CHAPTER II

LITERATURE REVIEW

2.1 Organic solvents

Solvents are substances that are capable of dissolving or dispersing one or more other substances. Organic solvents are carbon-based solvents (i.e., they contain carbon in their molecular structure). They have various chemical structures, and include aromatics, aliphatics, chlorinated hydrocarbons, alcohols, ethers, esters, etc. (Table 2.1). They share lipophilicity and volatility in common, although some of them are also hydrophilic (e.g. methanol) or less volatile (e.g. cresol). They are used regularly in both developed and developing countries, being used in industries for painting, printing, degreasing etc., and are present in home-use products as, e.g., propellants, coolants, and glue-constituents.

Table 2.1 Chemical classification of organic solvents

Chemical group	**Typical solvents**
Aromatics	Benzene, toluene, xylenes, ethylbenzene, styrene monomer
Chlorinated hydrocarbans	Trichloroethlene, tetrachloroethylene, methylchloroform (or 1,1,1-trichloroethane), dichloromethane (methylene chloride)
Alcohols	Methyl alcohol, isopropyl alcohol, butyl alcohol
Ethers	Diethyl ether, 1,4-dioxane
Esters	Methyl acetate, ethyl acetate, butyl acetate
Glycol derivatives	Etyleneglycol momomethl ether, ethyleneglycol monoethyl ether, ethyleneglycol monoethyl ether acetate, ethyleneglycol monobutyl ether
Chlorofluorocarbons	Fluorotrichloromethane (CFC-11), 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113)
Miscellaneous	*n*-Hexane, dimethylformamide, carbon disulfide

2.2 Use of organic solvent

The most organic solvent used in industry such as paints, inks, thinners and adhesives, contain more than one solvent. The percentage in Table 2.2 showed that toluene is the most popular solvent in all the products except for degreasers. The solvent of the 2nd popularity class varied dependent on the product category. Xylenes and ethylbenzene were detected at high percentages, but this high prevalence was due to the presence as impurities in commercial toluene. Ketones (e.g. methyl ethyl ketone and methyl isobutyl ketone) and ethyl acetate were frequently detected in paints, alcohols in inks and *n*-hexane in adhesives. In contrast, 3 chlorinated hydrocarbons of trichloroethylene (for metal degreasing), tetrachloroethylene (for dry-cleaning and cleaning of plastics) and methylchloroform (a surrogate of the former two solvents) were most popular degreasers and they were used unmixed except for the addition of stabilizing agents. Moreover, organic solvents are also commonly used as fuel additives. The prevalence of organic solvents in the environments means that exposure to solvents is generally unavoidable and humans are generally exposed for prolonged periods and often in combination with other compounds. They contaminate are harmful to living thing in environments.

Table 2.2 Popular solvents in solvent-containing products

Popularity	Product				
	Paint	**Ink**	**Thinner**	**Adhesive**	**Degreaser**
1	Toluene (80)*	Toluene (62)	Toluene (56)	Toluene (51)	Trichloro ethylene (14)
2	Xylenes (66)	Isopropyl alcohol (35)	Xylenes (33)	n-Hexane (27)	Methyl chloroform (14)
3	Methyl ethylketone (26)	Methanol (25)	Ethyl acetate (38)	Methyl ethylketone (23)	Methanol (14)
4	Methyl isobutyl ketone (26)	Methyl ethylketone (21)	Ethylbenzene (32)	Methanol (13)	Tetrachloro ethylene (12)
5	Ethyl acetate (22)	Ethyl acetate (17)	Methanol (31)	Acetone (13)	Toluene (8)

* in parenthesis: number of product tested.

2.3 Organic solvent tolerant bacteria

Environments having high concentrations (10 to 50%, vol/vol) of organic solvents are considered extreme (Aono,1998). Bacteria that are able to tolerate such environments have recently been recognized as a subgroup of the extremophiles (Aono,1998). Lipophilic hydrocarbons are harmful to bacteria because they accumulate in membrane lipid bilayers, thus affecting the structural and functional properties of the membranes, and cytoplasmic membranes are the primary site of cellular damage by both organic solvents. Because of this toxicity, organic solvents have in the past been used as permeabilization agents, disinfectants, food preservatives, and industrial solvents (Isken and de Bont,1998).

