Introduction
1. General introduction

There are over 70,000 synthetic organic chemicals, of which easily biodegradable organic chemicals are generally considered to be non-toxic, while non-biodegradable organic chemicals are considered as recalcitrant, persist in the environment, spread throughout the ecosystems and cause pollution problems. Among the first synthetic organic chemicals to create environmental problems were synthetic detergents (organosulfonates), developed in Germany during World War II because of lack of fats, the normal material from which soap is made.

Organosulfonates are the salts of strong acids, having widespread natural and xenobiotic origin. They have a common trait of a charged sulfonate group, which is regarded as Nature’s way to prevent a compound from crossing biological membrane. Naturally occurring sulfonates constitutes O-sulfonates, N-sulfonates and aliphatic C-sulfonates. These are few in number and have important biological functions (Table 1) where as anthropogenic sulfonates include aromatic C-sulfonates (Table 2).

1.2 Naturally occurring organosulfonates

1.2.1 O-Sulfonates: Compounds containing sulfonate moiety attached to the O atom (O-S bond) are termed as O-sulfonates, include tyrosine sulfate in eukaryotic proteins, cerebroside sulfate in human nervous tissue and estrone sulfate in humans (Dodgson et al., 1982)

1.2.2 N-Sulfonates: Compounds containing sulfonate moiety attached to the N atom (N-S bond) are termed as N-sulfonates, for example heparin (glycosylaminoglycan of anticoagulant) (Gatti et al., 1979).

1.2.3 Aliphatic C-sulfonates: Compounds containing sulfonate moiety attached to the aliphatic carbon atom (C-S bond) are termed as C-sulfonates, include taurocholate in mammalian gut, capnine a bacterial sulfolipid and few were listed in table 1.

1.2.4 Naturally occurring aromatic sulfonates

Aeruginosin B, a phenazine derivative synthesized by Pseudomonas aeruginosa (Herbert and Holliman, 1964), dihydroxyverdin-7-sulfonicacid is a bacterial metabolite (Budzikiewicz et al., 1998), bromoindole sulfonicacid of Echinodictyum an Australian marine sponge (Ovendenn and Capon, 1999) and petrobactin sulfonate present in the siderophore of Marinobacter hydrocarbonoclasticus (Hickford, 2004) are the naturally occurring aromatic sulfonates reported.
**Table 1: Naturally occurring organosulfonates**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Occurrence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurine</td>
<td><img src="image" alt="Structure" /></td>
<td>Insects, algae, arthropods and mammals</td>
<td>Huxtable 1992</td>
</tr>
<tr>
<td>Coenzyme M</td>
<td><img src="image" alt="Structure" /></td>
<td>Methanogenic archaea</td>
<td>White 1986</td>
</tr>
<tr>
<td>Cysteate</td>
<td><img src="image" alt="Structure" /></td>
<td>Weathering product in wool, spiders web</td>
<td>Cook <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Methanesulfonate</td>
<td><img src="image" alt="Structure" /></td>
<td>Atmospheric photo oxidation product of dimethylsulfide, marine algae</td>
<td>Baker 1991</td>
</tr>
<tr>
<td>Isethionate</td>
<td><img src="image" alt="Structure" /></td>
<td>Major anion in the squid nerve, red algae</td>
<td>Koechlin 1954</td>
</tr>
<tr>
<td>Sulfoacetate</td>
<td><img src="image" alt="Structure" /></td>
<td>Major spore component</td>
<td>Martelli and Benson 1964</td>
</tr>
<tr>
<td>Sulfolactate</td>
<td><img src="image" alt="Structure" /></td>
<td>Bacterial spores</td>
<td>Benson <em>et al.</em>, 1969</td>
</tr>
<tr>
<td>Sulfoquinovose</td>
<td><img src="image" alt="Structure" /></td>
<td>Plant sulfolipid</td>
<td>Benning 1998</td>
</tr>
<tr>
<td>Aeuginosin B</td>
<td><img src="image" alt="Structure" /></td>
<td>Bacteria <em>(Pseudomonas aeruginosa)</em></td>
<td>Herbert &amp; Hollaman 1964</td>
</tr>
<tr>
<td>Heparin</td>
<td><img src="image" alt="Structure" /></td>
<td>A representative of glycosaminoglycan</td>
<td>Kertesz 2000</td>
</tr>
<tr>
<td>Tyrosine sulfate</td>
<td><img src="image" alt="Structure" /></td>
<td>Eukaryotic proteins</td>
<td>Dodgson <em>et al.</em>, 1982</td>
</tr>
<tr>
<td>Cerebroside sulfate</td>
<td><img src="image" alt="Structure" /></td>
<td>Human nerve tissue</td>
<td>Kertesz 2000</td>
</tr>
<tr>
<td>Estrone sulfate</td>
<td><img src="image" alt="Structure" /></td>
<td>Humans</td>
<td>Kertesz 2000</td>
</tr>
</tbody>
</table>
1.3 **Anthropogenic sulfonates** include arylsulfonates or sulfonated aromatic compounds, in which a sulfonate functional group is covalently attached to the benzene ring by a covalent C-S bond. Aromatic sulfonates are highly acidic and strongly hydrophilic in nature. These are widely used as precursors for synthesis of dyes (e.g. orange II), optical brighteners, pharmaceuticals (e.g. sulfanilamide) (Hooper *et al.*, 1991), softening agents (e.g. phenolsulfonates), preservatives (e.g. sulfanilic acid) and super plasticizers i.e. water reducing admixtures in concrete (e.g. lignosulfonates), synthetic detergents (e.g. linear alkylbenzenesulfonates, LAS) (Schwitzguebel *et al.*, 2002). Environmental relevance of the anthropogenic sulfonates was listed in table 2. Anthropogenic sulfonates can be divided into two main groups; the first group comprises the linear alkylbenzenesulfonates. Their fate in the environment was studied while the second group comprises the aromatic sulfonates.

**1.3.1 Recalcitrance of anthropogenic (xenobiotic) sulfonates**

The sulfonate group attached to the benzene ring plays an important role in increasing the solubility and dispersion properties of the xenobiotic molecule thereby increases its recalcitrance, because of the thermodynamically stable carbon-sulfur bond. Poor biodegradability of arylsulfonates is primarily due to the quaternary carbon that attaches the aromatic structure to the alkyl part of the ABS molecule. Secondarily due to the sulfonate group and the high degree of branching in the alkyl side chain.
The chemical cleavage of C-S bonds requires high temperatures of 300-320 °C (Dudley and Frost, 1994). Owing to their excellent water solubility and hydrophilic properties, it is difficult to remove them completely from water in the wastewater treatment works. The problem cannot be solved by physical processes like adsorption because no chemical degradation occurs during these processes (Stoffler and Luft, 1999). As a result, they are discharged and accumulated into the aquatic (Altenbach and Giger, 1995) and terrestrial environments even after treatment (Ruff et al., 1999).

