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Utilization of detergent surfactant by bacteria isolated from freshwater bodies

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ABSTRACT: The utilization of detergent surfactants by bacterial species isolated from some freshwater bodies was studied using primary degradation under OECD screen test. the persistence of the detergent surfactant in the water bodies was also investigated using River water die-away test. Nineteen bacterial species were isolated in all. Three of the isolates designated as surfactant utilizing bacteria (SUB): *Citrobacter* sp., *Micrococcus luteus* and *Pseudomonas* sp. were found to utilize the detergent surfactants as a source of carbon. The highest rate of individual utilization (43%) was observed with *Pseudomonas*, while a consortium of the three SUB isolates achieved 62% reduction in 21 days. None of the isolates demonstrated co-metabolic activity. The surfactant persisted for 12 to 15 days at 50 – 50.35% concentration in the water bodies. The persistence of the surfactants is discussed and the use of the SUB species in bioremediation of the detergent effluent is suggested as a way of ameliorating the observed persistence of the surfactant in the water bodies.

Introduction

One of the consequences of our reliance on chemical technology is the pollution of the environment with chemical substances (Suflita *et al*., 1983), some of which are entirely novel. These xenobiotic substances are either biologically transformed, resulting in mineralisation, accumulation or polymerization, or are recalcitrant and hence persist in the environment (Hardman, 1989; Brock *et al*.; 1994). Their fate is conditioned on the presence of microorganisms, particularly bacteria, with the appropriate pathway to metabolize them either individually or in consortium. Detergents were introduced in the early 1930s but only began to gain prominence in the years following the World War II (Manaham, 1993). Houston (1997) pointed out that environmental complications for detergents actually began in the early 1960s when abnormal quantities of foam began to be noticed in streams in the United States. The race towards phosphate free detergents has occasioned an increase in the quantities of surfactants in commercial detergents and consequently an increase in the amount of surfactant entering the environment. In this study, the utilization of surfactant in a commercial detergent by bacteria isolated from fresh water bodies that regularly receive detergent bearing effluents were investigated as primary degradation using the OECD screen test (Fogel *et al*., 1982) The persistence of the surfactant in the water bodies was investigated using the river water die-away test.

Materials and Methods

Isolation and screening of Bacterial Species

The study involved two water bodies: River Asa and one of its tributaries; Okun stream. grab samples of the raw effluent-free water bodies and effluent-polluted water bodies were collected as described by ASTM (1985). The heterotropic bacteria were isolated on nutrient anar using the pour plate method (Olayemi, 1994) Detergent Mineral Agar (DMA) composed of 0.1% (w/v) of detergent and mineral salt medium described by Liu *et al* (1995) was used to screen for bacteria capable of degrading the surfactant. Incubation was done at 37^{o}C for 48 hours. The isolates were subsequently purified, characterized and identified as reported by Buccanhan and Gibbon (1974).

Utilization of Detergent Surfactant

One millilitre of 18-hour-old pure broth culture of each isolate that showed degradative potential was used to inoculate separate flasks containing 200 ml of detergent mineral broth. Different combinations of the isolates were also used to seed other set of flasks. The flasks were incubated at room temperature on an aorbital shaker for 21 days. At an interval of 3 days, 10 ml aliquots were taken from the flasks and the concentration of detergent using the Hyamine titration method (GSD, 1995). The growth of bacteria was monitored using the optical density method.

River Water Die-Away Test

One hundred and fifty millilitres of the efluent-polluted water bodies were collected into separate sterile flasks. The initial surfactant concentrations were determined using the Hyamine titration method (GSD, 1995). The flasks were then incubated at room temperature on an orbital shaker for 21 days. At an interval of 3 days, 10 ml aliquots were taken from the flasks and the concentration of detergent determined, using the Hyamine titration method (GSD, 1995).

Results

Nineteen bacterial species were isolated and identified. Their identity and distribution among the sample sources are shown in Table 1. The population of heterotropic bacteria in the samples is shown in Fig. 1. Three of them: *Citrobacter* sp.; *Micrococcus luteus* and *Pseudomonas* sp. showed ability to utilize the detergent durfactant. Their rates of surfactant utilization were 28%, 32% and 43% respectively and were accompanied by bacterial growth. Rates of surfactant utilization by the consortia of pure cultures were: 48% for *Citrobacter* sp. and *Micrococcus luteus*, 51% for *Citrobacter* sp. and *Pseudomonas*, 56% for *Micrococcus luteus* and *Pseudomonas* sp. and 62% for the consortium of the three. The percentage persistence of the surfactant in river Asa and Okun stream were 50.35% and 50% respectively, with persistence lasting for between 12 and 15 days.

Discussion

The ability of a microorganism to degrade chemical substances has been related to their previous exposures in the environment (Spain and Vanveld, 1983; Shimp and Pfeander, 1985a,b). Hence, it was anticipated that several of the bacterial species would be able to degrade the surfactant since they would have encountered it in the water bodies from which they were isolated. However, only three of the nineteen isolates (about 16%) demonstrated the ability to degrade the surfactant. Eniola and Olayemi (1999) have suggested that the presence of organic matter in the water bodies could be responsible for the non-utilization of the surfactant by many of the organisms.

Table 1: Bacterial isolates and their distribution among sample sources..

