Solubilization and Biodegradation of Naphthalene in Presence of *Sapindus Mukorossi* – Synthetic Mixed Surfactant Systems

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Thesis Submitted

by

K. JAGAJJANANI RAO

In partial fulfillment for the award of the Degree of

MASTER OF TECHNOLOGY (Research)

IN

CHEMICAL ENGINEERING

(Biochemical Engineering and Biotechnology)

Under the esteemed guidance of

Dr. SANTANU PARIA

DEPARTMENT OF CHEMICAL ENGINEERING
NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA - 769008
ORISSA, INDIA.
January - 2009

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CERTIFICATE

This is to certify that the project report titled “**Solubilization and Biodegradation of Naphthalene in Presence of *Sapindus Mukorossi* – Synthetic Mixed Surfactant Systems**”. has been done under my guidance is a bonafide record of work done by **Mr. K. Jagajjanani Rao** in partial fulfillment of the requirement for the completion of the Master in Technology in Chemical Engineering.

Date : 30/01/09

Santanu Paria

(Dr. Santanu Paria)

Assistant Professor

Department of Chemical Engineering

National Institute of Technology

Rourkela - 769008

ACKNOWLEDGEMENT

In pursuit of this academic endeavor, I feel that I have been singularly fortunate, inspiration, guidance, direction, cooperation, love and care all came in my way in abundance and it seems almost an impossible task for me to acknowledge the same in adequate term. Yes, I shall be failing in my duty if I do not record my profound sense of indebtedness and heart felt gratitude to my supervisor, **Dr. Santanu Paria**, Assistant Professor, Department of Chemical Engineering, NIT Rourkela, who guided and inspired me in pursuance of this work. His association will remain a beacon light to me through out my career.

I owe a depth of gratitude to **Prof. K.C. Biswal**., H.O.D, **Prof. G.K. Roy**., Chairman MSC, for all the facilities provided during the course of my tenure. I am thankful to **Dr. Madhusree Kundu** for her valuable suggestions and support.

I want to acknowledge the support of all the faculty and friends of Chemical Engg and Biotechnology Department, NIT, Rourkela.

A special thanks to my all lab mates Rajib Ghosh Chaudhuri, Nihar Biswal, Menaketan Ray for making a good atmosphere in the lab. I acknowledge the co-operation extended by Mr. Mohanthy.

I thank Kaleswar Rao and Chaitanya for their joyous company and for helping me in several ways. I wish to thank Ramakrishna and Sravanthi for their support and joyful company.

My heartfelt thanks to all my friends Ramesh, Jabes, Koti, Hari, Ashraf, Chandan, Yogi, Alok, Padma, Sirisha, Arathi, Kishore, Anand and Mallikarjun for their moral support.

I thank to the support, encouragement and good wishes of my parents and family members, without which I would not have been able to complete my thesis.

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Nomenclature

B = Empirical parameter
C_0, C_t = Concentration of naphthalene at day 0 and day t, in mM
C_1, C_{12} = Concentration of surfactant 1and mixed surfactant 12 in mM
C_{CMC} = Concentration of surfactant above CMC, in mM
C_S = Concentration of surfactant below CMC, in mM
f_1, f_2 = Activity coefficients
G_E = Gibbs free energy
H_E = Enthalpy of mixing
K = Kinetic degradation rate constant, day $^{-1}$
K_g = Growth rate constant, day^{-1}
K_m = Micellar partition coefficient
K_{m1}, K_{m2}, $K_{m1,2}$ = Micelle – water partition coefficients of a solute for surfactants 1, 2 and in mixture.
K_{ow} = Octonol water partition coefficient
N_2, N_1 = Concentration of cells, in mg/L
R = Universal gas constant
R = Deviation ratio
S = Apparent solubility of organic compound, in mM
S_{CMC} = Solbulity of organic compound at CMC, in mM
T = Absolute temperature
t_2, t_1 = Time in days
x_1 = Micellar mole fraction of Reetha
X_1, X_2 = Mole fraction of surfactant 1 and 2
X_a = Mole fractions of solute in aqueous phase
X_m = Mole fractions of solute in micelles
X_{m1} = Micellar mole fraction of surfactant 1
X_R = Mole fraction of Reetha

Greek letters

Δ = Change/Difference

α_1 = Mole Fraction of Reetha

B = Empirical Parameter

γ = Surface Tension, dyne/cm

ξ = Zeta potential, mV

Abbreviations

AOT = Dioctyl sodium sulfosuccinate

CMC = Critical Micellar Concentration

CMC_1, CMC_2, CMC_{12} = CMCs of Reetha, pure synthetic and mixed surfactant

CTAB = Cetyl trimethyl ammonium bromide

DNA = Deoxyribonucleic acid

DNAPL = Dense Non Aqueous Phase Lipid

EPA = Environmental Protection Agency

HOC = Hydrophobic Organic Contaminant

IARC = International Agency for Research on Cancer

KC = Known Culture

LNAPL = Light Non-Aqueous Phase Liquid

MSR = Molar Solubilization Ratio

NAPL = Non Aqueous Phase Lipid

PAH = Poly Aromatic Hydrocarbon

PCB = Polychlorobiphenyl

PCE = Polychloroethylene

RNA = Ribonucleic acid

SDBS = Sodium dodecyl benzne sulphonate

SDS = Sodiumdodecyl sulphate

SOS = Sodium octane sulfonate

TCE = Tetrachloroethylene

TCET = 1,1,1-Trichloroethane

TX–100 = Triton - 100

VOC = Volatile Oraganic Compund

WWC = Waste Water Culture

Abstract

Solubilization efficiencies of naphthalene by micellar solution of a plant–based surfactant extracted from fruit of *Sapindus mukorossi* (Reetha), and the synthetic surfactants like nonionic (TritonX–100 or TX–100), cationic (Cetyl trimethyl ammonium bromide or CTAB), anionic (Sodium dodecylbenzenesulfonate or SDBS; Dioctyl sodium sulfosuccinate or AOT; Sodium octanesulfonate or SOS) in their single and as well binary mixed (plant – synthetic) systems were measured and compared. The solubilization efficiency of single surfactants followed the order TX-100 > CTAB > Reetha = SDBS > AOT > SOS. The mixed surfactant systems show negative deviation in molar solubilization ratio (MSR) from the ideality. The ascending order of % change in MSR (Δ_{MSR}) are TX–100 – Reetha < CTAB – Reetha < SDBS – Reetha < AOT – Reetha < SOS – Reetha. The mixed micellar solution for a particular combination also exhibits Δ_{MSR} is more negative when interaction parameter for the mixed micelle (β) is more negative.

For the above mixtures mixed micellization and interaction of surfactant molecules were examined. Mixed critical micelle concentrations (CMCs) were obtained from surface tensiometry as well as from solubilization experiments. The mixed micellar compositons and the estimation of interacting forces were determined on the basis of a regular solution model along with thermodynamic potentials of micellization. The CMCs were reduced, although not substantial, and synergistic behaviour of the Reetha with synthetic surfactants was observed. The interaction parameter β for the mixed micellar system was calculated using regular solution model to see the nonideality of the system. The negative value of β for different Reetha – synthetic mixed surfactants systems indicate there is a synergistic effect in the mixed micellar systems.

The bioavailability and biodegradation of naphthalene was studied with Reetha, TX–100, CTAB, SDBS, and the mixture of these synthetic surfactants with Reetha. Biodegradation of naphthalene was more in the presence of surfactant mixtures indicate more bioavailability to microorganisms. Two mixed culture's (known culture (KC) and unknown culture (WWC)) naphthalene degrading ability in the presence of surfactants (pure/mixtures) were tested. Naphthalene biodegradation is explained with the help of growth rate constants (K_g), naphthalene degradation rate constants (K), surfactant (pure/mixtures) utilization (by % change in surface tension), and with zeta potential values of the microbes in surfactant combinations. Naphthalene degraded was more in CTAB, SDBS - Reetha mixtures and less in case of TX-100 - Reetha mixtures, while the order is opposite in mixed surfactant utilization. Addition of Reetha to the synthetic surfactants TX-100, CTAB and SDBS proved to be more beneficial to WWC than KC. Utilization of naphthalene along with surfactants (pure/mixtures) also explained and mixed micellar concentrations above critical micelle concentration were not toxic to the microorganisms used. Zeta potential for mixed cultures was monitored for surfactants (pure/mixtures) and its relation to K values explained. The results of this study may be useful for the applications of natural or natural – synthetic mixed systems in surfactant enhanced remediation and degradation.

Keywords: *Sapindus mukorossi*, Reetha, molar solubilization ratio, interaction parameter, Biodegradation, Zeta potential

Chapter 1

Introduction

1.1 Definitions of surfactant, micellar solubilization, and biodegradation

The term surface-active agent or "surfactant" represents a heterogeneous and long-chain molecule containing both hydrophilic (head) and hydrophobic (tail) moieties. Micellar solubilization. At a particular concentration (known as critical micellar concentration) surfactant monomers tend to form aggregates called micelles. *Micellar* systems have the unique property of being able to *solubilize* both hydrophobic and hydrophilic compounds (which entraps the undissolved molecules and get them dissolved) and then level off. This concentration is called as Micellar solubilization. Bioavailability is herein defined as the accessibility of a substrate by a microorganism. Biodegradation is the contaminant utilization by microorganisms as a substrate and turning down it to harmless products.

1.2 Hydrophobic organic contaminants

Hydrophobic organic compounds (HOC) are ubiquitous soil pollutants and cause many environmental problems. Among HOCs, polycyclic aromatic hydrocarbons (PAHs) are major components of crude oil, creosote, coal tar and wastes from the combustion of fossil fuel, coal gasification, and incineration of industrial wastes (Menzie et al., 1992). . PAHs are non-polar, neutral, and hydrophobic organic molecules comprised of two or more fused benzene rings. They have received much attention since they are known to be potentially mutagenic or carcinogenic to human being and other living organisms (Harvey, 1996). Sixteen PAHs are listed by EPA as priority pollutants due to their toxicity. Even though PAHs have low solubility in water, their slow dissolution can contaminate large amounts of ground water for a long period (Luthy et al., 1994). Physical, chemical and biological methods have been used for the remediation of hydrophobic organic compounds contaminated sites. Among many treatment methods for hydrophobic organic compounds contaminated soil, bioremediation has been used for the destruction of organic compounds in soil and has been considered as an economical option for contaminated soil and groundwater attenuation. However, conventional remediation methods, such as "pump and treat" and soil venting with nutrient solution, are often insufficient for PAHs contaminated soils because of their bioavailability in soils is often limited by their low solubility and strong sorption to the soil (Wilson and Jones, 1993; Makkar and Rockne, 2003).

1.3 Role of surfactants

The bioavailability of PAHs can be enhanced by solubilizing the contaminants in aqueous phase and by which the enzymes from microorganism catalyze the degradation step. In general, the surfactants are used to enhance solubilization of PAHs or any other hydrophobic compounds. Many studies employing single and mixed surfactants showed their remediation potential in solubilizing and mobilizing organic contaminants like PAHs (Paria, 2008; Edwards et al., 1991; Paria and Yuet, 2006). Although synthetic surfactants are efficient solubilizers of contaminants but surfactants used should be also biodegradable or food grade, else there will be another environmental problem due the presence of non degradable surfactants (Stelmack et al., 1999). Biosurfactants produced by microorganisms are also used to enhance bioavailability and biodegradation rates of PAHs in soil remediation. These are having advantages like easy degradability, production from renewable sources etc. than synthetic surfactants (Muller et al., 1993). But their usage in bulk is limited as their purification; recoveries are not economical and some are having antimicrobial properties (Fiechter, 1992). Surfactant enhanced degradation is also a complicated process; as surfactant presence some times adversly effect the innate microoorganisms growth and uptake of substrates. The presence of surfactants along with contaminant highly influences the ultimate degradation ability of microorganisms.

The synthetic surfactants are also some times expensive and its losses due to precipitation, adsorption, toxicity to microorganisms, and low biodegradability at higher CMC limitmg its usage. Plant-based natural surfactants which are cheap, abundent, eco friendly, are an effective alternative to use. Additional advantage of plant based natural surfactant application in remediation studies is that any residual surfactant left in the subsurface soils will be easily biodegraded. It is indeed beneficial to know the biological response of surfactant (pure/mixtures) addition on PAH degrading bacteria in order to develop practical surfactant enhanced bioremediation strategies.

Chapter 2

Background literature

2. Polycyclic aromatic hydrocarbons (PAHs)

2.1. General properties

PAHs are nonpolar and hydrophobic organic chemicals composed of two or more benzene rings. They have low solubility in water and are strongly bound to soil. Table-2.1 show properties of some polycyclic aromatic hydrocarbons (PAHs). PAHs are major components of crude oil, creosote, coal tar and wastes from the combustion of fossil fuel, coal gasification and liquefaction, and incineration of industrial wastes (Harvey, 1996; Wilson and Jones, 1993). These compounds are produced by industrial activities such as oil processing and storage, and largely by combustion. In urbanized areas, it has been reported that urban runoff also contains significant amounts of PAHs (Sansalone and Buchberger, 1995). Combustion products are the major sources of PAHs in storm water runoff from urbanized areas (Ngabe et al., 2000).

A benzene ring has six carbon atoms and a conjugated system of π electrons (Carey, 1987). The π electrons delocalization in the aromatic ring of cyclic $(4n+ 2)$ π-bond system causes cyclic compounds to be particularly stable compared to nonaromatic compounds (Schwarzenbach et al., 1993). From a remediation perspective, it is important to examine the environmental properties of these compounds. PAHs generally exist as solids in the environment, and some PAHs may exist as needles, plates, crystals, or prisms and range from colorless to golden yellow (NIEHS, 1997). Naphthalene has the lowest melting point (80.6ºC) and molecular weight (31.5 mg/L), and most soluble PAH in water (Schwarzenbach et al., 1993). The highest molecular weight PAH of environmental interest is coronene, and it has the lowest solubility in water, which is about 1.4×10^{-4} mg/L (Connell, 1997).

Table-2.1: Physio-chemical properties, solubility and cancer class of different PAH's (Wilson and Jones, 1993).

PAH	No. of Rings	Mol. wt. g $mole^{-1}$	Boiling point °C	Aq. Sol. [a] (µ g/L)	Log K_{ow}	Cancer Class	
						U.S EPA [b]	IARC[c]
Naphthalene	2	128	218	31000	3.50	D	3
Phenanthrene	3	178	339	1300	4.45	D	3
Anthracene	3	178	340	70	4.46	D	3
Fluoranthene	4	202	375	260	4.90	D	3
Pyrene	4	202	393	135	4.90	D	3
Benz [a] anthracene	4	228	435	14	5.61	B2	2A
Chrysene	4	228	448	2	5.90	B2	3
Benzo [a] pyrene	5	252	496	4	6.04	B2	2B
Dibenz [a,h] anthracene	5	278	535	0.5	7.20	B2	N/A

[a] Mackay et al. 1992

[b] Cancer class from U.S. Environmental Protection Agency (U.S EPA) weight-of-evidence classifications. D-not classifiable; B2-probable human carcinogen.

[c]-Cancer class from International Agency for Research on Cancer (IARC). 3-not classifiable; 2A-probabale human carcinogen; 2B-possible human carcinogen; N/A-not applicable.

(U.S. Environmental Protection Agency, 2002. Integrated risk information system (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC.)

Within the PAH family, many properties, such as solubility, melting and boiling point, vapor pressure, and octanol–water partition coefficient (K_{ow}), correspond to the molecular weight and structure of the compound. Octanol–water partition coefficient (K_{ow}) is a measure of solubility and defined as partition of the organic compound between octanol / water phase (Schwarzenbach et al., 1993). As shown in Table-2.1, the solubilities of PAHs decrease as the number of benzene rings increases. Even though

PAHs have low solubility in water, their dissolution can contaminate large amounts of ground water for long periods (Luthy et al., 1994).

2.1.2 Fate of PAHs in the soil and groundwater environment

As it is mentioned that PAHs have very low water solubility and high K_{ow} values, they tend to get sorbed preferably to the soil organic matter instead of being solubilized in the infiltrating water and through this be transported downwards to the groundwater reservoirs. The sorption process is therefore counteractive to efficient biodegradation process as it decreases bioavailability, and as these compounds are located in micro porous areas of the soil due to sorption which makes it inaccessible to the bacteria and the biodegradation will thus be controlled by the slow desorptive and diffusive mass transfer into the biologically active areas (Zhang et al., 1998). It has been claimed that a slow sorption following the initial rapid and reversible sorption lead to a chemical fraction that is very resistant to desorption (Hatzinger and Alexander, 1995). This phenomenon is called aging, and the existence of such desorption-resistant residues may increase the time for their removal as the compound stay in the soil dramatically. PAHs have also been shown to be partitioned or incorporated more or less reversibly into the humic substances of the soil after partial degradation and thereby be even more immobilised in the soil (Kästner et al., 1999).

At the same time they show very low aerobic degradability depending on the environmental conditions and the available concentration. Only two-and three-ringed compounds have been shown to be degraded under anaerobic conditions with nitrate or sulfate as the terminal electron acceptor (Mihelic and Luthy, 1988). Very low concentrations have a strong influence on the biodegradation of such hydrophobic compounds, and some studies have indicated that the process stops below a certain threshold concentration (Alexander, 1985). The low mobility and high persistence means that they can stay in the soil for decades, and even at sites with contaminations dating at least 50 years back, 4- or 5-ringed PAHs are found near the soil surface.

2.1.3 PAH Toxicity

Research into the toxicology of HOCs is ongoing, but presently many of these pollutants are considered to be mutagenic and/or known carcinogens (Harvey, 1991; Menzie et al., 1992; Connell, 1997; NIEHS, 1997). HOCs are found throughout a list of priority hazardous substances compiled by the U.S. EPA and the Agency for Toxic Substances and Disease Registry (ATSDR, 1999). In addition, in the 1997 report on carcinogens, the U.S. Department of Health and Human Services cited evidence that 15 PAHs caused various types of cancer in experimental animals (NIEHS, 1997). According to Lee et al. (1981), PAHs are the largest class of chemical carcinogens, and both Clar (1964) and Harvey (1991) also reported in detail about the evidence of PAH carcinogenicity in animals. Harvey (1996) reported that some PAH metabolites bind to protein, DNA, and RNA, and adducted compounds may cause damage to cells and cause carcinogenic effects. In Table- 2.1 the cancer classed of selected PAHs listed.

2.2 Bioavailability and Biodegradation of organic contaminants

Bioavailability and biodegradation are two important factors effect the ultimate fate of any contaminant. Bioavailability is governed by (1) the substrate concentration that the cell membrane "sees," (i.e., the "directly bioavailable" pool) as well as (2) the rate of mass transfer from potentially bioavailable (e.g., nonaqueous) phases to the directly bioavailable (e.g., aqueous) phase. The biodegradation process consists of several steps (Fig. 2.1). Consider a substrate that is initially present in soil or a porous matrix where it is inaccessible to microorganisms. The substrate may be adsorbed to the matrix or may be present in the liquid or solid phase. First, this substrate has to be transferred to sites where it can come in direct contact with microorganisms. This can occur by desorption, dissolution, or mobilization of the contaminant from the soil 'phase' to the aqueous phase, and eventually by transport, *i.e.* convection and dispersion (Fig. 2.1). Subsequently, the substrate has to be taken up by the cells. After the substrate is taken up, it is finally converted. Biodegradation of PAHs is restricted by their limited bioavailability, which is mainly associated with PAH hydrophobic nature and strong adsorptive capacity in soil (Thomas et al., 1986; Volkering et al., 1998). It has been reported that the mass transfer rate of PAHs into the aqueous phase is the rate-limiting step in their degradation (Stucki and Alexander, 1987; Pignatello and Xing, 1996).

The bioavailability of soil contaminants can be increased by stimulating the process that is limiting the rate of biodegradation (Chung et al., 1993). Stimulation of desorption and dissolution rates can be accomplished by all kinds of physical and chemical means. For instance, the temperature can be raised, soil might be pulverized to increase access and decrease diffusional distances, soil may be agitated, acoustic techniques may increase bioavailability, or soil organic matter may be oxidized using chemical agents (Bollag and Bollag, 1995). However, the most promising way to increase a contaminant's bioavailability is thought to be the addition of surface active agents such as surfactants that stimulate mass transfer rates.

