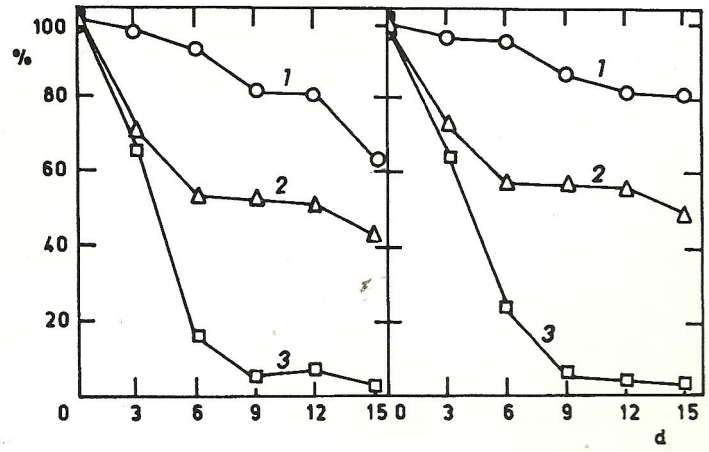

Fig. 1. Time course (d) of the degradation of surfactants (residual concentration, %) in detergents (*University of Lagos, left*; Marine beach, *right*); *1* — Omo, *2* — Teepol, *3* — SDS.

The total viable aerobic bacterial counts of water samples showed that highest numbers of bacteria occurred in the marine beach sample and the lowest in the tap water. Since the Lagos lagoon at the Marine beach area is more polluted than at the *University of Lagos*, it was not surprising that a higher microbial load was obtained at this point. The results of population counts appeared to corroborate the results of the physico-chemical parameters. The existence of bacteria in the tap water sample might be the result of contamination in the distribution network.

The highest number of detergent-utilizing organisms was isolated using SDS as the sole carbon source, followed by Teepol and last by Omo. Sodium dodecyl sulfate is an alkyl sulfate and a more easily degraded molecule than ABS or LAS. The very low number of bacteria growing on the control plates is an indication that the detergents provided the carbon and energy sources for growth (Okpokwasili and Nwabuzor 1988).

Primary biodegradation of a molecule occurs when a portion of it is sufficiently removed or transformed to change the identity or some particular property of the molecule (Swisher 1975). The degradation of the three test detergents by the native microorganisms of the Lagos lagoon water samples are presented in Fig. 1. Omo underwent the slowest primary biodegradation, being only about 30 % degraded in 12 d. Sodium dodecyl sulfate and Teepol proved more degradable with about 97 and 60 % degradation, respectively, in 12 d. This showed that the surfactant component of Omo is more difficult to degrade. It may therefore be inferred that Omo may still contain the surfactant of the branched chain alkylbenzene sulfonate (ABS) type. In view of the biodegradability potential of commercial detergents tested in this work, it may be necessary to carry out a proper scrutiny of the active surfactant components of the various detergents and shampoos in Nigeria by assessing their relative biodegradabilities with a view to enforcing regulatory guidelines as to their use.

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