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Degradation of Sodium Dodecyl Sulfate by the Bacterial Isolates Obtained from Polluted Aquatic Bodies

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Detergent is a product that contains a surfactant and other ingredients to clean fabrics in the wash. One of the major sources of water pollution involves the use of synthetic detergents in houses and industries. Detergents are susceptible to degradation by natural aquatic micro flora, though the rate of utilization differs with the type of detergent and type of flora. The present study aims at studying the growth pattern of bacteria in presence of sodium dodecyl sulfate. Eight bacterial isolates were isolated and identified from aquatic bodies and cultured on media containing 1% SDS as sole sources of carbon. All isolates were able to grow luxuriantly at 1% SDS concentration. Bacteria were not only able to tolerate SDS, but also use SDS for its growth. Effect of SDS on growth pattern of bacteria, viable count, and pH changes were studied for a period of 30 days.

Key words: Detergent, SDS (sodium dodecyl sulfate), Degradation, Aquatic micro flora.

Synthetic surfactants or detergent are used in many industries and in every household washing. The main hazards of detergent pollution lays in their effect on water ecosystem as a whole, but surfactant also affect micro algae at lowest a tropic level and impact on their function as major suppliers of oxygen to water bodies. Detergent concentrations greater than 0.1 g/m^3 are toxic to some marine organisms.[6]Sub lethal concentration affects the life in the different stages of marine organism's notably ovum and larval stages of different aquatic species. The main problem in aquatic waters is eutrophication and oxygen depletion. Analysis of wastewater phosphate showed that 50% originated from urban and industrial sources. Consequently, it is important that ways be found to prevent further inputs from these sources. Foaming due to detergents is a major operational problem in waste water treatment. A mixture of sludge waste water bacteria and grease get trapped in the foam, thereby inhibiting efficient biological treatment. Bogan and Sawyer in their studies showed that SLS is easily biodegraded by microorganisms in sewage. The ability of the bacteria to dissimilate many of the detergents provided apparently due to the capacity for producing enzymes for the oxidation of the series of water soluble or dispersible compounds with hydrocarbon chains[7]

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Thus, to ascertain the effect of detergents on the micro flora in the aquatic environments, this study was untaken.

MATERIAL AND METHODS

All media chemicals were of analytical grade from Hi Media. SDS was supplied by Sagar chemicals, Mumbai.

Sample collection

Water samples were obtained from three sources –1)Ulhas River, 2) Kalyan Lake (Kala talav) 3) water taken from Badlapur Lake.

Media for enrichment and isolation: The water was used for isolation of SDS degraders. Enrichment of SDS degraders was carried out in the St. Bushnell and Hass broth in 250ml flask (K_2HPO_4-100mg,$MgSO_4.7H_2O$-20mg,NaCl-20mg,$CaCl_2$-20mg,$(NH_4)_2SO_4$-190mg,pH-7.4,Distilled water-100ml) with 1% SDS as sole source of carbon and energy. The flasks were incubated at room temperature on a shaker. The SDS degraders were isolated on St. Nutrient agar medium after each round of enrichment and colony characteristics of isolates were studied.

Identification and preservation of the selected isolates: The isolated obtained after the final round of enrichment were identified on the basis of cultural, morphological and biochemical properties according to the Bergey's manual of systematic bacteriology and preserved at 4°C on St.Bushnell and Hass medium containing SDS.

Tolerance of organisms to SDS: The determination of tolerance of organisms to SDS. St.Nutreint broth with 2.0 g/ml of SDS.Culture-24 hrs old culture of each eight isolate. Range: 0.2-2.0 g/ml of SDS.

Enrichment was carried out on St.Bushnell and Hass medium incorporated with 1% SDS.

St.Bushnell and Hass medium containing 1% SDS was dispensed in 250 ml of flask. A total of 16 flasks were used .The flasks were in duplicates. Then each isolates with 5ml of 0.1 O.D was added. These flasks were incubated at ambient temperature (28 -31°C) for 30 days. The number of bacterial detergent-utilizes was determined by inoculating on Nutrient agar medium of serially diluted using spread plate techniques. These were kept at ambient temperature (28 -31°C) for 48-72 hr. Samples were taken at day 0, 5, 10, 20, 15, 25, 30 from flasks; this was to determine pH changes, absorbance's at 620 nm and total viable count.