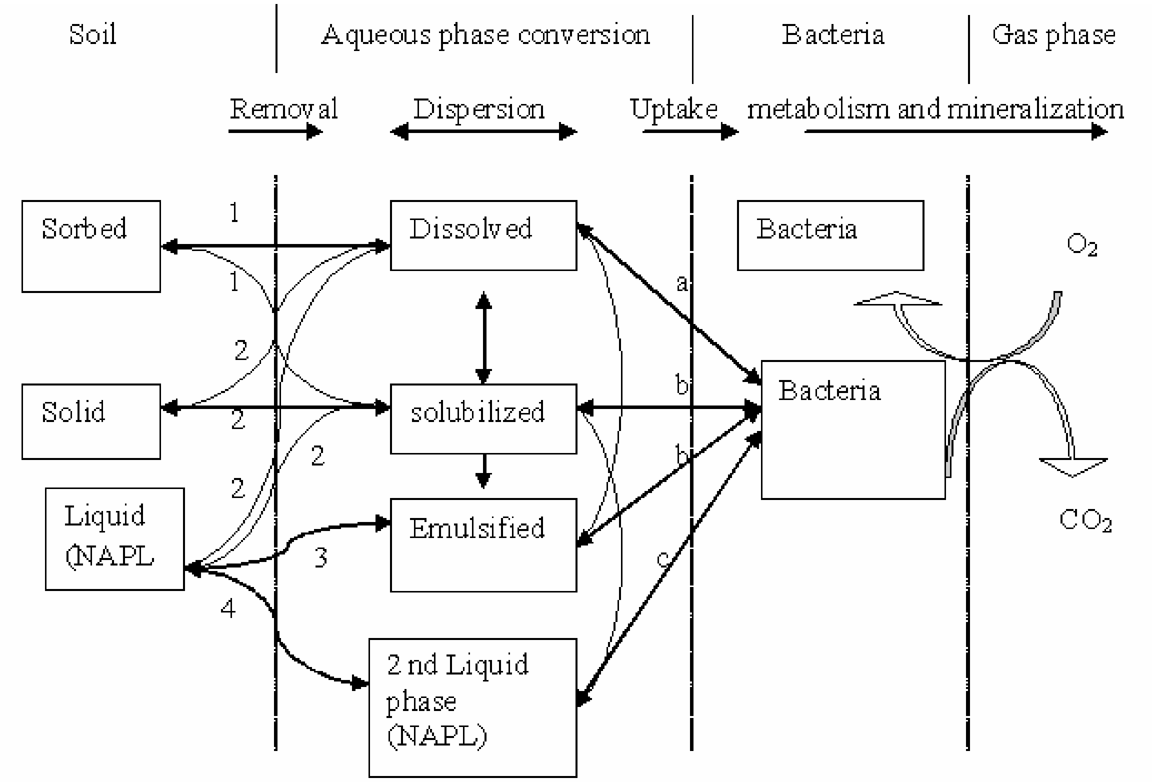

Figure 2.1: Processes involved in the biodegradation of contaminants that are initially present in soil. Processes involved in the transfer of compounds between the soil phase and the bulk aqueous phase: 1: desorption 2: dissolution 3: detachment 4: mobilization. Processes involved in the uptake of contaminants by cells: a: uptake of dissolved substrate; b: uptake of 'pseudo-solubilized' substrate; c: uptake of substrate by direct attachment of the organism to substrate droplets.

2.3 Surfactants

2.3.1 Characteristics of surfactants

Surfactants are amphiphilic compounds which contain hydrophobic and hydrophilic moieties. While hydrophobic groups (tail) have little affinity for polar solvents, hydrophilic groups (head) have strong affinity for polar solvents such as water and prevent the surfactant molecules from being completely separated from the polar solvent. At low concentration, surfactants are fully soluble in water. However, when the concentration is increased, in order to decrease the free energy of the system, hydrophobic groups begin to aggregate and build up the core of a cluster. This small aggregate is called a 'surfactant micelle' and the formation of micelles is called micellization. The lowest concentration at which micelles begin to form is called the 'critical micellization concentration' (CMC). The CMC becomes different according to temperature, surfactant type and structures. It was reported as a range of concentrations rather than a fixed value (Rosen, 1989).

2.3.2 Classification of surfactants based on origin

2.3.2.1 Synthetic surfactants

These are manufactured by the chemical routes. According to the nature of the head groups a surfactant can be classified into four types. The head group may have a positive charge (cationic), negative charge (anionic), both positive and negative charge (zwitterionic), or no charge (nonionic).

Anionic surfactants: Examples of anionic surfactant groups include sulfonic acid salts, alcohol sulfates, alkylbenzene sulfonates, phosphoric acid esters, and carboxylic acid salts. Anionic surfactants tend to be good solubilizers and are relatively nontoxic. They have been used in petroleum oil recovery operations as well as in contaminant hydrogeology remediation applications.

Cationic surfactants: Examples include polyamines and their salts, quaternary ammonium salts, and amine oxides. Cationic surfactants tend to be toxic and are therefore not widely used in environmental applications at this time. Cationic surfactants also tend to sorb to anionic surfaces and so can be severely retarded in groundwater systems.

Nonionic surfactants: Examples include polyoxyethylenated alkylphenols, alcohol ethoxylates, alkylphenol ethoxylates, and alkanolamides. Nonionic surfactants tend to be good solubilizers and are relatively nontoxic. They are usually easily blended with other types of surfactants (i.e., used as cosurfactants) and therefore have found widespread use in petroleum and environmental applications. The performance of nonionic surfactants, unlike anionic surfactants, is relatively insensitive to the presence of salts in solution. **Zwitterionic surfactants:** They can be anionic (negatively charged), cationic (positively charged) or non-ionic (no charge) in solution, depending on the acidity or pH of the water. They are compatible with all other classes of surfactants and are soluble and effective in the presence of high concentrations of electrolytes, acids and alkalis. These surfactants may contain two charged groups of different sign linked by a spacer which may be rigid or flexible, hydrophilic or hydrophobic, typically 2–8 bridging atoms (Menger et al., 1991, 1993). Whereas the positive charge is almost always ammonium, the source of the negative charge may vary (carboxylate, sulphate, sulphonate). These are mild with high foaming properties. An example of an amphoteric/zwitterionic surfactant is alkyl betaine.

2.3.2.2 Natural surfactants

Oleochemical surfactants: Oleochemical surfactants, also referred to as natural, are derived from plant oils such as palm, palm kernel or coconut oil or from animal fats such as tallow, lard or fish oil. Fish oil is no longer used as an oleochemical feedstock, and animal fats have lost ground in recent years. In contrast, vegetable oils have been gaining importance. But all these above surfactants are processed to gain the surface active property. These differ from the synthetic surfactants slightly by having linear carbon chains and even numbered, while synthetic surfactants may have branched carbon chains and contain even or odd numbers of carbon atoms. These differences may seem subtle, but they can have a significant impact on cleaning performance, especially in mixed surfactant systems.

Microbial surfactants: Many bacteria, yeasts, and fungi produce extracellular or membrane-associated surface active compounds, called ‘Biosurfactants’ (Hommel, 1990; Fiechter, 1992; Banat, 1995; Lin, S.C., 1996; Desai and Banat, 1997). The term biosurfactant refers to any type of compound produced by microorganisms with surface active or emulsifying properties (Rosenberg, 1986; Hommel, 1990; Fiechter,1992). Biosurfactants are of low and high molecular weight lipid complexes, which are normally produced by microorganisms by acclimation. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol (Mulligan, 2005). Most of the biosurfactants are anionic or nonionic and only a few are cationic such as those containing amine groups. The potential of biosurfactants in environmental applications has reviewed by some researchers (Volkering et al., 1998; Mulligan, 2005; Maier et al., 2000). Biosurfactants may be applied in foods and pharmaceutical products (Velikonja and Kosaric, 1993; Desai and Banat, 1997).

The structure of biosurfactant is a characteristic of the producing species and the availability of carbon source during the growth of the microorganisms (Zhang and Miller, 1995). The fact that many biosurfactants are produced even when the cells are not grown on hydrophobic substrates suggests that their function is not solely restricted to the stimulation of substrate availability (Bouchez-Naïtali et al., 1999). Another function might be to regulate cell adhesion to and cell detachment from surfaces, which controls mobility of the cells (Rosenberg, 1986; Busscher et al., 1996). Biosurfactants may function as antibiotics. The biocidic actions of rhamnolipid by Pseudomonas, mycolic acid-type biosurfactants from Actimomycetes, and of lipopeptides produced by Bacillus are well documented (Haferburg et al., 1986; Jenny et al., 1991).

Biosurfactants may also be used as anti-viral and anti-bacterial agents (Stanghellini and Miller, 1997; Vollenbroich et al., 1997; Lang and Wullbrandt, 1999). Biosurfactants might be used for enhancing the biotransformation rate of lipophilic compounds such as sterols or steroids when uptake by cells is rate limiting (Angelova and Schmauder, 1999). Advantages of biosurfactants over synthetic surfactants include the high structural diversity and concomitant diversity in properties, the possibility to produce these biosurfactants from renewable resources, and the fact that they are biodegradable. In addition, these compounds are natural, which facilitates the public acceptance of their application in the environment (Haferburg et al., 1986; Fiechter, 1992). Biosurfactants generally are more expensive than synthetic surfactants (Fiechter, 1992; Lang and Wullbrandt, 1999). Therefore, the use of purified biosurfactants is restricted to high added value products unless methods for cheap production and purification are developed.

Plant based surfactants: Surfactants from renewable sources like plant are gaining importance in recent years. The lipophile group of plant surfactants can come from oleochemical raw materials derived from rapeseed, sunflower, palm, or other plants. The hydrophile segment can come from co-products of the starch or sugar industries (sugar beets, derivatives of maize or other grain crops). They are used in hygiene products, cosmetics, pharmaceuticals, detergents and surfacing agents. The European market for surfactants represented 2.3 million tonnes in 2000, of which about 20 % were derived from plants. AGRICE (Agriculture for Chemicals and Energy) pertain to synthesis of new molecules with surfactant properties using environmentally friendly chemical processes and focusing on the development, simplification and optimization of chemical and microbiological synthesis of plant surfactants.

Natural plant derived surfactants mainly surface active agents like saponins without much processing are of main focus in recent years. Saponin glycoside from quillaja bark (Hong et al., 2002) and another palnt based nonionic surfactant derived from *Sapindus mukorossi,* commonly known as "Soapnut" or "Reetha" in the Indian subcontinent, has been tested to remediate contaminated soils (Kommalapati, 1995).

2.4 Surfactant solubilization

The surfactant aggregates allow partitioning of the hydrocarbons into the hydrophobic pseudophase of the micellar core (Miller and Bartha, 1989; Rouse et al., 1994). Surfactant micelles have received much attention because micelles are able to solubilize hydrophobic organic compounds which have low water solubility through incorporation of them into the hydrocarbon-like core of the micelle and partitioning of hydrophobic organic compounds between the micelle cores of surfactant and surrounding water. This phenomenon greatly enhances the total concentration of a compound in solution above its aqueous solubility, and is referred to as "solubilization" (Rouse et al., 1994). Micellar solubilization can be further enhanced as the hydrophobic chain length increases, and hence the size of micelle (Thangamani and Shreve, 1994). Solubilization also increases with reduction in the ionic charge of the hydrophilic end (Porter, 1991). But in some cases loose packing of micelle increase the solubility with bulk polar head group (Abe et al., 1987), more interaction results in solubility reduction due to tight packing of surfactants i.e allowing less solute into micelle core while surfactants enhance the removal of hydrocarbons by increasing their aqueous-plus-micellar “solubilities,” thereby facilitating transport across cell membranes and making them more available for degradation (Falatko and Novak, 1992). Studies have shown that application of surfactants result in a several-fold increase in the solubility of hydrocarbons (Zhang and Miller, 1992). Many of the most persistent contaminants especially PAH (poly aromatic hydrocarbons) exhibit low water solubility and hence, solubility of contaminants can often be improved by addition of surfactants.

2.5 Application of surfactants in remediation

The bioavailability of polycyclic aromatic hydrocarbons in soils is often limited by their low solubility and strong sorption to soil. As a way to increase the bioavailability of PAH contaminated soil, surfactant aided soil flushing has been considered for dissolving and mobilizing the soil bound hydrophobic contaminants (Moon et al., 2003; Ko et al., 1998; Yeom, 1996). Beginning with use by petroleum industries for oil recovery, surfactants have been applied in the field (Volkering et al., 1998) and research has been conducted on contaminated soil remediation applications (Willumsen and Karlson, 1998; Tsomides et al., 1995; Tiehmn et al., 1997). Three types of surfactants cationic, anionic and nonionic surfactants– have been considered for use in soil washing. Many researchers have reported that surfactants could increase the solubility and mass transfer of hydrophobic organic compounds (Volkering et al, 1998; Rothmel et al., 1998). The increased bioavailability with surfactant addition can be attributed to two main mechanisms based on the nature of contaminant. First, surfactants can reduce the interfacial tension between the aqueous phase and the non-aqueous phase liquid (NAPL). Therefore, the presence of the surfactant can disperse the NAPL and increase the contact area. Increased dispersion and contact area of the NAPL can give enhanced bioavailability for microorganisms. Second, the surfactant can increase the aqueous solubility of less soluble organic compounds significantly. Increased solubility of organic compounds can provide more available substrate for microorganisms. Schippers et al.

(2000) proposed three possible mechanisms to explain the enhanced biodegradation of PAH by the surfactant addition. The first mechanism is that bacteria might be able to utilize micellar solubilized / micelle portioned PAHs directly through the cell membrane. The second mechanism is that surfactant can increase mass transfer to the aqueous phase, and bacteria might subsequently use the aqueous phase PAHs. The third mechanism is that the surfactant might change the hydrophobicity of the cell surface, and the changed hydrophobicity might enhance the direct cell attachment to PAHs or NAPL.

In general, surfactants could enhance the apparent solubility of PAHs by micellar formation, which commences at the CMC and then solubility is proportional to surfactant concentration (Edwards et al., 1991). However, biodegradation of PAHs is not always correspondingly enhanced by surfactants. Some research groups have found that addition of surfactants stimulated PAH biodegradation (Aronstein and Alexander, 1992; Bury and Miller, 1993; Tiehm, 1994; Volkering et al., 1995; Boonchan et al., 1998), whereas others reported no effect (Laha and Luthy, 1991) or inhibition by surfactants (Guha and Jaffe, 1996; Boonchan et al., 1998). The contradictory results may be due to the varied interactions among PAH-degrading species, PAHs, and surfactants. Surfactants may be used as a growth substrate in preference to PAH compounds or toxic to some microorganisms, and hence PAH degradation would be reduced. If surfactants are neither toxic nor growth substrates, they can either enhance degradation of PAHs by solubilizing the PAHs inside the micelle that are accessible to microorganisms, or decrease degradation by preventing cells from directly contacting PAHs (Guha and Jaffe, 1996; Stelmack et al., 1999). By reducing surface and interfacial tension between liquids, solids and gases, allowing them to disperse readily as emulsions, chemical or biological surfactants may have variable effects on contaminant biodegradation (Banat et al., 2000).

2.6 Effects of surfactants on biodegradation of organic contaminants

2.6.1 Synthetic surfactants: uses and limitations

Numerous batch and column studies have indicated that surfactants enhance recoveries of non-aqueous phase lipids NAPL (Ellis et al., 1985; Soerens et al., 1992) by solubility enhancement or desorption. There have also been indications that pretreatment of a soil with surfactant washing (Igepal CA-720) to solubilize PAHs enhances biodegradtion of these contaminants (Joshi and Lee, 1995).

Table-2.2: Selected studies involving the use of synthetic surfactants to stimulate hydrophobic organic contaminant biodegradation (Makkar and Rockne, 2003).

Compound	Surfactant (b)	Medium	Surfactant conc.	Effect on biodegradation kinetics
Phenanthrene	Nonionic surfactants	Liquid	CMC	0
Phenanthrene	Nonionic surfactants	Liquid	> CMC	__
Phenanthrene, pyrene, fluoranthene, fluorene, and anthracene	Nonionic surfactants, sodium dodecyl sulfate	Liquid	>CMC	-
Naphthalene and phenanthrene	Triton X-100, Brij-35, Tergitol NPX, Igepal CA-720	Liquid	>CMC	+
Phenanthrene	Tween 80	Soil	>CMC	+
Naphthalene	Triton X-100, Brij-30,	Liquid	>CMC	+
Phenanthrene	Triton X-100, Triton X-102, Triton CF-21, Triton N-101, Brij-30, Brij-35, polyoxyethylene 10 laurylether, Tergitol 15-S-9	Liquid	>CMC	+
Phenanthrene	Tergitol NP-10, Tergitol 15-S-20, Tergitol TMN-10	Liquid	>CMC	-
Phenanthrene	Tergitol NP-10	Liquid	>CMC	+
Phenanthrene, pyrene, fluoranthene, fluorene, anthracene, and substituted naphthalene	Arkopal N-300, SapogenatT-300	Soil.	>CMC	+
Phenanthrene	Tween-40, Triton X-114, Brij-35	Soil slurry	<CMC	+
Naphthalene, phenanthrene, and pyrene	Triton X-100	Liquid	> CMC	+
Anthracene	Triton X-100, Dowfax 8390	Liquid	<CMC	-
Naphthalene and phenanthrene	Triton X-100	Liquid	>CMC	+/-
Total petroleum hydrocarbons	Igepal CO-630	Liquid.	>CMC	+/-/0
Pyrene	T10 and T15	Soil slurry	>CMC	+
Pyrene, chrysene, and Benzo [a] pyrene	Tween-80	Soil	>CMC	+
Aroclor 1242	L-Carvone, sodium dodecyl sulfate, sorbitan trioleate	Soil	100–180 mg/ml	+
Phenanthrene, acenaphthene, anthracene, fluorene, and pyrene	Triton X-100, Triton N-101, Brij-30, Brij-35	Liquid	>CMC	-

+ = beneficial effect defined as a significant increase in biodegradation rate and/or extent;

- = detrimental effect; 0 = no effect

Studies with synthetic nonionic and anionic surfactant additions have indicated that they can enhance/limit the biodegradation of soil xenobiotics and a range of other hydrocarbons (Table-2.2).Nonionic surfactants have also shown to inhibit biodegradation at concentrations above their CMC. Indeed many synthetic surfactants are known to exert an inhibitory effect on PAH-degrading microorganisms (Tsomides et al., 1995). However, the positive cases are counterbalanced by almost as many negative results.

Anionics and nonionic surfactants are less likely to be absorbed to the soil surface. Cationic surfactants have been used to lower aquifer permeabilities by sorption on to the aquifer materials (Westall et al., 1992). These are effective solubilizers, good desorption agents, emulsifying agents, suspending agents etc. In spite of above advantages these are having some limitations for use as some are toxic to soil microorganisms, more degradation time, some adsorb more to the soil, increased (pseudo) solubility (Singh et al., 2006).

2.6.2 Biosurfactants: uses and limitations

Bacteria that overproduce biosurfactants may have an important role in the biodegradation process (Ron and Rosenberg, 2002). In terms of physicochemical properties such as surface activity as well as pH and heat stability, many biosurfactants are comparable to synthetic surfactants (Georgiou et al., 1992). These have the following potential advantages i.e. these are made of cheap sources like microbes on cheap substrates / wastes, low critical micelle concentration (CMC), high biodegradability over synthetic surfactants and, therefore, are particularly well suited for environmental applications such as bioremediation and the dispersion of oil spills. (Leahy et al.,1990; Fiechter et al.,1992; Van Dyke et al.,1991). Some studies about solubility enhancement and biodegradation using biosurfactants raise a new alternative for the use of conventional synthetic surfactants in various environments (Table-2.3).

Due to some technical and/ or economic reasons biosurfactants have not been employed extensively in industry. Like most microbial metabolites, biosurfactants exist in fermentation broth of complex composition at relatively low concentrations, which often makes the costs associated with the isolation and purification of biosurfactants prohibitively high. To make the large-scale production of biosurfactants possible, it’s necessary to undergo the time consuming and labor-intensive strain improvement programs and medium optimization studies generally rely on the availability of efficient and specific analysis techniques for biosurfactants.

Table-2.3: Selected studies involving the use of biosurfactants to stimulate hydrophobic organic contaminant biodegradation (Makkar and Rockne, 2003).

