SCIOL BIOTECHNOLOGY

ISSN: 2631-4061

RESEARCH ARTICLE

Aerobic Biodegradation of the Anionic Surfactant Sodium Dodecyl Sulphate (SDS) at Sub and Supra Critical Micelle Concentrations

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Citation: Ambili PS, Jisha MS (2018) Aerobic Biodegradation of the Anionic Surfactant Sodium Dodecyl Sulphate (SDS) at Sub and Supra Critical Micelle Concentrations. SCIOL Biotechnol 2018;1:49-56

Accepted: July 18, 2018

Published: July 20, 2018

Abstract

The anionic surfactant Sodium dodecyl Sulphate (SDS), the core components of detergent and cosmetic product formulations, contributes significantly to the pollution profile of sewage and wastewaters of all kinds. Due to its high foaming capabilities which can cause numerous problems in sewage treatment facilities as well as the direct toxic effects on many different organisms in the ecosystem, it is generally considered to be a serious pollutant. In this study, 44 SDS degrading strains were isolated by soil enrichment methods and the utilization efficiency was assessed by methylene Blue Active Substances (MBAS) assay and HPLC method. The most efficient SDS degrading isolate was identified as *Pseudomonas aeruginosa* MTCC 10311 based on phenotypic features and 16S rDNA typing. The reduction of SDS in the synthetic wastewater contained subcritical micelle concentrations (CMCs) and supra CMCs of SDS by free cells and immobilized cells of the isolate *Pseudomonas aeruginosa* (MTCC10311) were investigated. Free cells could degrade up to 96 ± 1% and 80 ± 2% of SDS in the synthetic waste water contained sub CMCs and supra CMCs of SDS at a residence time of 48 hours, whereas with immobilized cells same result was obtained at a less residence time of 32 hours. In conclusion the isolate *Pseudomonas aeruginosa* can be exploited for the SDS removal from industrial sewage contained high concentration of SDS.

Keywords

Biodegradation, Critical micelle concentrations, HPLC, Sodium dodecyl sulphate, *Pseudomonas aeruginosa*

Introduction

Surfactant based processes are becoming increasingly important in pollution control. The use of surfactant can significantly enhance conventional groundwater pump-and-treat processes for the clean-up of contaminated soil and aquifers. Surfactants, in the form of conventional foams, micro-gas suspensions and micelles have also shown promise in wastewater treatment [1]. These surfactant-based technologies are at an expense of leaving residual surfactants in the produced water or the subsurface environment. Surfactant removal becomes a necessity for the successful application of these technologies.

Surfactants contain both strong hydrophobic and hydrophilic moieties. According to the charge of their hydrophilic moiety, surfactants can be classified into four categories: Anionic, non-ionic, cationic and amphoteric [2]. Anionic surfactants are one of the most frequently employed surfactants used in detergent formulations. The predominant classes of anionic surfactant are linear alkylbenzene sulfonate and linear alkyl sulfate [3].

There is an urgent need to search for new possibilities of enhanced degradation of surfactants. Bacterial degradation of pollutions has proved to be very efficient especially in combination with immobilization methods. Immobilization of bacterial cells offers several advantages such as prevention of cell losses in continual processes and allows working with high cell densities [4]. Furthermore, immobilized organisms are more resistant to adverse effects occurring during the degradation processes like changes of physico-chemical parameters such as pH, temperature, fluctuations of substrate concentration, presence of toxic substances etc.

It is well documented that most commercial surfactants in low concentration have rapid and extensive biodegradation in the aerobic environment. However, little is known about the biodegradation pattern under high concentrations (especially above the critical micelle concentrations (CMCs)) but below the toxic thresholds. The work has been largely motivated by concerns over the biodegradation of high concentration of surfactants employed in soil washing. To improve the biodegradation of surfactants at higher concentration efficient bacterial isolates has to be applied to bring out efficient solutions for biological cleanup of industrial wastewater. This study addresses surfactant removal by aerobic biodegradation. This work has been largely motivated by concerns over the biodegradation of SDS like surfactant at high concentration.

