# BACTERIAL UTILIZATION OF SODIUM DODECYL SULFATE

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**ABSTRACT:** SDS is widely used detergent in house holds and Industry. It is known to be toxic to fishes and also to animals. In the present review, we have emphasized on biodegradation of this detergent by bacteria, the pathway of biodegradation and also the enzymes involved in the degradation.

**Key words**: SDS, Bacteria, Biodegradation

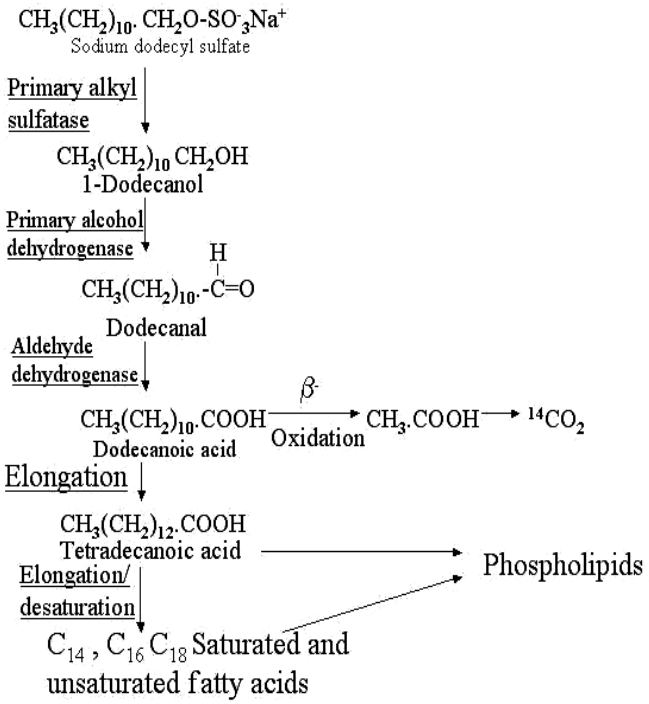
## INTRODUCTION

Due to widespread use of SDS in households and industry followed by its subsequent disposal in waterways, there is apprehension of alarming consequences on various living organisms (Chaturvedi and Kumar, 2010). Such a presumption has aroused interests of many workers to initiate works on biodegradation of SDS. Interestingly, now there are reports of several bacteria, which are able to degrade and metabolize SDS as a carbon source. The work on SDS biodegradation was first initiated by Payne and Feisal (1963). They did a detailed study on SDS biodegradation by *Pseudomonas* sp, including enzymes and kinetics of degradation.
Singh *et. al*. 1998, reported the biodegradation of SDS by *Bacillus cereus*. This is the first report of biodegradation of SDS, by a gram-positive bacterium. They reported that this bacterium apart from degrading SDS, could utilize other detergents as a carbon source. Biodegradation of SDS was also reported by consortia of *Acinetobacter calcoaceticus* and *pantoea agglomerans*. Notably biodegradation of SDS by facultative anaerobic bacteria is of rare occurrence (Abboud et. al., 2007). Shukor et. al. have isolated a strain belonging to *Klebsiella oxytoca* , which could degrade 1g/l SDS in 72 h incubation time. The rate of degradation of SDS by this isolate is very low as compared to our isolates. Hosseini, et al. has isolated two bacterial strains from activated sludge. These isolates were identified as *Pseudomonas betelli* and *Acinetobacter johnsoni* respectively. It was observed that these strains degraded 522 mg/L of SDS to the extent of 93.6% and 84.6% within 5 days respectively.

## Pathways of SDS biodegradation:

Thomas and White (1989) have done a detailed study on biodegradation of SDS by *Pseudomonas* sp. C12B. They employed $^{14}C$- radiotracer technique to study the fate of SDS during degradation by *Pseudomonas* sp. C12B. It was found that 70% of total radioactivity present in SDS evolved as $^{14}CO2$. This confirmed total biodegradation of SDS by this isolate.

Appearance of radiolabelled 1- dodecanol and 1-dodecanoic acid with consequent disappearance of SDS was also detected. This observation indicated the presence of primary alkyl sulfatase in the bacterium, which initiates the biodegradation of SDS and hence 1-dodecanol is formed. It is then oxidized to 1-dodecanoic acid by action of alcohol dehydrogenase. Finally it is metabolized by β- oxidation pathway. The pathway of SDS degradation is given below;




