Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Metabolic profile of sodium dodecyl sulphate (SDS) biodegradation by *Pseudomonas aeruginosa (MTCC 10311)*

P. S. Ambily[1] and M. S. Jisha[2]*

[1]Controllerate of Quality Assurance and Fire Fighting Equipment, Ministry of Defence, Pune-411 027, India
[2]School of Biosciences, Mahatma Gandhi University, Kottayam-686 560, India
*Corresponding Author's E-mail: jishams@mgu.ac.in

Publication Info

Paper received:
25 March 2012

Revised received:
23 January 2013

Accepted:
13 January 2014

Abstract

Sodium dodecyl sulfate (SDS) is one of the main components in the detergent and cosmetic industries. Its bioremediation by suitable microorganism has received greater attention. *Pseudomonas aeruginosa* MTCC 10311 was isolated from detergent contaminated soil which had degraded 96% of SDS in 48 hrs. Attempts were made to study the metabolic byproducts of SDS degradation using GC-MS analysis. Analysis of ether extracts of surfactant established the sequential production of Dodecanol, Dodecanal and Decanoic acid. At this point, the pathway diverged into the formation of acid residues through beta oxidation. This SDS degrading isolate, *Pseudomonas aeruginosa* can be exploited for decontamination of detergent contaminated waste water.

Key words

Biodegradation, GC-MS analysis, *Pseudomonas*, Sodium dodecyl sulfate

Introduction

Surfactants, due to their favorable physico-chemical properties are extensively used in many fields, like pharmacy, in cosmetics, textile industry, agriculture and biotechnology, of technology and research, (Dhuiib *et al.,* 2003). After use large quantities of surfactants and their derivatives are released in to aquatic and /or terrestrial environment (Sandbacka *et al*., 2000). Wastewater problem due to excessive use of detergents is becoming more pressing as detergents are known to have detrimental effects on aquatic life (Chukwu and Odunzeh, 2006; Kumar *et al*., 2007).Because many surfactants are ubiquitous, the potential toxic effects of these chemicals have attracted much research attention in the past several decades. (Ying *et al*., 2002; Venhuis and Mehrvar, 2004). Different tixicities mechanisms exist for different types of surfactants and one single surfactant can produce toxicity by more than one mechanism (Li, 2008).

Sodium dodecyl sulphate (SDS) is a widely applied anionic surfactant that accounts for approximately 25 to 30% of the world's total synthetic surfactants. SDS is a highly effective surfactant used for removal of oily stains and residues (Kowalska *et al.,* 2004). Its surface-active properties make it important in household and industrial cleaners, personal care products and cosmetics (Nasirudhin and Uzva, 2005). High prevalence and usage of this detergent thus demands knowledge on its degradation aspects.

Biodegradability of alkyl sulphate surfactants in such environments is well established however, little is known about the pathways involved in SDS degradation (Venkatesh and Ashok Kumar, 2010). Nevertheless, confirmation of ultimate biodegradation is important because primary biodegradation, loss of sulphate groups from surfactants, although leading to losses in surfactant properties may still yield recalcitrant and possible toxic metabolites (Roig *et al.,* 1998a). Hence, in the

Online Copy

present study attempt was made to describe the metabolic profile of biodegradation of SDS by *Pseudomonas aeruginosa* MTCC 10311 to ensure that all the degradative products were non toxic to the ecosystem.

Materials and Methods

Isolation of SDS degrading bacteria: *Pseudomonas aeruginosa* (MTCC 10311) capable of utilizing Sodium Dodecyl Sulphate (SDS) as sole carbon source was isolated from surfactant contaminated soil (Meenachil river basin, Kottayam, Kerala) (Ambily and Jisha,2012).

Analysis of the products of SDS degradation: Metabolic byproducts of SDS degradation were analyzed by GC-MS analysis. 1000 ml of basal salt SDS medium was taken and inoculated with *Pseudomonas aeruginosa*. MTCC 10311.The flasks were incubated for 48 hrs under optimal conditions for SDS degradation. The medium was centrifuged at 10000 rpm and the pellet was separated. The supernatant was repeatedly extracted in the solvent ether and the extract was concentrated by evaporation. The analysis was done by Varian CP-3800 gas chromatograph. The column used was VF-5 having a length of 30 m, internal diameter of 0.25 mm, thickness of the film 0.25 µm, the helium flow rate was 1ml min^{-1}., the injection port temperature was 250°C and the column temperature was increased from 60°C to 260°C at a rate of 8°C min^{-1}.