Materials and Methods

Isolation of SDS degrading bacteria

Soil enrichment method was used to isolate Sodium dodecyl Sulphate (SDS) degrading organisms from detergent contaminated laundry premises of Meenachil river shore, located in Kottayam, Kerala, India. Isolation was done by enrichment of the soil extract progressively with SDS. All the strains isolated at the final SDS concentration of 1500 mg/L were again subjected to enrichment with SDS in mineral salt SDS medium (MSSM). The composition of the mineral salt SDS medium (MSSM) used was KH_2PO_4, 1.5 g/L.; K_2HPO_4, 3.5 g/L.; $MgCl_2.6H_2O$, 0.15 g/L.; NaCl, 0.5 g/L.; Na_2SO_4, 0.14 g/L. SDS (1500 mg/L). The medium also contained the following trace elements (1 ml stock): $FeCl_2.6H_2O$, 0.24 g/L.; $CoCl_2.6H_2O$, 0.040 g/L.; $CuSO_4.5H_2O$, 0.06 g/L.; $MnCl_2.4H_2O$, 0.03 g/L; $ZnSO_2.7H_2O$, 0.31 g/L.; and $NaMoO_2.2H_2O$, 0.03 g/L. Incubation was carried out at room temperature (30 ± 2 °C) for 24 hours. At the final concentration of 1500 mg/L of SDS there was only 9 strains that could grow in MSSM and the most efficient strain was selected. MBAS assay [5] and HPLC were used for the determination of SDS reduction [6].

Inoculum preparation

One loopful of the culture was inoculated to 50 ml of mineral salt SDS medium. The flasks were incubated overnight at room temperature (30 ± 2 °C) at 150 rpm. From the culture the cells were harvested by centrifugation. The pellets were collected and diluted using physiological saline (0.86% NaCl) till the OD becomes 1. This was used as the inoculum for further studies.

Quantification of SDS

The concentration of SDS was checked by the Methylene Blue Active Substances (MBAS) assay of Hayashi [5] with minor modifications.

The biodegradation of SDS was confirmed by isocratic HPLC (Shimadzu) analysis using reverse phase C-18 column equipped with SP an isocratic mobile phase gradient of acetonitrile-water (95:5) conducted at a flow rate of 1 ml/min. Peak area at retention time of 2.740 min corresponds to SDS.

Identification of the isolate

The selected isolate was identified by conducting biochemical test as per Bergey's manual and confirmed by 16s rDNA sequencing. The sequences were analyzed using the gapped BLAST (www.ncbi.nlm.nig.gov) search algorithm and aligned to their nearest neighbors. The sequence was deposited in the NCBI gene bank data base (HM 214777) [6].

Effect of SDS concentration on SDS degradation

A culture of *Pseudomonas aeruginosa* was prepared in MSSM medium to investigate the effects of SDS concentration at range from 500 to 10000 mg/L.

Treatment of SDS at sub (1500 mg/L) and supra CMC level (2400 mg/L) with free cells of *Pseudomonas aeruginosa* (MTCC 10311)

In this study comparison in the degrading ability of *Pseudomonas sp.* has been

studied with two different concentration of SDS. CMC level of SDS is 1-8 mM (2310 mg/L). Here studies conducted at Supra-CMC level 2400 mg/L (8.311 mM), and sub CMC level (1500 mg/L). High surfactant concentrations were applied to simulate the conditions as found in industries where intense utilization of surfactants are usually taking place.

Cells were collected as per the procedure mentioned above. These cells were inoculated in SDS at supra CMC level and checked its performance in reducing SDS.

Immobilization of cell

*Pseudomonas aeruginosa (*MTCC 10311) was grown in mineral salt SDS medium for 24 hours and were harvested by centrifugation at 10,000 rpm for 30 minutes. These cells were resuspended in physiological saline (0.86% NaCl) at a cell concentration of 1 OD and were mixed with 4% sodium alginate in the ratio of 1:2. The mixture was added drop wise to excess 0.2 M $CaCl_2$ to get alginate entrapped cells. The beads were kept in the same solution for curing and activated later with MSSM.

Activation of immobilized viable cells

The immobilized viable beads were activated for achieving maximal activity using MSSM medium. Prepared immobilized beads were taken in large 500 ml beaker and immersed with SDS containing minimal medium for varying time intervals. Optimal activation time that promoted maximal activity, for immobilized cells was determined in terms of percentage reduction of SDS.