From the above pathway, it is evident that biodegradation is initiated by primary or secondary alkyl sulfatase enzymes, followed by the oxidation of the liberated primary or secondary alcohols by appropriate alcohol dehydrogenases. *Pseudomonas sp.* are capable of producing a multiplicity of alkyl sulfatases. For example *Pseudomonas* C12B can produce five such enzymes (Bateman et. al., 1986)), *Pseudomonas putida* FLA six (Lillis et. al., 1983)) and *Pseudomonas* DESI four (Matcham et. al., 1977) Of the five alkyl sulfatases produced by *Pseudomonas* C12B, two (designated P1 and P2) are active towards primary alkyl sulfates, whereas the other three (SI, S2 and S3) act on secondary alkyl sulfates (Cloves et. al., 1980). The latter enzyme exhibits positional and stereo-specificity, SI being active towards D-2- alkyl sulfates, S2 towards L-2-alkyl sulfates and S3 towards symmetrical and near symmetrical secondary alkyl sulfates. Collectively, they are able to initiate the degradation of commercial alkyl sulfate surfactant preparations, which generally contain vast range of positional and stereo isomers and alkyl chain lengths. All three enzymes operate by rupture of the C-O bond of the ester sulfate linkage. The P2 enzyme is inducible, appears in mid-exponential growth phase. In common with the secondary enzymes, it operates by fission of the C-O bond rather than the O-S bond. The P1 enzyme has been considered to be constitutive and appears during the early stationary phase of growth.

The librated alcohol is further oxidized to appropriate acids by alcohol dehydrogenases coded by the chromosomal *alc* or plasmid borne *alk* pathway (Thomas and White, 1986). These acids are finally used as substrates for β oxidation pathway.

**Alkyl sulfatases key enzyme involved in degradation:**

It is now known that alkyl sulfatases fall under three mechanistically distinct groups:
**Group I:** Aryl sulfatases, the best-studied group is predominantly from eukaryotic system. They are characterized by an active-site serine or cysteine post translationally modified to formylglycine that mediates the cleavage of the CO-S bond of sulfate esters, producing inorganic sulfate and the corresponding alcohol.
**Group II:** The Fe (II) α-ketoglutarate-dependent dioxygenase super family of enzymes constitutes a second group of sulfatases. These enzymes cleave sulfate esters into inorganic sulfate and the corresponding aldehyde and require α-ketoglutarate as a co substrate. The example is medium chain alkyl sulfatase *Ats*K of *P.putida*.
**Group III:** The third class of alkyl sulfatases contains the metallo-β-lactamase (MBL)-related enzymes. These enzymes cleave alkyl sulfate into corresponding alcohol and a water molecule is released. This group of enzymes has been reported in *P. aeruginosa*. The best example is *sds*A1 from *P. aeruginosa*.
Till date, detailed study on the structure and catalytic mechanism has been made on only two types of alkyl sulfatases, namely *sds*A1 (group III) of *P. aeruginosa* and *Ats*K (group II) from *P. putida*.

***i. sdsA1 from P. aeruginosa:***

Molecular studies on SDS biodegradation was first made by Davison *et al.* (1992). They cloned the gene of alkyl sulfatase from *Pseudomonas sp.* ATCC19151, the strain was able to use SDS as a carbon source. By NTG mutagenesis, *sds* mutants were developed, having defect in utilizing SDS as a carbon source. These mutants were complimented with clones from a *Pseudomonas* ATCC 19151 gene bank. A recombinant cosmid clone was identified by its ability to compliment the alkyl sulfatase negative mutants for growth on SDS as a carbon source, following conjugal transfer from *E. coli*. This clone was sequenced and matched with database. This sequence contained an open reading frame (ORF) which showed significant similarity to an N-terminal fragment of a possible protein from *Mycobacterium tuberculosis*. The function of this protein was unknown. They further cloned and expressed this fragment in a T7 promoter, but they didn't find any detectable enzyme activity. The conclusion was that since it is a periplasmic enzyme, there might be a possibility that it is not expressed in *E. coli* since in *E. coli* the signal sequence is not processed.
Hagelueken *et al.* (2006), found an ortholog of this enzyme in genome sequence of *P. aeruginosa* PA01, which had a 30% sequence identity with sdsA. They PCR amplified the gene, cloned and expressed this enzyme from *P. aeruginosa* PA01, using primers designed specifically for this enzyme. They purified the enzyme and did the structure elucidation by X-ray diffraction technique. From the observation it was concluded that it is the third class of alkyl sulfatase and a member of metallo-β-lactamase (MBL)-related enzymes. The N-terminal domain of SdsA1 and its homologues harbor a $Zn^{2+}$-binding motif (THxHxDHxGG-102-E-18-AE-44-H). Homologues of SdsA1 (sequence identities up to 37%) occur in numerous eubacteria. Eukaryotic homologues, presumably acquired from α-proteobacteria, are less frequent but include Bds1 (Yol164w) of *Saccharomyces cerevisiae* and EAL47917 of the human pathogen *Entamoeba histolytica* .
SdsA1 is a symmetric dimmer. The molecular weight of each monomer is 72 Kda. Each monomer consists of three domains: the N-terminal, catalytic, αββα-sandwich domain; an α-helical, dimerization domain, intricately interlocking the monomers and a mixed, C-terminal domain.