Mass Spectrometric analysis: The metabolites formed, as a result of degradation of SDS, were identified using Mass Spectrometric analysis of the extracted sample after 0, 12, 24 and 48 days of inoculation of *Pseudomonas aeruginosa* (MTCC 10311) isolate into basal salt media with SDS as carbon source. The sample preparation was same as that for GC-analysis. Conditions used in the MS were as follows: Mass scan- 40-400m/z; Ionization –EI; Trap temperature -230^0 C; Transfer line -270^0 C.

Results and Discussion

Pseudomonas aeruginosa (MTCC 10311), isolated from detergent contaminated soil, having the potential to degrade 96% of SDS in 48 hrs (Ambily and Jisha, 2012), was used to elucidate the possible pathway employed for the biodegradation of anionic surfactant SDS.

Gas chromatogram of uninoculated SDS extract showed few peaks at 7.902 and 9.707. There was no significant peak after 10 min (Fig.1). Mass spectroscopic analysis of the peak at 9.707min gave mass fragment pattern that corresponds to SDS fragments. (185(M^+ -SO_3Na), 168 (185-H_2O) and other $(CH2)_x$ mass peak (Fig 2.) However, the peak corresponding to SDS in the chromatogram of control (retention time 9.707min) was totally absent in the chromatogram of biodegraded SDS (Fig 3). This confirmed that SDS supplied in the medium was completely degraded by *Pseudomonas aeruginosa* (MTCC 10311).

Gas chromatogram of biodegraded SDS extract after 12 hrs of incubation showed many peaks at 8min, 8.279 min and 14.465 min. However, the peak at retention time of 7.902 min observed in the extract of uninoculated SDS was totally absent in this GC (Fig 3). Additional peak at 8.279 min gave mass fragment of 169(M^+-OH). It corresponds to dodecanol (Fig 4). It has been demonstrated that the initial biodegradation products of primary alkyl sulphatase were primary alcohol (Jovcic *et al*., 2009). From SDS, SO_4^{2-} was cleaved and formed dodecanol. However, the content of dodecanol decreased after 8 hrs most likely as a result of further metabolism and continued growth of culture (Singh *et al*., 1998). Venkatesh and Ashok Kumar (2010) reported the time course study of SDS biodegradation by monitoring the disappearance of SDS with time.There are several similar reports of other sulphate degrading bacteria (Abboud *et al.,* 2007; Hosseini and Malekzadeh, 2007; Shukor *et al.,* 2009; Venkatesh and Ashok Kumar, 2010).

Gas chromatogram of biodegraded SDS extract (after 24 hrs of incubation) showed many peaks at 7.911min, 8.333 min, 12.863 min, 15.831 min, 16.598 min and 17.983 min. (Fig. 5). The peak at 17.243 gave mass fragment pattern that corresponds to dodecanal, m/z is183(M- H^+) (not shown). Dodecanol was oxidized into dodacanal.

Retention time of 16.598 min gave a molecular ion at m/z 183(M-OH^+), conforming the presence of dodecanoic acid (Fig. 6). Aldehyde (dodecanal) was converted into acids (dodecanoic acid) *via* omega oxidation. Mass spectrum of dedecanoic acid is shown in Fig. 6. Venkatesh and Ashok Kumar (2010) reported the total biodegradation of SDS by *Pseudomonas sp* C12B and detected the appearance of dodecanol and its oxidation product dodecanoic acid.