Biodegradation of SDS by immobilized cells of *Pseudomonas aeruginosa* MTCC 10311

The MSSM medium was taken as 300 ml aliquots in 1 litre flasks and was inoculated with 300 activated immobilized beads. The flasks were incubated at room temperature on a shaker at 150 rpm under optimized conditions of SDS degradation. Samples were withdrawn at regular intervals of 4 hours for SDS estimations from the mineral salt SDS medium and a graph was plotted with % of SDS reduction against incubation period.

Treatment of the SDS at supra CMC level (2400 mg/L) with immobilized cell

Immobilized cells were used for the treatment of SDS at supra CMC level. The performance of immobilized cell in bringing SDS reduction was studied at different flow rates as mentioned above.

Growth pattern of free and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311 at supra CMC level of SDS (2400 mg/L)

Mineral salt SDS medium was inoculated with 3% of inoculum of 1 OD concentration in the case of free cells and 100 beads per 100 ml in the case of immobilized cells. The quantification of the growth of free cells was done by serial dilution of the sample followed by viable counts at regular intervals of 4 hours. In the case of alginate immobilized cells, to recover the bacteria for viable counts, known amounts of beads were immersed in phosphate buffer (1 M, pH -7) and dissolved by vigorous shaking [7].

Results

SDS degrading isolate (S9) was isolated from detergent contaminated soil. The optimum pH and temperature for the growth of S9 was 7.5 and 30 °C respectively [6]. The margin of the S9 isolate colony was round and entire. The colonies produced bluish green pigment on nutrient agar and showed fluorescence on King's B medium. The isolate was small rod-shaped gram-negative bacteria and showed single arrangement. The bacteria were non-spore forming and were found to be motile. Based on the morphological and biochemical characteristics as well as 16S rDNA gene sequencing, with nearest philological relatedness, the S9 was identified as *Pseudomonas aeruginosa* [6]. The Gene bank accession number for the 16S rDNA sequence generated in

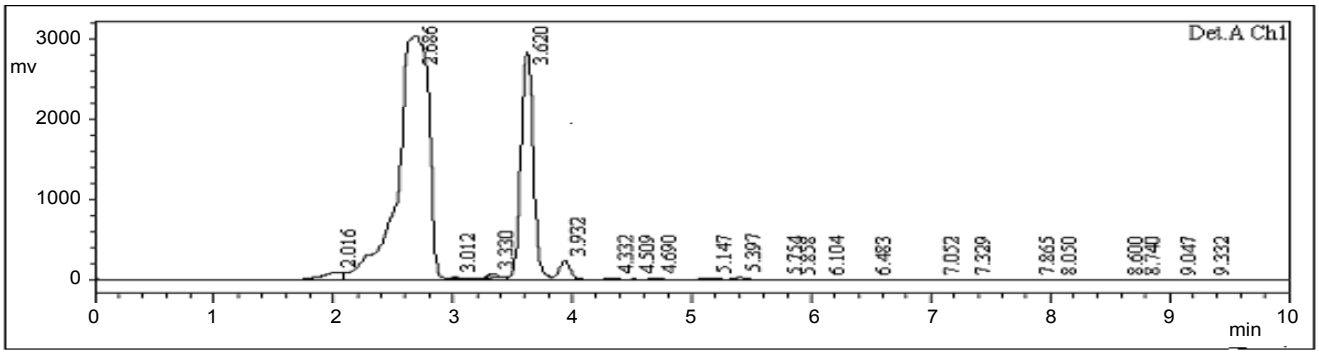

Figure 1: HPLC Analysis of SDS degradation at 0 hour of incubation.
Retention time: 2.686 min; Area: 56156126.