Compound(s)	**Surfactant**	**Medium**	**Organism(s)**	**Effect on biodegradation kinetics (b)**
Crude oil	Emulsan	Liquid	Acinetobacter calcoaceticusRAG-1	0
14-16 C alkanes, pristine phenyldecane, and naphthalene	Sophorose lipid	Liquid	Mixed population	+
Hexachlorobiphenyl	Rhamnolipid	Soil slurries	Pseudomonas aeruginosa UG12	+
Octadecane	Rhamnolipid	Soil	Pseudomonas aeruginosaATCC 9027c	+
Hexachlorobiphenyl	Rhamnolipid, Emulsan	Soil	Pseudomonas aeruginosa, Acinetobacter calcoaceticusRAG-1	+
Aliphatic and aromatic hydrocarbons	Rhamnolipid	Soil	Pseudomonas aeruginosaUG12	+
Phenanthrene	Rhamnolipid	Soil slurries	Pseudomonas aeruginosa UG12	+
Metals, phenanthrene, andPCBs	Rhamnolipid	Soil	Pseudomonas aeruginosa ATCC 9027	+
Mixture of alkanes and naphthalene	Rhamnolipid and oleophilic fertilizer	Soil	Pseudomonas aeruginosa	+
4,4'-dichlorobiphenyl	Rhamnolipid	Soil	Pseudomonas aeruginosa	+
Phenanthrene, pyrene, and B[a]PSodium dodecyl sulfate and	Rhamnolipid	Soil	Pseudomonas aeruginosa UG2	--
Naphthalene	Rhamnolipid	Soil	Pseudomonas aeruginosa 19SJ	+
Naphthalene and phenanthrene	Rhamnolipid	Soil	Pseudomonas aeruginosaATCC 9027	+
Naphthalene and methyl naphthalene	Glycolipid andTween 80	Liquid	Rhodococcus sp H13A	+
Hexadecane and kerosene oil	Crude surfactin	Soil	Bacillus subtilis ATCC 2423	+
Crude oil	Biosurfactant	Liquid		0
Phenanthrene and hexadecane	Rhamnolipid	Soil	Pseudomonas aeruginosa UG2	+
Phenanthrene, fluoranthene, pyrene, B[a]P, and pentachlorophenol, endosulfan	Rhamnolipid, Crude surfactin	Soil Soil	Pseudomonas aeruginosa #64, Bacillus subtilis ATCC 2423 1, 1	+ +

Phenanthrene, fluoranthene, and pyrene	Alasan	Liquid	Acinetobacter radioresistensKA53	+
Aliphatic and aromatic hydrocarbons	Crude surfactin	Sea water	Bacillus subtilis O9	+
Phenanthrene	Sophorolipid	Soil	Candida bombicola ATCC22214	+
Phenanthrene and cadmium	Rhamnolipid	Soil	Pseudomonas aeruginosa ATCC 9027	+
Naphthalene and cadmium	Mono-rhamnolipid	Soil	Pseudomonas aeruginosaATCC 9027	+
Toluene, ethyl benzene,and butyl benzene	Di-rhamnolipid	Liquid	Pseudomonas	+

Unfortunately, for the most biosurfactants reported so far the techniques frequently employed for the detection of biosurfactants have been surface/interfacial tension measurements (Akit et al., 1981; MacDonald et al., 1981; Ramsay et al., 1988; Hommel et al., 1987; Persson et al., 1988). Many researchers have investigated the effects of biosurfactants on bioremediation and it is acknowledged that precise mechanisms are not easily elucidated in bioremediation systems because multiple variables are typically in play.

These studies that were performed with strains that take up dissolved substrate indicate that the effect of surfactants on biodegradation rates in these cases was exclusively caused by their effect on dissolution rates. When substrate is not taken up from the dissolved aqueous phase but by direct attachment of the cells to the substrate, surfactants may negatively affect uptake and biodegradation (Churchill, P.F. and Churchill, 1997). Surfactants render these substrate droplets hydrophilic and inhibit bacterial attachment (Efroymson and Alexander, 1991; Stelmack et al., 1999). The description of biodegradation rates based on independently determined mass transfer processes is more difficult for cells that take up substrate by attachment of the cells to the substrate than for cells that take up dissolved or solubilized substrate. The quantitative analysis of degradation kinetics for strains using the former uptake mode deserves further attention. Several studies have investigated the effect of surfactants on biodegradation of contaminants in field-contaminated soil, as reviewed by Volkering et al (1998). Volkering concludes that more positive results generally were found during experiments with field contaminated soil than with soil contaminated in the laboratory (Volkering et al., 1998). This might indicate that surfactants generally perform better during situations with lower bioavailability of contaminants. Few field scale applications of surfactants during bioremediation have been reported in Table-2.4 The ultimate objective of any surfactant enhanced remediation study should be beneficial to environment with easy biodegradability after the application, economical, must enhance the degradation of contaminants etc. Plant based surfactants are a novel alternative from conventional resources with satisfying the above objectives.

Table-2.4:. Summaries of surfactant soil flushing field tests.

Group	Process	Contaminant removed	Reference
SUNY-Buffalo with Dupont at Corpus Christi Site	Addition of 1% surfactant (approved in food preparation) Witconol 2722 and Tergitol 15-S012	73 gal carbon tetrachloride successfully removed in comparison to pump and treat	Fountain (1993)
Canadian Air Forces Base at Borden (SUNY)	2% mixture of surfactant added to sandy aquifer	80% of PCE recovered	Fountain and Hodges (1992)
General Motors NAO Research and Development Center	0.75% nonionic ethoxylated surfactant washing with recovery	10% of PCBs, oils removed in first test and 14% in second exceeding expectations	Ang and Abdul (1991)
University of Michigan	Injection of 4% Witconol and Tween 80 and modeling	Removal of 10% dodecane required 0.7 1 surfactant and 130,0001 water	Abriola et al. (1993)
Eckenfelder, Inc.	Injection of 2.5% SDS and recycle/reuse of surfactant	Injection of 7.7 pore volumes of 2.5% SDS removed as much PCB as 20 to 40 pore volumes of water (90% waste volume reduction)	Underwood et al. (1993)
US DOE Gaseous Diffusion SitePortmouth, OH	4% sodium dihexyl sulfosuccinate surfactant/4% isopropyl alcohol as cosolvent and 2% electrolyte (1:1 NaCl and $CaCl_2$)	DNAPLs, TCE with some PCBs and other chlorinated solvents, >90% removed by solubilization	Jafvert (1996)
Ecosites Inc., Estrie Reg. Shop, Que., Canada	Biodegradable nontoxic surfactant	Hydrocarbons, LNAPLs, "Cutting Oil", 160,000 kg hydrocarbon recovered in 12 months, US$ 1.2 million Cdn for fulll scale, commercial completed project	Jafvert (1996)
Fredicksburg, VA Wood treating site	Alkaline agent (0.5% Na_2CO_3), nonyl phenol surfactant with 10 mol ethylene oxide (0.1 % wt Makon-10) and polymer (1500 mg/1 xanthan gum)	Creosote-based wood treating oil (DNAPL), unable to inject fluid, poor site evaluation	Rice University (1997)

TWCC Biosolve Group	Patented non-hazardous, biodegradable surfactant (1-2% concentration)	80% removal of NAPL, 90% removal of transmission fluids in Northern California, increases biodegradation by 30%. Cost US$ 0.35-0.85 per gallon	USEPA (1998)
Hill Air Force Base, Layton, UT Cell 5, Surfactant Mobilization	6.6 PV of surfactant (2.2% Aerosol OT/ 2.1%Tween) and Electrolyte ($CaCl_2$) injected	LNAPL (JP-4 jet fuel, chlorinated and nonchlorinated VOCs, naphthalene, pesticides, PCBs, dioxins)	Jafvert (1996)
Cell 6, surfactant Solubilization	10 PV of 4.3% surfactant (Dowfax)	Mixture of VOCs, naphthalene, pesticides, PCBs, dioxins, JP-4 fuel	Jafvert (1996)
Cell 8, Surfactant/cosolvent solubilization	Surfactant (3.5% wt Brij 91) and cosolvent (2.5% wt *n*-pentanol), < 10 PV injected	LNAPL (JP-4 jet fuel, chlorinated and nonchlorinated solvents, PCBs), 72% average reduction	AATDF (1998)
OU2-Micellar Flood	0.6 PV of surfactant (7.5% sodium dihexyl sulfosuccinate), cosolvent (3.75% isopropyl alcohol) and electrolyte (7000 mg/1 NaCl)	chlorinated solvents (TCE, TCA, PCE, and TCET), petroleum hydrocarbons, DNAPL (70% TCE), 99% recovery of DNAPL	Jafvert (1996)
Volk Air National Guard Base, WI	9-14 PV of Adsee 799 and Hyonic PE-90 (50:50) blend	Hydrocarbons, chlorinated hydrocarbons (Dichloromethane, chloroform, TCA, TCE)	Nash (1988)

2.6.3 Plant surfactants: uses and limitations

Plant based surfactants with less purification steps are under spot light; efficient and found to be economical for contaminant remediation processes. Saponins of this class are glycosides widely distributed in the plant kingdom. They include a diverse group of compounds characterized by their structure containing a steroidal or triterpenoid aglycone and one or more sugar chains.Saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors (Price et al., 1987; Oakenfull, 1981; Fenwick et al., 1991; Hostettmann and Marston, 1995; Oakenfull and Sidhu, 1989) with their physicochemical (surfactant) properties (San Martin and Briones, 1999) and mounting evidence on their biological activity (such as anticancer and anticholesterol activity) (Gurfinkel and Rao, 2003; Kim et al., 2003b). Purified saponins and saponin mixtures resulted in both enhancements and reductions in water solubility of test compounds like quercetin (Schpke and Bartlakowski, 1997), digitoxin (Walthelm et al., 2001), rutin (Walthelm et al., 2001), and aesculin (Walthelm et al., 2001), the extent of which was determined by concentration of saponin and the model compound. For example solubility enhancement of quercetin obtained by pure saponins at concentrations > cmc values can be attributed to micellar solubilization, whereas solubilization effect of some saponin mixtures at concentrations < cmc points to an alternative mechanism (Schpke and Bartlakowski, 1997).

Solubilization properties of saponins well discussed in some remediation studies. Saponin from quillaja bark reported having potential for heavy metal removal from contaminated sites (Hong et al., 2002). While palnt based nonionic surfactant derived from *Sapindus mukoross,* has been tested to remediate contaminated soils.. Laboratory studies revealed its potential for remediation of contaminated soils by desorption and solubilization (Kommalapati et al., 1997). The recorded use of this product as common soap does not cite any toxic effects on human skin and eyes (Windholz, 1983). Along with all this surfactant showed better microbial growth with rapid degradation property both in aerobic and anaerobic conditions (Kommalapati and Roy, 1996, 1997). Limited use of these surfactant compounds are mainly due to their region specificity and are not available in all parts of the world.

2.7 Concluding remarks

Conventional bioremediation processes for PAHs contaminated soil have been known to have limitations because of the low solubility and strong sorption of PAHs to soil. In order to overcome the limitation of bioavailability, surfactant aided PAH bioremediation has been considered over the last few decades. However, even though much research has been conducted on surfactant aided PAH remediation; bacterial responses to synthetic surfactants have not produced any robust results. Bisurfactants may be an alternative, but are used limited in bulk and practical applications. From economical and engineering points of view, it is crucial to know the biological response of surfactant addition on PAH degrading bacteria in order to develop practical surfactant enhanced bioremediation strategies. Natural surfactants from plant origin are cheap, abundant, eco friendly and are easily biodegradable than synthetic surfactants.Much more research is required to make the application of natural plant surfactants a standard tool in biological soil and water remediation.

2.8 Motivation

Surfactants from renewable sources are of increasing interest in recent years and the potential of plant based surfactants from various sources are not fully explored. *Sapindus mukorossi* known as Reetha is widely grown in tropical countries like India and has surface active properties. Although its incorporation is seen in some herbal and toilet formulations; its use in remediation of organic contaminants like PAHs focused very little. It will be healthy if it's PAHs solubilizing and easy degradation properties are effectively utilized. The incorporation of this cheap surfactant with synthetic surfactants can limit the use of synthetic surfactants which are costly and are environmental hazards if used in bulk scale.

2.9 Research objectives

The overall objective of the project is to study the mixed micellar, solubilization, and PAH biodegradation behavior of plant based natural surfactant from *Sapindus mukorossi* (Reetha) with synthetic surfactants for the application of polycyclic aromatic hydrocarbons remediation.

The specific objectives of this study are:

- To know naphthalene solubilization efficiency of natural surfactant and comparative studies with synthetic pure surfactants.
- To elucidate the mixed micellar behavior of Reetha – synthetic mixed surfactants.
- To know the solubilization behavior of naphthalene in presence Reetha – mixed surfactant systems and to correlate that with mixed micellar behavior.
- To observe the biodegradation of naphthalene in presence of Reetha, synthetic and Reetha – synthetic surfactant mixtures.
- Evaluate and estimate the degradation potential of mixed cultures in presence of Reetha – synthetic surfactant systems along with pure surfactants.

Chapter 3

Solubilization of naphthalene in presence of Sapindus mukorossi – synthetic mixed surfactant systems.

3.1 Introduction

Surfactants flushing emerged as new alternative to treat PAH contaminated soil and water systems and many laboratory studies are reported (Zhu and Feng, 2003). Surfactants in aqueous solution solubilize the water insoluble hydrophobic compounds in the core of the micelles (Edwards et al., 1991). The effectiveness of a surfactant in solubilizing a particular solute is known as MSR, expressed by (Edwards et al.,1991).

$$\mathrm{MSR} = (S - S_{CMC})/\,(C_{S-CMC}) \qquad (3.1)$$

Where S and S_{CMC} are the apparent solubility of an organic compound at surfactant concentration C_S (C_S > CMC) and that at the CMC respectively. Thus, when solute concentration in solvent phase is plotted against surfactant concentration above the CMC, MSR can be determined from the slope of the linearly fitted line.

Mixed surfactants systems for the remediation of organic contaminants gaining more and more importance over the single surfactants in recent years, due to its better performance than the single surfactants (Paria and Yuet, 2006; Zhu and Chiou, 2001). The solubilization performances of organic compounds in mixed surfactants aiming for remediation application are mostly laboratory scale and limited than that of single surfactants (Zhu and Feng, 2003; Paria and Yuet, 2006; Zhu and Chiou, 2001; Dar et al., 2007; Mohamed and Mahfoodh, 2006; Zhou and Zhu, 2004). However, till now there is a very little information available on their fate in sub surface. The surfactants left in sub–surface after the remediation process may have a negative or positive influence on the biodegradation of the organic compounds (Liu et al., 1995). The non–biodegradable synthetic surfactants along with organic contaminants become hazard to the environment. In this case, the surfactant toxicity and it's potential to be carefully considered prior to the selection of surfactants for soil and water bodies cleanup (De Oude, 1992). Biosurfactants are found to be promising recently, due to its environmentally friendly nature; however, their use has been limited in industry due to some technical and/or economic reasons. Natural surfactants from plant origin may gain more importance in future, but very few studies have been done employing them for hydrophobic contaminants removal from the soil (Kommalapati et al., 1997).

Plant based natural surfactant derived from *Sapindus mukorossi*, commonly known as "soapnut" or Reetha is a saponin, a non–ionic glycoside contains sugars such as D–glucose, D–xylose, L–arbinose, L–rhamnose and glucoronic acid (Row and Rukmini, 1966). Solubilization studies using Reetha surfactant exposed its remediation potential on different hydrophobic contaminants in soil and water systems (Balakrishnan et al., 2006; Kommalapati and Roy, 1997) along with efficient biodegradable property under both aerobic and anaerobic conditions (Kommalapati and Roy, 1997; Kommalapati and Roy, 1996).

In this study, we have studied the solubilization of naphthalene in presence of Reetha in different proportions with cationic, nonionic and anionic synthetic surfactants, which has not been reported to the best of our knowledge. The objective of this paper is to evaluate the efficiency of Reetha surfactant and synthetic–Reetha surfactant combinations to solubilize naphthalene in aqueous systems using batch studies for the application of surfactant enhanced remediation soil and other applications like detergency and industrial cleaning etc. As the synthetic surfactants hinders biodegradation process (Guha et al., 1998; Yuan et al., 2000), that used in remediation process or in laundry cleaning creates lots of environmental problem. The use of only natural surfactant or synthetic–natural surfactant mixture enables easy degradation. Nowadays, many countries are trying to focus on the biosurfactants from microorganism as a substitute of synthetic surfactants, but in our view, production of plant based surfactant is very easy in compare to biosurfactants and huge amount of plant surfactant production is possible to substitute synthetic surfactants. In that respect, it is worthy to give more insight in this area.

3.2 Experimental section

3.2.1 Materials: The surfactants TX–100, CTAB, AOT, and SOS were obtained from Loba chemie Pvt Ltd, India, Sodium dodecyl benzene sulfonate (SDBS) from Sigma Aldrich, Germany; Naphthalene from Spectrochem Pvt. Ltd, India. All the chemicals were used as received. Dry fruit (Reetha or *S. mukorossi*) was purchased from the local market, Rourkela, Orissa, India. The structures of surfactants used in this study are shown in the Figure-3.1 Ultra pure water (Sartorius AG Gottingen, Germany) was used for the experiments of 18.2 mΩ resistivity and pH 6.8 – 7. Surface tension of surfactant solutions were determined by Whilmey plate method using a surface tensiometer, Data Physics, Germany (DCAT–11EC). Naphthalene concentrations were measured by UV–Vis. spectrophotometer (Jasco, Japan, V–530). The organic content of the plant surfactant solution was measured by total organic carbon analyzer (Shimadzu, Japan, TOC–VCPN).

3.2.2 Extraction of Reetha surfactant from the fruit: After removing seed from fruit, the pericarp was dried in an oven at 50 °C for about 48 hours and then powdered in a grinder. The powdered sample was extracted with methanol gave brown syrup. The suspended particles were separated by centrifugation at 8000 rpm for 15 – 20 min. and followed by vaccum evaporation and drying of the extract at 60 – 80 °C which gave a light yellowish white colored powder.

3.2.3 Methods: All the solubility experiments were done in the poly propylene falcon tubes of capacity 15ml. A stock solution of naphthalene (400mM) was prepared by dissolving naphthalene in pure methanol is preferred over higher alcohols because of its negligible effect on solubilization and CMC values (Rosen, 2004). For experiment 0.1ml naphthalene from the stock was added to10ml surfactant solution of a specified strength in falcon tube, as 1% methanol will not affect the solubility of PAH (Edwards et al., 1991; Paria and Yuet, 2006). The concentration and volume of the stock naphthalene solution used in each experiment was selected to (i) keep the methanol concentration in the test solution to below 1 vol% and (ii) to give an excess of naphthalene in the test solution. The tube was sealed with a screw cap to prevent volatilization loss of naphthalene from water. For the study 24 hr equilibration time was taken as kinetics of solubilization show no change in solubility of naphthalene (Paria, 2006), after 24 hr equilibration period and subsequent centrifugation at 5000 rpm for 30 min to separate the suspended solids, if any, the concentration of naphthalene was determined by measuring the absorbance at 274 nm wave length with a quartz cuvettes of 10mm path length using an UV–Vis spectrophotometer. Note that subsequent dilutions were made where ever needed and surfactant concentration was kept the same in both the reference and the measurement cells to minimize the effect of surfactant on UV absorbance. All experiments were performed at room temperature, 25 – 28 °C. Experiments were done in triplicate and the average values are reported.

3.3 Results and discussion

3.3.1 Structure and physical properties of Reetha solution

The structure of Reetha is most important to know before doing the experiment. Huang et al., 2003 have proposed the structure of saponin isolated from *S. Mukorossi* is shown in Figure-3.1 They have mentioned that depending on the R group seven different structures are possible. In our sample total carbon content was analyzed using TOC analyzer and found the value of 152.5 ppm for 0.03 wt. % solution. Back calculating the TOC of all the probable structure proposed by Huang et al., 2003 our value shows less error when we consider the R group given in Figure-3.1 and with a molecular weight of 966. We have also compared our TOC value with the reported value by Kommalapati and Roy, 1997 was 41 g/l for 10 % solution that is close to our value 49.24 g/l. The CMC of Reetha solution found was around 0.05 wt % (0.510 mM) as measured by surface tensiometer which is comparable with the reported value 0.045 wt % (Balakrishnan et al., 2006). The CMC value obtained from solubilization curve (0.517 mM) is also shows good agreement with the surface tension data. pH of 1 % solution was 4.34 close to the reported value of 4.5 (Kommalapati and Roy, 1997).