1.3.2 Environmental accumulation of anthropogenic (xenobiotic) sulfonates and its effects

Organosulfonates are produced in large amounts as surfactants (2.5 x 10^6 metric tons of linear alkylbenzenesulfonates per annum [Schulze 1996]), dyestuffs (3 x 10^5 metric tons per annum [Anliker, 1977]), dyestuff precursors and additives in oils and inks (Elvers et al., 1994). The major compound is 4-toluene sulfonate (about 2.7 x 10^4 metric tons per annum in Europe (Behret, 1991) used in household detergent formulations, preparation of foundry molds and syntheses of pharmaceuticals. These compounds were detected in forest soils and marine sediments (Lie et al., 1996), rivers (Zerbinati et al., 1997), waste dump leachates (Riediker et al., 2000) and sewage sludge amended soils. Dudley et al (1994) reported that synthetic detergents pose approximately 10 % of the pollution load in the Rhine River and Italian river Bromida was polluted by sulfonated azo dyes (Zerbinati et al., 1997). Vairavamurthy et al (1994) have reported the accumulation of organosulfonates in marine sediments to a level of 20–40 % of the total organic sulfur, while sulfonate sulfur exceeded 40 % of total S in the O1 horizon of forest soils (Autry and Fitzgerald 1990). It was found that 4-toluene sulfonate was accumulated in Swiss landfill leachates; benzenesulfonate and naphthalenesulfonates were also found in groundwater samples contaminated by percolating leachates (Reidiker et al., 2000).

Anthropogenic sulfonates are nontoxic to higher organisms but toxic to bacteria, algae invertebrates and fish even at a concentration of 1mg/l (Lewis 1993), hence they bioaccumulate and concomitantly cause adverse environmental problems which include destruction of the external mucus layer of fish protecting from bacteria and pathogens, severe damage to the gills, lowering of the surface tension of the water and decrease in the...
breeding ability of aquatic organisms. These are of particular environmental concern in purification of drinking water and the development of biological methods to remove them completely during water treatment would be desirable because microbial metabolism provides a safer, more efficient, and less expensive alternative to physico-chemical methods for pollution abatement. Microbial degradation of the arylsulfonates is superior to physical and chemical treatment owing to their more expensive way of physical and chemical treatment; hence there is a need for a better understanding of mechanisms of arylsulfonates biodegradation. Cationic pharmaceuticals were formulated with both aromatic and aliphatic sulfonates (Neil 2001), used as a major aid in assisting patients to abstain from alcohol is dosed at 2 g day^{-1} (Cook et al., 2006). Candidate drugs (sulfonates) for stroke and Alzheimer’s disease are also dosed at high levels (Cook et al., 2006).
### Catabolism of 4-toluenesulfonate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Environmental relevance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAS (linear alkyl benzenesulfonate)</td>
<td><img src="image1" alt="Structure" /></td>
<td>Surfactant</td>
<td>Schwitzguebel et al., 2002</td>
</tr>
<tr>
<td>Benzenesulfonate</td>
<td><img src="image2" alt="Structure" /></td>
<td>Optical brightener</td>
<td>Cook et al., 1999</td>
</tr>
<tr>
<td>4-Toluenesulfonate</td>
<td><img src="image3" alt="Structure" /></td>
<td>Hydrotropic agent</td>
<td>Cook et al., 1999</td>
</tr>
<tr>
<td>Sulfophenylbutyrate</td>
<td><img src="image4" alt="Structure" /></td>
<td>Intermediate in LAS biodegradation</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>Sulfophenyloctane</td>
<td><img src="image5" alt="Structure" /></td>
<td>LAS detergent</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>DATS</td>
<td><img src="image6" alt="Structure" /></td>
<td>By product in LAS synthesis</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>Orange II</td>
<td><img src="image7" alt="Structure" /></td>
<td>Dyestuff</td>
<td>Hooper et al., 1991</td>
</tr>
<tr>
<td>Tiron</td>
<td><img src="image8" alt="Structure" /></td>
<td>Chelating agent</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>Acid red I</td>
<td><img src="image9" alt="Structure" /></td>
<td>Dyestuff</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>4,4’-Bis(2-sulfo-styryl) biphenyl</td>
<td><img src="image10" alt="Structure" /></td>
<td>Optical brightener</td>
<td>Kertesz et al., 1994</td>
</tr>
<tr>
<td>Naphthalene sulfonate</td>
<td><img src="image11" alt="Structure" /></td>
<td>Concrete admixtures Contd. in page 7</td>
<td>Cook et al., 1999</td>
</tr>
</tbody>
</table>
### Table 2: Environmental relevance of anthropogenic or synthetic (xenobiotic) sulfonates.

<table>
<thead>
<tr>
<th>Sulfanilate</th>
<th>Pesticides and azodyes</th>
<th>Schwitzguebel <em>et al.</em>, 2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfanilamide</td>
<td>Pharmaceuticals</td>
<td>Hooper <em>et al.</em>, 1991</td>
</tr>
<tr>
<td>Aminonaphthalene sulfonate</td>
<td>Dye precursor</td>
<td>Alonso and Barcelo 2000</td>
</tr>
<tr>
<td>5-Sulfosalicylate</td>
<td>Integral color anodiser</td>
<td>Cook <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Saccharine</td>
<td>Food stuffs, sweetener</td>
<td>Cook <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Tartrazine</td>
<td>Dyestuffs</td>
<td>Cook <em>et al.</em>, 1999</td>
</tr>
<tr>
<td>Alachlor</td>
<td>Herbicides</td>
<td>Cook <em>et al.</em>, 1999</td>
</tr>
</tbody>
</table>
1.4. Biodegradation of anthropogenic sulfonates

Early studies of arylsulfonates degradation indicated that these compounds were recalcitrant to normal degradation and were reported as non biodegradable (Bretcher 1981), or poorly biodegradable (Schwitzguebel et al., 2002). LAS compounds were not mineralized in anaerobic sediment (Federle and Schwab, 1992), likewise there was no anaerobic mineralization of various amino aromatic sulfonates (Tan et al., 2005). There is little evidence for degradation of aromatic sulfonates under anaerobic conditions where desulfonation of arylsulfonates was reported (Shcherbakova et al., 2003).

Bacterial metabolism of anthropogenic (xenobiotic) sulfonates

1.4.1 Benzenesulfonate (Phenylsulfonate)

Phenylsulfonate used as optical brightener and in the preparation of pharmaceutical drugs known as besylates or besilates. Degradation of benzenesulfonate was studied in Pseudomonas aeruginosa A, where degradation was channeled through catechol 2, 3-dioxygenase meta pathway with 2-hydroxymuconic semialdehyde, acetaldehyde and pyruvate as products (Fig 2) (Cain and Farr 1968). Ripin et al (1971) proposed a degradative pathway (ortho pathway) of benzenesulfonate in Comamonas testosteroni H-8 to catechol, 2-hydroxy muconate semialdehyde, formate, 4-hydroxy -2- oxovalerate, acetaldehyde and pyruvate. In Alcaligenes sp. CAT498 and Comamonas testosteroni H-8 sulfate accumulation was reported in benzenesulfonate degradation (Johnston 1975). Utilization of benzenesulfonate as a carbon source was reported in a bacterium isolated from activated sludge from Sosei river sewage purification plant at Sapporo (Endo et al., 1977).

