GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
*(**Published By**: Global Institute for Research & Education)*
www.gifre.org

STUDIES ON THE BACTERIAL FLORA OF SYNTHETIC-DETERGENT EFFLUENT AND THEIR BIODEGRADATION POTENTIALS

Amengialue, O. O.[1], Ibeh, I. N.[2], Egharevba, A. P.[1], Omoigberale, M. N. O.[3] & Edobor, O.[1]

[1]Department of Biological Sciences, College of Natural and Applied Sciences, Wellspring University, Benin City, Edo State, Nigeria.

[2]Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

[3]Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria.

Abstract

Soapy detergents constitute a major part of anthropogenic liquid waste that is regularly discharged into the environments which may have a deleterious effect if not completely degradable. Hence, the potential utilization of Ariel and Omo detergents by bacterial isolates from laundry effluents from Unique, Superkleen and Mega laundries all in Benin City, Edo State, Nigeria were investigated. Physico-chemical parameters such as temperature, pH, BOD, COD, DO, total hydrocarbon and ionic components were determined following the APHA outlined methods. Bacteria isolates characterized from the laundry effluents includes: *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Enterobacter liquefasciens* and *Citrobacter koseri*. The mean total heterotrophic bacterial counts of the laundry effluents were 8.4×10^5 cfu/ml, 2.2×10^5 cfu/ml and 4.1×10^5 cfu/ml for Unique, Superkleen and Mega laundries respectively. Biodegradation potential of the test detergents by the indigenous bacterial isolates was optimal at pH range of 5.0 – 7.4 with *Pseudomonas aeruginosa* showing the highest utilization potential.

Keywords: Linear alkylbenzene sulfonate (LAS), biodegradation, detergent, laundry effluent, physico-chemical

Introduction

Detergents are synthesized cleaning products derived from chemicals. They are any soap or non-soapy powder which has a wide-range of both industrial and domestic usage (Swisher, 1972; Holding, 2005). However, the cheapness of detergent production from petrochemical sources with its ability to foam when used in acid or hard water gives it's usage an edge over soaps (Okpokwasili and Nwabuzor, 1988). Basically, the detergency property of any detergent is owned to its surfactant components which are between 10% – 20% in commercial detergents. Other components designed to enhance the quality and cleaning action of the detergent include abrasive, pH modifier, oxidants, enzymes, foam boasters, anti-caking agents, viscosity enhancer, preservatives, optical brighteners, colour, perfumes, anti-corrosion agent, water and inorganic filler (Swisher,1975; Okpokwasili and Nwabuzor, 1988; Ojo and Oso, 2008).

In recent years, several reports have shown the occurrence of linear alkylbenzene (LAB) residues in municipal and domestic wastes and in marine and riverine sediments which were considered to arise from detergent bearing wastes (Eganhouse *et al*., 1983; Ishiwatari *et al*., 1983). Today, linear alkylbenzene sulphonates (LAS) is the most commonly used anionic surfactant in detergents and it is easily biodegraded compared to non-linear alkylbenzene sulphonate (ABS) even though total biodegradation still requires several days (Gledhill, 1975; Nomura *et al*., 1998).

With the increasing usage of synthetic detergents and the releasing of organic pollutants by industries, detergent compounds have become an important compound in wastewater as an environmental pollutant causing a number of health-related problems. Hence, interest now centers on microbial degradation of these detergent compounds (Goodnew and Harrison Jr., 1972). A considerable level of detergents biodegradation activity takes place in the environment (Lawson and Payne, 1980), but only the easily degradable detergent chemicals are used up by microorganisms while those not readily degradable bio-accumulate and concomitantly cause adverse environmental problems (Swisher, 1973). Such problems have been reported to include: destruction of the external mucus layer that protects fish from bacteria and pathogens, severe damage to the gills, lowering of the surface tension of the water, algal blooms that releases toxins and decreases oxygen in waterways and decrease in the breeding ability of aquatic organisms (Holding, 2005). However, increased awareness of the harmful effects of the 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 an eye opener that microbial metabolism provides a safer, more efficient and less expensive alternatives to physico-chemical methods for pollution abatement (Hebes and Schwall, 1987).