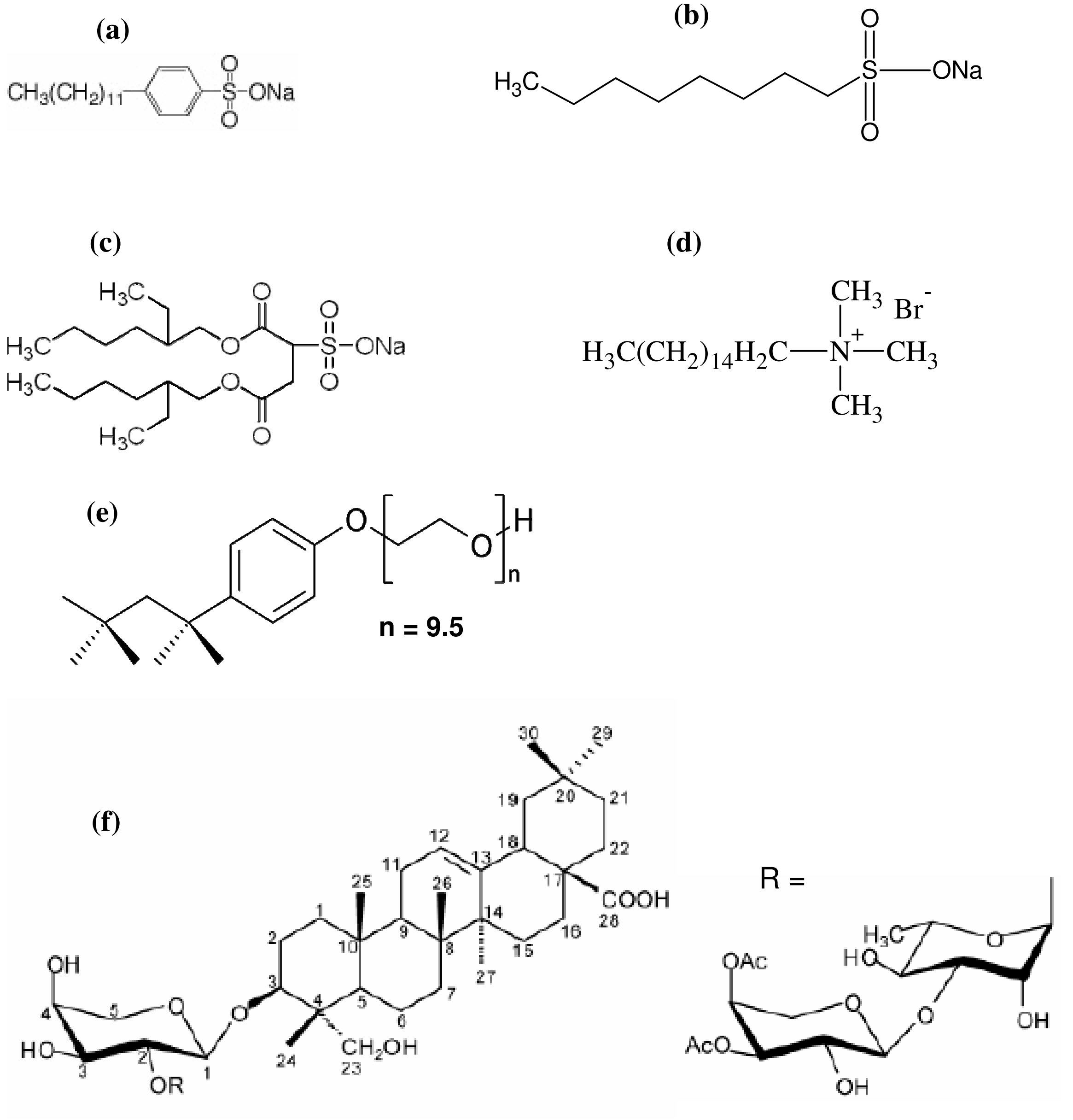

Figure-3.1: Structures of surfactant molecules used in this study (a) SDBS, (b) SOS, (c) AOT, (d) CTAB, (e) TX -100, and (f) Reetha.

3.3.2 Solubilization by single surfactants

In this section we have mainly focused on the solubilization behavior in presence of mixture of Reetha and different synthetic surfactants. Before studying the mixed systems, single surfactants were studied to get an idea about the effectiveness of different synthetic surfactants (TX–100, CTAB, SDBS, SOS, and AOT) with respect to Reetha. The solubility of naphthalene in presence of synthetic surfactants and Reetha is shown in

Figures-3.2 (a) - (d). MSR values of the single surfactant systems are calculated from the slope of the linear portion of the experimental data (Surfactant concentration in mM vs. naphthalene concentration in mM) given in Table-3.1. The effectiveness of solubilization can also be expressed in terms of the other parameter like partition coefficient (K_m). The partition coefficient is defined as partition of the organic compound between micelles and the aqueous phase, can be written as

$$K_m = X_m/X_a \tag{3.2}$$

where X_m and X_a are the mole fractions of solute in micelles and the aqueous phase, respectively. The value of X_m can be calculated as X_m = MSR/(1 + MSR), and X_a can be expressed as $X_a = S_{CMC}V_w$, where V_w = 0.01805 L/mol is the molar volume of water. Consequently, eq. (2) can be rearranged to yield

$$K_m = \frac{MSR}{S_{CMC}V_w(1+MSR)} \tag{3.3}$$

The following order of MSR and log K_m values are observed: TX–100 > CTAB > Reetha > SDBS > AOT > SOS, shown in Table-3.1. The difference between Reetha and SDBS is found very close. The increasing order of CMC values of these surfactants are: TX–100 < Reetha < CTAB < SDBS < AOT < SOS. In general, lower the CMC values higher the solubilization efficiency of the surfactants. Here, except Reetha, all the synthetic surfactants studied are followed the CMC rule. Efficiency of Reetha is expected after TX–100 but practically it is just after CTAB. In general, it is reported, non ionic surfactants are better solubilizing than ionic surfactants, because of their lower CMC. Even, solubilization capacity of a nonionic surfactant of polyoxyethylene type for water insoluble materials is much greater than that of an ionic surfactant having the same hydrocarbon chain length as that of the nonionic surfactant, this fact suggests that the polyoxyethylene part in the molecule also plays an important role in solubilization (Tokiwa, 1968). Between cationic and anionic surfactants with similar chain length cationic surfactant shows greater solubilization efficiency of PAHs due to adsorption at micellar water interface in addition to solubilization in the inner core of the micelle (Paria, 2006). Here, though the CMCs of CTAB and SDBS are very close, due to chain length difference and cationic nature, CTAB shows higher solubilization efficiency. Less solubilization efficiency of Reetha than expected based on the CMC value is attributed in terms of complex structure of the micellar core due to bulkiness of both the hydrophobic (tail) and hydrophilic (head) group. Hydrophobic part is consisting of five six member cyclic hydrocarbon rings resulting shorter effective chain length and less hydrophobic in nature. As a general rule, incorporation of a phenyl group in the alkyl group increases its hydrophobicity to a much smaller extent than increasing its chain length with the same number of carbon atoms (Tedros, 2005). Another reason may be the presence of carboxylic group in the hydrophobic region reduces the solubilization of naphthalene molecule in the inner core due to repulsion between the carboxylic group and π–electron cloud of naphthalene ring. Among the synthetic surfactants TX–100 and AOT have branched chain hydrocarbon tail and others are having straight chains, as a result, their core region is not as complicated as Reetha and more hydrophobic in nature due to presence straight or branched chain hydrocarbon tail. Finally, the micellar aggregation number also plays an important role in the solubilization. The decreased aggregation number in the micelles causes reduced solubilization of nonpolar substances (Rosen, 2004). The reported values of micellar aggregation number of the surfactants TX-100, CTAB, SDBS, AOT, SOS, and Reetha are ~ 145 (Tummino and Gafni, 1993), ~ 60 (Saroja et al., 1999), ~ 28 (Cheng and Gulari, 1982), ~22 (Umlong and Ismail, 2005), ~ 25 (Tarter, 1955), ~ 21 (Balakrishnan et al., 2006) respectively. All the synthetic surfactants show the increasing solubility order according the order of micellar aggregation numbers. Although Reetha is having lowest micellar aggregation number but showing higher solubility than SDBS, AOT, and SOS due to its low CMC value. Lower micellar aggregation number indicates lower solubilization capacity per micelle, but at the same time lower CMC indicates more number of micelles present at a particular concentration. Since SDBS, AOT, and SOS are showing higher CMC than Reetha, they are having lower solubilization efficiency.

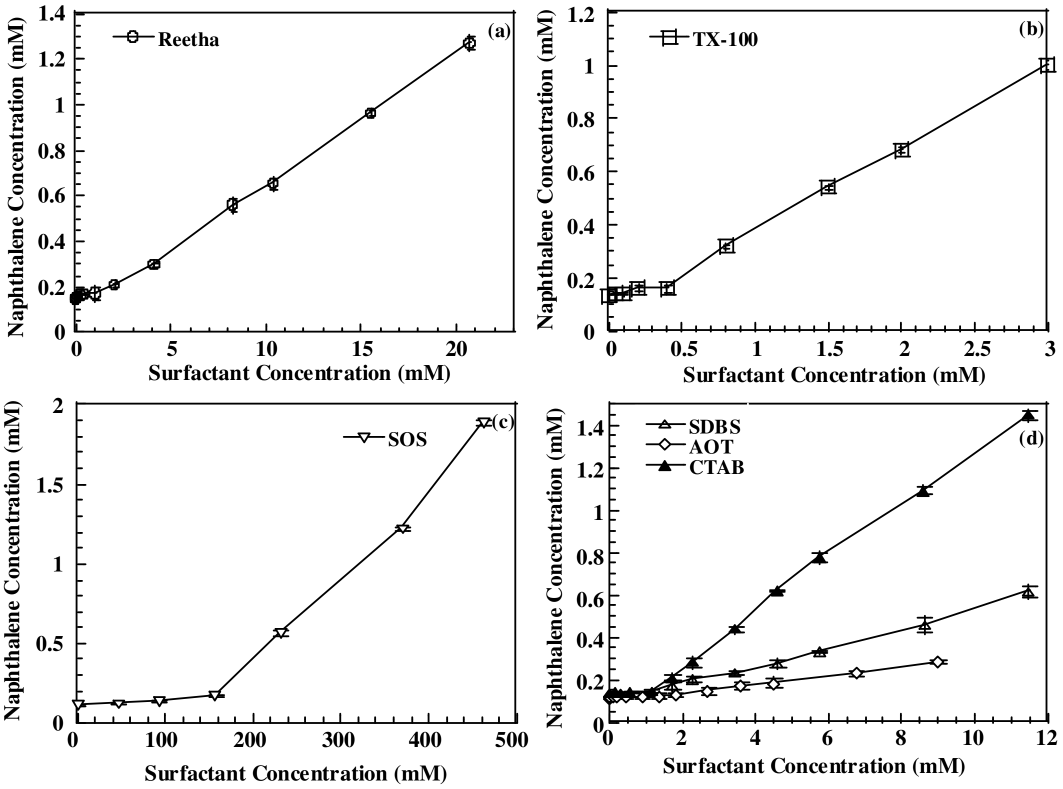

Figure-3.2: Solubilization of naphthalene by using surfactants (a) Reetha, (b) TX–100 (c) SOS, (d) SDBS, CTAB, and AOT.

3.3.3 Solubilization by surfactant mixtures

Solubilization of naphthalene by aqueous surfactant mixtures composed of Reetha with TX–100, CTAB, SDBS, AOT and SOS are done by taking different mole proportions of surfactants and compared with those by single surfactants. The plots on change of naphthalene solubilization in presence of mixed surfactant systems are shown in Figure-3.3. Table-3.1 summarizes different parameter values of mixed surfactant systems like mixed CMC, MSR, and log K_m. Mixed CMC values reported here are obtained from the surface tension data; there is also a good agreement with the values obtained from solubilization plot. It is observed from the Table-3.1 that mostly the MSR values of mixed systems are in between that of the single surfactants. Figure-3.4 (a) depicts the effect of change in MSR values of different synthetic surfactants in presence of Reetha. The Figure clearly indicates the MSR values changing nonlinearly with the change of mole fraction of Reetha (X_R), in further, that is an indication of nonideal behavior of mixed surfactant systems. To measure the nonideality and also to see the nature of deviation (positive or negative) we have calculated the deviation ratio. The Deviation ratio is calculated by

$$R = MSR_{exp}/MSR_{ideal} \tag{3.4}$$

Where, MSR_{exp} is the experimentally determined MSR and MSR_{ideal} is the MSR of naphthalene in a binary surfactant mixture based on ideal mixing, i.e.

$$MSR_{ideal} = MSR_1X_1 + MSR_2X_2 \tag{3.5}$$

Where, X_1, X_2 mole fraction of surfactant 1 and 2 respectively, MSR_1, MSR_2 are MSR values of pure surfactants 1 and 2 respectively. The values of $R > 1$ indicate positive deviation and $R < 1$ indicate negative deviation. The change in R with the mole fraction of Reetha is shown in Figure-3.4 (b).

3.3.3.1 Mixing effect of nonionic – Reetha surfactants: The mixing behavior of TX–100 – Reetha systems are depicted in Figures-3.4 (a), (b), and Table-3.1. The MSR and log K_m values with different mole fractions of Reetha show the values are decreasing with the increase in mole fraction of Reetha (X_R). The results indicate solubilization follow almost ideal behavior with small deviation for this combination of surfactants. From the table it is clear the deviation ratio (R) is close to 1 for most of the compositions and % change in MSR (Δ_{MSR}, $(MSR_{exp} - MSR_{ideal}) \times 100/MSR_{ideal}$ or $(R-1) \times 100$) is less than ~ –14%. The maximum Δ_{MSR} is observed at $X_R = 0.07$. It is also observed that MSR values are changing linearly (with correlation coefficient of linear fit above 0.97 and above 0.99 if the point $X_R = 0.07$ is excluded) for this combination with the change of Reetha mole fraction, an indication of ideal behavior.

3.3.3.2 Mixing effect of cationic – Reetha surfactants: Similar to the previous combination the MSR and K_m values for CTAB – Reetha combination also mostly decreased with the increase in mole fraction of Reetha (see Figures-3.4 (a), (b) and Table 3.1). Initially, with very low concentration of Reetha Δ_{MSR} is positive and above Reetha mole fraction of 0.14 the value becomes negative. The maximum values of positive and negative change occurred ~ 2 % ($X_R = 0.14$) and ~ –18 % ($X_R = 0.77$) respectively. Similar trend is also observed for log K_m.

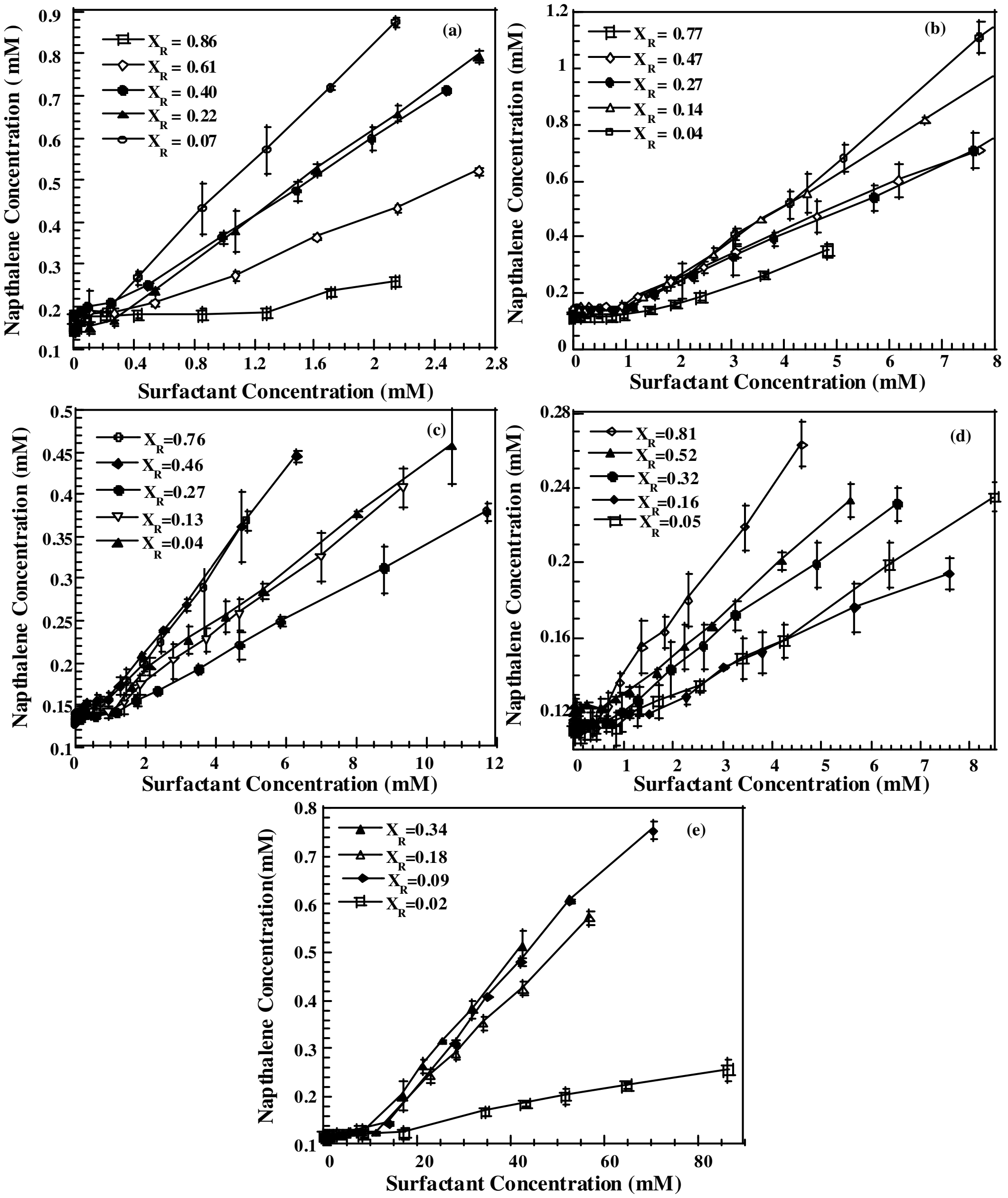

Figure-3.3: Solubilization of naphthalene in presence of (a) TX–100 + Reetha, (b) CTAB + Reetha, (c) SDBS+Reetha, (d) AOT+Reetha, (e) SOS+Reetha mixed systems.

3.3.3.3 Mixing effect of anionic – Reetha surfactants: We have studied three anionic surfactants SDBS, AOT, and SOS with a variation of chain length and structure. The change in MSR values with X_R is not following linear relationship indicating the nonideal behavior for all the cases. It is observed from the Figure-3.4 (b) that the nature of change in R for SDBS is in reverse order to that of CTAB with respect to mole fraction of

Reetha. We will explain this in detail in the section 3.5. The negative deviation for SDBS is maximum (–39.68 %) at X_R = 0.04. Negative deviation is observed up to ~ 0.5 mole fraction, after that the deviation is positive with a very low value of maximum 1.48 %, i.e. close to ideal behavior. In contrast to previous surfactants (TX–100, CTAB, and SDBS) AOT shows overall negative deviation for all the compositions with more negative values and a maximum of – 53 % at X_R = 0.16. Similar to AOT, SOS also shows over all negative deviation. Among all the surfactants studied here SOS shows highest negative deviation of – 78.43 % at X_R = 0.09.

Now, by comparing all the results of mixed surfactant systems we can conclude that ascending order of Δ_{MSR} are TX–100 – Reetha < CTAB – Reetha < SDBS – Reetha < AOT – Reetha < SOS – Reetha, which is the reverse order of sequence that of single surfactants for solubilization or same sequence of CMC. From the Table-3.1 it is worthy to note that when the synthetic surfactants are having higher MSR than Reetha, the mixed systems are showing lower MSR than only synthetic surfactant. Similarly, when the synthetic surfactants are having lower MSR than Reetha then the mixed systems are showing higher MSR than only synthetic, but the negative deviations indicate lower MSR than expected or calculated considering ideal mixing.

3.3.4 Effect of surfactant mixtures on micelle–water partition coefficient

Treiner et al., 1998 have suggested the following relationship for the partitioning of a neutral nonpolar organic solute between micelle and aqueous phase in a mixed surfactant systems as

$$\ln K_{\mathrm{m1,2}} = X_{\mathrm{m1}} \ln K_{m1} + (1 - X_{\mathrm{m1}}) \ln K_{m2} + B X_{\mathrm{m1}}(1 - X_{\mathrm{m1}}) \qquad (3.8)$$

Where K_{m1}, K_{m2}, and $K_{\mathrm{m1,\,2}}$ are the micelle – water partition coefficients of a solute for the single surfactants constituting the mixed micelle and mixed systems respectively, X_{m1} is the micellar mole fraction of surfactant 1. B is an empirical parameter includes surfactant – surfactant (like β) and surfactant – solute interactions. The value, $B = 0$ indicates there is no mixing effect of surfactants on the partition of the solute (Zhou and Zhu, 2004), if $B > 0$ or $B < 0$ implies that K_{m} in the mixed surfactant system is larger or smaller respectively than predicted by ideal mixing rule (Dar et al, 2007). X_1 can be calculated using mixed micellar theory discussed in the next section.