Benzenesulfonate, 4-hydroxybenzenesulfonate, 3-nitrobenzenesulfonate were desulfonated to corresponding phenols by Pseudomonas sp. S-313 (Zurrer et al., 1987) (Fig 2) and Klebsiella oxytoca KS3D (Dudley and Frost 1994) (Fig 2). Alcaligenes sp. O-1 was reported to degrade benzenesulfonate to catechol by benzenesulfonate dioxygenase system or benzenesulfonate: NADH: oxygen oxidoreductase (Thurnheer et al., 1990) and also by 2-aminobenzenesulfonate dioxygenase system / orthanilate dioxygenase system (Cook et al., 1998) (Fig 2).

Mixed bacterial culture from the River Elbe, Hamburg, degraded benzene 1, 3-disulfonate to catechol 4-sulfonate. Catechol 4-sulfonate was further metabolized to 3-sulfomuconate and 4-carboxymethyl-4-sulfobut-2-en-4-ol (Contzen et al., 1996).
Rhodococcus opacus ISO-5 utilized benzenesulfonate as a sulfur source (David et al., 2003). Clostridium pasteurianum DSM 12136 utilized benzenesulfonate, benzene 1, 3-disulfonate as sole source of sulfur (Chih-Ching Chien 2005).

Fig 2: Degradative pathways of benzenesulfonate
(I) Alcaligenes sp. O-1 (Thurnheer et al., 1990)
(II) Pseudomonas aeruginosa A (meta pathway) (Cain and Farr, 1968),
(III) Comamonas testosterone H-8 (ortho pathway) (Ripin et al., 1971)
(IV) Pseudomonas sp. S-313 (Zurre et al., 1987).
1.4.2 4-Toluenesulfonic acid: (Tosic acid, 4-methylbenzenesulfonicacid, p-toluenesulfonicacid, Tolysulfonate)

4-Toluenesulfonate has a methyl group para to the sulfonate group. It is a white crystalline, hygroscopic solid used in manufacture of dyes, hydrotropes, used as a catalyst in textile industries, stabilizer for pharmaceuticals intermediates, cleaning agents and plating additive. These compounds enter the environment through the domestic wastes and wastes generated from textile, dye, pharmaceutical industries etc. In humans, ingestion may cause oesophageal burns, dermal contact produce severe burns, inhalation may result in dyspnea, pleuritic chest pain, pulmonary edema, hypoxemia, bronchospasm, pneumonitis, tracheobronchitis and persistent pulmonary dysfunctions, eye contact may cause corneal erosions (http://www.chemadvisor.com)

1.4.2.1 Abiotic degradation

Direct photolysis of 4-toluenesulfonate is not known, since the compound does not absorb light beyond 290 nm. Advanced oxidation processes (AOP) are rapid and non-selective methods for the quantitative chemical breakdown of compounds at high temperature and pressure. The degradation mechanism of 4-toluenesulfonate through AOP is a complex process, involve the generation of highly reactive hydroxyl radicals generated by thermal activation of hydrogen peroxide, where desulfonation occurs at a high temperature and pressure of 180 °C and 2 M Pa respectively (Stoffler and Luft, 1999).

1.4.2.2 Biotic degradation

1.4.2.2.1 Bacterial metabolism of 4-toluenesulfonate

(i) Aerobic biodegradation of 4-toluenesulfonate

Aerobic degradation of 4-toluenesulfonate was studied in various bacteria, especially those belonging to the genus Pseudomonas. Different pathways have been proposed for different strains of Pseudomonas, Pseudomonas aeruginosa A, Comamonas testosteroni H-8, Pseudomonas putida S-313 (Fig 3). Overall five pathways were proposed for aerobic degradation of 4-toluenesulfonate in various chemotrophic bacteria. These pathways
Involve an initial reaction comprising the oxidation of 4-toluenesulfonate catalyzed by oxygenases.

In Pseudomonas aeruginosa A 4-toluenesulfonate was degraded by catechol 2, 3-dioxygenase pathway with intermediates 4-methylcatechol and 2-hydroxy, 5-methylmuconic semialdehyde followed by ring cleavage resulted in formate, propionaldehyde and pyruvate (Cain et al., 1968) (Fig. 3). Pseudomonas sp. was reported to degrade 4-toluenesulfonate involving 2, 3 dihydro 4-sulfonate as an intermediate to 3-methyl catechol (Focht and Williams 1970). In Comamonas testosteroni H-8 reported that the overall oxidation system for 4-toluenesulfonate is inducible (Ripin et al., 1971).

Comamonas testosteroni T-2 completely degraded 4- toluenesulfonate to cell material, CO₂ and sulfate with a series of intermediates 4-sulfobenzylalcohol, 4-sulfobenzaldehyde, 4-sulfobenzoate, protocatechuate followed by meta ring cleavage products pyruvate and succinate (Locher et al., 1989) (Fig 3). In Comamonas testosteroni T-2 a novel outer membrane anion channel (porin) for 4-toluenesulfonate was identified (Mample et al., 2004). The enzymes involved in 4-toluenesulfonate degradation such as 4-toluenesulfonate methyl monoxygenase (EC 1.12.4) and 4-sulfobenzoate 3,4 dioxygenase (EC 1.14.12.8) were purified and characterized (Locher et al., 1991a and Locher et al., 1991b) in Comamonas testosteroni T-2. These two enzymes encoded catabolic IncP1b plasmid pTSA was also mapped by subtractive analysis (Tralau et al., 2001). Achromobacter xylosoxidans TA12-A and Ensifer adhaerens TA12-B were identified as the 4-toluenesulfonate degraders showed the same pathway and contain tsa genes from the Tntsa cluster as described previously in Comamonas testosteroni T-2 (Tralau et al., 2011).