Materials and Method

Sources of wastewater sample

Laundry effluent samples were collected from three laundry houses, namely Unique Laundry at Ugbowo, Superkleen Laundry in G.R.A. and Mega Laundry at Upper Sokponba all in Benin City, Edo State, Nigeria.

Sample collection

Sampling was done with sterile screw bottle (2L) and collection of the untreated laundry effluent was randomly done at the point of discharge of effluent along the production line and stored at 4^0C and transported to the laboratory immediately for analysis.

Test detergents used

Domestic detergents used in this study (Ariel detergent produced by Procter and Gamble Nigeria Ltd. and Omo detergent produced by Unilever Nigeria Plc.) were purchased from Oba Market in Benin City, Edo State, Nigeria.

Determination of the physico-chemical parameters

The physico-chemical parameters of the laundry effluents collected from the different laundry houses were determined using standard methods for the examination of water and wastewater (APHA, 1985; 1992).

Aerobic heterotrophic bacterial counts

The laundry effluent samples collected from the different laundry houses were serially diluted and inoculated onto nutrient agar plate amended with nystatin in duplicates. The plates were then incubated at 37^0C for 24 hours after which colony count were taken (Okpokwasili and Nwabuzor, 1988; Larson and Payne, 1981)

Viable counts of detergent-utilizing bacteria

The number of bacteria detergent-utilizers in each laundry effluent collected was determined by inoculating minimal salt agar medium amended with test detergent at 0.01% (w/v) with 0.1ml of the serially diluted laundry effluent sample using pour plate technique. The inoculation were done in duplicates and incubated at 28 ± 2^0C for 72 hours while the control plates were not inoculated with the laundry effluent (Thysse and Wanders, 1972; Okpokwasili and Nwabuzor, 1988). Bacterial isolates were characterized using standard and conventional methods according to Gerhardt *et al*. (1981) and Bergey's manual of systematic bacteriology (Holt and Krieg, 1984).

Bacterial screening test for detergent-utilization ability

The ability of the isolated bacteria to utilize the laundry effluent as their source of carbon and energy were tested by the determination of growth turbidity. This was done by dispensing 9ml of mineral salt medium (MSM) into different test tubes and 1ml of the laundry effluent was added into each test tube (Zajic and Supplison, 1972). Thereafter, 0.1ml of 10^{-3} dilution of each bacterial isolate was inoculated into each test tube separately. The test tubes were then incubated at room temperature (28 ± 2^0C) for 7 days. At the end of the incubation, the optical density (OD) of each culture was measured at 520nm using HACH DR/2010 Portable Data logging Spectrophotometer. In this case, the OD was an index of growth, reflecting the potential for the biodegradation of detergent in the laundry effluent by the respective bacterial isolates.

Shake flask biodegradation test

Bacteria which showed high turbidity in the screening test were selected for shake flask degradation experiment. One hundred milliliter (100ml) of mineral salt medium was dispensed into conical flasks. The prepared medium was then autoclaved at 121^0C for 15 minutes. The sterilized medium in the conical flask was amended with 0.01% (w/v) of test detergents (Ariel and Omo) separately. The amended mineral salt medium in each conical flask was then inoculated with 1ml each of the 10^{-3} dilution of each test bacteria isolate. For each detergents, a control was set up in which no organism was seeded into the conical flasks. The experimental flasks were incubated at room temperature on a rotary shaker operated at 120rpm. This experiment was monitored over a period of 30 days with samples withdrawn at day0, day5, day10, day15, day20, day25 and day30 for absorbance reading (optical density) at 520nm, total viable counts and pH determination.

Result

The result of this study reflects the physico-chemical properties of the different laundry effluents, the indigenous micro-flora and their degradation potential of the synthetic detergents. The indigenous bacteria species isolated and characterized from the laundry effluent/wastewater samples were *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Enterobacter liquefasciens* and *Citrobacter koseri*.