Gas chromatogram of biodegraded SDS extract, after 48 hrs of incubation, showed many peaks (Fig 7). Mass spectrum of different peaks gave a molecular ion at m/z 169, 155.141, 127, 113, 99, 85, 71, 57 and 43 (M-OH^+) which corresponds to C_{11}, C_{10}, C_9, C_8, C_7, C_6, C_5, C_4, C_3 and C_2 acid residues, respectively. Dodecanoic acid through beta oxidation produced acid metabolites of low molecular weight ($C_{11\ to}$ C_1). Mass spectrum of C_6 and C_3 acids are shown in Fig. 8 and 9 respectively. Thomas and White (1989) reported that *Pseudomonas sp* C12B produced dodecanoic acid from SDS and these acids were used as substrate for beta oxidation pathway. Beta oxidation is the main mechanism and leads to the production of various short acids. The above study with *Pseudomonas aeruginosa* MTCC 10311 showed possible routes for primary biodegradation of SDS *via* Sulphatase and ω - / β oxidation.

Online Copy

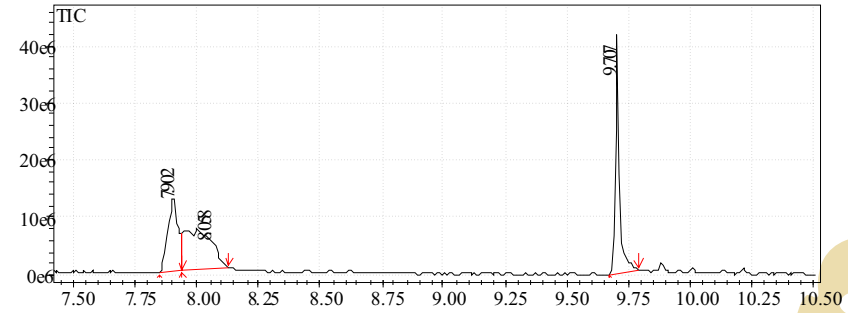

Fig. 1 : Gas chromatography Analysis of the extract of uninoculated basal medium containing SDS (SDS Control)

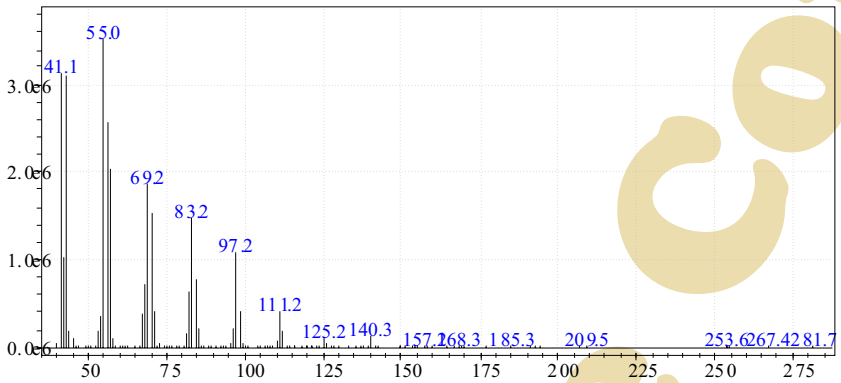

Fig. 2 : Mass spectroscopic analysis of the peak at 9.702 min of the Gas chromatogram of the extract of uninoculated SDS (m/z 185 (M^+-$NaSO_3$)

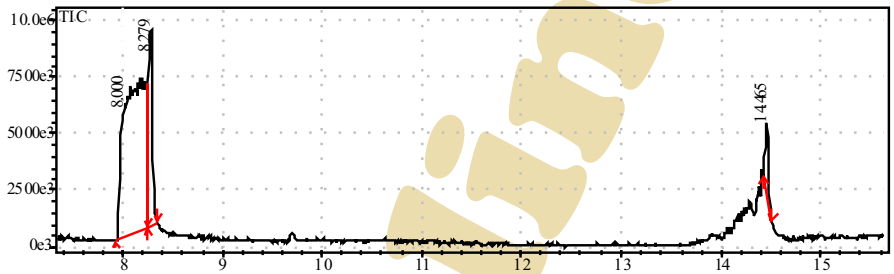

Fig. 3 : Gas chromatography analysis of the extract of inoculated basal medium contains SDS after 12 hours of incubation