Alcaligenes sp. O-1 was reported to desulfonate 4-toluenesulfonate by toluenesulfonate dioxygenase or 4-toluenesulfonate: NADH: oxygen oxidoreductase to 4-methylcatechol (Thurnheer et al., 1990; Junker et al., 1994) (Fig 3). However Pseudomonas putida S-313 (DSM 6884), Klebsiella oxytoca KS3D were also reported to desulfonate 4-toluenesulfonate to p-cresol when used as a sulfur source (Kertesz et al., 1994; Dudly and Frost, 1994). Rhodococcus opacus ISO-5 utilized 4-toluenesulfonate as a sulfur source for growth (David et al., 2003), however products were not identified.
(ii) Aerobic biodegradation of toluene (methyl benzene)

Similarly the structural and substrate analogue of 4-toluene sulfonate such as toluene degradation under aerobic conditions was studied in various bacteria, especially those belonging to the genus *Pseudomonas*. Five different pathways were proposed for different strains of *Pseudomonas*, *P. putida* mt-2 (Worsy *et al.*, 1975), *P. mendocina* KR (Whited *et al.*, 1991), *P. cepacia* G4 (Mars *et al.*, 1996), *P. picketti* PK01 (Ronald *et al.*, 1994) and *P. putida* F1 (Zylstra *et al.*, 1988). These pathways involve an initial reaction comprising the oxidation of toluene catalyzed by oxygenases. The aerobic toluene degradation pathway in *P. putida* mt-2 involves oxidation of the methyl group of toluene to produce benzoic acid. Subsequently, cis-benzoate dihydrodiol dehydrogenated to catechol. Aerobic toluene degradation in *P. mendocina* KR, *P. cepacia* G4 and *P. picketti* PK01, involves oxidation of toluene to produce cresol isomers (*p*-cresol, *o*-cresol and *m*-cresol, respectively) as intermediate products. *p*-Cresol is further converted into protocatechuate, while *o*-cresol and *m*-cresol are transformed into 3-methylcatechol. *P. putida* F1 can metabolize toluene by producing cis-toluene dihydrodiol, which was converted to 3-methylcatechol. Catechol, protocatechuate and 3-methylcatechol were undergo aromatic ring cleavage by either ortho-cleavage or meta-cleavage pathways, to produce non-aromatic intermediates (like acetate, pyruvate and succinate) which are metabolized by the Tricarboxylic Acid Cycle (TCA cycle).

(iii) Anaerobic biodegradation of 4-toluene sulfonate

Anaerobic degradation of 4-toluene sulfonate was reported in few of the chemotrophic bacteria belongs to the genus *Clostridium*, *Methanosarcina*, *Desulfovibrio* and *Cupriavidus*.

Anaerobic desulfonation of 4-toluene sulfonate was reported in *Clostridium* sp. EV4, where metabolic intermediates were not identified (Denger *et al.*, 1996). *Methanosarcina mazei* MM demethylate 4-toluene sulfonate to benzenesulfonate, forming methane under anaerobic conditions (Shcherbakova *et al.*, 2003). Pure cultures of three strains of *Clostridium* sp. 14 (VKM B-2201), 42 (VKM B-2202), 21 (VKM B-2279), two methanogens, *Methanobacterium formicicum* MH (VKK B-2198) and *Methanosarcina mazei* MM (VKK B-2199) and one sulfate-reducing bacterium, *Desulfovibrio* sp. SR1
(VKM B-2200) were isolated from an anaerobic microbial community reported to degrade 4-toluenesulfonate. 4-Toluenesulfonate was desulfonated to toluene by Clostridium sp. VKM B-2202 (Fig 3). The sulfate-reducing strain Desulfovibrio sp. SR1 utilized 4-toluenesulfonate as an electron acceptor (Shcherbakova et al., 2003). 4-Toluenesulfonate was used as sole source of sulfur by Clostridium pasteurianum DSM 12136 (Chih-Ching Chien 2005), Cupriavidus metallidurans and Variovorax paradoxus T (Schmalenberger 2007).

(iv) Conjugative metabolism of toluene (methylbenzene)

Anaerobic toluene degradation has been reported in a wide range of bacteria including methanogenic bacteria, iron reducing bacteria, sulfate reducing bacteria and phototrophic bacteria (Table 3). Benzylsuccinate synthase is an oxygen sensitive, glycyl-radical enzyme that catalyzes the first step of anaerobic toluene degradation by adding fumarate or succinate to the methyl group of toluene (Beller and Spormann. 1997; Biegert et al., 1996; Krieger et al., 2001; Leuthner and Heider 2000; Leuthner et al., 1998). The enzyme has been purified from Thauera aromatica K172 and Azoarcus sp. strain T (Krieger et al., 2001; Leuthner et al., 1998). Electronic paramagnetic spectroscopy (EPR) has indicated the presence of an oxygen-sensitive, stable organic radical in benzylsuccinate synthase of Azoarcus sp. strain T (Krieger et al., 2001). Benzylsuccinate synthase is comprised of three subunits (αβγ) which are responsible for its heterohexameric structure (Krieger et al., 2001; Leuthner et al., 1998).

Benzylsuccinate synthase shows a significant homology with other well characterized enzymes viz. pyruvate formate lyase (PFL) and anaerobic ribonucleotide reductase (ARR) from E.coli (Krieger et al., 2001; Leuthner et al., 1998). PFL is a key enzyme that is involved in anaerobic glucose metabolism (Knappe et al., 1984). ARR is required for bacterial DNA replication under anaerobic conditions (Sun et al., 1993).

Biochemical analysis of the purified enzyme benzylsuccinate synthase comprises three subunits; alpha, beta, and gamma, in Thauera aromatica T1 are encoded by the tutD, tutG, and tutF genes, respectively. The large alpha-subunit contains the essential glycine and cysteine residues that are conserved in all glycyl radical enzymes. However, the
function of the small beta- and gamma-subunits has remained unclear (Li et al., 2009). FeS clusters in the glycol-radical enzyme benzylsuccinate synthase were identified through EPR and Mossbauer spectroscopy (Hilberg et al., 2012).

Further, benzylsuccinate synthase is also involved in the degradation of \( m \)-xylene and cresol isomers in *Azoarcus* sp. T and cresol isomers but not xylene in *T. aromatica* K172 (Verfurth et al., 2004). Analysis of a *bssA* defective mutant in *Azoarcus* sp. T indicated that *bssA* is essential for metabolism of both toluene and \( m \)-xylene (Achong et al., 2001).