Isolation was done on nutrient agar plate. The organisms were identified on the basis of cultural, morphological and a biochemical property according to the Bergey's manual of systematic bacteriology and preserved at 4^0C on St.Bushnell and Hass medium containing SDS.

RESULTS AND DISCUSSION

Water sample were obtained from three sources – ulhas river, kala talav(kalyan),badlapur lake. These water bodies are highly polluted and thus a greater chance of obtaining the strains which could have higher resistance to many chemical pollutants. The organisms growing in these waters may be using substances for their metabolism and survival in this polluted environment. Different water samples were screened for degradation of detergents using St.Bushnell and Hass medium with 1% SDS (sodium dodecyl sulfate). The organisms were grown on shaker at R.T for week and isolated on St Nutrient agar plate. Different isolates obtained were then identified on the basis of their cultural and biochemical characteristics according to Bergey's manual of systematic and determinative bacteriology.

The native microbial consortium of wastewater ecosystem found to utilize detergent components were characterized using standard and conventional methods and they were identified as *Escherichia coli, Enterobacter agglomerans, Pseudomonas aeruginosa, Enterococcus majodoratus,, Enterobacter liquefasciens, Klebisiella liquefasciens, Proteus* spps, *Staphylococcus albus* .

Several organisms from sludge waste-water treatment plant, detergent manufactures, and industries using LAS have also been isolated. The organisms identified were *Enterococcus* spps, *Klebsiella* spps, *Enterobacter* spps, *Staphylococcus albus, Proteus* spps, *Pseudomonas* spps, *Brevibacterium, Myceliophythora thermophila, Geomyces* spps, *Alternaria alternata, Aspergillus flavus, Trichoderma* spps. Alkaline pH and mesophilic temperature range (33.9 – 34.3°C) was found to be supportive of the metabolic activities of the

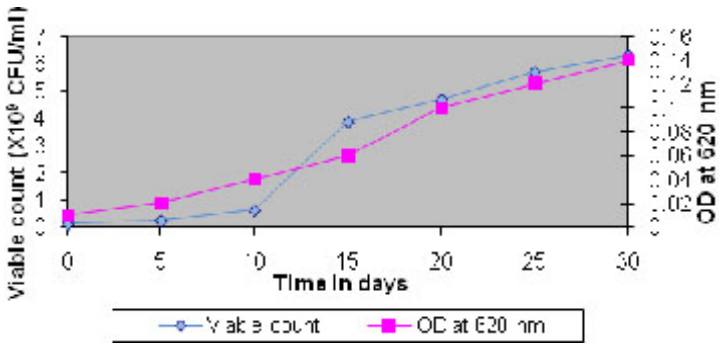

Fig. 1. Viable count and O.D at 620nm V/S time in days (*Enterococcus majodoratus)*

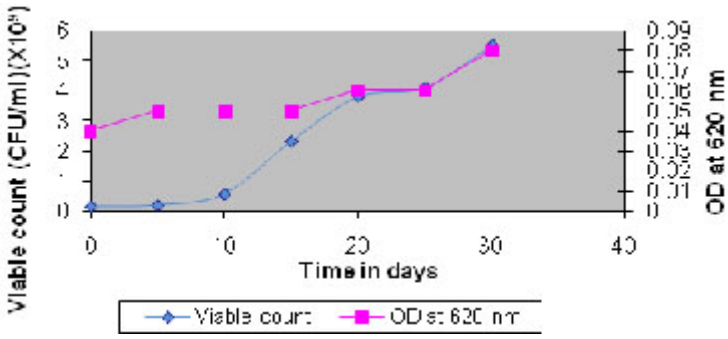

Fig. 2. Viable count and O.D at 620nm V/S time in days (*Escherichia coli*)