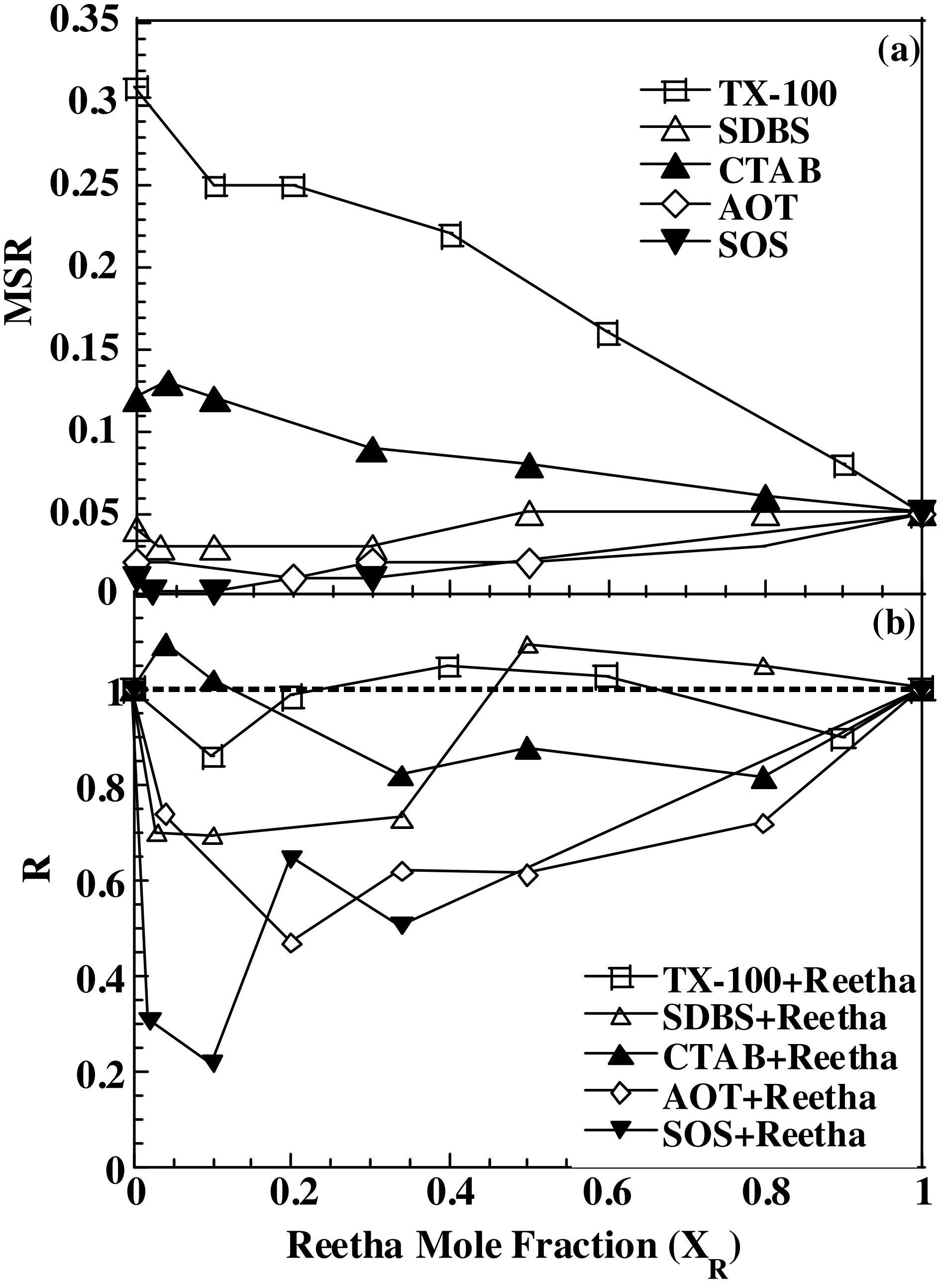

Figure-3.4: Comparison of (a) MSR, (b) Deviation ratio, R of naphthalene as a function of mole fraction of Reetha for different mixed surfactant systems.

The *B* values are calculated from the above relation for nonionic – Reetha, cationic – Reetha, and anionic – Reetha mixtures and presented in Table-3.1. The average *B* values obtained for napthalene in TX–100 – Reetha, CTAB – Reetha, SDBS – Reetha, AOT – Reetha, and SOS – Reetha mixed surfactant systems are 0.77, 0.40, −1.86, −3.21, and −10.701 respectively. The *B* values are found to be negative except TX–100 and CTAB – Reetha mixtures and the positive values are also less than one. According to the values of *B*, the mixing effect of anionic – Reetha surfactants on the partition of naphthalene is negative in SDBS – Reetha, AOT – Reetha, SOS – Reetha mixed systems and consistent with the result of negative deviation of MSRs from the ideal, which can be used to explain the mixing effect of anionic – nonionic surfactants on the solubilization of naphthalene. Similarly, that of solubilization of pyrene reported by Zhou and Zhu, 2004 for anionic – nonionic systems as they got positive *B* value and positive deviation of MSR. However, mixtures of TX–100 – Reetha, and CTAB – Reetha show the partition of naphthalene is positive, which are contradictory with the some negative deviation of MSRs for some combinations as shown in Table-3.1 with different mole fraction of Reetha from the ideal mixture. Similar contradictory results were also found before in other studies (Zhou and Zhu, 2004; Edwards et al., 1994; Tokuota et al., 1994). Hence, these results indicate some times the mixing effect of surfactants on the $K_{m1,2}$ is contrary to that on the solubilization of organic compounds in mixed surfactants.

3.3.5 Solution properties of mixed surfactant systems

The results of solubilization in the mixed surfactant systems indicate the behavior is complex in nature and mostly follow nonideal path. Since solubilization is closely associated with the solution properties of the surfactant micelles, to explain the above results we have studied mixed micellar properties of all the combinations. The mixed CMC values obtained from the experiments are reported in Table-3.1. To know whether the mixed solution follows ideal or nonideal behavior mixed CMC values were calculated using ideal solution theory (Clint, 1975).

$$\frac{1}{CMC_{12}} = \frac{\alpha_1}{CMC_1} + \frac{1-\alpha_1}{CMC_2} \tag{3.6}$$

Where CMC_1, CMC_2, CMC_{12} are the critical micelle concentrations of Reetha, pure synthetic surfactant, and mixed surfactant. α_1 mole fractions of component 1 in mixed surfactant solutions. The Table-3.1 clearly indicates that there is a deviation between the ideal and experimental CMC values. The mixed CMCs of TX–100, CTAB, SDBS and AOT with Reetha surfactant are intermediate between the respective individual surfactants used in the combination. In the case of TX–100 – Reetha mixture the experimental values are close to ideal, and in the other cases experimental CMC values are lower than the ideal values, indicating negative deviation from the ideal behavior for mixed micelle formation. The data for the other surfactants show there is a difference between the experimental and theoretical CMC values and the deviation is highest for SOS – Reetha mixture. In further, for nonideal binary mixture of surfactants Rubingh's equation of regular solution theory can be used to calculate the micellar mole fraction (Rubingh, 1977).

$$\frac{x_1^{\,2}\ln\left(\dfrac{\alpha_1 C_{12}}{x_1 C_1}\right)}{(1-x_1)^2\ln\left(\dfrac{(1-\alpha_1)C_{12}}{(1-x_1)C_2}\right)}=1 \tag{3.7}$$

The micellar mole fraction (x_1) can be calculated from equation 3.7 by solving iteratively. To show the interaction between two surfactants, β is an interaction parameter which indicates the interaction between two surfactant molecules in the mixed micelle and is a measure of deviation from the ideal behavior. The parameter β can be now calculated by substitiuting the value of x_1 in the equation below

$$\beta=\frac{\ln\left(\dfrac{\alpha_1 C_{12}}{x_1 C_1}\right)}{(1-x_1)^2} \tag{3.8}$$

The β parameter quantitatively captures the extent of non–ideality for a mixed surfactant system. The negative value of β indicates synergism in mixed micelle formation, a positive value indicates antagonism, and if zero, then mixed micelle formation is ideal. The larger the absolute value of β, stronger the mixing non–ideality. The negative deviation indicates a reduction in free energy of micellization over that predicted by ideal solution theory. The larger negative value of β denotes the greater negative deviation of CMCs from ideal mixture. All the surfactants used here show a synergistic effect in the mixture as it satisfied the conditions (i) β must be negative (ii) $\ln(\mathrm{CMC}_1/\mathrm{CMC}_2) < \beta$ (Zhou and Zhu, 2005). The extent of deviation from ideality is different for various surfactant combination used as mentioned in Table-3.1. From the Table it is very clear that β values are not constant for all the compositions in a particular binary mixture and some compositions the variation is more. In addition, it is also observed that β values are very much sensitive to x_1, even; small change in second or third decimal level in x_1 can give a very significant change in the β parameter. The average β values for TX–100, CTAB, SDBS, AOT, and SOS are –0.560, –0.458, –0.511, –1.022, and –4.178 respectively indicating the ascending deviation order as CTAB < SDBS < TX–100 < AOT < SOS. These sequences are almost following the same as of Δ_{MSR} except TX–100. Actually, the values are very close for TX–100, CTAB, SDBS and since the β parameter is very sensitive to x_1 value, the change in sequence from the expected may be due to that. In summary, for the combinations studied here indicate if the β parameter is more negative, Δ_{MSR} also more negative. From the β parameter values it can be attributed that AOT and SOS are having more interaction with Reetha in the mixed micelle, as a result surfactants molecules are more closely packed in the mixed micelle than the pure micelle. Since it reported that looser packing of surfactant molecules in the mixed micelle may increase the solubilization of organics in the micellar phase (Venable and Nauman, 1964; Abe et al., 1987; Roy et al., 1995; Schott, 1967), in our study we have also found similar observation. More closely packed surfactant micelle resist to enter the naphthalene molecule from aqueous phase to micellar phase by diffusion that ultimately may reduce the solubilization efficiency. When the interaction parameter is more, micelles are closely packed and lead to less solubilization and greater deviation from the predicted values. It is also observed, for the ionic surfactants deviation is more than nonionic, may be due to closer packing of ionic micelle in presence of nonionic Reetha due to reduction in head group repulsion.

To explain the results of Figures-3.4 (a), (b) we have plotted β and Δ_{MSR} vs. mole fraction of Reetha are shown in Figures-3.5 (a), (b). For CTAB – Reetha mixture decreasing Δ_{MSR} at high Reetha mole fraction is attributed in terms of the following reasons. (i) With increasing the Reetha mole fraction as interaction parameter value (β) increases, micelles are closely packed, (ii) presence of more nonionic group in the micelle shield the cationic charge of the micelle at water micelle interface and in turn, reduce the solubilization by adsorption at the micellar surface, (iii) micelle consists of more Reetha molecules which has lower slolubilization capacity. For SDBS – Reetha composition we have observed the opposite trend that of CTAB is also attributed: (i) The

MSR values of SDBS and Reetha are very close, so ideally there should not be much deviation of solubilization in the mixture. However, at low concentration of Reetha repulsion between the head groups of SDBS molecules in the micelle decreases, which may cause close packing of the molecules in the micelle and ultimately reduce the diffusion of naphthalene molecules inside the micellar core. (ii) With increasing Reetha mole fraction when the micelle is dominating by Reetha (x_1 ~ 0.5) the deviation is less and becoming close to ideal. When the micelle is dominating by Reetha, solubilization is close to that of pure Reetha and the presence of more Reetha molecule in the micelle may shield the negative charge of the SDBS as a result repulsion between the mixed micelle and π – electrons of naphthalene molecule also reduced. For the other two anionic surfactants probably due to similar reason initially there is a deep minimum and with increasing Reetha concentration as x_1 increases, the solubilization of the mixture is close to Reetha and ultimately deviation decreases.

Previously some researchers (Dar et al, 2007; Mohamed and Mahfoodh, 2006; Zhu and Feng, 2003) have reported opposite trend in MSR in the mixed surfactant systems, like higher MSR in the mixed systems than the individual surfactants. The solubilization efficiency depends on many factors like type and structure of surfactants, type of solubilizates, mixing ratio of surfactants and so on (Rosen, 2004). For those reported studies the nonionic surfactants mixed with the ionic surfactants were higher MSR values than the ionic surfactants, and different PAHs were used. Whereas, in contrast to that, for our study MSR values of TX-100 and CTAB are higher than Reetha, SDBS is close to that, SOS and AOT are lower than that, as a result we got little positive deviation in MSR for last two surfactants. In addition to that it is note worthy, in the mixed surfactant systems when the synergism is there (more negative β) the micelle will be more densely packed and solubilization may decrease; but at the same time the mixed micellar CMC also will be low. In that case, at a particular concentration, since the number of micelle will be more the solubilization also will enhance. The amounts of positive or negative deviation will depends on the factor which is predominant.

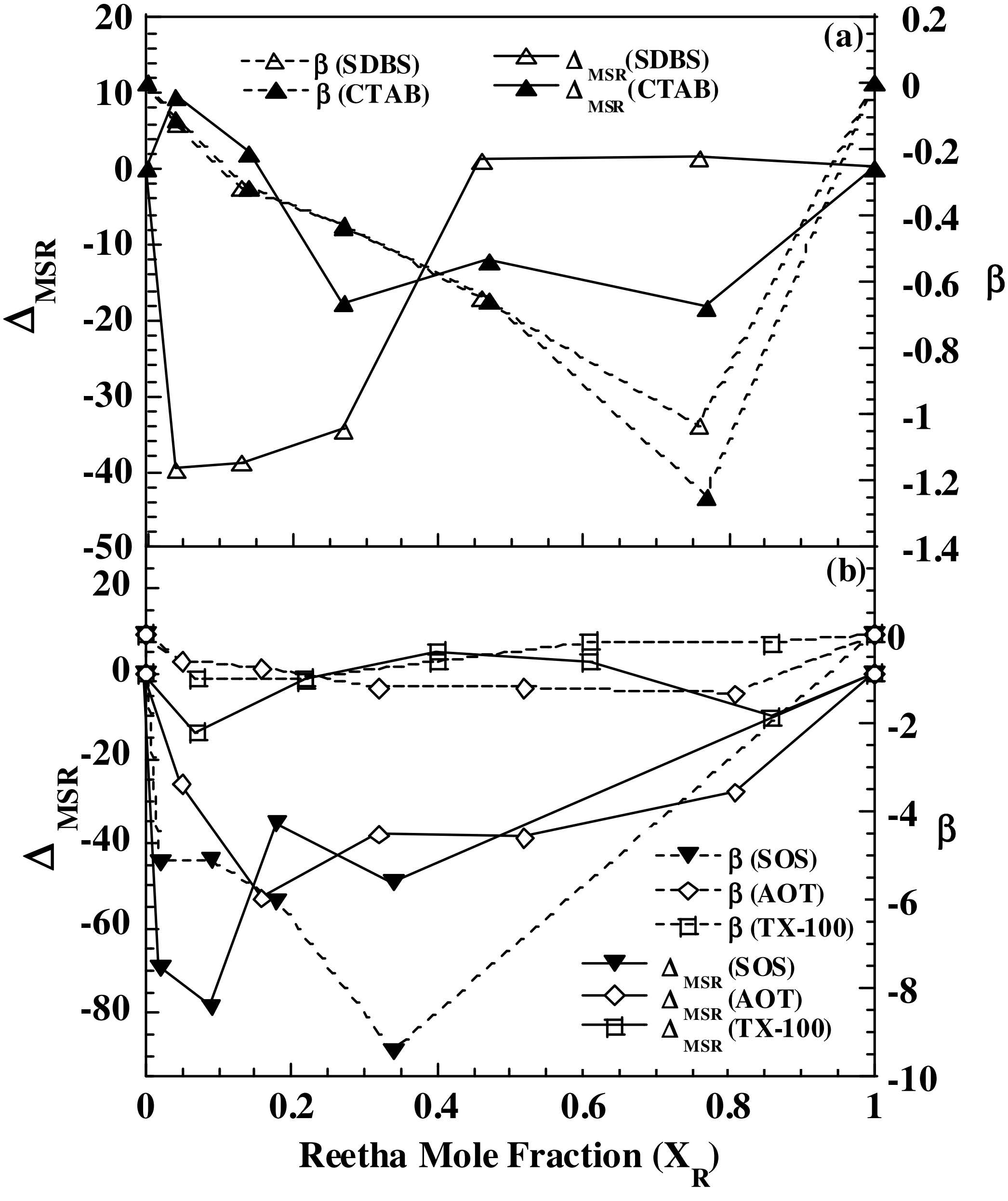

Figure-3.5: Comparison of β and Δ_{MSR} vs. mole fraction of Reetha for (a) SDBS, CTAB (b) SOS, AOT, TX–100.

Table-3.1: Values of X_R, MSR, R, Δ_{MSR}, log K_m, B, β, CMC, and x_1 for mixed surfactant systems.

Surfactant	X_R or α_1	MSR $_{ideal}$	MSR $_{exp}$	log $K_{mExp.}$	R	Δ_{MSR} (%)	B[b]	β[a]	CMC $_{ideal}$ (mM)	CMC$_{exp}$(mM)	x_1
TX–100	0.00	0.312	0.312	4.937	1.000	0.00	0.000	0.000	0.309	0.309	0.000
	0.07	0.294	0.253	4.866	0.859	–14.09	0.878	–1.009	0.318	0.239	0.197
	0.22	0.255	0.251	4.863	0.985	–1.46	1.018	–0.984	0.339	0.292	0.227
	0.40	0.209	0.219	4.815	1.049	4.95	1.029	–0.625	0.368	0.322	0.331
	0.61	0.155	0.159	4.698	1.028	2.78	0.779	–0.179	0.409	0.392	0.485
	0.86	0.091	0.081	4.437	0.897	–10.26	0.128	–0.194	0.437	0.456	0.765
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.472	0.517	1.000
CTAB	0.00	0.124	0.124	4.630	1.000	0.00	0.000	0.000	1.097	1.097	0.000
	0.04	0.121	0.133	4.629	1.096	9.59	1.552	–0.106	1.050	1.029	0.097
	0.14	0.114	0.117	4.579	1.021	2.08	0.802	–0.318	0.949	0.897	0.283
	0.27	0.105	0.086	4.459	0.821	–17.92	0.072	–0.430	0.839	0.755	0.455
	0.47	0.091	0.080	4.430	0.878	–12.22	0.319	–0.653	0.719	0.619	0.617
	0.77	0.070	0.057	4.292	0.817	–18.30	–0.705	–1.247	0.588	0.480	0.770
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000
SDBS	0.00	0.051	0.051	4.249	1.000	0.00	0.000	0.000	1.148	1.148	0.000
	0.04	0.051	0.031	4.038	0.699	–39.68	–5.339	–0.124	1.096	1.069	0.102
	0.13	0.052	0.032	4.045	0.695	–38.99	–2.377	–0.318	0.987	0.927	0.283
	0.27	0.052	0.034	4.077	0.730	–34.57	–1.681	–0.429	0.867	0.781	0.455
	0.46	0.052	0.053	4.262	1.093	1.06	0.004	–0.652	0.737	0.634	0.617
	0.76	0.053	0.054	4.270	1.049	1.48	0.060	–1.036	0.594	0.544	0.820
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000
AOT	0.00	0.020	0.020	3.853	1.000	0.00	0.000	0.000	2.700	2.700	0.000
	0.05	0.022	0.016	3.758	0.739	–26.08	–2.415	–0.591	2.240	1.724	0.310
	0.16	0.026	0.012	3.634	0.469	–53.07	–3.955	–0.758	1.593	1.319	0.506
	0.32	0.031	0.019	3.831	0.620	–38.02	–2.848	–1.207	1.159	0.886	0.635
	0.52	0.038	0.023	3.912	0.613	–38.67	–3.166	–1.208	0.848	0.699	0.754
	0.81	0.047	0.034	4.077	0.720	–28.01	–3.686	–1.347	0.614	0.566	0.896
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000
SOS	0.00	0.005	0.005	3.257	1.000	0.00	0.000	0.000	157.203	157.2	0.000
	0.02	0.006	0.002	2.838	0.307	–69.28	–10.598	–5.141	18.831	6.849	0.637
	0.09	0.009	0.002	2.861	0.216	–78.43	–14.849	–5.102	5.715	3.524	0.762
	0.18	0.014	0.009	3.511	0.646	–35.38	–9.403	–6.021	2.788	2.069	0.827
	0.34	0.022	0.011	3.597	0.506	–49.42	–7.955	–9.440	1.498	1.061	0.826
	0.67							–6.720	0.773	0.525	0.832
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000

[a] β_{av}: TX-100 = –0.560; CTAB= –0.458; SDBS= –0.511; AOT= –1.022; SOS= –4.178.

[b] B_{av}: TX-100 = 0.766; CTAB= 0.408; SDBS= –1.866; AOT= –3.214; SOS= –10.701.

