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DEGRADATION OF ANIONIC SURFACTANT BY *PSEUDOMONAS AERUGINOSA*

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ABSTRACT

The anionic surfactants, especially linear alkyl benzene sulphonate (LAS) has been the most important industrially produced surfactant detected in urban sewage. Because of its poor biodegradability, LAS accumulates in the environment. In the present investigation, *Pseudomonas aeruginosa*, able to grow luxuriantly at LAS concentration of 150 µg/mL, was isolated from the urban sewage by enrichment culture technique. Its ability to degrade LAS was studied over a period of six days along with changes in biomass and pH. The estimation of the residual LAS by methylene blue method showed 98% degradation of LAS. The biologically treated waste water showed its potential to be used as irrigation water.

INTRODUCTION

Twentieth century ushered in an era of industrial development that changed the entire lifestyle of man. But, these developments have come up with newer and severe problems related to each area of progress, pollution being one of them. Among the first synthetic organic chemicals to create environmental problems, were the surfactants. Surfactants have an impact on all aspects of our daily life due to their use in the household detergents, soaps, personal care products, textiles, paints, and pulp and paper industry etc. (Sodhi 1987).

Anionic surfactants like linear alkyl benzene sulfonate (LAS) was introduced in 1960s, in contrast to the branched chain products like tetrapropyl benzene sulfonates, which were very difficult to degrade. LAS has become the major cleaning agent for laundry detergents in most parts of the world. About 2.5 million tons of LAS is produced worldwide accounting for an estimated 28% of all synthetic surfactants**.** LAS enters the environment through municipal sludge applied to land as a soil conditioner and the effluents of sewage treatment plants, which are discharged in rivers, lakes and estuaries (Gledhill 1982).

A steady increase in surfactant use has been accompanied by problems such as foaming in waste water plants, reduced efficiency of water purification, eutrophication and imparting an objectionable taste to water etc. Concern has been raised about the possible toxic effects of LAS on susceptible biota in agricultural soils receiving high loads of these anionic surfactants from recycled sewage sludge or contaminated irrigation water (Brandt et al. 2001). Since LAS exerts so many ill effects on the aquatic as well as the terrestrial environment, its removal is of utmost importance. The present research has, therefore, sought to isolate and identify organisms that can utilize and degrade LAS and the use of this water for irrigation purposes in agriculture.

MATERIALS AND METHODS

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

Determination of anionic surfactant content of the collected waste water: Influent waste water was obtained from a sewage treatment plant, Mumbai in a clean sterile glass bottle. The anionic surfactant content of the collected waste water was determined by methylene blue method (Longman 1975).

Isolation and selection of LAS degraders: The influent waste water was used for isolation of LAS degraders. Enrichment of LAS degraders was carried out in the mineral salt medium (K_2HPO_4 - 100mg, $MgSO_4.7H_2O$ - 20 mg, $FeCl_3$ - 2mg, NaCl - 20mg, $CaCl_2$ -20mg, $(NH_4)_2SO_4$ - 190mg, pH 7.4, Distilled water 100ml) containing LAS. Five successive rounds of enrichment were carried out with increasing concentrations of LAS ranging from 50 to 200 µg/mL, each for 1 week. The flasks were incubated at room temperature on a shaker at 200 rpm. The LAS degraders were isolated on solid medium after each round of enrichment and the colony characteristics of the isolates were studied.

Identification and preservation of the selected isolates: The isolates 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 4ºC on solid mineral medium containing LAS.

Degradation of LAS by the selected isolate: Degradation of LAS was carried at a concentration supporting luxuriant growth of the isolate in 250 mL flasks. 100 mL of mineral salt medium was prepared using the influent waste water diluted appropriately to get the required concentration of LAS. It was inoculated with 10mL of 18 hour old cell suspension (10^8 cells/mL) of the isolate. The flasks were incubated on shaker at room temperature for a period of 6 days. After every 24 hours, aliquots were removed and assessed for biomass, pH and residual LAS content after centrifugation at 8000 rpm for 20 min.

Effect of the treated and untreated waste water containing LAS on the germination and growth of wheat seeds: The effect of the treated and untreated waste water on the germination of wheat seeds was studied by using garden soil that had been kept under organic rotation and had not received any domestic waste product or sludge. N-K-P fertilizers were added to the soil to avoid deficiency of the nutrients. Fifty healthy and undamaged wheat seeds were sown in pots containing 500 g of soil each. A set of ten pots each was watered regularly with tap water, which served as a control (C), treated waste water (T1) and untreated waste water (T2). The germination and growth of seeds were monitored along with the length of roots and shoots after growth of ten days.

RESULTS AND DISCUSSION

Anionic surfactants like LAS represent the major fraction of the widely used surfactants in use today. They are usually present in the urban sewage in high concentrations, especially in areas of high population densities. The anionic surfactant content of the collected influent sewage water was estimated by methylene blue method, which is the only convenient method to measure surfactants and their gratuitous degradation without special equipment. Anionic surfactant concentration in the influent was found to be 700mg/L, which was higher than the reported average concentrations of 1-10 mg/L for municipal waste water treatment facilities, probably due to tremendous population density of Mumbai.