2.4 Physiological basis of organic solvent toxicity and the concept of organic solvent tolerance

The primary site of action of organic solvents is the cell membrane. The cytoplasmic membrane of bacterial cells, a phospholipid bilayer, is a matrix in which various enzymes and transport proteins are embedded. It plays a vital role in solute transport, maintaining the energy status of the cell, regulation of the intracellular environment, turgor pressure, signal transduction and energy transducing processes. Solvents partition into and disrupt the lipid bilayer, thus compromising cell viability (Sikkema *et al.*,1994; Sikkema *et al.*,1995). It has been proved that it is not the chemical structure of the solvent, but the concentration to which it accumulates in the cell membrane that plays a crucial role in determining toxicity (Isken and de Bont,1998).

Physiological investigation of microbes has revealed a correlation between solvent toxicity and its log P_{ow} value (Table 2.3). The parameter log P_{ow} is defined as the partition coefficient of the given solvent in an equimolar mixture of octanol and water (Inoue and Horikoshi,1989). The greater the polarity, the lower the log P_{ow} value and the greater the toxicity of the solvent. Organic solvents with log P_{ow} values between 1 and 5 (Cruden *et al.*,1992) or log P_{ow} 1.5-3 (Ramos *et al.*,1997), are highly toxic to microorganisms. Each organism has its own intrinsic tolerance level for organic solvents, which is determined genetically and is also influenced by environmental factors. Organic solvent tolerance is believed to be a strain specific property (Ramos *et al.*,1997).

Table 2.3 Organic solvents and their log P_{ow} values

Solvent	log P_{ow} value	Solvent	log P value
n-Decane	5.6	*p*-Xylene	3.1
Decalin	4.8	Styrene	3.0
n-Octane	4.5	Octanol	2.9
Diphenyl ether	4.3	Carbon tetrachloride	2.7
Cyclooctane	4.2	Toluene	2.5
n-Heptane	4.1	Heptanol	2.4
Propyl benzene	3.8	Dimethyl phthalate	2.3
Tetralin	3.8	Fluorobenzene	2.2
Methyl cyclohexane	3.7	Benzene	2.0
Hexane	3.5	Chloroform	2.0
Diethylphthalate	3.3	Cyclohexanol	1.5
Cyclohexane	3.2	Buty acetate	1.8
Ethyl benzene	3.1	*n*-Butanol	0.8
o-Xylene	3.1	Ethyl acetate	0.7
m-Xylene	3.1		

2.5 Isolation of organic solvent tolerant bacteria

Organic solvents are known to be extremely toxic to microbial cells. Solvents are known to accumulate in and disrupt the bacterial cell membrane thus affecting the structural and functional integrity of the cell (Inoue and Horikoshi,1989; Sikkema *et al.*,1995). Although there are some microorganisms which can assimilate these toxic organic solvents, they do so only when the solvent concentration is very low. For example *P. putida*, *P. aeruginosa*, *P. fluorescens*, etc. *P. putida* IH- 2000 is of soil origin (Inoue and Horikoshi,1989), and *P. putida* DOT-T1E was isolated from wastewater (Ramos *et al.*,1995), others are laboratory strains that have gradually adapted to solvents, e.g., *P. putida* S12 (Weber *et al.*,1993), *P. putida* Idaho to *p*-xylene and *P. putida* S12 to styrene (Cruden *et al.*,1992; Weber *et al.*,1993). It has been found that *Pseudomonas* strains growing in the presence of short chain fatty acids like acetate have a lower membrane fluidity which prepares them for growth in the presence of supersaturating

amounts of toxic non-metabolisable solvents like toluene. *P. putida* S12 could adapt to grow on styrene in a two phase styrene-water system.