In table 1 is shown the physico-chemical parameters of laundry effluent/wastewater from three (3) different laundry houses.

In table 2 and 3 are shown the biodegradation pH and turbidity reading of ariel and omo detergents respectively.

Total viable count of bacteria-degraders for a period of 30 days for ariel and omo detergents are shown in table 4 and 5 respectively.

Table 1: Mean physico-chemical parameters of laundry wastewater.

PARAMETERS	Unique Laundry	Superkleen Laundry	Mega Laundry
Appearance	Cloudy	Cloudy	Cloudy
Colour	Gray	Gray	Gray
Odour	Soapy smell	Soapy smell	Soapy smell
pH	9.000	9.100	8.300
Temperature (^{0}C)	28.800	29.000	30.400
Conductivity (µm/cm)	174.000	197.000	208.000
Specific gravity	1.006	1.008	1.019
Total hydrocarbon(mg/l)	4.800	9.600	5.400
TDS (mg/l)	158.140	180.560	295.300
TSS (mg/l)	121.800	164.200	185.2
DO at day 0 (mg/l)	2.600	3.400	4.800
DO at day5 (mg/l)	20.600	20.800	30.400
BOD (mg/l)	30.800	55.700	40.600
COD (mg/l)	54.800	106.400	141.900
Chloride (mg/l)	18.100	34.600	20.800
Sulphate (mg/l)	84.700	64.900	58.600
Phosphate (mg/l)	22.500	37.800	25.300
Nitrate (mg/l)	2.540	13.460	8.300
Ammonium (mg/l)	19.700	22.500	17.800

Table 2: Measurement of pH and turbidity reading during bacterial degradation of ariel detergent.

pH and OD MEASUREMENT FOR ARIEL DETERGENT							
Bacterial isolates	**Day 0**	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**
Escherichia coli	7.2 (0.003)	6.6 (0.008)	6.2 (0.014)	6.0 (0.023)	5.7 (0.034)	5.6 (0.031)	5.3 (0.028)
Pseudomonas aeruginosa	7.1 (0.003)	6.4 (0.009)	6.1 (0.017)	5.9 (0.029)	5.6 (0.038)	5.4 (0.035)	5.2 (0.031)
Enterobacter liquefasciens	7.2 (0.003)	6.6 (0.005)	6.3 (0.011)	6.1 (0.017)	5.9 (0.021)	5.6 (0.020)	5.5 (0.019)
Klebsiella aerogenes	7.3 (0.003)	6.8 (0.004)	6.3 (0.009)	6.2 (0.015)	6.0 (0.018)	5.7 (0.018)	5.4 (0.017)
Bacillus subtilis	7.4 (0.003)	7.1 (0.005)	6.7 (0.010)	6.5 (0.017)	6.3 (0.023)	6.0 (0.025)	5.7 (0.022)

Note: values in bracket represent optical density reading while values not in bracket represent pH reading

Table 3: Measurement of pH and turbidity reading during bacterial degradation of omo detergent.

pH and OD MEASUREMENT FOR OMO DETERGENT							
Bacterial isolates	**Day 0**	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**
Escherichia coli	7.1 (0.004)	6.8 (0.010)	6.6 (0.018)	6.2 (0.032)	5.7 (0.039)	5.6 (0.036)	5.1 (0.034)
Pseudomonas aeruginosa	7.1 (0.004)	6.7 (0.013)	6.4 (0.019)	6.2 (0.035)	5.5 (0.041)	5.3 (0.038)	5.0 (0.035)
Enterobacter liquefasciens	7.2 (0.004)	6.9 (0.009)	6.7 (0.016)	6.4 (0.024)	6.0 (0.029)	5.9 (0.026)	5.4 (0.024)
Klebsiella aerogenes	7.3 (0.004)	6.9 (0.007)	6.7 (0.011)	6.5 (0.023)	6.1 (0.027)	5.9 (0.024)	5.5 (0.020)
Bacillus subtilis	7.3 (0.004)	7.1 (0.008)	6.8 (0.013)	6.5 (0.031)	6.2 (0.031)	5.9 (0.030)	5.6 (0.027)

Note: values in bracket represent optical density reading while values not in bracket represent pH reading.