Goodnow & Harrison (1972) used LAS concentrations of 4µg/mL, 20µg/mL and 40 µg/mL for isolation of LAS degraders. However, in the present study, a much higher concentration of LAS was

selected ranging from 50 µg/mL to 200 µg/mL. On the third day of each enrichment, the colourless mineral salt medium turned yellowish brown probably due to accumulation of phenolic compounds like catechol, the intermediate compounds in the breakdown of LAS by the organisms, as shown by Horvath & Koft (1972) using *Pseudomonas* spp HK-1.

Luxuriant growth of the isolates was obtained up to the surfactant concentration of 150 µg/mL and the concentrations above 175 µg/mL were found to be inhibitory for the growth of the organisms. Hence, the concentration of LAS was maintained at 150 µg/mL for further work. Gram staining of the broth revealed presence of gram negative short rods. The enriched broth when used for the isolation of the organisms on solid media after every round of enrichment, initially showed presence of five isolates based on their colony characteristics. However, with increasing LAS concentration, the mixed population was replaced by a pure culture at 150 µg/mL of LAS.

Based on the cultural and morphological characteristics of the isolate, the organism was identified as *Pseudomonas aeruginosa* based on its bluish green pigmentation on nutrient agar, oxidative fermentation of glucose, nitrate reduction, citrate utilization and oxidase positive nature according to Bergey's Manual of Systematic Bacteriology (Krieg & Halt 1984).

The genus *Pseudomonas* and its members occupy a prominent place in the surfactant biodegradation since the earlier days (Gledhill 1982). Along with *Pseudomonas* the names of certain other genera have recurred again and again in surfactant biodegradation studies like *Aerobacter, Alcaligenes, Achromobacter, Escherichia, Micrococcus, Bacillus, Corynebacterium, Proteus,* and *Flavobacterium* etc. Each of these was isolated from culture media containing alkyl benzene sulfonate (ABS) as sole carbon source, after inoculation with acclimated activated sludge (Swisher 1982).

Knaebel et al. (1994) reported that five or six days were required for disappearance of LAS in unacclimated river water, but only one to two days were required upon addition of acclimated river water, in which the compound had already been degraded. The results of the present investigation showed a linear increase in the rate of degradation of LAS over a period of five days, reaching a plateau on the sixth day as seen in Fig. 1. A total of 98% of the surfactant was degraded at the end of the fifth day. With longer acclimation, the isolate will be able to degrade LAS much more rapidly.

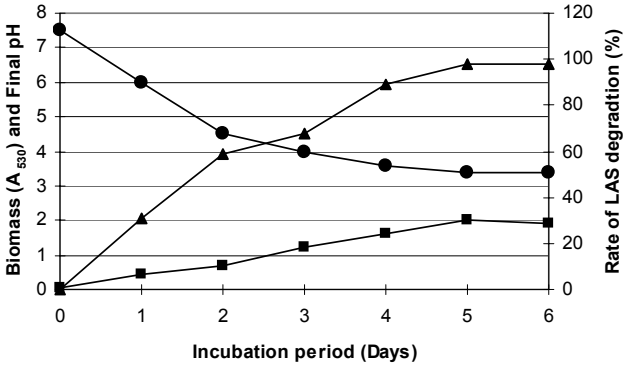

Fig. 1: Degradation of LAS content of the wastewater by *P. aeruginosa*. The degradation experiment was carried out at LAS concentration of 150 µg/mL and parameters like increase in Biomass A_{530} (-■-), final pH (-●-) and rate of LAS degradation (-▲-) were recorded over a period of 6 days.

During degradation of LAS, the biomass measured in terms of optical density at 530 nm, showed gradual increase and reached its maximum on fifth day of incubation, which correlated with the rate of degradation. As the organisms utilized LAS as the sole carbon source, acidic end products were produced, as was observed by shift in pH of the medium from alkaline to acidic range with pH falling sharply in the initial stages. Another significant aspect of the study was

Table 1: Effect of treated and untreated waste water on germination and growth of wheat seeds.

Pot	No. seeds sown/pot	No. of seeds germinated*	Measurement of growth after 10 days (cm)* Shoot length	Root length
Control	50	45 ± 1.38	22.5 ± 1.15	6.1 ± 0.50
Test I	50	38 ± 1.41	21.5 ± 1.20	5.6 ± 0.60
Test II	50	16 ± 5.09	8.3 ± 2.50	2.3 ± 0.90

Control pot: Irrigated with tap water; Test I pot: Irrigated with treated wastewater
Test II pot: Irrigated with untreated wastewater; *: Mean ± Standard deviation

the feasibility of use of microbially treated waste water containing LAS for irrigational purposes. In an agricultural country like India, there is an acute shortage of irrigation water. If recycled sewage effluent can be used for irrigation, it can cut down on the cost of irrigation water to a great extent.

In the present investigation when the microbially treated waste water was used for the irrigation purpose, it was observed that germination and growth of wheat seeds was comparable with seeds watered with tap water (control). The germinability of the seeds in the control pots was 90%, while that in Test 1 pots (watered with treated water) was 84.4% and in Test 2 pots (watered with untreated water) was only 35.5% (Table 1). The measurement of growth carried out on the tenth day in terms of average shoot and root length is shown in Table 1. In Test 2 pots, the height of the plants was markedly reduced with an average height of 8.3 cm and root length of 2.3 cm as opposed to shoot length of 21.5cm and root length of 5.6 cm in the Test 1 pots.

Thus in conclusion, the prospect of using *Pseudomonas aeruginosa* isolated from sewage water shows a great potential in removal of LAS from the environment. The rate of degradation can be increased further by acclimation 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.

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