Several gram-positive bacteria such as *Bacillus, Rhodococcus* and *Arthrobacter* have been reported from natural habitats (Kato *et al.*,1996; Paje *et al.*,1997; Sardessai and Bhosle,2002). Kato et al. (1996) have described the following method for isolation of organic-solvent-tolerant bacteria that degrade crude oil, polyaromatic hydrocarbons or cholesterol or utilize sulphur compounds from deep sea sediment. Benzene was added to artificial sea-water containing deep-sea sediment to a concentration of 50% v/v and the cultures were incubated at room temperature. After incubation, the benzene layers were carefully separated from the sea-water layers, and a portion of each benzene layer was spread on a suitable nutrient medium. Colonies that grow on the medium after incubation for 2 days at 25 or 30°C were isolated and purified (Kato *et al.*,1996).

Table 2.4 Examples of organic solvent tolerant bacteria

Organism	Tolerated solvents	Reference
Pseudomonas putida IH-2000	Heptanol, toluene	(Inoue and Horikoshi,1989)
Pseudomonas putida PpG1 (mutant)	Toluene	(Sikkema *et al.*,1995)
Pseudomonas putida Idaho	Dimethylphthalate, toluene	(Cruden *et al.*,1992)
Pseudomonas aeruginosa ST-001	Heptanol, toluene	(Aono *et al.*,1994)
Pseudomonas patida S12	Dimethylphthalate, toluene	(Weber *et al.*,1993)
Pseudomonas aeruginosa LST-03	Toluene	Ogino et al. 1995
Pseudomonas putida DOT-T1E	Toluene	(Ramos *et al.*,1995)
Sphingomonas aromaticivorans B0695	Toluene, naphtalene, xylenes, p-cresol, fluorene, biphenyl, dibenzothiophene	Fredrickson et al. 1995
Arthrobacter ST-1	Benzene	(Kato *et al.*,1996)
Rhodococcus strain 33	Benzene	(Paje *et al.*,1997)
Bacillus	Toluene	(Isken and de Bont,1998)
Pseudomonas putida GM62, GM73 *Pseudomonas* sp. Strain GM80	Toluene	(Kim *et al.*,1998)
Bacillus cereus strain R1	Toluene	(Matsumoto *et al.*,2002)
Bacillus sp. BC1	Chloroform	(Sardessai and Bhosle,2004)
Rhodococcus opacus	Benzene, toluene, styrene, xylene, ethylbenzene, propylbenzene, octane, decane	(Na *et al.*,2005)
Staphylococcus sp. Strain ZZ1	Toluene	(Zahir *et al.*,2006)

Table 2.5 Solvent tolerance of Gram-negative bacteria (Inoue and Horikoshi,1991)

Type strains			Limiting log P values for growth
Pseudomonas aeruginosa	IFO	3924	3.4
"	IFO	3755	3.3
Pseudomonas fluorescens	IFO	3507	3.4
"	IAM	12022	3.4
Pseudomonas putida	IFO	3738	3.1
"	IFO	1506	3.1
Pseudomonas pseudoalcaligenes	ATCC	12815	3.4
Pseudomonas chlororaphis	IFO	3904	3.1
Pseudomonas syringae	IFO	3310	3.1
Pseudomonas stutzeri	IFO	3773	3.4
Escherichia coli	IFO	3806	3.8
"	IFO	3366	3.4
"	IFO	3545	3.8
"	HB 101		3.4
"	JM 101		3.4
"	JM 109		3.4
Aeromonas hydrophila	JCM	1027	4.5
"	IFO	3820	4.2
Alteromonas putrefaciens	IFO	3908	4.2
Achromobacter delicatulus	IAM	1433	3.9
Achromobacter calcoaceticus	IFO	12552	3.9
Agrobacterium tumefaciens	IFO	3058	4.8
Alcaligenes faecalis	JCM	1474	4.5
Serratia marcescens	IFO	3406	3.4
"	IFO	3736	3.4
Proteus vulgaris	IFO	3167	4.2
Proteus mirabilis	IFO	3849	3.8
Proteus morganii	IFO	3838	4.2
Klebsiella pneumoniae	IFO	3317	3.4
"	IFO	3321	3.4
Flavobacterium lutescens	IFO	3084	4.2
Chromobaterium chocolatum	IFO	3758	7.0

