Research Journal of Applied Sciences 2 (2): 156-159, 2007

Effectiveness of Some Aerobic and Anaerobic Bacteria in Primary Biodegradation of Anionic Synthetic Surfactant in Detergents

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Abstract: The efficiency of biodegradation of surface active agents by five bacteria strains was carried out on four anionic synthetic detergents found in the Nigerian Market. Biodegradation was carried by some strains of bacteria namely, *Bacteroides fragilis*, *Peptostreptococcus*, *Clostridium botulinum*, *Staphylococcus aureus* and *Escherichia coli*. Anaerobic and aerobic treatment systems were both effective in reducing the concentration of surfactants, however, anaerobic treatment generally produced the greatest removal. The results showed a removal rate of 99.9% by *Bacteroides fragilis*. The order of efficiency of the bacteria used was found to be: *B. fragilis*>*C. botulinum*>*Peptostreptococcus* sp., *E. coli*>*S. aureus*

Key words: Biodegradation, *Bacteroides fragilis, Peptostreptococcus*

INTRODUCTION

The ever-expanding utilization of materials, energy and space has increased the flux of anthropogenic organic chemicals into the environment. One of the set of transformation that removes organic compounds from the environment is that group of reactions mediated by organisms. In detergents, though other organic ingredients such as enzymes may also be biodegradable, the term is most often applied to the surface active agents-surfactants-contained in the product.When detergents first came into general use in the 1950s there were problems of foaming on rivers. In this first generation of synthetic detergent washing powders, branched chain Alkyl Benzene Sulphonate (ABS) was extensively used as the surfactant. The replacement of ABS by LABS, straight chain Linear Alkyl Benzene Sulphonatewhich was more easily biodegraded, came into use in early 1960s (Hon-nami, 1980).

LABS is commonly used in many household detergent including laundry powders, liquids and tablets, laundry bleach additives, hand dish washing liquids and all purposed in some industrial applications such as in the field of textiles and fibres, chemical and agriculture. Industrial facilities that use detergents to clean machinery can discharge anionic detergents into the water supply (Tebbutt, 1998). There they combine with other wastes for treatment in either a municipal treatment plant, an on site chemical treatment process or a household septic tank system During treatment, microorganisms biodegrade surfactants and other organic materials, ultimately breaking them down into carbon dioxide, water and minerals (Hammer J.M., 1996). Any small amounts of surfactants that remain after treatment continue to biodegrade in the environment. Environmental analysts often include a determination of anionic detergents when assessing surface water pollution because they are poisonous to fish and other types of aquatic life, thus reducing availability of aquatic food (Painter and Zabel, 1987). Sewage effluents mainly affect water bodies which could also seep into and pollute underground water. Conventional activated sludge treatment of wastes is often an effective and highly economic system for reducing organic pollutants in wastewater (Gray, 1994).

Biodegradation of detergents starts as soon as the dirty laundry water is washed down the drain and is completely biodegraded under oxic conditions (KOPS-Datenbank, 2004). Micro-organisms (or bacteria) thrive naturally in sewers, sewage treatment plants, waterways and soil. These bacteria break down surfactants, using them as food for energy and growth. Since there are very large numbers of bacteria at the sewage treatment plants, biodegradation takes place there very rapidly and substantially complete biodegradation is usually achieved within a few hours.The first steps in the biodegradation process will often produce an intermediate product or metabolite and this stage is referred to as Primary Biodegradation (AISE/CESIO). Subsequent action by bacteria may then result in further decomposition of the metabolites to produce simple inorganic compounds

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such as carbon dioxide, water and mineral salts. This complete breakdown of the compound is known as Ultimate Biodegradation. Primary biodegradation of a surfactant usually results in the loss of environmentally undesirable properties such as foaming ability. It is therefore very important that this stage of biodegradation takes place rapidly if undesirable effects are to be avoided.

This study is aimed at determining the effectiveness of different aerobic and anaerobic bacteria strains, (*S. aureus* and *E. coli*) and (*B. fragilis*, Peptostreptococcus, *C. botulinum*) respectively, to biodegrade surfactants in various type of detergents found in the Nigerian Market.

MATERIALS AND METHODS

Sample collection and preparation samples: Detergents referred to as A, B and C is produced in Nigeria and D is an imported detergent; all were bought from a supermarket in Zaria. Samples were prepared by dissolving detergents that would give approximately 5 mg of surfactant in separate 1 dm^3 volumetric flask.

Description and culturing of bacteria

***Bacteriodes fragilis*:** These are strictly anaerobic grain positive short rods bacteria; they grow mostly on brain heart infusion agar and blood agar. Their natural habitat is upper respiratory tract genitals and intestinal track of humans.

***Peptotreptococcus* sp:** These are anaerobic grain positive coli in chain arrangement when stained. They are frequently the cause of urinary tract, in the mouth and the intestine.

***Clostridium botulinum*:** These are grain positive anaerobic rods which form spores, they decompose protein and produce toxin, their natural habitat is soil or the intestine.