Table 4: Total viable count of bacteria-degraders of ariel detergent.

Isolates	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Mean	SE
A	1.1×10^4	3.3×10^5	5.3×10^5	7.0×10^4	9.6×10^4	9.1×10^4	8.8×10^4	1.7×10^6	7.0×10^6
B	1.5×10^4	4.2×10^4	6.6×10^4	9.1×10^4	1.10×10^5	1.06×10^5	1.02×10^5	7.6×10^5	1.3×10^6
C	6.0×10^3	2.7×10^4	4.0×10^4	6.2×10^4	8.9×10^4	8.4×10^4	8.1×10^4	5.6×10^5	1.2×10^6
D	5.0×10^3	2.2×10^5	3.7×10^4	5.3×10^4	8.3×10^4	7.8×10^4	7.4×10^4	7.9×10^5	2.5×10^6
E	9.0×10^3	3.6×10^4	4.9×10^4	5.9×10^4	7.7×10^4	8.6×10^4	9.6×10^4	5.9×10^5	4.3×10^6

A = *Escherichia coli*
B = *Pseudomonas aeruginosa*
C = *Enterobacter liquefasciens*
D = *Klebsiella aerogenes*
E = *Bacillus subtilis*
SE = Standard Error

Table 5: Total viable count of bacteria-degraders of omo detergent.

Isolates	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30	Mean	SE
A	1.7×10^4	4.5×10^4	6.9×10^4	9.7×10^4	1.20×10^5	1.17×10^4	1.05×10^4	5.3×10^5	1.6×10^6
B	1.9×10^4	5.1×10^4	7.7×10^4	1.08×10^4	1.31×10^5	1.24×10^5	1.16×10^5	7.6×10^5	1.9×10^6
C	9.0×10^3	2.3×10^4	4.6×10^4	7.1×10^4	1.10×10^5	1.04×10^5	9.7×10^4	6.6×10^5	1.5×10^6
D	6.0×10^3	2.2×10^5	4.0×10^4	6.4×10^4	9.7×10^4	9.1×10^4	8.8×10^4	8.7×10^5	2.5×10^6
E	1.0×10^4	3.6×10^4	5.1×10^4	8.2×10^4	1.16×10^5	1.09×10^5	1.03×10^5	7.2×10^5	1.5×10^6

A = *Escherichia coli*
B = *Pseudomonas aeruginosa*
C = *Enterobacter liquefasciens*
D = *Klebsiella aerogenes*
E = *Bacillus subtilis*
SE = Standard Error

Discussion

The physico-chemical parameters of the laundry effluent from each of the three laundry houses (Unique Laundry, Superkleen Laundry and Mega Laundry) used for this study showed a slightly higher biological oxygen demand for Unique laundry and a significantly higher biological oxygen demand for Superkleen and Mega laundry when compared to Federal Environmental Protection Agency (FEPA) and World Health Organization (WHO) standard (Table 1). Also, the ionic components phosphate and ammonium significantly exceeded the WHO/FEPA standard (Table 1). The implication is that the laundry effluents studied were capable of producing deleterious effects on the receiving environment. This is because high concentrations of nitrogen and phosphorus could be toxic to microorganisms (Ojo and Oso, 2009). In temperate climate, mineralization of synthetic detergent products in wastewater has been achieved within 25days (WWI, 2004, 2005), whereas studies carried out in the tropical climate showed that some commercial synthetic detergent takes more than 30days to be mineralized by microorganisms which could be related to absence of optimal physico-chemical parameters in the system.