Table 2.6 Solvent tolerance of Gram-positive bacteria (Inoue and Horikoshi,1991)

Type strains			Limiting log P values for growth
Bacillus subtilis	AUH	1219	4.9
"	AUH	1390	4.5
"	IFO	3009	5.1
Bacillus brevis	IFO	3331	6.0
Bacillus cereus	IFO	3131	4.9
Bacillus circulans	IFO	3329	7.0
Bacillus macerans	IFO	3490	6.0
Bacillus polumyxa	IFO	3020	5.1
Bacillus thuringiensis	IFO	3951	4.8
Micrococcus luteus	IFO	3333	4.8
Micrococcus roseus	IFO	3764	4.8
Micrococcus varians	IFO	3765	4.8
Staphylococcus aureus	IFO	3183	4.8
Staphylococcus epidermidis	IFO	3762	4.8
Streptococcus alcalophilus	IFO	3531	4.9
Streptococcus faecalis	IFO	3826	5.1
Streptococcus faecium	IFO	3181	5.1
Corynebacterium glutamicum	JCM	1318	7.0
Corynebacterium flavesvens	IAM	1642	6.0
Corynebacterium herculus	ATCC	13868	6.0
Brevibacterium ammoniagenes	IFO	12072	7.0
Brevibacterium roseum	ATCC	13825	6.0
Brevibacterium flavum	ATCC	13826	7.0
Rhodococcus erythropolis	IFO	12320	6.0
Rhodococcus eque	IFO	3730	7.0
Leuconostoc medenteroides subsp. *dextran*	IFO	3349	5.1
Leuconostoc medenteroides subsp. *mesenea*	IFO	3832	5.1
Lactobacillus casei subsp. *rhamnosus*	IFO	3425	5.1

2.6 Adaptive mechanisms of organic-solvent tolerance

"Adaptation" is defined as a change in cell physiology and/ or composition to adjust to the environment without the means of genetic modifications (mutations). Mechanisms enabling bacteria to survive toxic of organic solvents comprise (Isken and de Bont,1998; Sikkema *et al.*,1995) (Fig. 2.1):

1) Morphological adaptation	5) Modifying surface properties (charge and hydrophobicity)
2) Changes of the energetic status	6) Transformation or degradation of the solvent
3) Adaptation of the cell membrane	7) Active transport of solvents from the membrane into the environment by energy-consuming efflux systems
4) Changes in the cell wall and outer membrane	8) Modifying membrane proteins

However, it is a combination of diverse mechanisms that eventually leads to efficient solvent tolerance ((Isken and de Bont,1998; Kabelitz *et al.*,2003).

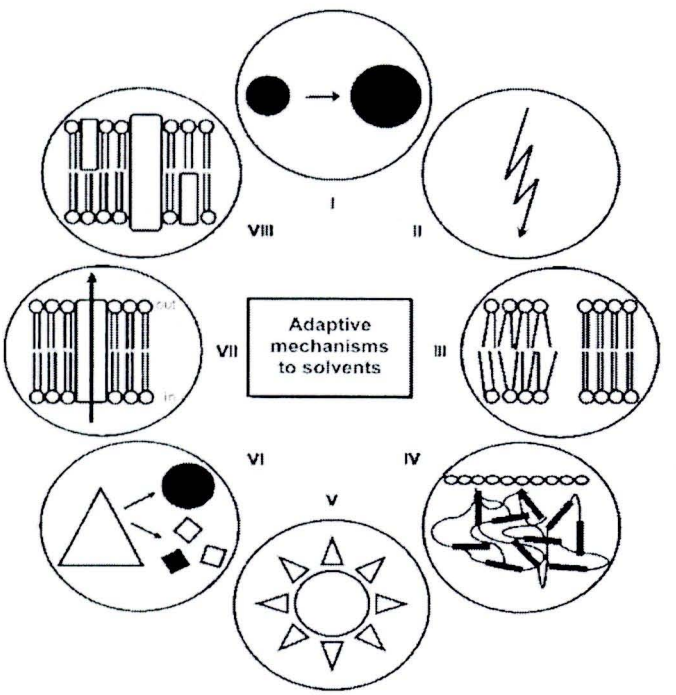

Fig.2.1 Adaptive mechanisms protecting cells against toxic effects of organic solvents.