Bacterial isolate	Sample Source				
	Raw Effluent	Effluent-free water body		Effluent-polluted water body	
		RA	OK	RA	OK
Enterobacter hafnia	–	+	+	+	+
Enterobacter cloacae	+	+	+	+	+
Citrobacter freundii	–	+	+	+	+
Escherichia coli	–	+	+	+	+
Proteus mirabilis	–	+	+	+	–
Proteus vulgaricus	–	+	+	+	+
Shigella sp.	–	+	+	–	–
Salmonella sp.	–	+	+	–	–
Micrococcus albus	–	+	+	+	+
Staphylococcus aureus	–	+	+	–	–
Streptococcus faecalis	–	+	+	–	–
Bacillus subtilis	+	+	+	+	+
Bacillus sp.	+	+	+	+	+
Serratia sp.	–	+	+	+	+
Pseudomonas sp.	+	+	+	+	+
Citrobacter sp.	–	+	+	+	+
Micrococcus luteus	–	+	+	+	+
Pseudomonas aureginosa	+	+	+	+	+

RA – River Asa; OK – Okun Stream; +: Present; –: Absent.

The three bacteria: *Citrobacter* sp.; *Micrococcus luteus* and *Pseudomonas* sp., designated as surfactant utilizing bacteria (SUBs) in this work, have been similarly reported as capable of degrading surfactant (Gledhill, 1974). Their status as SUBs is strengthened by their attendant proliferation with the surfactant as the only source of carbon. Absence of bacterial growth with disappearance of the surfactant would have signified co-metabolism which is defined as the transformation of a non-growth substrate in the obligate presence of a growth substrate or other transformable substance (Dalton and Stirling, 1982). Anderson *et al*. (1988) also recognised *Pseudomonas* as a genus that is vastly involved in degradation of alkyl sulphates, and that the presence of the surfactant sodium dodecyl sulphate (SDS) degrading alkylsulphatase is synonymous with the ability to accomplish complete mineralization of SDS. This is because the alkylsulphatase enzymes that initiate the degradation of alkyl sulphates remove inorganic sulphate from the substrate to give an alcohol that is readily assimilated by central metabolic pathways present in bacteria. It has, however, been pointed out by Kertesz *et al*. (1994) that there are other pathways by which the surfactant can be degraded. It thus remains to ascertain whether or not the transformation of the detergent surfactant by the SUBs result in the accumulation of metabolites in the environment.

Since it is generally accepted that the persistence of a compound will, in most cases, be most significantly affected by its degradability, it is surprising that the detergent surfactant, though biodegradable persisted at such concentration in the water bodies for the length of time observed.

Bartholomew and Pfaender (1983) have reported that the ultimate breakdown of any substrate is dictated more by environmental factors than by the nature of the chemical or the degradative agents that may be present. Gray (1989) also indicated that for any waste to be biologically treatable (i.e. for it to be removed by biochemical means) it must satisfy a nutrient/element ratio of 101:15:1, C:N:P. Hence, the nutrient/element in-balance in the water bodies reported by Eniola (1996) and the depletion of oxygen identified by Eniola and Olayemi (1999) could be expected to affect the rate of removal of the surfactant consequently resulting in the persistence of the surfactant in the water bodies found in this study.

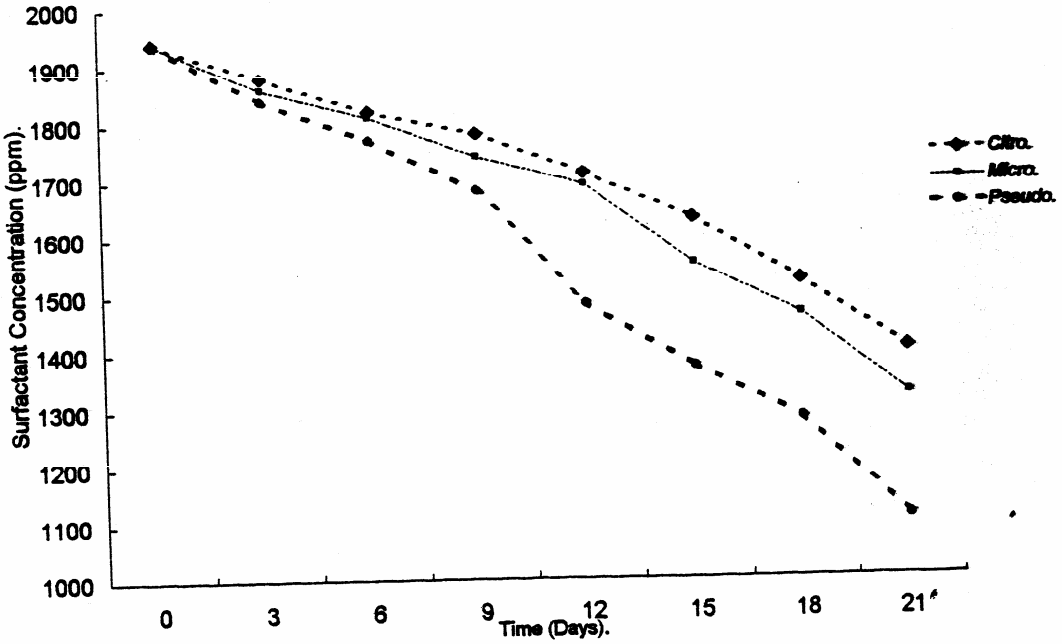

Fig 1. Rate of Utilization of Detergent Surfactant by Pure Cultures of SUB Isolates.

The persistence of the surfactant in the water bodies may be taken as a confirmation of the inability of the majority of the resident flora to utilize it due to the reason earlier highlighted. Ameliorating the pollution of the water bodies requires a proper management of the aquatic environment by monitoring the discharge of waste into them. Since chemical treatment of the effluent will also mean introducing more chemical substances into the environment, bioremediation using the SUBs is a ready option, once the conditions for the degradation are optimized.

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