However, under natural conditions, the degradation of pollutants is most controlled by a variety of physico-chemical parameters such as temperature, pH and available substrate, and not only by the presence or absence of the appropriate microbial consortium. When these physico-chemical parameters are in optimal level, this will favour the eventual evolution and growth of the best adapted microbial population (WWI, 2004, 2005). However, the availability of

competent synthetic detergent degraders will be an added advantage. The laundry effluents analyzed showed that the pH was slightly alkaline and it was of mesophilic temperature range which favours the growth and proliferation of mesophiles. Following this knowledge of the laundry effluents, the fate of the detergent surfactant could be projected considering that the slow degradation of surfactants in a 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) may also dictate the rate of mineralization of xenobiotics in wastewater (Kertesz *et al*., 1994; Willets, 1973a, 1973b). Hence, the total suspended solid and total dissolved solid of the laundry effluents was significantly higher compared to FEPA/WHO standard value for wastewater and effluents (Tables 1). These results show the fact that the laundry effluents were being polluted with both soluble and insoluble organic compounds.

Detergent-utilizer bacterial isolated from the laundry effluents used for this study comprises of *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Enterobacter liquefasciens* and *Citrobacter koseri* This finding agrees with earlier reports on the microbiota of detergent effluents (Ojo and Oso, 2008; 2009 and Ogbulie *et al*, 2008). The mean total heterotrophic bacterial counts of the laundry effluents were 8.4×10^5cfu/ml, 2.2×10^5cfu/ml and 4.1×10^5cfu/ml for Unique Laundry, Superkleen Laundry and Mega Laundry respectively. The adaptability nature of microbial isolates in wastewater to detergent components could be attributed to the success of utilizing linear alkylbenzene sulfonate components in effluent, since the physico-chemical properties of the laundry effluents was supportive of these microorganisms (Spain and van Veld, 1983).

From the 30days shake-flask biodegradation study, it was seen that the pH of the degradation medium of the bacteria detergent-utilizer was adjusted between the ranges of 5.2 – 7.4 and 5.0 – 7.3 for Ariel and Omo test detergent respectively (Tables 2 and 3). However, there was a steady decrease in pH of the ariel and omo detergent biodegradation set-up with no remarkable difference between both detergent pH reading (Tables 2 and 3). The gradual decrease in pH values is suggestive of the production of the acidic metabolite (SO_4^{2-}). There have been similar reports by Hales *et al*.,(1986), Okpokwasili and Olisa, (1991). These physico-chemical factors were particularly important for the survival of the detergent-utilizing microorganisms in the biodegradation set-up. This finding is in collaboration with the findings of Okpokwasili and Olisa (1991).

The absorbance turbidity of the shake flask biodegradation set-up monitored for the 30-days duration of study revealed significant changes in optical density of the test detergent-mineral salt broth challenged with the test microorganisms. A steady increase in the optical density among the bacteria detergent-utilizers were observed up to day 20 of the study period for both Ariel and Omo detergent (Tables 2 and 3). Comparatively, the changes observed showed *Pseudomonas aeruginosa* among other bacterial detergent-utilizers to have more ability to utilize Ariel and Omo detergent (Tables 2 and 3).

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). In this study, bacterial chemo-taxis for Ariel detergent were in this order: *Escherichia coli* > *Klebsiella aerogenes* > *Pseudomonas aeruginosa* > *Enterobacter liquefasciens* > *Bacillus subtilis* (Table 4), while the bacterial chemo-taxis for Omo detergent were in the order of *Klebsiella aerogenes* > *Pseudomonas aeruginosa* > *Escherichia coli* > *Bacillus subtilis* > *Enterobacter liquefasciens* (Table 5). The initial increase of the microbial cell population in the shake-flask biodegradation set-up could be attributed to the availability of carbon source and sulphate in the test detergent for energy and growth (Kertesz *et al*., 1994; Zurrer *et al*.,1987) since the mineral culture media lacks carbon C and sufficient sulphate (SO_4^{2-}) source. 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 detergent-degraders (Konopka *et al*., 1996), This supported 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 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). Consequently, the fall in growth phase, that is death phase among the detergent-utilizers could also be attributed to the depletion of carbon source from the test detergent and other essential nutrient components in the medium.