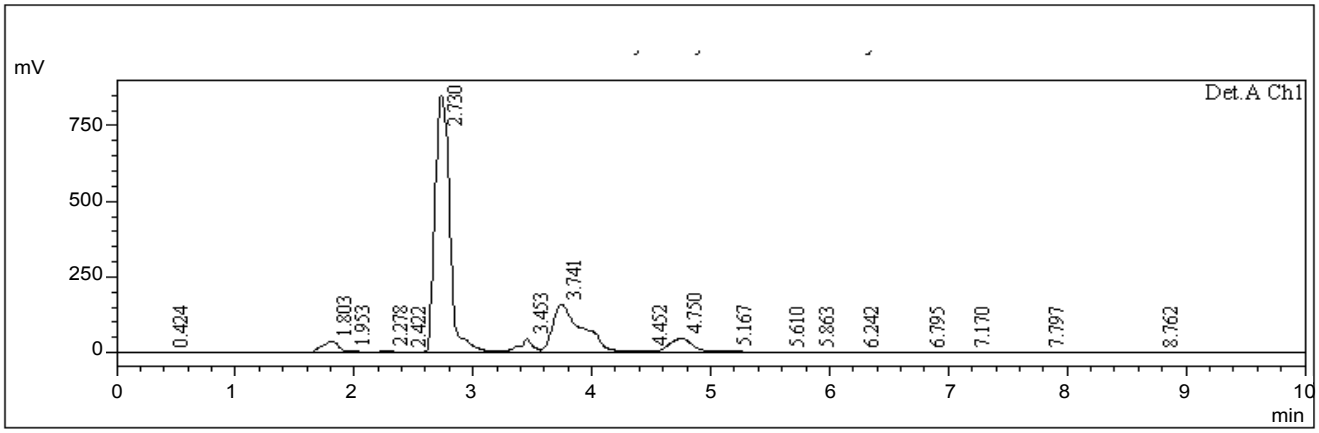

Figure 2: HPLC Analysis of SDS degradation at 48 hours of incubation.
Retention time: 2.730 min; Area: 743158.

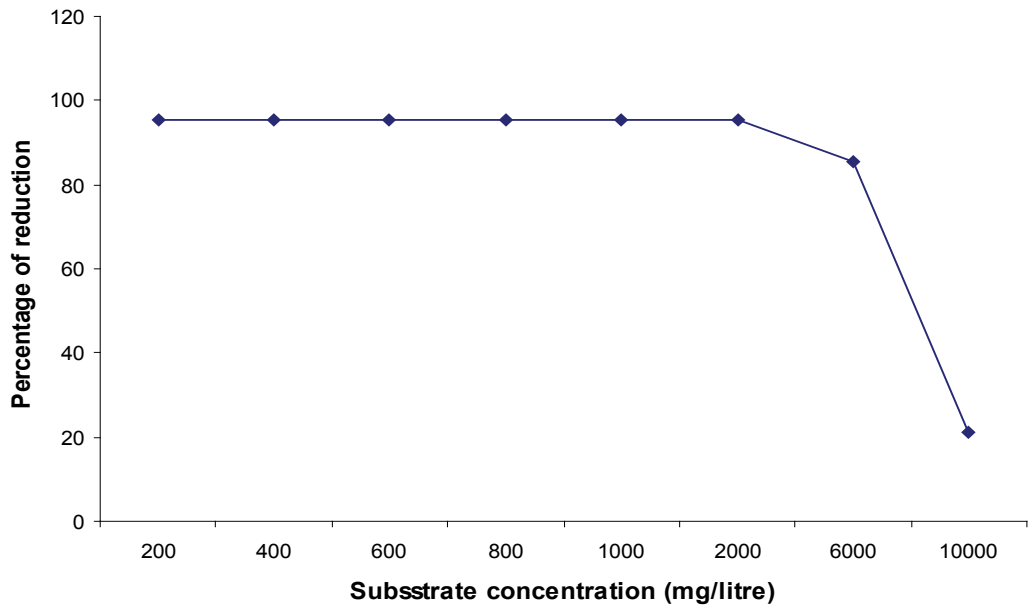

Figure 3: Effect of SDS concentration on SDS biodegradation.

this study was HM 214777. The isolate was capable of degrading 96% of 1500 mg SDS within 48 hours of incubation. HPLC analysis showed that the area of biodegradation of SDS in mineral salt medium was decreased from 56156126 to 432439 (2.758 min) within 48 hours of incubation (Figure 1 and Figure 2).