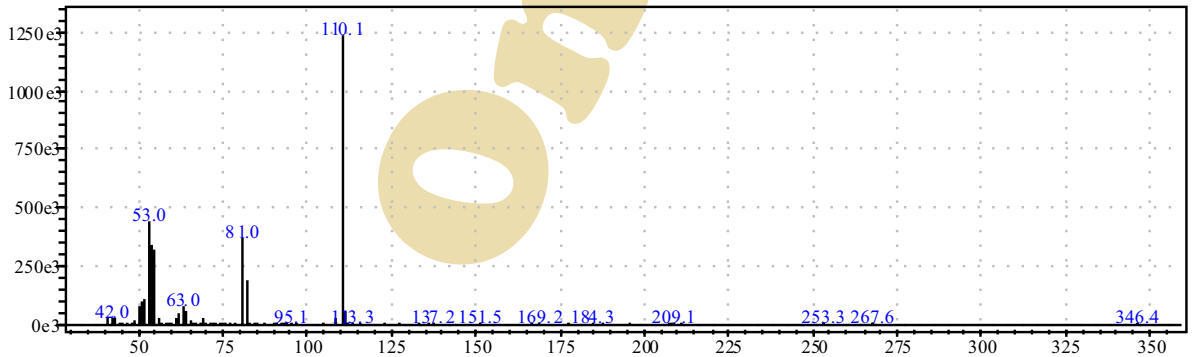

Fig. 4 : Mass spectroscopic analysis of the additional peak at 8.279 min of the gas chromatogram of the extract of biodegraded SDS showing Mass spectrum for Dodecanol (m/z 169(M-OH)]

Online Copy

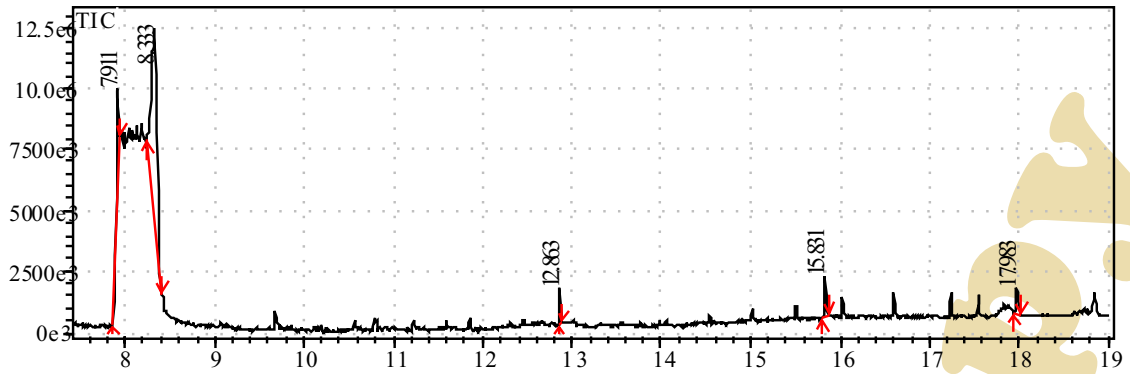

Fig. 5 : Gas chromatography analysis of the extract of inoculated basal medium contains SDS, after 24 hours of incubation

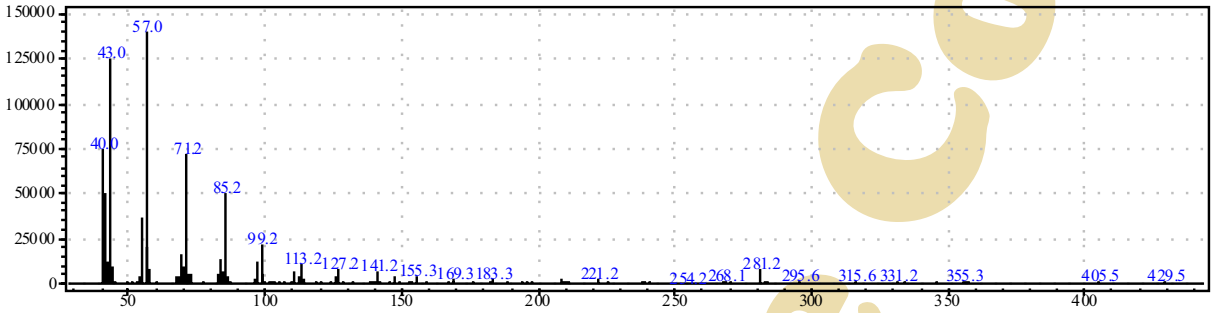

Fig. 6 : Mass spectroscopic analysis of the additional peak at 17.983 min of the gas chromatogram of the extract of biodegraded SDS showing mass spectrum for Dodecanoicacid (m/z 183 ($M-OH^+$))]