<table>
<thead>
<tr>
<th>Nutritional mode</th>
<th>Organism</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfate-reducing</td>
<td>Strain PRTOL1, <em>Desulfobacula toluolica</em>, <em>Desulfobacterium cetonicum</em>, <em>Desulfosarcina cetonica</em>, <em>Desulfotomaculum</em> sp.</td>
<td>Beller et al., 1992 Rabus et al., 1993 Muller et al., 1999 Winderl et al., 2007 Winderl et al., 2007</td>
</tr>
<tr>
<td>Iron reducing</td>
<td><em>Geobacter metallireducens</em>, <em>Geobacter grbiciae</em>,</td>
<td>Lovely and Lonergan, 1990 Lovely et al., 1993 Winderl et al., 2007</td>
</tr>
<tr>
<td>Phototrophic</td>
<td><em>Blastochloris sulfoviridis</em> strain ToP1</td>
<td>Zengler et al., 1999</td>
</tr>
</tbody>
</table>

**Table 3: Bacteria involved in anaerobic degradation of toluene**
Introduction

Fig 3: Degradative pathways of 4-toluenesulfonate in

I. *Alcaligenes* sp. O-1 (Junker et al., 1994),
II. *Pseudomonas aeruginosa* A (Cain and Farr 1968),
   (A) 2-Amionbenzencesulfonate dioxygenase (2ASDOS) or Benzenesulfonate dioxygenase (BSDOS)
   (B) Catechol 2, 3 dioxygenase
   (C) 2-Hydroxymuconate semialdehyde hydrolase
   (D) 2-oxopent-4-enolate hydratase
   (E) 4-hydroxy-2-oxovalerate aldolase
III. *Comamonas testosteroni* T-2 (Locher et al., 1989),
    (a) 4-toluensulphonate methyl-monoxygenase system (TSMOS)
    (b) 4-Sulphobenzyalcohol dehydrogenase, (SOLDH)
    (c) 4-sulphobenzaldehyde dehydrogenase (SYDDH)
    (d) 4-sulphobenzoate 3,4-dioxygenase system (PSBDOS)
IV. *Klebsiella oxytoca* KS3D (Dudly and Frost 1994),
V. *Pseudomonas* sp. (Focht and Williams, 1970),
VI. *Methanosarcina mazei* MM (Shecherbakova et al., 2003) and
VII. *Clostridium sp.* EV4 (Denger et al., 1996)
1.4.3 Aminobenzenesulfonates (ABS) are widely used as precursors in the manufacture of dyes and optical brighteners

1.4.3.1 2-Aminobenzenesulfonate: orthanilic acid (2ABS)

2-Aminobenzenesulfonate was reported in Pseudomonas, Alcaligens and bacterial consortium having Acinetobacter and Flavobacterium. Pseudomonas sp. O-1 completely mineralized 2-aminobenzenesulfonate (as carbon source) to cell material and CO₂ (Thurnheer et al., 1986). Alcaligens sp. O-1 degrading 2-aminobenzenesulfonate to sulfite, ammonia and 3-sulfocatechol (Thurnheer et al., 1990) (Fig 4I). 2-Aminobenzenesulfonate dioxygenase system/ orthanilate dioxygenase system catalyzed the first reaction and 3-sulphocatechol 2,3-dioxygenase (EC 1:13:11:2) catalyzed the second reaction. 3-Sulphocatechol 2,3-dioxygenase (EC 1:13:11:2) was purified (Junker et al., 1994) and Mampel et al (1999) purified the oxygenase component of the 2-aminobenzenesulfonate dioxygenase system from Alcaligenes sp. O-1. Rhodococcus opacus ISO-5 utilized 2-aminobenzenesulfonate as a sulfur source for growth (David et al., 2003).

A bacterial consortium degrading 2-aminobenzenesulfonate was reported by Singh et al (2008). Acinetobacter and Flavobacterium in a bacterial consortium utilized 2-aminobenzenesulfonate as the sole carbon and energy sources under aerobic conditions, (Awasti et al., 2009).

1.4.3.2 3-Aminobenzensulfonate (3-ABS): Metanilic acid is commonly used as a mild oxidant

Pure cultures of putative pseudomonads and two unidentified rods strains M-1 were completely degraded 3-aminobenzenesulfonate and 3-nitrobenzenesulfonate to biomass, SO₄²⁻, NH₄⁺ and CO₂ (Locher et al., 1989, Thurnheer et al., 1988). Pseudomonas sp. S-313 utilized 3-aminobenzenesulfonate as the source of sulfur yielding the product 3-aminophenol (Zurrer et al., 1989) (Fig 4II). Similarly Rhodococcus opacus ISO-5 utilized 3-aminobenzenesulfonate as a sulfur source for growth (David et al., 2003), however products were not identified.
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Pseudomonas aeruginosa CLRI, BL22 were able to degrade 3-aminobenzensulfonate to aniline and β-keto adipic acid with dioxygenase (Valli Nachiyar et al., 2007). 3-Nitrobenzenesulfonate (used in reduction as resistant agent for printing thickeners in textile dyeing factories and as a precursor of synthetic dyes in dye producing factories) mineralization and desulfonation was reported in Alcaligenes sp. GA-l (Takeo et al., 1997). Rhodococcus opacus ISO-5 utilized 2-, 3- and 4-nitrobenzenesulfonate as a sulfur source for growth (David et al., 2003).

1.4.3.3 4-Aminobenzensulfonate (4-ABS) or Sulfanilic acid

4-ABS is an intermediate in the production of pharmaceuticals, pesticides and is a component of many azo dyes. A co culture of Hydrogenophaga palleronii S1 and Agrobacterium radiobacter S2 degraded 4-aminobenzensulfonate where strain S1 deaminated sulfanilate to catechol-4-sulfonate, which served as growth substrate for strain S2 (Burkhard et al., 1988; Burkhard et al., 1993 and Dange man et al., 1996) (Fig 4III). 4-Aminobenzensulfonate degradation was reported in Hydrogenophaga intermedia S1T (= DSM 5680) (Contzen et al., 2000), Pseudomonas paucimobilis ([sulfanilate as sole source of carbon and nitrogen to biomass] (Pieri et al., 2001)), Rhodococcus opacus ISO-5 [(as a sulfur source for growth] (David et al., 2003)) and Clostridium pasteurianum DSM 12136 [(as sole source of sulfur] (Chih-Ching Chien 2005)). Agrobacterium sp. PNS-1 was reported to completely mineralize sulfanilate with stoichiometric release of sulfite and ammonia (Singh et al., 2004; 2006 a & b). Pannonibacter sp. W1 degraded 4-aminobenzensulfonate as sole carbon as well as energy, nitrogen and sulfur source to 4-sulfocatechol and followed by ring cleavage (QingWanga et al., 2009).
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Fig 4: Degradative pathways of isomers of aminobenzenesulfonate

(I) 2-Aminobenzenesulfonate by Alcaligenes sp. O-1 (Thrunheer et al., 1990)
   a. 2-aminobenzenesulfonate dioxygenase system / orthanilate dioxygenase system
   b. 3-sulfocatechol 2,3-dioxygenase

(II) 3-Aminobenzenesulfonic acid by Pseudomonas sp. 313 (Zurrer et al., 1989)

(III) 4-Aminobenzenesulfonate by a mixed culture of Hydrogenophaga palleronii S1 and Agrobacterium radiobacter S2 (Feigel and Knackmuss 1993).
1.4.4 Substituted naphthalene sulfonates