Conclusion

Considering the possible and environmental effects that could arise from the inevitable use and the poor disposing pattern of synthetic-detergent, this study has demonstrated a sound methodology for revealing the susceptibility of synthetic detergent to microbial degradation. Though, the rate of mineralization of different detergents differs among microbial detergent-utilizers.

References

American Public Health Association (APHA). (1985). *Standard methods for the examination of water and wastewater*. 6th Ed. American Public Health Association. Washington, D. C. 1220pp.

American Public Health Association. (1992). *Standard methods for the examination of water and wastewater*, 18th Ed. American Public Health Association, Washington, D. C. 1644pp.

Bren, A., and Eisenbanch, M. (2000). How signals are heard during bacterial chemotaxis: protein-protein interactions in sensory signal propagation. *Journal Bacteriology*, **182**, pp. 6865 – 73.

Eganhouse, R. P., Blumfied, D. L. and Kaplan, I. R.(1983). Long chain alkyl benzenes as molecular tracers of domestic waste in

the marine environment. *Environmental Science Technology*, **17**, pp. 523 – 30.

Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. and Philips, G. B. (1981). Preservation In: *Manual of Methods of General Bacteriology*. ASM Washington, D. C. 435pp.

Gledhill, W. E. (1975). Screening test for assessment of ultimate biodegradability: Linear alkylbenzene sulfonates. *Applied Environmental Microbiology*, **30**, pp. 922 – 29.

Goodnow, R. A. and Harrision, A. P. Jr. (1972). Bacterial Degradation of Detergent Compounds. *Applied Microbiology*, **24(2)**, pp. 555 – 60.

Hales, S. G., Watson, G. K., Dodgson, K. S. and White, G. F. (1989). A comparative study of the biodegradation of the surfactant Sodium dodecyltriethoxy sulphate by four detergent degrading bacteria. *Journal General Microbiology*, **132**, pp. 953– 61.

Hebes, S. E., Schwall, I. R. (1987). Microbial degradation of polycyclic aromatic hydrocarbons in pristine and petroleum contaminated sediments. *Applied Environmental Microbiology*, **35**, pp. 306 – 16.

Higgins, I. J. and Burns, R. G. (1975). *The chemistry and microbiology of pollution*. Academic Press, London. 686pp.

Holding, B. V. (2005). Detergents occurring in freshwater. *African Journal Biotechnology*, **7(6)**, pp. 824 – 30.

Holt, J. G. and Krieg, N. R. (1984). *Bergey's Manual of Systematic Bacteriology.* Williams and Wilkins Coy., Baltimore. M. D. 1445pp.

Ishiwatari, R. Takada, H., Yun, S. J. and Matsumoto, E. (1983). Alkylbenzene pollution in Tokyo Bay sediment: *Nature*, **301**, pp. 599 – 600.

Kertesz, M. A., Kolbener, P., Stocinger, H., Beil, S., Cook, A. M. (1994). Desulfonation of linear alkylbenzene surfactand and related compounds by bacteria. *Applied Environmental Microbiology*, **60(7)**, pp. 2296 – 303.

Konopka, A., Zakharova, T., Oliver, L., Camp, D., Turco, R. F. (1996). Biodegradation of organic waste containing surfactants in a Biomass Recycler Reactor. *Applied Environmental Microbiology* **62(9)**, pp. 3292 – 97.

Lawson, R. J. and Payne, A. G. (1980). Fate of the Benzene Ring of Linear Alkylbenzene Sulfonate in Natural Waters. *Applied Environmental Microbiology*, 41, pp. 21 – 22.

Lawson, R. J. and Payne, A. G. (1981). Fate of the Benzene Ring of Linear Alkylbenzene Sulfonate in Natural Waters. *Applied Environmental Microbiology*, **41(3)**, pp. 626 – 27.