3.3.6 Conclusion

The results of this study can be summarized as:

The solubilization efficiency of naphthalene by Reetha in terms of MSR is less than TX–100 and CTAB close to SBDS, and higher than AOT, SOS. The MSR values of all the mixed systems are in between that of single surfactants and are nonlinearly changing with mole fraction of Reetha, the negative change in MSR than expected (Δ_{MSR}) are in the sequence of TX–100 – Reetha < CTAB – Reetha < SDBS – Reetha < AOT – Reetha < SOS – Reetha, which is the reverse order of the sequence that of single surfactants for solubilization.. The interaction parameter for mixed micellization (β) is negative for all the mixed surfactant systems indicate the synergistic behavior. The average interaction parameter for mixed micellization, β_{av}, for the mixtures of TX–100, CTAB, SDBS, AOT, and SOS with Reetha is –0.560, –0.458, –0.511, –1.022, and –4.178 respectively indicating the same order as of Δ_{MSR} except TX–100 mixture. When the value of interaction parameter is more negative, micelle are densely packed and solubilization is less. From the application point of view, although the plant surfactant is environmentally friendly due to its fast biodegradable nature, but in terms of solubilization efficiency, commonly used synthetic surfactants like TX-100, SDBS, CTAB are better than Reetha. So, use of plant - synthetic mixed surfactant systems may increase the solubility than only plant surfactant and reduce the environmental problem by replacing a certain fraction.

Chapter 4

Mixed micellar behavior of plant surfactant with synthetic surfactants

4.1 Introduction

Surfactants have many applications in diversified areas such as coatings to improve wettability of surfaces, as emulsifiers, as detergents etc and the role of mixed surfactants proved to be more advantageous than single surfactant systems to fulfill various industrial applications. In the economy and performance point of view mixed surfactants preferred as they contain more than one type of surfactant and are essentially important because most of the commercially available surfactants are mixtures with better properties than pure surfactants. The properties of a pure surfactant are mainly determined by its chemical structure and its geometrical arrangement within a micelle. But as known for a long time, surfactant mixtures can show a very different behavior in comparison to their components. The interaction between different surfactants can lead to synergism or to antagonism, depending on the kinds of surfactants.

Some surfactant mixtures present synergistic properties and produce a high degree of reduction in surface tension and a low critical micelle concentration (CMC) than each pure surfactant does. Several studies have been there with different combinations of anioinic – cationic, nonionic – anionic, nonionic – nonionic, cationic – nonionic, anionic – biosurfactants (Rodenas et al., 1999) which are having an advantage to limit the use of pure surfactants in wide applications like soil washing, detergency and many other phenomena. Among the more important aspects to be studied in mixed surfactant systems, there are two points particularly interesting, namely: (i) the effect of the polar headgroup and (ii) the influence of the hydrophobic chain length of one component of the mixture.

Here in this study we investigate the mixed micellar behaviour of nonionic natural plant surfactant (Reetha) with cationic (CTAB), nonionic (TX-100), and anionics (SDBS, AOT, SOS) surfactants. For the nonideal behavior of surfactants, mixed micellar compositions and the estimation of interacting forces, we used the most common model, Rubingh's regular solution theory. Thermodynamics of the various types of solutions that are formed from mixing above components in different proportions also examined by calculating and correlated the excess enthalpy of micellization with activity coefficients as described in (Kang et al., 2001).

4.2 Experimental section

4.2.1 Materials: The surfactants SOS, AOT, CTAB and TX–100 were obtained from Loba chemie Pvt Ltd, India. Sodium dodecyl benzene sulfonate (SDBS) has been from Sigma Aldrich, Germany. All the chemicals were used as received. Ultra pure water (Sartorius, Germany) was used for the experiments of 18.2 mΩ resistivity and pH 6.8 – 7. Surface tension of surfactant solutions were determined by whilmey plate method using a surface tensiometer, Data Physics, Germany (DCAT–11EC).

4.2.2 Extraction of Reetha surfactant from the fruit: Dry fruit (*S. mukorossi*) was purchased from the local market, Rourkela, Orissa, India. And method of extraction was same as described in earlier chapter.

4.2.3 Surface tension measurements: Surfactant solutions surface tension measured by Wilhelmy plate method using surface tensiometer, Data Physics, Germany (DCAT–11EC). The temperatures (±0.1ºC) were maintained by circulating thermostatic water by a circulator through jacketed vessel containing the solution. The platinum plate used for the measurements are cleaned with acetone and burned for each reading. The measured surface tension values were plotted as a function of logarithm of surfactant concentration and the critical micelle concentration (CMC) was estimated from the break point in the resulting curve. Representative plots of surface tension (γ) vs logarithm of surfactant concentration ($\log_{10} C$) are shown in Figure-4.1. The reproducibility of the surface tension (γ) vs concentration curve was checked by duplicate runs. All the samples done in triplicate and average value has taken for the plots.

4.3 Results and Discussion

4.3.1 CMC behavior of mixtures

The critical micelle concentration (CMC) was determined by the surface tension reduction method as shown in Figure-4.1 for pure nonionic (TX-100), cationic (CTAB) anionics (SDBS, AOT, SOS) surfactants with and with out Reetha and are compared with our earlier results presented in Chapter 3.

Results had good correlation and the mixed CMC values obtained from the experiments are reported in Table-4.1. To know whether the mixed solution follows ideal or nonideal behavior mixed CMC values were calculated using ideal solution theory (Clint, 1975).

$$\frac{1}{CMC_{12}} = \frac{\alpha_1}{CMC_1} + \frac{1-\alpha_1}{CMC_2} \tag{3.6}$$

The Table-4.1 and Figure-4.2 clearly indicates that there is a deviation between the ideal and experimental CMC values.

The mixed CMCs of TX–100, CTAB, SDBS and AOT with Reetha surfactant are intermediate between the respective individual surfactants used in the combination. In the case of TX–100 – Reetha mixture the experimental values are close to ideal, and in the other cases experimental CMC values are lower than the ideal values, indicating negative deviation from the ideal behavior for mixed micelle formation. The data for the other surfactants show there is a difference between the experimental and theoretical CMC values and the deviation is highest for SOS – Reetha mixture (Figure-4.2).

4.3.2 Intraction between surfactants

Rubingh's equation of regular solution theory can be used to calculate the micellar mole fraction. And the nature and the strength of the interaction between two surfactant molecules in the mixed micelle were determined by calculating the values of their β parameter using equation 3.7 and 3.8 as described before.

The micellar mole fraction (x_1) can be calculated from equation 3.7 by solving iteratively. To show the interaction between two surfactants, β is an interaction parameter which indicates the interaction between two surfactant molecules in the mixed micelle and is a measure of deviation from the ideal behavior. The parameter β can be now calculated by substituting the value of x_1 in the equation 3.8.

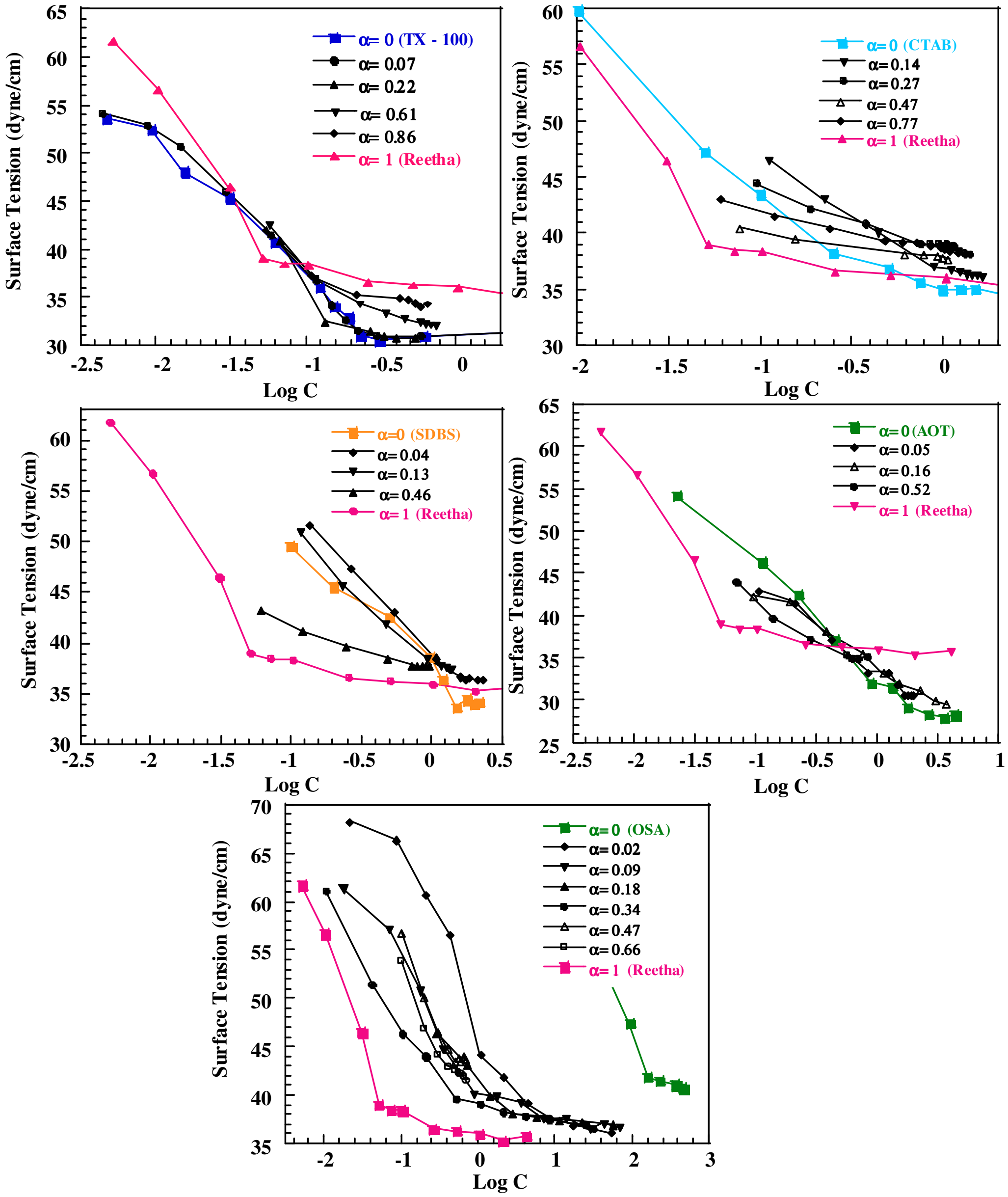

Figure-4.1: Surface tension vs. logarithm of total surfactant concentration at 28°C for the surfactants TX-100, CTAB, SDBS, AOT, SOS with Reetha.

The β parameter quantitatively captures the extent of non–ideality for a mixed surfactant system. The negative value of β indicates synergism in mixed micelle formation, a positive value indicates antagonism, and if zero, then mixed micelle formation is ideal. The larger the absolute value of β, stronger the mixing non–ideality. The negative deviation indicates a reduction in free energy of micellization over that predicted by ideal solution theory. The larger negative value of β denotes the greater negative deviation of CMCs from ideal mixture. All the surfactants used here show a synergistic effect in the mixture as it satisfied the conditions (i) β must be negative (ii) $\ln(CMC_1/CMC_2) < \beta$ (Zhou and Zhu, 2005).

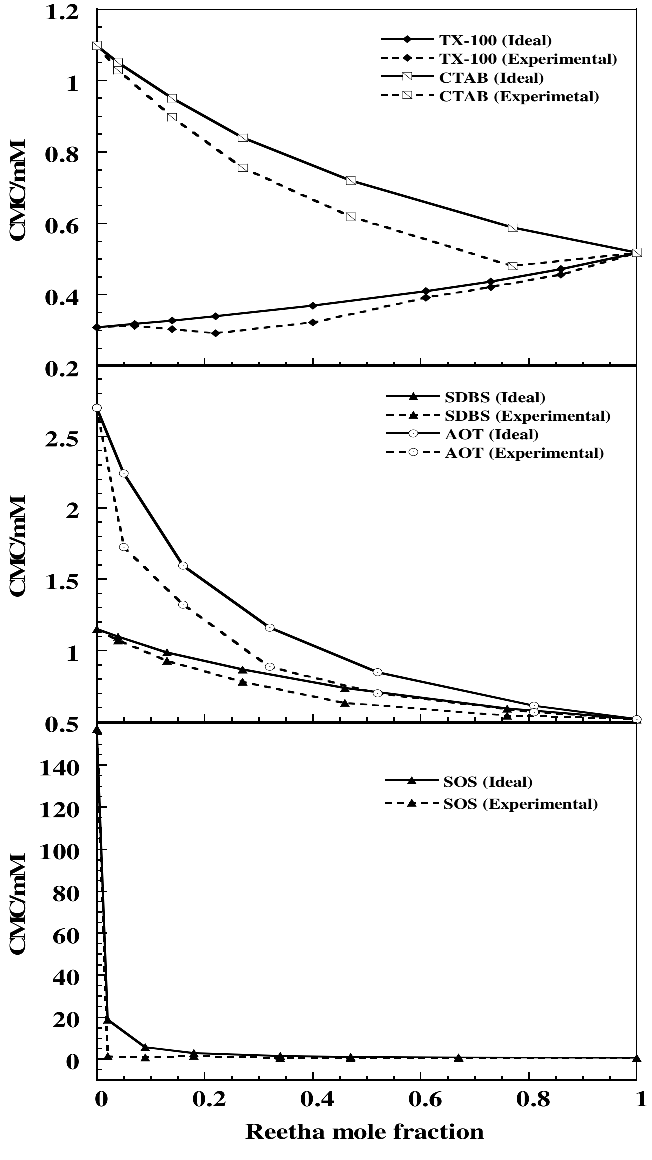

Figure-4.2: Mixed CMCs of surfactants TX-100, CTAB, SDBS, AOT, SOS with increase of mole fraction of Reetha compared to the theoretically predicted CMCs.

The regular solution approximation can be introduced to treat the nonideality of mixing by assuming that the excess entropy of mixing is zero. This condition allows the excess enthalpy of mixing to be directly substituted for the excess free energy of mixing. In a binary mixture, the excess enthalpy of mixing is ordinarily represented by Holland, 1986.

$$H_E = X_1(1\text{-}X_1)\,\beta\, RT = G_E \tag{4.1}$$

Where $\beta\ RT$ represents the difference in interaction energy between mixed and unmixed systems. Equation 4.1 corresponds to the leading term in the lattice model description for the enthalpy of mixing in liquid mixtures (Munster, 1974). H_E of the mixed micellization was calculated by this equation and the results are presented in Table-4.1. H_E observed was negative for all combinations. These negative values signify that the mixed micellization of the used combinations is exothermic. Since it is assumed that the excess entropy is zero in the RST, the excess Gibbs free energy G_E is indistinguishable from H_E, and also is given by eq. 4.1.

The activity coefficients f_1 (Reetha) and f_2 (non-Reetha) are related to G_E by the equation (Holland, 1986)

$$G_E/RT = X_1 \ln f_1 + (1\text{-}X_1) \ln f_2 \tag{4.2}$$

Substitution of Eq. 4.1 for G_E to Eq. 4.2 yields the following expressions for f_1 and f_2.

$$f_1 = \exp[\beta\ (1\text{-}X_1)^2] \tag{4.3}$$

$$f_2 = \exp[\beta\ X_1^2] \tag{4.4}$$

With these equations f_1 and f_2 were calculated and the results are tabulated in Table-4.1. The activity coefficients were less than 1 for all the surfactants with different combinations, and f_1 was smaller than f_2 in some cases and vice versa. The value which is far to 1 will be more responsible for non-ideality in mixtures.

4.3.3 Effect of CMC ratio and x_1 on β

Mixing of various surfactants with Reetha were tested to see the interaction. As mentioned above positive β means there is a net repulsion between the surfactant molecules in the micelle, whereas a negative β from the Table-4.1 indicates a net attraction. To get an idea about the nonideal behavior of binary surfactant mixtures from the CMC difference of the pure surfactants the average interaction parameters (β_{avg}) of different pairs of surfactants mixtures is plotted against % ratio of the CMCs of the pure surfactants ($CMC_{Reetha/synthetic} \times 100$).

Table-4.1: Mixed CMCs with interaction parameter (β), activity coefficients (f) relation with Gibbs free energy at 28 °C at relative mole fractions α of Reetha to TX-100, CTAB, SDBS, AOT, SOS.

Surfactant used with Reetha	mole fraction α (Reetha)	CMC_{Th} in mM	$CMC_{Expt.}$ in mM	$CMC_{Reetha/synthetic}$	β	x_1	f_1	f_2	G_E/RT
	0	0.309	0.309	1.673	0	0	1	1	0
	0.07	0.318	0.239		-1.009	0.197	0.522	0.962	-0.1596
	0.22	0.339	0.292		-0.984	0.227	0.555	0.951	-0.1727
TX-100	0.4	0.368	0.322		-0.625	0.331	0.756	0.934	-0.1384
	0.61	0.409	0.392		-0.179	0.485	0.954	0.959	-0.0447
	0.86	0.437	0.456		-0.194	0.765	0.989	0.893	-0.0349
	1	0.517	0.517		0	1	1	1	0
	0	1.148	1.148	0.349	0	0	1	1	0
	0.04	1.096	1.069		-0.124	0.102	0.905	0.999	-0.0114
	0.13	0.987	0.927		-0.318	0.283	0.849	0.975	-0.0645
	0.27	0.867	0.781		-0.429	0.455	0.88	0.915	-0.1064
SDBS	0.46	0.737	0.634		-0.652	0.617	0.909	0.78	-0.1541
	0.76	0.594	0.544		-1.036	0.820	0.967	0.498	-0.1529
	1	0.517	0.517		0	1	1	1	0
	0	1.098	1.097	0.471	0	0	1	1	0
	0.04	1.05	1.029		-0.106	0.0977	0.917	0.999	-0.0093
	0.14	0.949	0.897		-0.318	0.283	0.849	0.975	-0.0645
	0.27	0.839	0.755		-0.43	0.455	0.88	0.915	-0.1066
CTAB	0.47	0.719	0.619		-0.653	0.617	0.909	0.78	-0.1543
	0.77	0.588	0.48		-1.247	0.770	0.936	0.477	-0.2208
	1	0.517	0.517		0	1	1	1	0
	0	2.7	2.7	0.191	0	0	1	1	0
	0.05	2.24	1.724		-0.591	0.3101	0.755	0.945	-0.1264
	0.16	1.593	1.319		-0.758	0.506	0.831	0.824	-0.1895
	0.32	1.159	0.886		-1.207	0.635	0.851	0.615	-0.2798
	0.52	0.848	0.699		-1.208	0.754	0.93	0.503	-0.2241
AOT	0.81	0.614	0.566		-1.347	0.896	0.986	0.339	-0.1255
	1	0.517	0.517		0	1	1	1	0
	0	157.204	157.204	0.003	0	0	1	1	0
	0.02	18.831	6.849		-2.256	0.637	0.743	0.4	-0.5217
	0.09	5.715	3.524		-1.93	0.762	0.896	0.326	-0.35
	0.18	2.788	2.069		-4.08	0.827	0.885	0.061	-0.5837
SOS	0.34	1.498	1.061		-5.37	0.826	0.85	0.026	-0.7718
	0.67	0.773	0.525		-7.255	0.832	0.815	0.007	-1.0141
	1	0.517	0.517		0	1	1	1	0

The effect of CMCs ratio on β_{avg} for anionic,cationic and nonionic surfactants shown in Figur-4.3. It was observed that β_{avg} decreased with increase in CMC ratio and up to a ratio value 35 %. And a constant change in β_{avg} also observed beyond a ratio value 35 %. This indicates below 35 % of CMC % of other surfactants irrespective of head group with Reetha will result in mixtures with good net interaction and the order of increase shown in the figure. Since the CMCs of TX – 100 and Reetha are very close the interaction parameter also very low. So, from the CMC values of the pure surfactants we can get a rough idea about their mixed micellar behavior.

The change in interaction parameter with the change in micellar molefraction (x_1) for Reetha – synthetic surfactants mixtures are plotted in Figure- 4.4. It was observed that β values tend to increase for all the surfactant combinations CTAB, SDBS, AOT, SOS with increase of micellar mole fraction of Reetha, but the order of increase in β varied for each surfactant. This indicates net interaction of mixed micelle increasing with increase in micellar contribution of Reetha. Where as β values of TX-100 – Reetha combinations followed the reverse order and showed the increasing trend is up to (x_1) = 0.5 and above that there is no change.