Isomers of sulfonated naphthalene are used as an intermediate in industrial production of a wide range of chemicals, including dispersants, detergents, azo dyes, and wetting agents. Naphthalene-1-sulfonate and naphthalene-2-sulfonate were desulfonated by dioxygenation to gentisate by *Pseudomonas* sp. A3 and *Pseudomonas* sp. C22 (Brilon *et al.*, 1981). *Sphingomonas xenophaga* BN6 was mineralized naphthalene-2-sulfonate (2NS) to salicylate (Fig 4), 5-hydroxynaphthalene- 2-sulfonate to 6-hydroxysalicylate, 6-hydroxynaphthalene- 2-sulfonate to 5-hydroxysalicylate (gentisate) and 7-amino- and 7-hydroxynaphthalene-2-sulfonate to the corresponding 3-substituted salicylate (Nortemann *et al.*, 1986). 1,2-Dihydroxynaphthalene dioxygenase (DHND) was purified, the amino terminal amino acid sequence was determined and characterized in *Sphingomonas xenophaga* BN6 (Soltz 1999). The genes encoding the 1, 2-dihydroxynaphthalene dioxygenase, 2-hydroxycromene-2-carboxylate isomerase and 2- hydroxybenzalpyruvate aldolase of the naphthalenesulfonate pathway were identified on the chromosomal DNA of *Sphingomonas xenophaga* BN6 (Keck 2006). *Pseudomonas* sp. S-313 degraded 1-naphthalenesulfonic acid, 2-naphthalenesulfonic acid, 5-amino-1-naphthalenesulfonic acid to l-naphthol, 2-naphthol and 5-amino-l-naphthol respectively (Zurrer *et al.*, 1987).

Disulfonated naphthalenes are more resistant to biodegradation. However, *Pigmentiphaga daeguensis* ASL4 (formerly *Moraxella*) completely metabolized naphthalene-1,6-disulfoante and naphthalene-2,6-disulfonate (Wittich *et al.*, 1988). *Pigmentiphaga* sp. NDS-2 metabolized naphthalene-2,6-disulfonate to gentisate via 5-sulfosalicylic acid, gentisate, maleylpyruvate, fumarate and pyruvate as the products (Fig 5) (Uchihashi *et al.*, 2003). *Rhodococcus opacus* ISO-5 utilized naphthalene-2-sulfonate as a sulfur source for growth (David *et al.*, 2003). *Pseudomonas* sp., *Arthrobacter* sp. and an unidentified bacterium were desulfonated sixteen different sulfonated aromatic compounds, none of which served as a carbon source (Song 2005).
Fig 5: Degradative pathway of 2-naphthalenesulfonate and 2, 6-naphthalenedisulfonate by *Pseudomonas* sp. BN6 (I) (Nortermann *et al*., 1986) and *Pigmentophaga* sp. NDS-2 (II) (Uchihashi *et al*., 2003) respectively.
1.5 Algal metabolism of anthropogenic sulfonates

*Chlorella fusca* utilized linear sulfonic acids and arylsulfonates as sources of sulfur such as 1-naphthalene sulfonate and 2-naphthalene sulfonate (Biedlingsmeier and Schmidt 1983) and 1-naphthol was identified as a metabolite of 1- naphthalene sulfonate (Soeder et al., 1986). *Scenedesmus obliquus* was reported to utilize 1,2-naphthoquinone-4-sulfonic acid, 1-naphthalenesulfonic acid, 2-naphthalenesulfonic acid, 1,5-naphthalenesulfonic acid, 1,6-naphthalenesulfonic acid, 2,6-naphthalenesulfonic acid, 2,7-naphthalenesulfonic acid, 1-hydroxynaphthalene-5-sulfonic acid, 1-hydroxynaphthalene-6-sulfonic acid, 2-hydroxynaphthalene-5-sulfonic acid, 2-hydroxynaphthalene-6-sulfonic acid, 2-hydroxynaphthalene-7-sulfonic acid and naphthalenetrisulfonic acid as sulfur sources (Luther et al., 1991). Under sulfate limitation, the green alga *Scenedesmus obliquus* metabolized 1- naphthalenesulfonic acid and partially used the sulfonate as a source of sulfur. The main metabolite, 1-hydroxy-2- naphthalenesulfonic acid, which was not metabolized further in the algal culture, was formed by hydroxylation of the substrate in position 1 and by migration of the sulfonic acid group to position 2 of the naphthalene ring (NIH shift). A smaller amount of 1-naphthalenesulfonic acid was desulfonated. The resulting 1-naphthol was mostly transformed into 1-naphthyl b-D-glucopyranoside (Kneifel et al., 1997).

1.6 Fungal metabolism of anthropogenic sulfonates

The degradation of sulfonated dyes like Tropaeoline {4-[(2,4-dihydroxyphenyl)azo]benzenesulfonic acid}, Orange II {4-[(2-hydroxy-1-naphthyl) azo] benzenesulfonicacid}, and Congo Red {3,3’-[[1,1’biphenyl]- 4,4’diylbis-(azo)]bis[4-amino-1-naphthalenesulfonic acid]} was reported in *Phanerochaete chrysosporium* (Cripps et al., 1990). Sulfanilic acid, 4-(3-methoxy-4-hydroxyphenylazo)-benzenesulfonic acid, Acid yellow 9 {4-(3-sulfo-4-aminophenylazo)-[benzenesulfonic acid}, 4-(2-sulfo-3'-methoxy-4-hydroxyazobenzene-4-azo)-benzenesulfonic acid, Orange II {4-(2-hydroxynaphthylazo)-benzenesulfonic acid} and Orange I {4-(4-hydroxynaphthylazo)-benzenesulfonic acid} were mineralized to CO₂ by *Phanerochaete chrysosporium* (Paszczynski et al., 1992). Desulfonation of 3, 5-dimethyl-4- hydroxyl and 3, 5-dimethyl-4-aminobenzenesulfonic acid was reported in *Phanerochaete chrysosporium*.
The enzymatic mechanism for the oxidation of sulfonated azo dyes by lignin peroxidases from *Phanerochaete chrysosporium* was studied by Goszczynski *et al.* (1994) (Chivukula *et al.*, 1995). These degradations have been attributed to extra cellular peroxidases (Muralikrishna and Renganathan, 1993; Goszczynski *et al.*, 1994).

*Phanerochaete chrysosporium* oxidize and shortens the side chain of LAS resulting in the formation of sulfophenyl carboxylates (Jagjit *et al.*, 2001). Some of the fungi like *Phanerochaete chrysosporium*, *Geotrichum candidum*, *Trametes versicolor*, *Bjerkandera adusta*, *Penicillium* sp., *Pleurotus ostreatus*, *Pycnoporus cinnabarinus*, and *Pyricularia oryza* were decolorized sulfonated dyes such as Direct Blue 1 (Chicago Sky Blue 6B), reactive dye Reactive Black 5. Comparisons of different fungi suggested that *Trametes* or *Bjerkandera* species were superior compared to *Phanerochaete chrysosporium* for the decoloration of different dyes (Stolz 2001).