Nomura, Y., Ikebukuro, K., Yokohama, K., Takeuchi, T., Arikawa, Y., Ohno, S. and Karube, I. (1998). Application of a linear alkylbenzene sulfonate biosensor to river water monitoring. *Biosensor Bioelectronics*, **13**, pp. 1047 – 53.

Ogbulie, T. E., Ogbulie, J. N. and Umezuruike, I. (2008). Biodegradation of detergents by aquatic bacterial flora from Otamiri river, Nigeria. *African Journal Biotechnology*, **7(6)**, pp. 824 – 30.

Ojo, O. A. and Oso, B. A. (2008). Isolation and characterization of synthetic detergent-degraders from Wastewater. *African Journal Biotechnology*, **7(20)**, pp. 3753 – 760.

Ojo, O. A. and Oso, B. A. (2009). Biodegradation of synthetic detergents in wastewater. *African Journal Biotechnology*, **8(6)**, pp. 1090 – 1109.

Ojo, O. A. and Oso, B. A. (2009). Isolation of Plasmid-DNA from Synthetic Detergent Degraders in Wastewater from Tropical Environment. *African Journal Microbiology Research*, **3(3)**, pp.123 – 27.

Okpokwasili, G. O. and Nwabuzor, C. N. (1988). Primary biodegradation of anionic surfactants in laundry detergents. *Chemosphere*, **17**, pp. 2175 – 182.

Okpokwasili, G. O. and Olisa, A. O. (1991). River-water biodegradability of surfactants in liquid detergents and shampoos. *Water Research* **25**, pp. 1425 – 429.

Spain, J. C. and Van Veld, P. A. (1983). Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects of concentration, exposure, time, inoculum and chemical structure. *Applied Environmental Microbiology*, **45**, pp. 428 – 435.

Swisher, R. D. (1972). Linear Alkylbenzene Sulphonate (LAS) Benzene Rings: Biodegradation and Acclimation Studies. *Yukagaku*, **21(3)**, pp. 328 – 32.

Swisher, R. D. (1973). Anionic Surfactants and the Environment: Discussion. *Progress Water Technology*, **3**, pp. 303 – 06.

Swisher, R. D. (1975). Surfactant from recalcitrant to docile 126pp. In: *Proceedings of International Biodegradation Symposium* (Eds.) Sharpely, J. M. and Kaplan, A. M. Hysse, G. J. E. and Wanders, T. H. (1972). Degradation of n-alkane-1-sulphonates by *Pseudomonas. Antonnie van Leeuwenhoek*, **38**, pp. 53 – 63.

Water and Wastewater International (WWI) (2004). Decentralized treatment removes nitrogen from septic effluent In: *EU legislation lights up sludge-power potential.* PennWell Pub. Ltd., UK. 306pp.

Water and Wastewater International (WWI) (2005). Sharing Risks and Rewards alliance contracts profit desalination project. In:*MBR helps Breschia company with EU regulations.* PennWell Pub. Ltd, UK. **20(4)**, pp. 23 – 5.

Willets, A. J. (1973a). Microbial aspects of the biodegradation of synthetic detergents: *A Review of International Biodeterioration Bulletin*, **9(1-2)**, pp. 3 – 10.

Willets, A. J. (1973b). Microbial metabolism of alkylbenzene sulphonates: Fungal metabolism of 1- phenylundecane - p –sulphonate and 1- phenyldodecane - p – sulphonate. *Antonie van Leeuwenhoek*, **39**, pp. 585 – 97.

Zajic, J. E. and Supplison, B. (1972). Emulsification and degradation of Bunker C. fuel oil by microorganisms. *Biotechnology and Bioengineering*, **14**, pp. 331 – 343.

Zurrer, D., Cook, A. M. and Leisinger, T. (1987). Microbial desulfonation of substituted naphthalene sulfonic acids and benzene sulfonic acids. *Applied Environmental Microbiology*, **53**, pp. 1459 – 63.