***Starphylococcus aureus*:** This is highly pathogenic aerobe and causes diseases such as boils, urinary track infection etc. These organisms grow rapidly in most media, but the media of choice is the blood agar, its grain positive coli that is arranged in a duster.

***Escherichia coli*:** These are grain negative short rods when stained, the bacterium is also pathogenic, among the diseases caused by this bacterium is urinary track infection diarrhea etc.

Culturing was done at National Research Institute of Chemical Technology (NARCT) Zaria. The isolates for the anaerobic bacteria were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria. The isolates were local isolates; they were checked for purity and maintained in a nutrient agar slants. The isolates were subculture on nutrient agar plates and incubated at 37°C for 24 h (Singleton, 1999; Ajibola *et al*., 2005). A single colony from the cultured growth was picked and incubated at 37°C for a period of time.

Bacteroides fragilis, *Peptostreptococcus* sp. *Clostridium botulinum* being anaerobes were collected in an anaerobic condition. The slant culture was maintained in an anaerobic jar with Gas Park and every subculture was with a gas park to maintain the CO_2 condition. Each colony of the microbes was introduced into the 10 cm^3 of the sample and incubated in an anaerobic jar at 37°C for a given period of time, at the end of this period the bacteria strains were all destroyed.

Extraction of surfactant: The extraction of surfactant was carried out by chloroform extraction described in the American Public Health Association (APHA, 1995) the Methylene Blue Active Stain (MBAS) method for monitoring biodegradable organic component in a compound were employed to determine the biodegradability. Each extraction was carried out in duplicate.

Colorimetric method: The absorbance was taken at 652nm using a spectrophotometer Sp6 B 400 UV/Visible Spectrophotometer. The Methylene Blue Active Substances (MBAS) method is used in a 3-min procedure to measure anionic detergents in the 0-5 (mg dm^{-3}) range. Results are expressed in (mg MBAS dm^{-3}) as Linear Alkylbenzene Sulphonate (LAS), equivalent weight 324.5.

RESULTS AND DISCUSSION

Extensive laboratory testing and real-world monitoring studies have shown that the major surfactants biodegrade quickly and thoroughly and do not present a risk to organisms living in the environment (KOPS-Detenbank, 2004, The Soap and Detergent Association). Biodegradation though only one aspect of the environmental profile, is an important factor influencing the fate of the components of products after their disposal to the environment, other processes such as photodegradation, chemical degradation and absorption may also have an influence.This biodegradation stage corresponds to the disappearance of the parent molecule and to the loss of interfacial activity and of toxicity towards organisms present in the environment. The result obtained in this study shows the effectiveness of different strains of bacteria in biodegrading the selected detergents (Fig. 1).