When SDS was tested on different SDS concentrations, maximum SDS degradation occurred at 1500 mg/L level within 48 hours of incubation, whereas higher

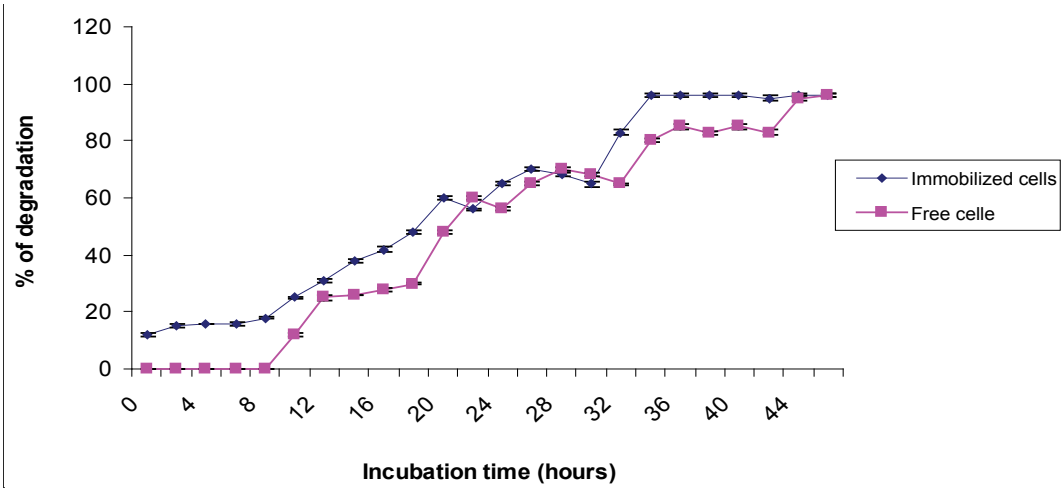

Figure 4: Comparison of degradation of free cells and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311 in Mineral salt medium contain SDS at sub CMCs level (1500 mg/L).

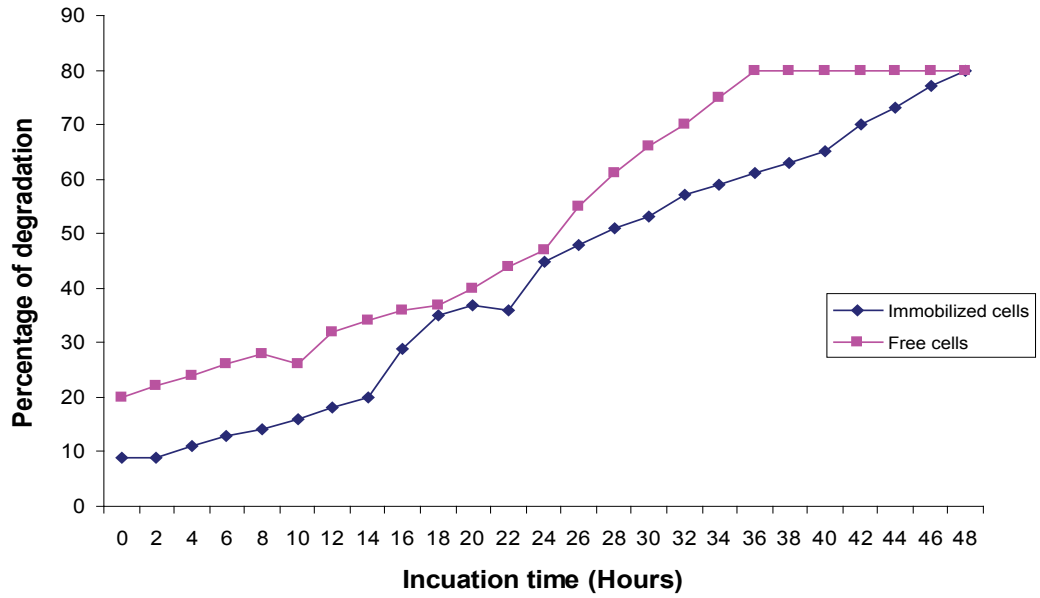

Figure 5: Comparison of degradation of free cells and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311 in mineral salt medium with supra CMCs SDS concentration (2400 mg/L).

concentration of SDS (10000 mg/L) showed only 20% degradation (Figure 3). The immobilized cells of *Pseudomonas aeruginosa* was able to degrade 96% of SDS within 36 hours of incubation (Figure 4). The mineral salt medium with supra CMCs SDS concentration (2310 mg) was treated with free cells and immobilized cells of *Pseudomonas aeruginosa* (MTCC 10311). The mineral salt medium with supra CMCs SDS concentration revealed that 80 ± 2% SDS removal was affected by 36 hours of treatment with immobilized cells whereas the same performance was achieved by 48 hours of treatment in the case of free cells (Figure 5).