N-terminal domain, which was first described for metallo-β-lactamases, has been observed in other metalloenzymes. These enzymes are mostly hydrolases, inter alia cleaving β-lactams, phosphoesters, and N-acyl homoserine lactones but also include a rubredoxin oxidase. The metal-binding end (except for the $Zn^{2+}$- binding loops) is responsible for substrate recognition and, hence, unique to each enzyme. Correspondingly, the extended loops and secondary structure elements covering the active-site of SdsA1 are much shorter or absent in related structures. Despite sequence identities of only 10%, the C-terminal domain of SdsA1 is structurally similar to several eukaryotic sterol-binding domains such as the SCP-X domain of human peroxisomal multifunctional enzyme type 2 and sterol-carrier protein 2 from rabbit or yellow fever mosquito. In this enzyme Zn ions form the active site. In similar hydrolytic enzymes bearing a binuclear metal cluster, a hydroxyl ion bridging the metal ions or a $Zn^{2+}$-bound water is thought to generally function as the nucleophile. Such a mechanism is widely accepted for phosphate ester hydrolysis.

### *ii. AtsK from P. putida:*

A miniTn*5* mutagenesis experiment led to the identification of various mutants of *P. putida* S-313 that were no longer able to grow with aliphatic or aromatic sulfate esters as sulfur sources (Kahnert and Kertesz, 2000). Application of transposon rescue techniques revealed that some of these mutants carried transposon insertions in a gene cluster displaying a high level of sequence identity to the *ats* gene cluster of *P. aeruginosa*, which is required for the utilization of organic sulfate esters in that species. Alike *P. aeruginosa* homologue, the *P. putida ats* cluster contains the A*ts RBC* genes, which presumably encode an ABC-type transport system (*P. putida* AtsB was 40–50% identical to known bacterial permeases, and AtsC was 45–55% identical to ATP-binding proteins of ABC-transporters). In one of the *P. putida* mutants, strain PH3, the transposon was inserted 100 bp upstream of the translational stop codon of the *AtsR* gene, which encoded a putative periplasmic sulfate ester-binding protein (59% identical to the *P. aeruginosa* sulfate ester-binding protein *AtsR*). PH3 was not able to desulfurize *p*-nitrocatechol sulfate (a representative of the aromatic sulfate esters), nor did it grow with the aliphatic sulfate esters hexyl sulfate and SDS as the sulfur source. Growth with all other sulfur sources tested (including cysteine, methionine, and aliphatic or aromatic sulfonates) was not affected in strain PH3. The *Ats*R gene was introduced into strain PH3 on the medium-copy plasmid pME4562, where it was expressed from a *lac* promoter. PH3 (pME4562) was found to be able to grow with *p*-nitrocatechol sulfate but not with hexyl sulfate or SDS as sulfur sources, suggesting that the loss of alkyl sulfate utilization was not directly caused by the mutation in *Ats*R, but might be due to a polar effect of the transposon insertion on downstream genes. Indeed, 39 bp downstream of *Ats*R, another open reading frame (903 bp) was located, which was named *Ats*K. It was preceded by a good consensus ribosome binding site, and its predicted gene product showed similarity to members of the α-ketoglutarate-dependent dioxygenase superfamily. The most similar characterized protein to AtsK (38% protein identity) is the α-ketoglutarate-dependent taurine dioxygenase (TauD), which was first purified from *E. coli*. Taurine dioxygenase catalyzes the desulfonation of 2-aminoethanesulfonate (taurine) to aminoacetaldehyde and sulfite, which is then channeled into the sulfate assimilation pathway, enabling *E. coli* to grow with taurine as a sulfur source. *P. putida* S-313 is also able to utilize taurine as a sulfur source, but this ability was not affected in strain PH3. When PH3 (pME4562) was additionally provided with the *Ats*K gene on pME4596, growth with both hexyl sulfate and SDS was restored, although this was not the case for PH3 (pME4596), which still lacks a functional *Ats*R gene.

Hence, it was concluded that the *Ats*R protein was required for growth with all sulfate esters as sulfur sources and that the *Ats*K protein was specifically required for the utilization of aliphatic sulfate esters but not aromatic sulfate esters. It was further over expressed and characterized using gel filtration chromatography, the molecular weight of native AtsK was estimated to be 121 kDa. The calculated molecular mass of the *Ats*K gene product was 33.5 kDa, suggesting that AtsK is present as a tetramer. The biochemical properties of the oxygenative alkyl sulfatase AtsK and sequence analysis of the *Ats*K gene demonstrated that it belongs to the α-ketoglutarate-dependent dioxygenase superfamily of enzymes. These enzymes catalyze a variety of significant metabolic reactions including hydroxylations, desaturations, and epoxidations and require an α-keto acid cosubstrate. One oxygen atom from molecular oxygen is incorporated into the a-keto acid, which subsequently decomposes to give succinate and $CO_2$.

In conclusion, available data suggest that the use of SDS in various industry and household products is increasing at an alarming rate. The consequences arising from its overuse and subsequent disposal in waterways are of serious concern especially for health of humans. However certain bacteria capable of degrading this detergent have been reported. Pathways and enzymes involved in SDS degradation have been deciphered but there is a need of detailed study. These enzymes might be useful in developing appropriate remediation technology for SDS present in any habitat.

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