**1.7 Anthropogenic sulfonates biodegradation by plants**

The cultured cells of *Rheum rabarbarum* (rhubarb) were reported to accumulate and biotransform 2-chloro-5-nitrobenzenesulfonate, 2-hydroxy-4-sulfonaphthalenediazonium, 2-hydroxy-4-sulfo-6-nitro-naphthalene-diazonium and 1, 3-naphthalene disulfonates, containing either an amino or a nitro group in position 7, without releasing the metabolites (Romain Duc *et al.*, 1999). Schwitzguébel et al (2008) reported that *Rheum rabarbarum* (rhubarb) accumulate and transform sulfonated anthraquinones. The biochemical mechanisms involved in the metabolism and detoxification of sulfonated anthraquinones in rhubarb (*Rheum rhaponticum*), maize (*Zea mays*) and celery (*Apium graveolens*) were reported (Valerie *et al.*, 2009). The role of antioxidant and detoxification enzymes of *Phragmites australis*, in the degradation of an azo dye, acid orange 7 (AO7), was studied. Increase in activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), and glutathione S-transferase (GST) was observed in crude extracts of leaves of *Phragmites australis* during degradation of AO7. *Blumea malcolmii* was found to decolorize Malachite green, Red HE8B, Methyl orange, Reactive Red 2 and Direct Red 5B. The phytotransformation of Direct Red 5B products identified were 4-(4-amino-phenylazo)- benzene sulfonic acid, 3-amino-7-carboxyamino-4-hydroxy-
naphthalene-2-sulfonic acid and 7-carboxyamino-naphthalene-2-sulfonic acid (Anuradha et al., 2009). *Portulaca grandiflora* Hook was reported to decolorize and degrade a sulfonated diazo dye Navy Blue HE2R (NBHE2R) NBHE2R into metabolites viz. N-benzylacetamide and 6-diazenyl-4-hydroxynaphthalene-2-sulfonic acid (Rahul et al., 2011).

### 1.8 Anoxygenic phototrophic bacteria

Anoxygenic phototrophic bacteria (APB) are physiologically and phylogenetically heterogeneous group of bacteria perform photosynthesis under anoxic conditions in the presence of light with the help of bacteriochlorophyll without the liberation of oxygen. Unlike oxygenic phototrophs (cyanobacteria, algae and green plants), they use only one photosystem and are unable to use water as an electron donor (Imhoff, 1995). They require electron donors of lower redox potential than water like mainly sulfide and other reduced sulfur compounds (thiosulfate, tetrathionate, sulfite etc), and also molecular hydrogen and many small organic compounds (Imhoff, 1995) or sometimes-ferrous iron (Heising et al., 1999; Heising and Schink, 1998) and instead of oxygen, the corresponding oxidized products are sulfate, protons (which are not observed because they are reused for the reduction of CO₂ to cell material), and organic compounds and CO₂, respectively.

Classification of APB was conventionally based on a number of morphological and physiological properties (Trüper and Pfennig, 1981; Imhoff and Trüper, 1989) with some important characteristics like dissimilatory sulfur metabolism, deposition of sulfur granules inside or outside the cells, oxidation of sulfur used for the differentiation major groups of phototrophic purple and green bacteria (Imhoff 1984; Pfennig 1989). Based on phenotypic characteristics, anoxygenic phototrophic bacteria were classified into the green sulfur bacteria, the green nonsulfur bacteria, the purple sulfur bacteria, the purple non sulfur bacteria and the heliobacteria. Based on 16S rRNA gene sequence analysis, they were distributed in five distantly related phyla viz., phylum *Proteobacteria* (which includes the purple bacteria), *Chloroflexi* (which includes the filamentous green nonsulfur bacteria), *Chlorobi* (which includes the green sulfur bacteria), *Firmicutes* (which includes the Gram-positive heliobacteria); the phototrophic members were also discovered in the
phylum Acidobacteria which was represented by a single species “Candidatus Chloracidobacterium thermophilum” (Bryant et al., 2007).

The Phototrophic purple bacteria are separated into purple sulfur bacteria with two families, Chromatiaceae, Ectothiorhodospiraceae, BChl b-containing anoxygenic phototrophic bacteria, aerobic anoxygenic phototrophic bacteria and purple non sulfur bacteria. The photosynthetic pigments and the photosynthetic apparatus in all purple bacteria are located in intracytoplasmic membranes, which are considered to be originating from, and being continuous with the cytoplasmic membrane. These intracytoplasmic membranes are in the form of small fingerlike intrusions, vesicles, tubules or lamellae. Chromatiaceae family members are able to grow with sulfide and elemental sulfur as e- donor and deposit globules of elemental sulfur inside the cells (Imhoff 1984). Ectothiorhodospiraceae members deposit elemental sulfur outside the cells (Imhoff et al., 1991). Based on the pufLM gene sequence analyses of the metagenome from samples of salt lakes in Chile, South America Thiel et al (2010) indicated the existence of purple sulfur bacteria outside the well-recognized families of Chromatiaceae and Ectothiorhodospiraceae. However, this is only based on the metagenome (uncultured) analysis, which needs to be confirmed by pure cultures.

1.8.1 Photometabolism of aromatic compounds by anoxygenic phototrophic bacteria

Anoxygenic phototrophic bacteria (APB) are diverse and important members of microbial communities (Imhoff, 2001a; Morris et al., 2004; Yutin et al., 2007), play a major role in anaerobic nutritional cycles. APB are metabolically highly versatile organisms capable of growth on substances ranging from simple aliphatic organic acids to complex polysaccharides (Hiraishi et al., 1989).

Rhodopseudomonas palustris (Harwood and Gibson 1988), Rhodospirillum fulvum (Pfennig et al., 1965, renamed Phaeospirillum fulvum by Imhoff et al., 1998), Rhodocyclus purpureus (Pfennig et al., 1965), Rhodomicrobium vannielii (Wright and Madigan 1991), Rubrivivax gelatinosus, Rhodobacter capsulatus (Blasco and Castillo 1992) Rhodopseudomonas acidophilus (Yamanaka et al., 1983 renamed Rhodoblastus acidophilus in Imhoff 2001b), Rhodobacter blasticus and Rhodospirillum rubrum are
known to metabolize monocyclic aromatic compounds. Many of the reported *Rhodopseudomonas* spp. were grown on methylbenzenes, amino benzenes and phenolics, while few of the *Rhodopseudomonas* spp. were reported for degradation of halo carboxylic acids, polychlorinated biphenyls, dinitrophenols, pyrazines and chlorobenzoates (Blasco and Castillo 1992; Harwood and Gibson 1988; Kamal and Wyndham 1990; Khanna *et al.*, 1992; McGrath and Harfoot 1997; Montgomery and Vogel. 1992; Sasikala *et al.*, 1994, Kusalatha *et al.*, 2010). Zengler et al (1999) reported that *Blastochloris sulfoviridis* ToP1 is the first phototrophic bacterium shown to utilize an aromatic hydrocarbon, toluene.