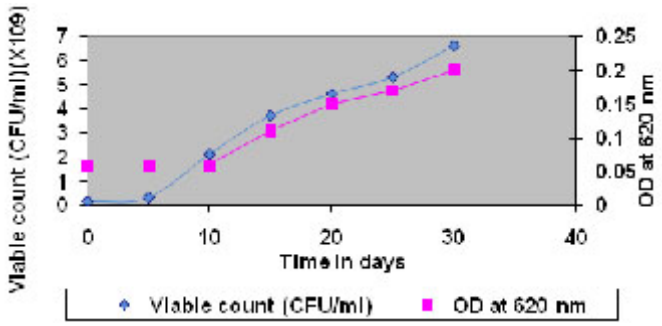

Fig. 3. Viable count and O.D at 620nm V/S time in days (*Enterobacter liquefasciens)*

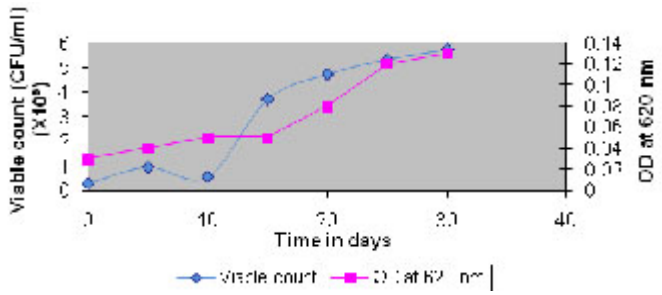

Fig. 4. Viable count and O.D at 620nm V/S time in days (*Enterobacter agglomerans)*

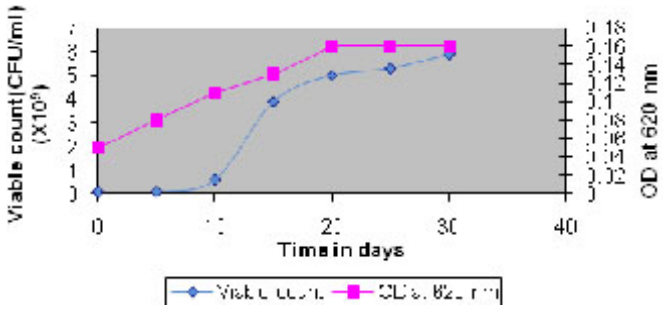

Fig. 5. Viable count and O.D at 620nm V/S time in days (*Pseudomonas aeruginosa)*

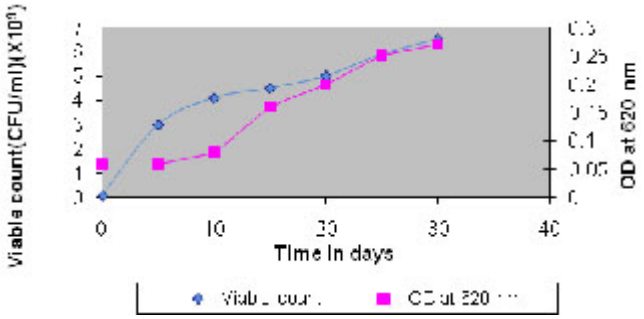

Fig. 6. Viable count and O.D at 620nm V/S time in days(*Klebisiella liquefasciens)*

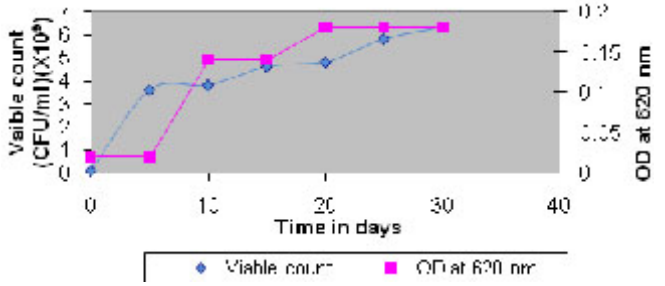

Fig. **7.** Viable count and O.D at 620nm V/S time in days (*Proteus spps)*

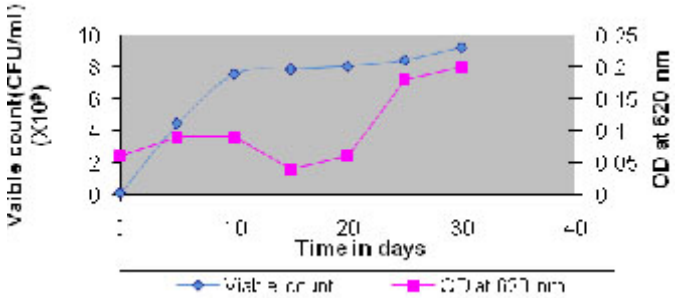

Fig. 8. Viable count and O.D at 620nm V/S time in days (*Staphylococcus albus)*

detergent-degraders in the tropical wastewater ecosystem. The bacterial detergent-degraders were more of gram-negative than gram-positive.[1]