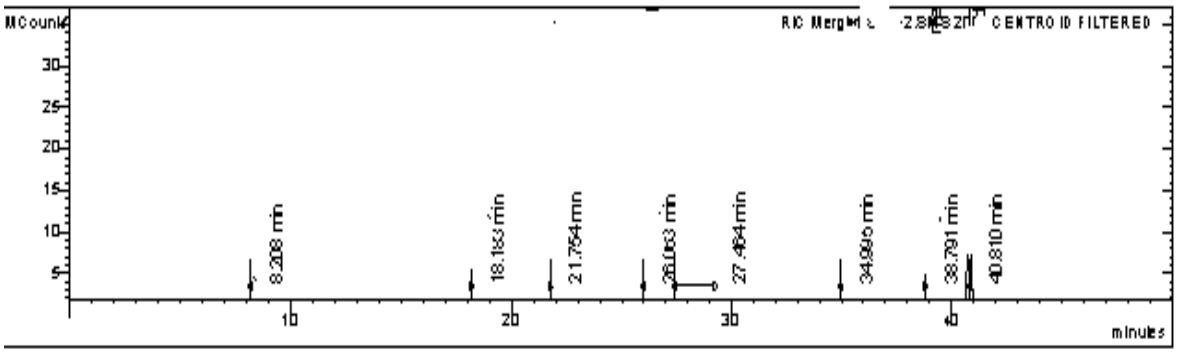

Fig. 7 : Gas chromatography analysis of the extract of inoculated basal medium contains SDS after 48 hours of incubation

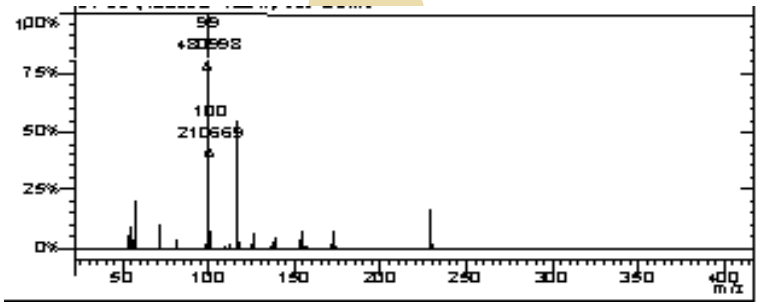

Fig. 8 : Mass spectroscopic analysis of the additional peak at 21.754 min in the gas chromatogram of the extract of biodegraded SDS showing mass spectrum for C_6 acid (m/z 99 ($M-OH^+$))]

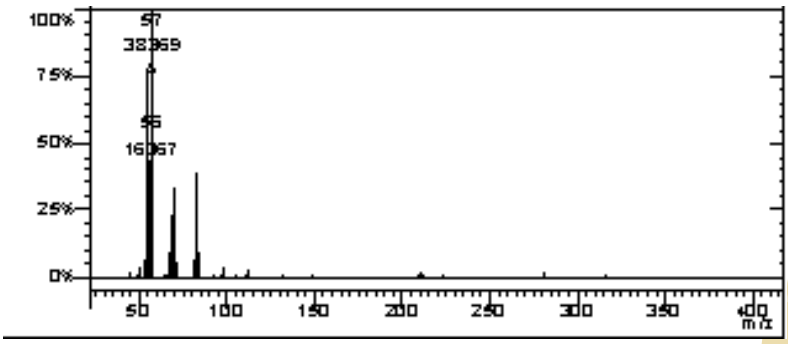

Fig. 9 : Mass spectroscopic analysis of the additional peak at 8.208 min in the gas chromatogram of the extract of biodegraded SDS showing mass spectrum for C_3 acid (m/z 57 ($M\text{-}OH^+$))]

Acknowledgment

The authors are grateful to the Kerala State Council for Science and Technology, Thiruvananthapuram for financial assistance.