A comparison of the growth curve of free cells of *Pseudomonas aeruginosa* in mineral salt SDS medium with 1500 mg/litre revealed that the logarithmic phase of the strain in MSSM was from 26 hours to 36 hours (The maximum biomass yield was 10^7 at 36 hours) and in supra CMCs SDS concentration, it was from 10 hours to 46 hours. The maximum biomass yield was 10^8 at 46 hours. Growth curve of the immobilized cells revealed that the supra CMCs SDS concentration, offered a better growth condition with a longer logarithmic phase and enhanced growth rate (maximum cell yield of 10^{16} in 42 hours in mineral salt medium with supra CMCs SDS concentration and 10^{10} in 24 hours in mineral salt medium) (Figure 6 and Figure 7).

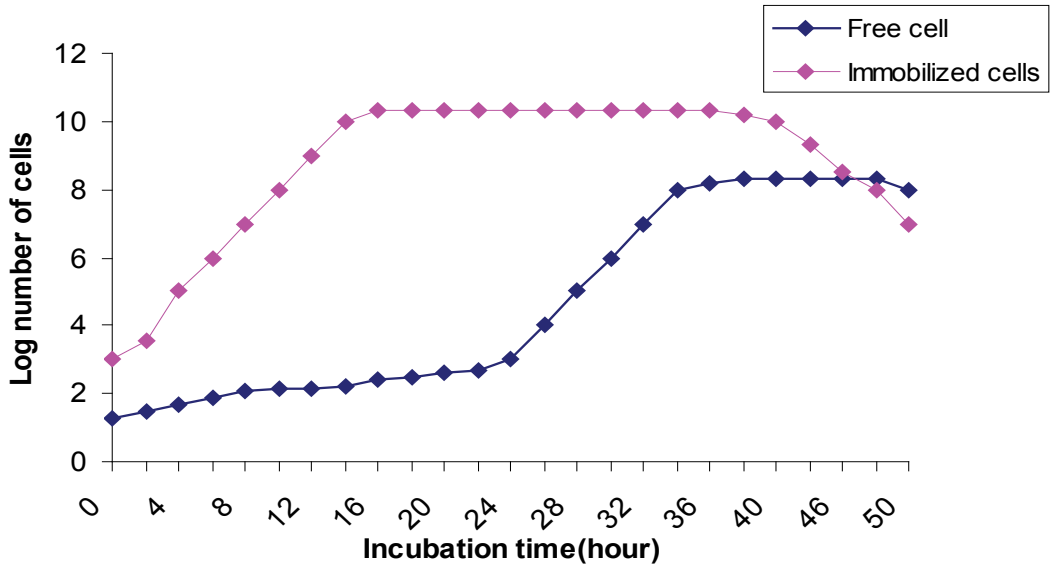

Figure 6: Comparison of growth pattern of free cells and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311 in Mineral salt medium contain SDS at sub CMCs level (1500 mg/L).