2.6.1 Morphological adaptation

Little is known about morphological changes in bacteria as a response to solvents, although there are a number of reports that cell sizes increase when exposed to toxic organic compounds. Major factor responsible for these changes seems to be the surface-to-volume ratio of the cells. Two bacteria, *P. putida* and *Enterobacte*r sp., increased in size when exposed to phenol, 4-chlorophenol, and butanol (Neumann *et al.*,2005). Presumably minimizing the surface-to-volume ratio is favorable, as it minimizes the target for toxic solvents. However, decrease in cell size has been reported for *Enterobacter* sp. When grown on 1-butanol up to 1.5% (v/v)as the sole carbon and energy source (Veeranagouda *et al.*,2006). The enhanced surface might enhance uptake of potential growth substrates, as in glucose-grown cells (which were not solvent-adapted), the addition of butanol led to the opposite effect: a decrease in the surface–volume ratio.

2.6.2 Changes of the energetic status

For energy transduction, the barrier properties of the cytoplasmic membrane are of utmost importance. Due to the presence of solvents, permeability of the membrane for ATP molecules increases protons or other ions such as potassium leading to dissipation of the proton motive force regularly affecting energy transduction. Next to that, numerous energy-consuming adaptation mechanisms are known (de Bont,1998; Isken and de Bont,1998; Ramos *et al.*,2002). Nevertheless, the solvent-tolerant bacterium in *P. putida* DOT-T1E displayed no significant differences in both the ATP content as well as the adenylate energy charge during fermentations with and without a second phase of 1-decanol (Neumann *et al.*,2006), implying complete solvent adaptation of the cells on the energetic level. Although the bacteria needed additional energy for adaptation to the

solvent, they were able to maintain or activate electron transport phosphorylation, allowing homeostasis of the ATP level and energy charge in the presence of solvent.

2.6.3 Adaptation of the cell membrane

As the membrane constitutes the main target for toxic effects of solvents, it is not surprising that, along with the discovery of solvent-tolerant bacteria, changes in the membrane composition were considered to play a pivotal role.

Adaptation systems routinely deal with the reestablishment of fluidity and rigidity of the membrane after solvent exposure (Weber *et al.*,1993). The most important and, for a long time known, adaptive mechanism concerns the change in the saturation degree of membrane lipid fatty acids. It was reported in the 1970s that *E. coli* adapts the level of membrane lipids fatty acid's saturation upon treatment with alcohols, antibiotics, and food additives (Ingram,1976), similar to those induced by changed temperatures (Ingram and Buttke,1984).

Cis-trans isomerisation of unsaturated fatty acids was proven to be independent of energy, cell growth, and *de novo*-fatty acid biosynthesis, thus allowing to adapt to toxic concentration of solvents, even under environmental conditions not permitting growth and, hence, synthesis of new fatty acids (Heipieper *et al.*,1992). *Cis-trans* isomerisation was proven to be caused by solvents such as toluene (Heipieper *et al.*,1992). There is a correlation between the amount of unsaturated trans-fatty acids and survival of solvent-tolerant bacteria in the presence of a second phase of toluene (Ramos *et al.*,2002; Ramos *et al.*,2001) A direct correlation was observed between the hydrophobicity of organic compounds (log P_{ow}), concentration-dependent growth inhibition, and the *trans–cis* ratio of unsaturated membrane fatty acids. However, *cis–trans* isomerisation alone does not provide an adequate protection against organic

solvents. In fact, there are bacterial strains able to perform such isomerisation, but they are still solvent sensitive. Starvation as well as the presence of antibiotics or heavy metals was also shown to provoke the activation of the *cis–trans* mechanism (Heipieper *et al.*,2003). Stress due to the presence of an organic solvent (or other stress factors) can be quantified by determination of the *trans–cis* ratio of unsaturated fatty acids in the membrane; thus, it may serve as a general stress biomarker (de Bont,1998; Heipieper *et al.*,2003)