*Rhodobacter sphaeroides* OU5 was reported to metabolize 2-aminobenzoate (Nanda *et al.*, 2000; Sunayana *et al.*, 2005), *trans*-cinnamate (Usha *et al.*, 2007), L-phenylalanine, tyrosine (Ranjith *et al.*, 2007a), and photo transform aniline to indole esters (Shankaer *et al.*, 2006). The novel compounds with biotechnological applications were identified in strain OU5 include Sphestrin (a novel indole ester) (Sunayana *et al.*, 2005a), Rhodestrin (a novel indole terpenoid phytohormone) (Sunayana *et al.*, 2005b) Rhodethrin (novel indole terpenoid ether has cytotoxic and phytohormonal activities) (Ranjith *et al.*, 2007b) were identified. Phenols and gallate esters were identified in *Rhodobacter sphaeroides* OU5 in presence of l-phenylalanine used as sole source of nitrogen (Ranjith *et al.*, 2010). *Rhodobacter spheroides* Z08 was effective in ameliorating hazardous pollutants in pharmaceutical wastewater with over 80 % COD reduction (Madukasi *et al.*, 2010).

Ramana et al (2006) reported that *Rubrivivax benzoatilyticus* JA2 grown on benzoate, 2-aminobenzoate (anthranilate), 4-aminobenzoate, 4-hydroxybenzoate, phthalate, phenylalanine, *trans*-cinnamate, benzamide, salicylate, cyclohexanone, cyclohexanol and cyclohexane-2-carboxylate as carbon sources and/or electron donors. Aromatic aminoacids viz. l- tryptophan, l-phenylalanine catabolism and production of tryptophan and indole derivatives in presence of aniline (Mujahid *et al.*, 2010) was reported in *Rubrivivax benzoatilyticus* JA2, where L-tryptophan was metabolized through 2-oxoglutarate, indole-3-pyruvic acid, indole-3-acetaldehyde, indole-3-acetic acid, isatin, benzaldehyde, gallic acid and pyrogallol as intermediates (Ranjith *et al.*, 2011) and l-phenylalanine with l-phenylpyruvic acid and l-phenyllactate as metabolites (Prasuna *et al.*, 2012). Mujahid et al (2011a) reported that *Rubrivivax benzoatilyticus* JA2 produces
indole-3-acetic acid and related indole derivatives from L-tryptophan. Rubrivivaxin, a cytotoxic and cyclooxygenase-I inhibitory phenol terpenoid ester was reported (Ranjith et al., 2011). Due to these properties, PNSB have been utilized to treat different types of wastewaters such as concentrated latex wastewater (Choorit et al., 2002), odorous swine wastewater (Myung et al., 2004), tuna condensate (Prasertsan et al., 1997), oil-containing sewage wastewater, and latex rubber sheet wastewater (Kantachote et al., 2005). Kasomu and Obst (2009) studied the influence of photosynthesis on calcite precipitation.

1.8.2 List of anoxygenic phototrophic bacterial genomes sequenced

1) Allochromatium vinosum DSM 180
2) Chlorobaculum tepidum TLS
3) Chlorobium phaeovibrioides DSM 265
4) Chlorobium limicola DSM 245
5) Chloroflexus aggregans DSM 9485
6) Chlorobaculum parvum NCIB 8327
7) Chloroflexus aurantiacus J-10-fl
8) “Candidates chloracidobacterium thermophilum B”
9) Chloroherpeton thalassium ATCC 35110
10) Chlorobium phaeobacteroides DSM 266
11) Chlorobium phaeobacteroides BS1
12) Chlorobium chlorochromatii CaD3
13) Chlorobium luteolum DSM 273
14) Heliobacterium modesticaldum Ice1
15) Pelodictyon phaeoclathratiforme BU-1
16) Rhodopseudomonas palustris CGA009
17) Rhodopseudomonas palustris TIE-1
18) Rhodopseudomonas palustris BisA53
19) Rhodopseudomonas palustris BisB18
20) Rhodopseudomonas palustris BisB5
21) Rhodocymbium vannielii ATCC 17100
22) Rhodospirillum rubrum ATCC 11170
23) Rhodospirillum centinum SW
24) Rhodobacter sphaeroides ATCC 17029
25) Rhodobacter sp.SW2
Among the phototrophic bacterial genomes sequenced listed above, only few of the bacterial genomes have the genes for aromatic hydrocarbon metabolism. *Allochromatium vinosum* DSM 180\(^T\) complete genome sequence verified that aromatic hydrocarbon degrading genes were not identified (Thomas *et al.*, 2011). *Rhodomicrobium vannielli* ATCC 17100, complete genome indicated the genes for aromatic hydrocarbon degradation. (NCBI Reference Sequence: NC_014664.1). *Rhodospirillum rubrum* ATCC 11170, *Rhodobacter sphaeroides* KD131, *Rhodobacter sphaeroides* ATCC 17025, *Rhodobacter sphaeroides* ATCC 17029 complete genome sequence showed the genes for 4-chlorobenzoate degradation. Fusaricacid resistance proteins, multidrug resistance protein coding genes were reported in *Rhodospirillum rubrum* ATCC 11170. Among *Rhodopseudomonas palustris* strains 70% of the genes in each genome, shared by four or more strains. Between 10% and 18% of the genes in each genome are strain specific (Oda *et al.*, 2008). The complete genome sequence of *Rubrivivax benzoatilyticus* JA2 revealed many multidrug-resistant transporter, organic solvent resistance, and aromatic compound metabolizing genes (Mujahid *et al.*, 2011b). *Rhodopseudomonas palustris* CGA009, *Rhodopseudomonas palustris* TIE-1, *Rhodopseudomonas palustris* BisA53, *Rhodopseudomonas palustris* BisB18 and *Rhodopseudomonas palustris* BisB5 whole genome sequence analysis indicated the genes for benzoate degradation (Larimer *et al.*, 2004).
1.9 Definition of the problem

Aerobic metabolism of 4-toluenesulfonate is largely studied in chemotrophic bacteria and the enzymes, genes involved in 4-toluenesulfonate metabolism were well reported. Though there are reports on anaerobic desulphonation and demethylation of 4-toluenesulfonate, the work has not been extended for the elucidation of metabolic pathways, the enzymes and genes involved in anaerobic degradation of 4-toluenesulfonate were not studied. Though anoxygenic phototrophic bacteria are well known for aromatic hydrocarbon metabolism, the published information is only on purple non sulfur bacterial metabolism of aromatic hydrocarbons and alkylsulfonates. The present research work was initiated to explore the metabolic potential of purple sulfur bacteria by opting 4-toluenesulfonate as a test compound, with the following objectives.

OBJECTIVE

1. Polyphasic taxonomic characterization of a 4-toluenesulfonate degrading anoxygenic purple sulfur bacterium
2. Catabolic studies of 4-toluenesulfonate by an isolated strain