References

Ambily, P. S and M. S. Jisha : Biodegradation of the anionic surfactant, sodium dodecyl sulphate by *Pseudomonas aeruginosa* MTCC 10311 . *J. Environ. Biol .,* **33,** (2012).

Chukwu, L.O. and C.C. Odunzeh : Relative toxicity of spent lubricant oil and detergent against benthic macro-invertebrates of a West African estuarine lagoon. *J. Environ. Biol .,* **27**, 479-484 (2006).

Dhuiib, A., N. Hamad and I .Hassairi: Degradation of anionic surfactants by *Citrobacter braakii. Process Biochem.*, **38**, 1245-1250 (2003).

Hosseini, F., F. Malekzadeh., N. Amirmozafari and N Ghaemi: Biodegradation of anionic surfactants by isolated bacteria from activated sludge. *Int. J. Environ. Sci. Technol.,* **4**, 127-132 (2007).

Jovcic, B., Jelena Bego Vić., Jelena Lozo, L. Topisiro Vić and Milan Koji: Dynamics of sodium dodecyl sulfate utilization and antibiotic susceptibility of strain *Pseudomonas* sp. ATCC 19151. *Arch. Biol. Sci.,* **61**, 159-164 (2009).

Kowalska I., M. Kabsch-Korbutowicz., K. Majewska-Nowak and T. Winnicki: Seperation of anionic surfactants on ultrafiltratio memberanes. *Desalination,* **162**, 33-40 (2004).

Kumar, M., S.P. Trivedi .,A. Misra and S. Sharma : Histopathological changes in testis of the freshwater fish, *Heteropneustes fossilis* (Bloch) exposed to linear alkyl benzene sulphonate (LAS). *J. Environ. Biol.,* **28**, 679-684 (2007).

Li, M.H.: Effects of nonionic and ionic surfactants on survival, oxidative stress and cholinesterase activity of planarian. *Chemosphere,* **70**, 1796-1803 (2008).

Nasirudhin M. and Z. Uzva : Sand sorption process for removal of sodium dodecyl sulphoate from water. *Hazard Mattern.,* **33**, 1-7 (2005).

Roig, M. G., M. A. Dedraz and J. M. Sanchez : Sorption isotherms and kinetics in the primary biodegradation of aionic surfactants by immobilized bacteria, *Pscudomonas C12B. J. Mol. Catal. B: Enzym.,* **4**, 253-270 (1998).

Sandbacka, M ., I. Chistianson and B. Isoma : The acute toxicity of surfactants on cells of *Daphnia magna*a comparative study. *Toxicol Vitro.*, **14** , 61–68 (2000).

Shukor, M .Y., W.S.W. Husin, M.F.A. Rahman, N.A. Shamaan and M.A. Syed: Isolation and characterization of an SDS-degrading *Klebsiella oxytoca. J. Environ. Biol.*, **30,** 129-134, (2009).

Singh, K.L ., A. Kumar and A.Kumar : *Bacillus cereus* capable of degrading SDS shows growth with a variety of detergents. *World. J. Microb. Biot.,* **14**, 777-779 (1998).

Thomas, O.R.T. and G. F. White: Metabolic pathway for biodegradation of sodium dodecyl sulfate by *Pseudomonas sp.* C12B. *Biotechnol. Appl. Biochem.*, **11,** 318–27 (1989).

Venkatesh Chaturvedi and Ashok Kumar: Bacterial utilization of sodium dodecyl sulfate., *Int. J. Appl. Biol. Pharma. Technol.,* **1,** 1126-1131 (2010).

Venhuis, S. H. and M. Mehrvar: Health effects, environmental impacts and photochemical degradation of selected surfactants in water. *Int. J. Photoenergy,* **6**, 115-125 (2004).

Ying, G.G.,B. Williams and R. Kookana: Environmental fate of alkylphenols and alkylphenolethoxylates: E*nviron. Int.,* **28**, 215-226 (2002).

Online Copy