2.6.4 Changes in the cell wall and outer membrane

Most solvent-tolerant bacteria described until now are Gram-negative, which suggests that their outer membrane plays a critical role in solvent adaptation. Due to their relatively high impermeability, the outer membrane is an important barrier against intrusive and toxic compounds. The outer membrane consists mainly of lipopolysaccharides (LPS), a heteropolymeric layer of lipids and sugars (Makin and Beverdge,1996).

2.6.5 Modifying surface properties (charge and hydrophobicity)

The quantity and type of LPS found on the cell surface has a profound effect on cell surface properties, such as hydrophobicity, as well as on the interactions with solid surfaces (Makin and Beverdge,1996). Nearly all bacterial surfaces possess an electronegative charge, mainly caused by phosphate as the most important charged component of the outer cell surface. Cells of *P. putida* grown on phenanthrene were much more negatively charged than glucose-grown cells, suggesting that the low water soluble compounds such as polycyclic aromatic hydrocarbon induced modifications of the physical properties of bacterial surfaces (Wick *et al.*,2002).

In gram-positive bacteria, such as *Mycobacterium frederiksbergense*, an increased hydrophobicity was observed when grown with anthracene instead of glucose as the sole carbon source. Probably, the increased hydrophobicity resulted in high affinity of the cells for anthracene, which favoured uptake of the compound in soils displaying low anthracene bioavailability (Wick *et al.*,2002).

2.6.6 Transformation or degradation of the organic solvent

Biodegradation may be part of long-term adaptive responses but is not a main mechanism leading to solvent tolerance (Isken and de Bont,1998). Biodegradation was postulated to be a major adaptive response for a benzene-tolerant *Rhodococcus* strain (Paje *et al.*,1997). However, many solvent-tolerant bacterial strains can grow abundantly in the presence of solvents without degrading or modifying them (Ramos *et al.*,2002).

2.6.7 Active transport of solvents from the membrane into the environment by energy-consuming efflux systems

The toluene concentration in the membrane can be reduced with the help of a proton motive force-dependent toluene transporting system directly proving that solvents themselves could be excreted from the cell (Isken and de Bont,1996). Both compounds are structurally unrelated and are known to be energy-coupling inhibitors, suggesting the requirement of energy for excreting solvent molecules (Isken and de Bont,1996). In *P. putida* DOT-T1E, three efflux pumps (TtgABC, TtgDEF, and TtgGHI) located in the membrane mediating an efficient toluene tolerance were identified (Segura *et al.*,2005). Two pumps, designated as TtgABC and TtgGHI, are constitutively expressed, and next to toluene, they "extrude" also four different solvents from the membrane. The third pump, TtgDEF, is induced by aromatic hydrocarbons such as toluene and styrene, and the three

encoding genes are linked to the chromosomal *tod* genes for toluene metabolism (Segura *et al.*,2005).

2.6.8 Modifying membrane proteins

Protein complexes embedded in the outer membrane constitute water-filled channels; the majority of which are so-calledporins. As solvent molecules can pass through them, mutants lacking porins or bacteria displaying smaller porins than normal should be more tolerant to toxic effects of organic solvents(Isken and de Bont,1998; Weber *et al.*,1993). As another long-term response to toluene in *P. putida* DOT-T1, changes in the phospholipid polar headgroups were reported: an enrichment of cardiolipin up to 22% of the total phospholipids and a decrease in phosphatidylethanolamine (Ramos *et al.*,2001).