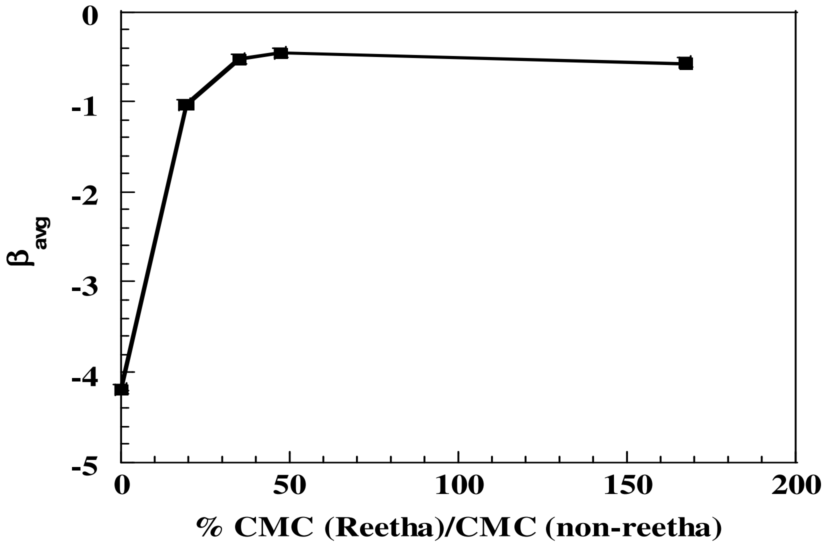

Figure-4.3: % CMC ratio (Reetha/non-Reetha) vs. β_{avg} of surfactant combinations (TX-100, CTAB, SDBS, AOT, SOS) with Reetha.

4.3.4 Conclusion

The mixed CMCs of nonionic Reetha with nonionic, cationic and anionic in varying mole fraction were measured using surface tensiometry. Reduction in CMC was observed more for ionic surfactants with Reetha. And the synergism found varied with different surfactants used. The interaction parameter β estimated by regular solution model for each surfactant combinations are negative indicates synergism in the mixed micelle. The excess enthalpy of micellization was also negative implying that the mixed micellization was exothermic. When the % of CMC ratio is 35% or less the value of interaction parameter is high.

Chapter 5

Biodegradation of Naphthalene in presence of *Sapindus mukorossi* - synthetic mixed surfactant systems.

5.1 Introduction

Removal of hazardous compounds from the soil and ground water systems are gaining more and more importance due to worldwide increasing public and regulatory concerns with the problems of groundwater contamination and resultant impact on human health and ecological systems. Polycyclic aromatic hydrocarbons (PAHs) as pollutants are of major concern and may adversely impact health and environmental quality, making remediation necessary (Wild et al., 1991; Haeseler et al., 1999). Bioremediation is a cheaper technology available, which uses the natural plants, or microorganisms usually bacteria and fungi to convert contaminants into less toxic compounds, ideally CO_2 and water (Riser-Roberts, 1998; Alexander, 1999). The application of this technology suffers from several bottlenecks, one of which is the low availability of hydrophobic organic contaminants like PAHs to the organisms. Contaminants poor bioavailability is due to low mass transfer rates to the microorganisms from sites where they are inaccessible (Grimberg et al., 1996; Miller and Bartha, 1989).

The bioavailability of PAHs described as the solubilized contaminant reaching the organisms by which their enzymes catalyze the conversion step. The interfacial tension of hydrophobic contaminants can be reduced by using compounds such as surfactants which are capable of solubilizing wide range of PAHs. Many studies employing single and mixed surfactants showed their remediation potential in solubilizing and mobilizing organic contaminants like PAHs (Edwards et al., 1991; Zhang et al., 1997; Paria and Yuet, 2006; Treiner et al., 1987; Mohamed et al., 2006). Although synthetic surfactants are efficient solubilizers of contaminants some times hinder their biodegradation by limiting bioavailability, itself accumulate and non benign to environment and degrading organisms (Guha and Jaffe, 1996; Stelmack et al., 1999). Biosurfactants produced by microorganisms also used to enhance bioavailability and biodegradation rates of PAHs in soil remediation. These are having advantages like easy degradability, production from renewable sources etc., than synthetic surfactants (Muller et al., 1993). But their usage in bulk is limited as their purification; recoveries are not economical and some are having antimicrobial properties (Haferburg et al., 1986; Jenny et al., 1991; Fiechter, 1992). Different microorganisms whether individual or mixed culture, have different modes of uptake in the presence or absence of surfactant systems. The presences of surfactants along with contaminant highly influence the ultimate degradation ability of microorganisms.

Surfactants from renewable plant sources which are environmental friendly surely other option for contaminant remediation studies. They are only few studies concerning the application of plant based natural surfactants from sapindus mukorossi (Kommalapati et al., 1997; Row, 1966) and no study regarding their blend with synthetic surfactants reported. The solubilization and degradation of PAHs in synthetic – plant based surfactant mixtures may be useful in reducing the amount of synthetic surfactants usage making them easy removal from the environment. More than that, to date, most research for elucidating the surfactant effect on bioavailability of PAHs has been conducted with suspended growth systems; employing mixed cultures will be advantageous as currently, individual microbial responses to synthetic surfactants haven't shown any robust results. There is no study reported employing the mixed culture with synthetic – natural surfactant mixtures and their ability to degrade PAHs. It is crucial to know the biological response of surfactant addition to PAH degrading microbial cultures in order to develop practical surfactant enhanced strategies. Much more research is required to make the application of natural surfactants a standard tool in biological soil and water remediation.

5.2 Materials and methods

5.2.1 Chemicals

The surfactants CTAB and TX–100 were obtained from Loba chemie Pvt Ltd, India. Sodium dodecyl benzene sulfonate (SDBS) has been from Sigma Aldrich, Germany, Naphthalene, Carbinol from Spectrochem Pvt Ltd, India. All the chemicals were used as received. Ultra pure water (Sartorius, Germany) was used for the experiments of 18.2 mΩ resistivity and pH 6.8 – 7. Dry fruit (*S. mukorossi*) was purchased from the local market, Rourkela, Orissa, India. The surfactant was extracted from the fruit according to the method mentioned in Chapter 3.

5.2.2 Microorganisms and medium

Microorganisms with no biosurfactant producing activity were purchased by National Collection of Industrial Microorganisms (NCIM), Pune. The different type of simple to complex carbon compound degrading bacteria used for the study are *Pseudomonas aeruginosa 2074, Acinetobacter calcoaceticus 2886*, *Acinetobacter calcoaceticus 2890, Mycobacterium sp. 2984*, *Pseudomonas desmolyticum 2028*, *Pseudomonas desmolyticum 2112*. Each stain maintained in the nutrient medium and appropriate proportions mixed before experiment to get a known mixed culture.

Waste water obtained from Chromite mines in Sukinda area, Jajpur district, Orissa; microorganisms isolated and cultured in nutrient media. Two mixed cultures prepared and used in equal amounts for the experiment. Autoclaved mineral salt media (MSM), composed of KH_2PO_4 (1.5 g), Na_2HPO_4 (4 g), NH_4Cl (1 g), $MgSO_4.7\ H_20$ (0.2 g), $CaCl_2$ (0.01 g), $FeCl_3$ (0.005 g) per liter of deionized water used for the experiment. Surfactant solutions were prepared from the respective stocks added to autoclaved MSM and the overall concentration in each flask maintained was 10mM. All the flasks were shaken well to ensure that the surfactant was completely mixed and pH adjusted to 7 - 7.2.

5.2.3 Experimental design

All the solubility and degradation experiments were done in the Erlen meyer conical flasks of capacity 250 ml. A stock solution of naphthalene (100 mM) was prepared by dissolving naphthalene in pure methanol is preferred over higher alcohols because of its negligible effect on solubilization and CMC values (Edwards et al., 1991). For experiment 1ml naphthalene from stock added to flasks with TX-100 and its mixtures, where as 0.5ml to other all flasks with surfactants CTAB, SDBS and its mixtures with

Reetha. Flasks were dried to remove solvent. Here the medium used is mineral salt medium (MSM) and at about 50 ml in each flask and 10 ml kept as reference without addition of naphthalene or culture. Except for control all the flasks supplied with pure and with different combination of surfactant mixtures. Naphthalene degradation was monitored by adding 0.1ml waste water culture (WWC) and known culture (KC) throughout a period of 18 days in the flasks containing MSM. Mixed cultures employed shown in Figure-5.1 and naphthalene left was measured periodically. Flasks were properly plugged with cotton plugs and covered. Aseptic conditions followed where ever required and all experiments were performed at room temperature, 25 – 28 °C and flasks were agitated at 130 rpm.

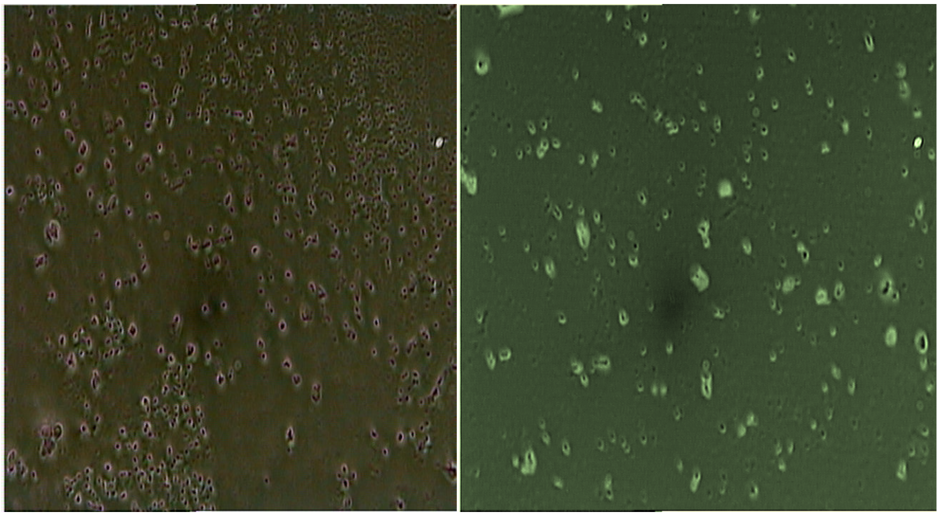

Figure-5.1: Mixed cultures KC and WWC at 40X.

5.2.4 Analytical methods

Surfactant solutions surface tension was measured by Wilhelmy plate method using surface tensiometer, Data Physics, Germany (DCAT–11EC). The temperatures (28 ± 0.1 ◦C) were maintained by circulating thermostatic water by a circulator through jacketed vessel containing the solution. The platinum plate used for the measurements was cleaned with acetone and burned after each reading. The measured surface tension values were plotted for each composition in different time intervals in days. Representative plots of surface tension (γ) vs various combinations are shown in Figure- 5.2. The reproducibility of the results was checked by duplicate runs. Microbial growth measurements were done by UV–Vis. spectrophotometer (Jasco, Japan, V–530) at an absorbance of 600nm. All the samples were treated with 10% formaldehyde prior to analysis to kill the microorganisms. Samples measured for residual naphthalene in each flask measured by HPLC with C_{18} reversed phase (RP) column and methanol as mobile phase (Jasco, MD2015*plus*). Experiments were done in triplicate and the average values are reported.

5.3 Results and Discussion

5.3.1 Surface tension and growth measurements

In the degradation experiments, better result in degradation is expected when the growth of culture in the medium is higher. If the growth is more there are both possibilities of degradation of naphthalene and surfactant is there. Hence, before knowing the amount of naphthalene degraded first we observed the change in growth of the cultures and surface tension in the system. All the combinations used showed static values as shown in Figure-5.2 indicates surfactants and not degraded.

The culture growth in the presence of naphthalene and surfactants is reported in terms of growth rate constant (K_g). That is defined as (Thomas et al., 1993).

$$K_g = \ln \frac{N_2 - N_1}{t_2 - t_1} \tag{5.1}$$

Where N_2 and N_1 are concentration of cells in mg/L at time t_2 and t_1 in days. Growth rate constant (K_g) of KC and WWC calculated for the pure surfactants (Reetha, TX-100, CTAB, SDBS) are shown in Table-5.1. The higher the values of (K_g) indicate faster growth of microorganisms in the respective surfactant. By comparing the K_g values for WWC and KC we can see the values are little higher for WWC but the change is not very significant, but the increasing order of K_g values are also similar for both the cultures. The ascending order of growth by KC was CTAB < TX-100 < SDBS < Reetha; and by WWC was TX-100 ≈ < CTAB < SDBS < Reetha with negligible difference between TX-100 and CTAB.While comparing the mixtures of synthtetic surfactants with Reetha in different mole fractions, Figure-5.3 shows that with increase in mole fraction of Reetha, (K_g) values of all the mixed combinations are increased. This indicates since pure Reetha is having higher K_g value than the synthetic surfactants in presence of naphthalene, so its addition to synthetic surfactants enhanced the growth of both the cultures than the synthetic surfactants alone. It is reported that the known cultures are not producing surfactants in presence of hydrophobic compounds.

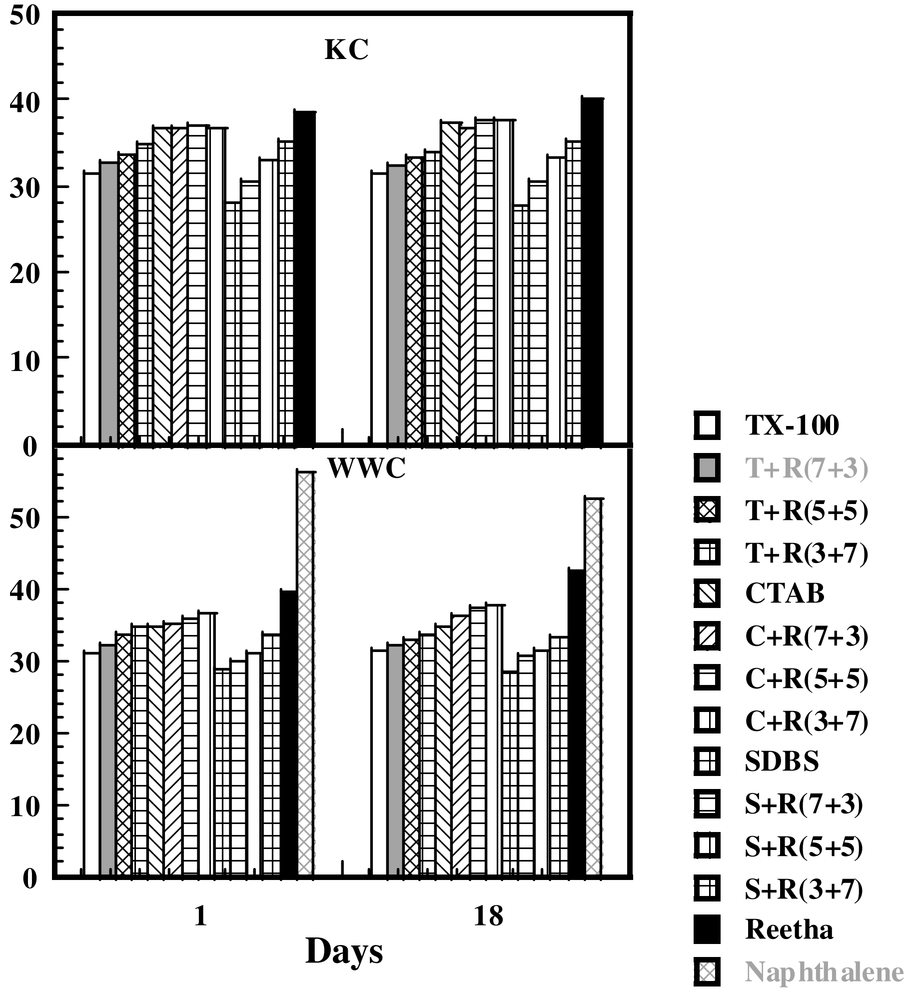

Figure-5.2: Surface tension comparison of various surfactants with Reetha by KC and WWC.

As KC is devoid of having biosurfactant production ability; to test the surfactant production ability of WWC, we have monitored the growth and surface tension in presence of naphthalene without surfactant (Figure-5.2). The K_g value found was 0.277 for WWC and 0.209 for KC, no change in surface tension even after 18 days indicates WWC are utilizing naphthalene in absence of surfactant but not producing any surfactant.

The K_g value without surfactants is lower than all the K_g values in the presence of surfactants (pure/mixtures) by WWC/KC. This indicates the presence of surfactant (pure/mixtures) showing increase in growth of respective mixed culture. Different K_g values show varying growth of mixed cultures in the presence of surfactants.

Surface tension depletion throughout the period of 18 days for individual and mixed surfactants of CTAB, SDBS, TX-100 was checked and no difference found with the initial value for both the cultures. The constant values in surface tension measurements through out the study may be due to preferably only naphthalene utilization by mixed cultures with out degrading surfactants or if surfactants are degraded the rate is very slow, so that even after 18 days remaining concentration of surfactants are still above CMC.

Table-5.1: Growth rate constant (K_g) for various surfactants by KC and WWC. CTAB, Reetha, SDBS, and TX – 100 are abbreviated as C, R, S, T respectively.

Surfactant	**K_g Values**	
	KC	**WWC**
R	0.424	0.43
T	0.338	0.293
C	0.264	0.297
S	0.332	0.371
T + R (30:70)	0.399	0.378
T + R (50:50)	0.347	0.378
T + R (70:30)	0.341	0.331
C + R (30:70)	0.314	0.373
C + R (50:50)	0.276	0.333
C + R (70:30)	0.273	0.291
S + R (30:70)	0.392	0.397
S + R (50:50)	0.352	0.377
S + R (70:30)	0.336	0.366

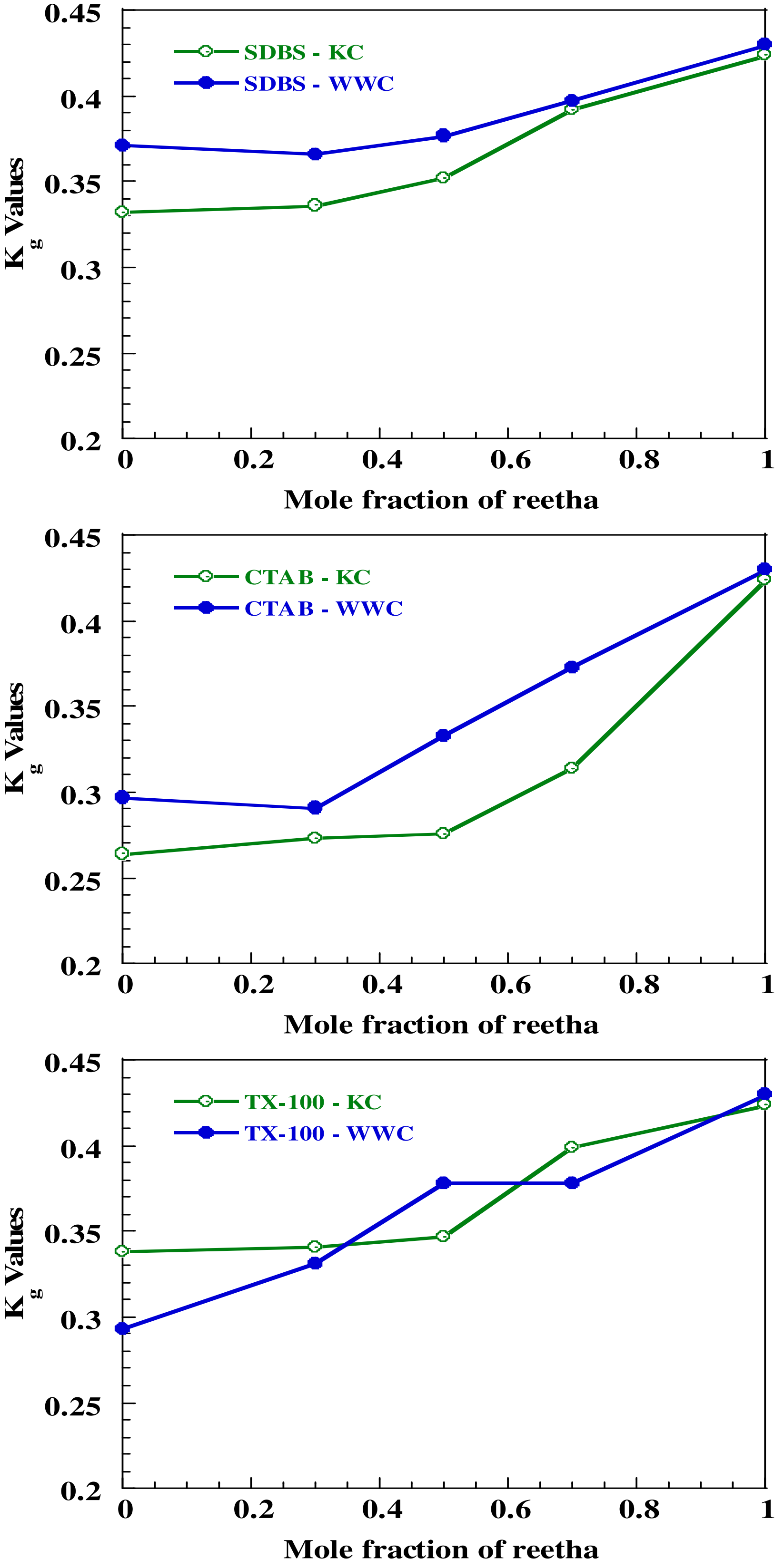

Figure-5.3: Compariosn of growth rate constants (K_g) of cultures KC and WWC with TX-100, CTAB, SDBS with different mole fraction of Reetha

5.3.2 Solubilization of naphthalene by single and mixed surfactants

As it is mentioned before the availability of the PAH is an important step in biodegradation, so the solubility limits of naphthalene in different surfactant solution is worthy to know in biodegradation study. In our study we found the aqueous solubility of naphthalene is 0.234 mM at room temperature in the absence of surfactants. The solubility of naphthalene in single and mixed surfactant systems we have reported in Chapter 3. We found the solubility order of the pure surfactants are TX-100 > CTAB > Reetha > SDBS. In the mixed systems for TX-100 and CTAB solubility is more when mole fraction of synthetic surfactant is more, but reverse for SDBS since Reetha is having little more solubilization capacity. From Figure-5.4 TX-100 – Reetha mixtures showed a linear decrease in solubilization with increase in mole fraction of Reetha similar to that in absence of MSM as reported before (Rao and Paria, 2009), indicates as the both the surfactants are nonionic shows ideal behavior with negligible effect of MSM on micellization or solubilization. But our study (Rao and Paria, 2009) in aqueous system with MSR (molar solubilization ratio)values reported the solubility of naphthalene by SDBS mixtures showed low efficiency i.e below Reetha solubility up to 0.5 mole fractions but later on increased; where as CTAB showed increased value in solubility than pure Reetha for all mole fractions.