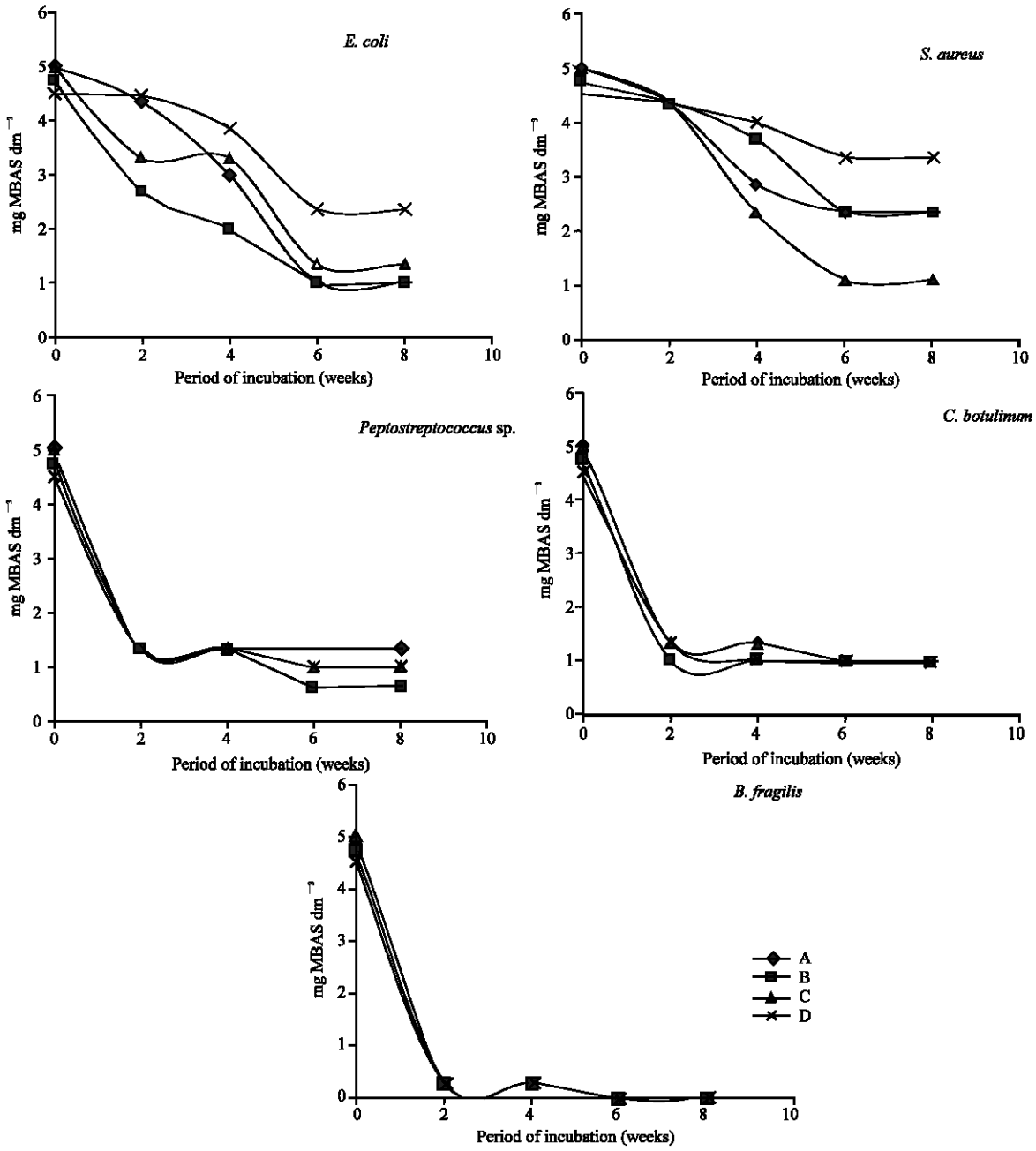

Fig. 1: Primary degradation of surfactants with period of incubation with strain of bacteria

Subsequent action by bacteria may then result in further decomposition of the metabolites to produce simple inorganic compounds such as carbon dioxide, water and mineral salts. This complete breakdown of the compound is known as Ultimate Biodegradation.

The effectiveness of each bacterium in biodegradation of surfactants in the detergents is shown in Fig. 1. The result obtained for *E. coli* shows that the surfactants were not totally degraded by this aerobic bacteria. The different detergents were degraded to different extents and the sample D seemed to be the least degraded with 52.2% surfactant left after 8 weeks of incubation (Table 1). *Staphylococcus aureus* degraded sample C more than all other detergent samples used in this investigation. The degradation process was slow and similar to that observed for *E. coli* except that the concentration of surfactant left after the investigation period was lower for *E. coli* than for *S. aureus* for all the

Table 1: Concentration (%) of surfactants left after ten weeks of incubation with different bacteria strains

	A	B	C	D
Escherichia coli	20	21	27	52.2
Staphylococcus aureus	47	49.5	22	74.4
Clostridium botulinum	20	21	20	22
Peptostreptococcus	27	13.6	20.0	22.0
Bacteroides fragilis	0.0	0.0	0.0	0.0

detergent samples used. In both research, the imported sample (D) was the least degraded. There was an initial rapid degradation by *Peptostreptococcus* sp. of all the detergent samples used. Less than 20% of the surfactant was left after just two weeks of incubation and remained so till the end of incubation period. At the end of the fourth week, there seemed to be no further degradation of sample A, but the remaining samples were further degraded especially sample B (Fig. 1). The result obtained for *C. botulinum* was similar to that obtained for *Peptostreptococcus* sp., however, this strain of bacteria seemed to show similar degradation pattern on all the detergent samples. The concentrations of surfactant left by the eighth week of incubation were similar. *Bacteroides fragilis* was the most effect in degrading all the detergent samples. Over 90% removals were observed at the end of the first week and no surfactant was detected at the end of incubation period for all the detergent samples. Primary biodegradation of a surfactant usually results in the loss of environmentally undesirable properties such as foaming ability and this was found to be independent of water type (Odokuma and Otokunefor, 2003). It is therefore very important that this stage of biodegradation takes place rapidly if undesirable effects are to be avoided; in this work *B. fragilis* was found to be the most effective. Thus bacteria break down surfactants, using them as food for energy and growth. Since there are very large numbers of bacteria at the sewage treatment plants, Biodegradation takes place there very rapidly and substantially complete biodegradation is usually achieved within a few hours (Biodegradation on Surfactants Directive).

CONCLUSION

The anaerobic organisms (*Peptostreptococcus* sp. *C. botulinum* and *B. fragilis*) were found to be most effective in primary biodegradation of all the detergents used in this study, while the aerobic were less effective (*E. coli* and *S. aureus*). The results showed a drastic reduction in surfactant concentration in all the detergents within two weeks of incubation by the anaerobes with *B. fragilis* being the most effective. The aerobes were found to be slow in action with more than half the concentration of surfactants remaining after eight weeks of incubation. In the natural environment however, any residual material or metabolites released to waterways before ultimate biodegradation is complete will continue to biodegrade.

ACKNOWLEDGEMENTS

We thank Mallam Mikail Abdullahi of Department of Microbiology, National Research Institute for Chemical Technology, Zaria-Nigeria, for culturing and inoculating the microbes into the various detergent samples. We also appreciate Ahmadu Bello University, Zaria-Nigeria for providing part of the funds used for this work.

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