2.7 Application of organic solvent-tolerant bacteria

Bacterial cells with enhanced solvent tolerance would be extremely useful in the fields of applied microbiology and biotechnology. In the industrial production of fine chemicals, organic solvent tolerant bacteria would be better able to withstand extraction of the chemical end-product with a second phase of organic solvent. Organic-solvent tolerant bacteria used in the environmental bioremediation of toxic wastes could be enhanced to withstand higher concentrations of the pollutants. The further characterization of these specialized organic solvent-tolerant bacteria will provide us with the knowledge required for their use in biotechnological applications.

2.7.1 Biotechnology application

Organic solvent tolerant bacteria can serve as invaluable agents in catalyzing the biotransformations of water-insoluble substances in organic aqueous biphasic systems. For bioconversion of organic compounds with low solubilities in water, large volumes of appropriate medium are required for solubilization of the compounds. This consumption of media and water and the inevitable treatment of wastewater constitute one of the major cost factors in bioconversion fermentation. If the water-insoluble compounds were suspended in a small volume of the medium, it would take a very long time to complete the bioconversion.

Another significant aspect is the use of organic solvent tolerant bacteria to scavenge toxic substrates/products from the cells using a second liquid extraction phase in the production process. An important advantage of having an organic phase in the system lies in elimination of toxic products from the fermenter. Product toxicity of fine chemicals is a problem in several biotechnological processes. In many instances, a second phase of the organic solvent can extract the toxic product from the aqueous phase during the fermentation. *Pseudomonas oleovorans* can convert 1, 7-octadiene into both 7, 8-epoxy-1-octene and 1, 2, 7, 8-diepoxyoctane when grown on octane (Schwartz and McCoy,1977). The use of organisms such as *P. oleovorans*, *E. coli*, or *Nocardia corallina* in production of lipophilic compounds in 2-phase systems is limited by the range of solvents that the organism can withstand as a second phase (Schwartz and McCoy,1976). It is here that solvent-tolerant bacteria play a vital role by allowing a new degree of freedom in coping with toxic products. They can tolerate solvents that are much less lipophilic, and these can be used to partition out toxic products from the aqueous phase. Many important fine chemicals including catechols, phenols, aldehydes and ketones, low

molecular weight epoxides and diepoxides, medium-chain alcohols, and terpenoids are in low lipophilicity range (de Bont,1998)

2.7.2 Bioremediation

Environmental pollution can harm the flora and fauna of affected habitats, resulting in the uptake and accumulation of toxic chemicals in food chains, causing serious health problems and/or genetic defects in humans. Although many aromatic hydrocarbon degrading strains have been isolated, they are usually solvent sensitive and degradation of aromatic hydrocarbons occurs only when these compounds are supplied at low concentrations (Sikkema *et al.*,1995). There is considerable interest in the isolation of microbes able to thrive in high concentrations of organic solvents, because such organisms can be used as vehicles in the elimination of low molecular weight aromatics that are highly carcinogenic even in ppm amounts. Since most natural contaminated sites are saturated with solvents such as benzene, toluene, etc., organic solvent tolerant bacteria with the requisite catabolic potential can be of vital importance in cleanup operations. For instance, a *Rhodococcus* sp. strain 33 isolated from a contaminated site can degrade benzene at concentrations of 200 ppm and tolerate high concentrations of benzene, which used in cleaning up marine oil spills (Paje *et al.*,1997). This led to the isolation of several strains such as *Flavobacterium* sp. DS-711, which degrades crude oil, *Bacillus* sp. DS-994, which utilizes sulfur compounds, *Bacillus* sp. DS-1906, which degrades polyaromatic hydrocarbons, and *Arthrobacter* ST-1, which degrades cholesterol (Kato *et al.*,1996). Abe et al. (1995) isolated an organic solvent tolerant bacterium from deep sea sediment samples after treatment with 50% v/v benzene. This strain, *Bacillus* DS- 1906, showed polyaromatic hydrocarbon degrading ability in the presence of organic solvent. It

degraded 48% of naphthalene solubilized in *n*-hexane and the amount degraded was more in the presence of solvent (Abe *et al.*,1995).