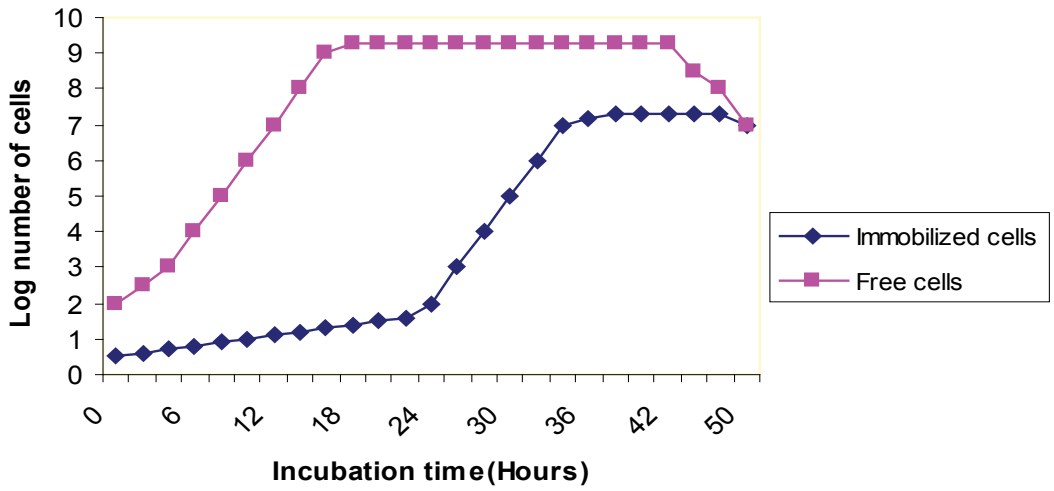

Figure 7: Comparison of growth pattern of free cells and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311 in Mineral salt medium with supra CMCs SDS concentration (2400 mg/L).

Discussion

Biodegradation is the destruction of a chemical by the metabolic activity of microorganisms [8]. Past experiences have demonstrated that anionic surfactants biodegradation are exclusively conducted by bacteria. Shukor, et al. [9] isolated *Klebsiella oxytoca* from soils and water contaminated with detergent from a car wash outlet in Serdang. Many researchers used detergent contaminated activated sludge in order to isolate bacteria that are able to degrade anionic surfactants. Schleheck, et al. [10] isolated *Proteobacterium* strain DS-1, that could utilize commercial Linear alkyl sulphate in aerobic culture. In this study SDS degrading isolates was isolated from detergent contaminated laundry premises of river shore. MBAS assay method was used for determination of anionic surfactant biodegradation. This chromatographic method was originally proposed in 1975 (Hayashi) and was subsequently used by many other investigators [11,12]. The results of HPLC analysis agrees with MBAS assay.

Pseudomonas sp. has been reported many times as a potent biodegrading isolate [11,13,14]. In most of the earlier cases *Pseudomonas sp.* could degrade higher SDS concentration with 80% efficiency with long hours of incubation time [9]. Our isolate *Pseudomonas aeruginosa* (MTCC 10311) strain was capable of degrading compara-

tively higher SDS concentrations of 2.4 gl_1 in a short incubation period while 0.1% to 1% is found to be optimum concentrations of SDS degradation as per published studies [13]. This isolate could degrade, 96% of 1500 mg SDS within 48 hours and it could degrade 80% of 2310 mg (supra CMC level) within 48 hours.

The increasing surfactant concentration from sub to supra-CMCs significantly decreased primary biodegradation and foam degradation. This decrease may attribute to the limited bioavailability of surfactants in the micelle phase as compared to the monomeric surfactants [15]. Supra-CMC level of SDS cause excessive foaming and causes operational difficulties in wastewater treatment plants and may also lead to health hazardous in the form of airborne pathogens carried on windblown foams [15].

In immobilized systems, as the cells were entrapped inside the alginate matrix greater time might have taken to overcome the diffusion limitation. Hence the system took longer time for the complete removal of the SDS (34 hours) even after getting the maximum biomass at 16 hours. The immobilized cells could degrade 80 ± 2% of high concentration of SDS (2310 mg) within 32 hours of treatment. Reduced incubation time for the reduction of surfactant was a possible indication of better activity of the immobilized cells (Figure 5) [9]. Immobilization of *Pseudomonas aeruginosa*, enabled it to remediate SDS at a greater level than its free state [4]. The immobilization on adsorbents indirectly promotes the formation of biofilms in the bacteria and cell aggregation helps bacteria to overcome the stress caused by SDS loaded environment [13].

A high concentration of surfactant in cosmetic industry water significantly inhibits microbial growth and produces foam. These two phenomena are very problematic and require judicious intervention in order to reduce their impact on the good running of the industrial wastewater treatment. The use of bacteria possessing high catabolic ability for degrading SDS is a cost-effective solution for biological treatment of wastewater in detergent, cosmetic and cleaning industries.

Conclusion

The data presented here represent the first report about the comparison study of degradation of sub and supra CMC concentration of SDS with the free and immobilized cells of *Pseudomonas aeruginosa* MTCC 10311. This could be a unique bacteria in the degradation of high concentration of SDS.

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