Similar observation of different genera have also been seen from otamiri river and the organisms reported are *Bacillus, Micrococcus, Escherichia, Enterobacter, Klebsiella, Pseudomonas, Actinomyces, Corynebacterium, Serratia, Staphylococcus*.[2]

Minimum inhibition concentration (MIC) was with SDS different isolates to determine the tolerance of the isolates to SDS. The MIC of carried out *Escherichia coli, Enterobacter agglomerans, Pseudomonas aeruginosa* was found to be 1.4 mg/ml, while *Enterococcus majodoratus, Enterobacter liquefasciens, Klebisiella liquefasciens, Proteus* spps, *Staphylococcus albus* was found to be 1.6 g/ml.

Growth reduction of two unknown bacterial isolates (C12 and C12B) in SLS (sodium lauryl sulfate) have been studied with concentration above 0.15 molarity, indicating its toxic effect on the survival of micro-organisms.The ability of these detergent degrading bacteria to dissimilate many of the detergents provided is apparently due to the capacity for producing enzymes for the oxidation of any series of compounds with hydrocarbon chains.Both the isolates destroyed the foaming capacity of cultures containing dodecyl sulfate but C12B, which could grow on dodecyl benzene sulfonate, whereas C12 could not destroy the foaming capacity of this surfactant.[3]

To determine the effect of detergents on growth of bacteria, viable count was studied for 30 days; the change in pH was also estimated. It was noticed that most of the cultures showed a drop of pH, though there were no drastic changes in H+ ion concentration. It was also observed that viable number of cells in the broth showed slow growth and an extended lag phase for 10 days before they started multiplying and distinct rise was oberseved, only after the initial lag phase of 10 days. *Klebsiella liquifasciens* and *Proteus* spps showed a striking increase in the viable count after five days. OD values were stationary for the following isolates *Pseudomonas aeruginosa, Proteus spps* even after 20 days of incubation while the other isolates like *Enterococcus majodoratus, Escherichia coli, Enterobacter agglomerans, Enterobacter liquefasciens, Klebisiella liquefasciens* showed increase in cell number with the days of incubation. *Staphylococcus albus* showed sudden drop of O.D after 15 -20 days, but showed increase in viable count.

Flask containing St. Bushnell and Hass medium with detergent and culture and before treatment at 0 hrs contain foams. Flasks containing St.Bushnell and Hass medium with detergent and culture after 96 hrs treatment showed complete disappearance of foam. The foaming property of the detergents depends on the type of ingredients in the detergents but after biodegradation it is seen that no foam was observed in the SDS flask after degradation. Therefore this could be considered one of the easiest parameters for the degradation of detergents in the effluent. Different parameter like pH changes, viable count, and temperature has been reported for 30 days. In the past overall increases in microbial numbers in the biodegradation is mainly due to availability of carbon source and sulphate in the detergent product for energy.

Anionic synthetic detergent is detected in all surface waters and ground water samples in Greater Kolkata. Relatively higher load in the Hugli River indicates that the river receives untreated domestic sewage from the urban centers located along the banks. The tanks and the ponds are showing higher values than ground water as they are used for ablution and washing of clothes.[4]

The prospect of using *Pseudomonas aeruginosa* isolated from sewage water show a great potential in removal of LAS from the environment.The rate of degradation can be increased further by acclimatization of the culture to LAS for a longer period of time.With this positive effect of biodegradation the treated waste water can be safely and effectively used in agricultural soils.[5]

The present study investigates that the organisms isolated from the aquatic bodies not only can use SDS as a sole source of carbon but they have also been able to sustain their growth in this for almost 30 days. These organisms can thus be exploited for the treatment of waste waters from various washing industries, and bioremediated before it can be discharged into the water bodies.

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