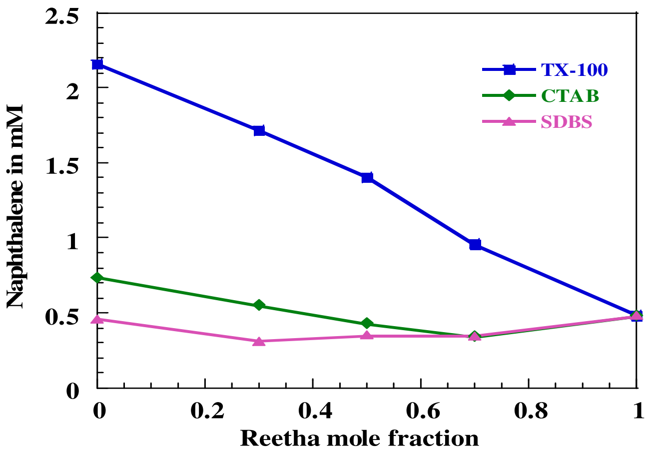

Figure-5.4: Naphthalene solubility in MSM by surfactants TX-100, CTAB and SDBS with Reetha.

The variation in solubility's here may be due to MSM effect on solubilization of naphthalene. The presence of nonionic surfactant Reetha in mixed micelle reduced repulsion between ionic heads of surfactants and similarly mono, di, tri valence ions of MSM resulted in tight packing of mixed micelle resulting in less solubility of naphthalene.

5.3.3 Naphthalene Biodegradation

To see the efficiency of degradation of organic compound in presence of mixed surfactant systems using Reetha as one of the components, naphthalene biodegradation was studied in the presence of various surfactants with two different mixed cultures. The degradation of naphthalene in percent by mixture of KC and WWC on different single and mixed surfactants is shown in Figure-5.5(a) and Table-5.2. Naphthalene depletion periodically measured for surfactants used and indicated in Figure-5.5(b). The maximum degradation time given was 18 days, for all the single surfactants and their mixtures show after 18 days negligible naphthalene left except for TX-100 and its mixtures with Reetha.

The degradation of PAHs in presence of surfactant can be described as first order kinetics (Wong et al., 2004). To have a clear understanding of the extent of naphthalene degradation in the presence of each surfactant and surfactant combinations used, the kinetic rate constant (*K)* of naphthalene are presented in Table-5.3 calculated according to

$$K = \ln\left(\frac{C_0}{C_t}\right)/t \tag{5.2}$$

Where C_0 and C_t are the concentration of naphthalene at day 0 and day t respectively. The low K values indicate lower degradation rate. From Table-5.3 the K values followed following order for the pure surfactants: SDBS > Reetha > CTAB > TX-100 for KC and WWC. The comparison of two mixed cultures in presence of pure surfactants reveals that both mixed cultures degrade naphthalene effectively in presence of SDBS and very slow in TX-100. Comparisons of both the cultures show the rate constant values are very close for pure surfactants.

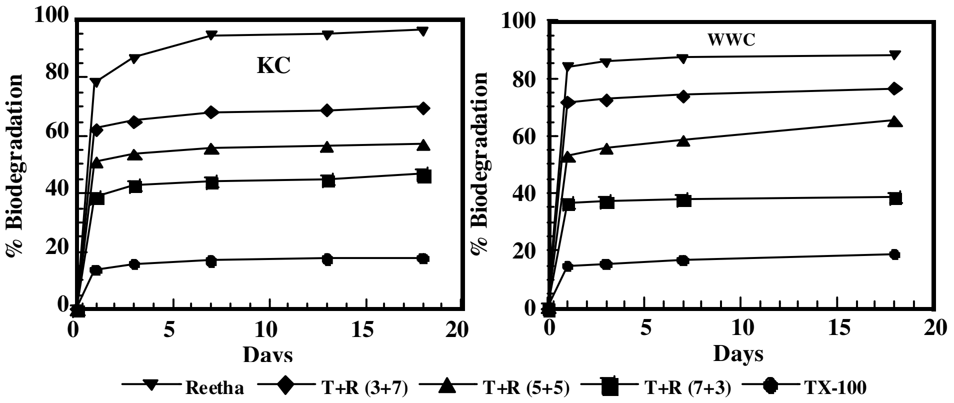

Figure-5.5 (a): Biodegradation % by KC and WWC for TX-100 – Reetha mixture.

Table-5.2: Biodegradation % by mixed cultures with CTAB and SDBS mixtures with Reetha.

Mixed cultures		CTAB with different mole fraction of Reetha in %					SDBS with different mole fraction of Reetha in %				
	Days	0.0	0.3	0.5	0.7	1.0	0.0	0.3	0.5	0.7	1.0
	1	54.49	77.81	74.34	74.88	78.20	82.64	88.24	76.78	80.48	78.20
	3	87.87	90.40	85.94	91.36	86.80	90.73	93.07	87.06	89.95	86.80
KC	7	89.48	91.16	89.57	92.39	94.57	93.18	95.13	92.99	92.90	94.57
	13	89.55	91.18	92.94	93.21	94.79	94.49	96.64	93.68	95.43	94.79
	18	91.62	93.25	93.59	93.32	96.33	96.22	97.24	95.94	96.04	96.33
	Days	0.0	0.3	0.5	0.7	1.0	0.0	0.3	0.5	0.7	1.0
	1	72.89	96.10	93.47	92.45	83.65	78.81	96.96	97.14	94.95	83.65
	3	79.76	97.68	97.60	94.00	85.33	87.60	97.36	97.60	96.40	85.33
WWC	7	82.38	98.09	99.18	94.25	86.80	92.87	97.52	98.04	97.60	86.80
	18	90.96	98.95	99.58	94.86	87.86	95.81	98.80	98.43	98.52	94.40

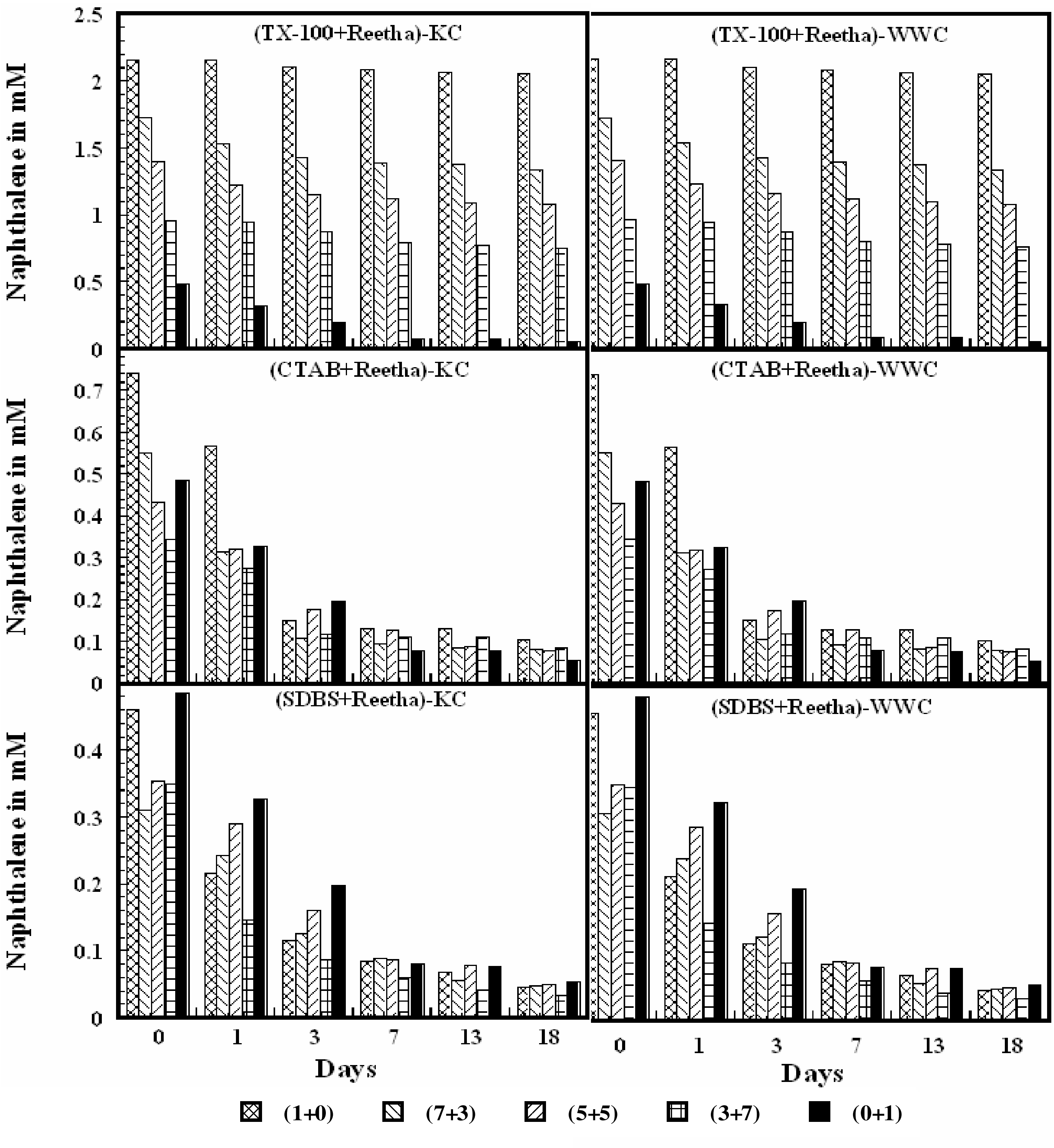

Figure-5.5 (b): Naphthalene depletion in 18days for surfactants TX-100, CTAB, SDBS with reehta (pure/mixtures); 2.5 mM .naphthalene taken for TX-100 and its mixtures with reetha, where as it was1.25 mM for all other cases.

From Table-5.3, Figure-5.6 the mixed surfactant systems show little complex behavior than the single systems. TX-100 – Reetha mixtures showed increase in - values with increase in mole fraction of Reetha. But are less than pure Reetha and more than TX-100 alone, this indicates naphthalene degradation increased with addition of Reetha to TX-100 by both mixed cultures.

Table-5.3: Degradation rate constant (*K*) values of napthalene by two mixed cultures.

Surfactant	*K*	
	KC	WWC
R	0.174	0.160
T	0.011	0.012
C	0.138	0.134
S	0.182	0.177
T + R (30:70)	0.066	0.080
T + R (50:50)	0.047	0.058
T + R (70:30)	0.035	0.027
C + R (30:70)	0.150	0.254
C + R (50:50)	0.153	0.307
C + R (70:30)	0.151	0.165
S + R (30:70)	0.200	0.246
S + R (50:50)	0.178	0.230
S + R (70:30)	0.180	0.233

In presence of CTAB – Reetha mixtures for KC; *K* values are almost constant and lied above pure CTAB and below Reetha surfactants for KC. Whereas order of *K* values followed by WWC for CTAB – Reetha mixtures is as follows: 50:50 > 30:70 > 70:30 and are interestingly greater than pure surfactants CTAB and Reetha. This indicates addition of Reetha increased the degradation of naphthalene in ascending order except 50:50 combinations by WWC which are also greater than CTAB – Reetha mixtures by KC. Coming to SDBS – Reetha mixtures; for both the cultures *K* values of 30:70 is higher than their other combinations. *K* values are equal to or more than pure surfactants by KC and totally more than their pure SDBS, Reetha surfactants by WWC.

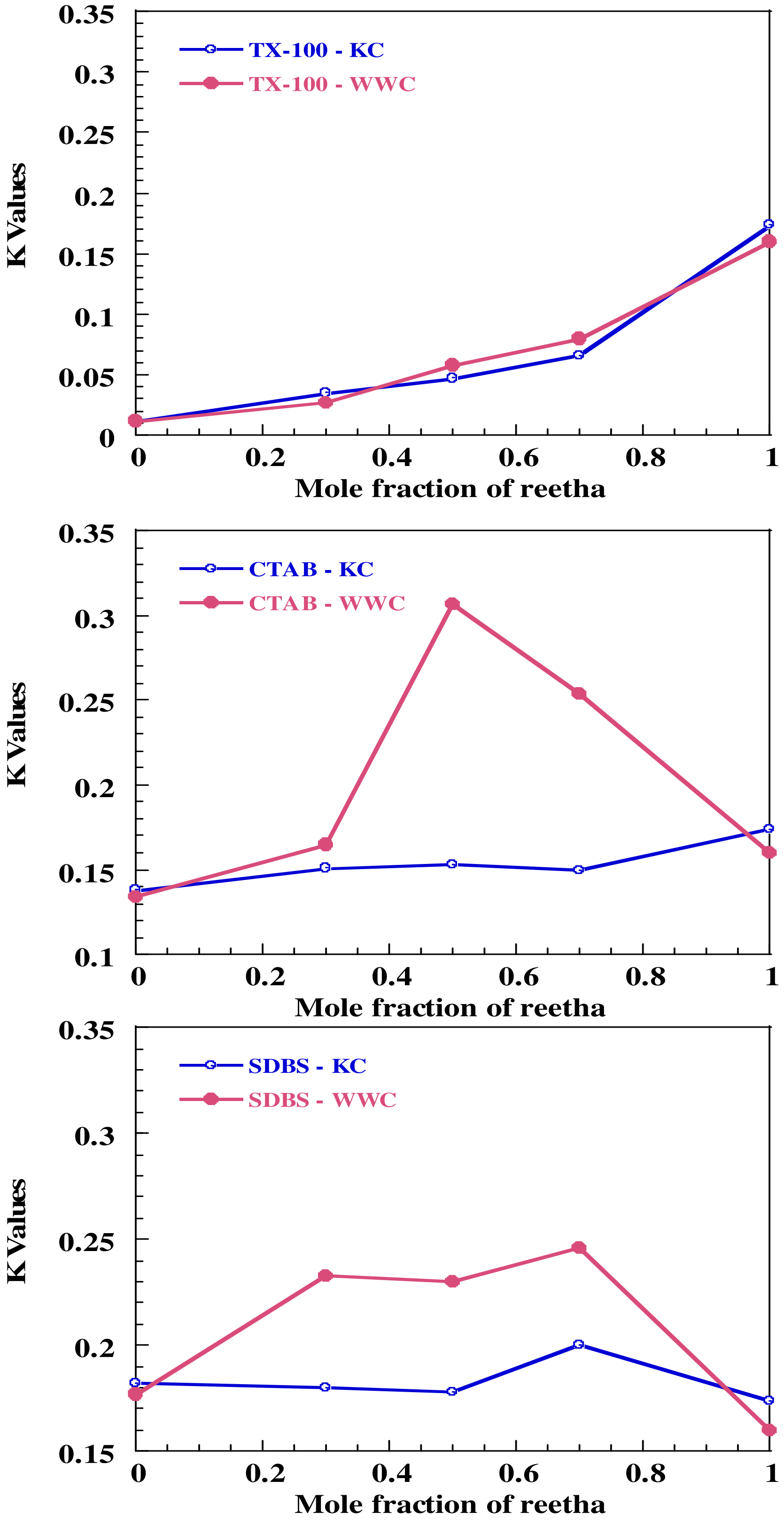

Figure-5.6: Kinetic rate constant (K) values compariosn of cultures KC and WWC with TX-100, CTAB, SDBS with different mole fraction of Reetha

This indicates addition of Reetha enhanced naphthalene degradation by KC and WWC in SDBS – Reetha mixtures. *K* value comparison for pure and surfactant mixtures by two mixed cultures from Table-5.3, Figure-5.7 states that addition of Reetha surfactant increased rate of degradation of naphthalene in surfactant mixtures. SDBS – Reetha mixtures by KC and, SDBS, CTAB – Reetha mixtures by WWC stand with high *K* values greater than their pure surfactants; indicating faster naphthalene biodegradation. In addition from the Figure-5.7 with increase in *K* order for KC and WWC, simultaneous increase in growth rate constant (K_g) not observed. This indicates surfactant mixtures with Reetha also enhanced the growth of mixed cultures apart from naphthalene.

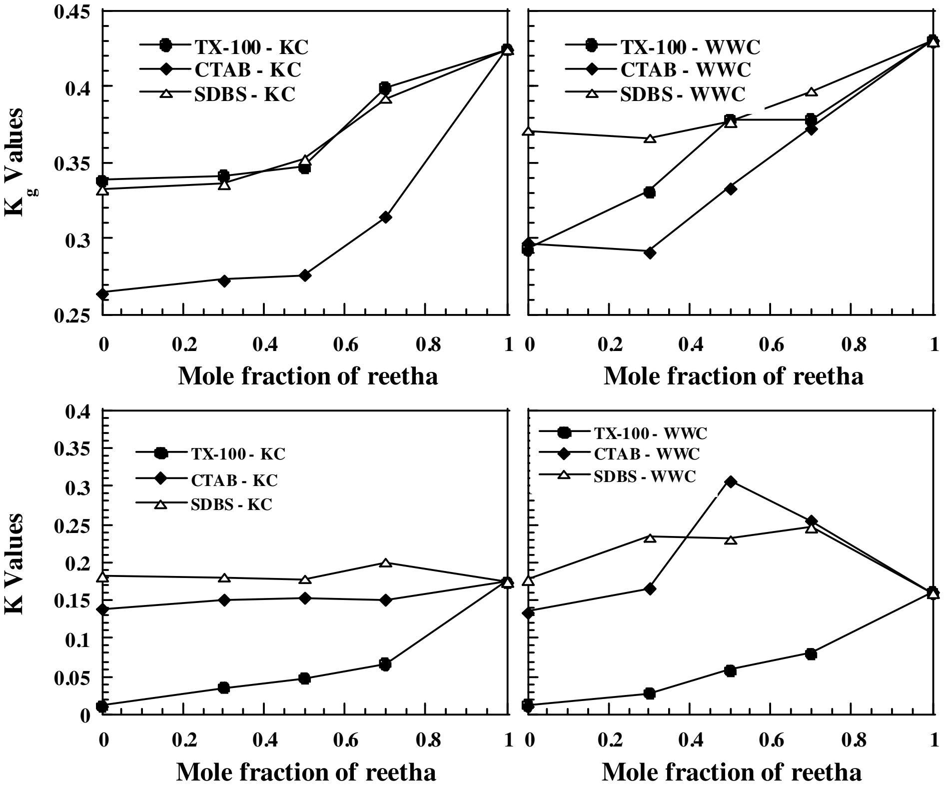

Figure-5.7: Kinetic degradation rate constant (K) and growth rate constant (Kg) values comparison for KC and WWC by surfactant (TX-100, CTAB, SDBS) – Reetha mixtures

5.3.4 Degradation of surfactants by mixed cultures

Degradation of surfactants TX-100, CTAB, SDBS, Reetha pure and synthetic – Reetha surfactant mixtures was studied to know the utilization of surfactant by mixed cultures. Qualitative study was performed by observing the change in surface tension by KC and WWC in surfactants (pure/mixed). This gives the ease or probability degradation of surfactants by the cultures in addition to naphthalene.

The degradation of surfactants monitoring the surface tension values for a period of 60 days by mixed cultures KC and WWC. Table-5.4, Figure-5.8 shows the percentage increase in surface tension by pure surfactants TX-100, SDBS, CTAB, and Reetha in the following order: Reetha (16.4 %) > TX-100 (14.3 %) > SDBS (0.1 %) > CTAB (0.0 %) > for KC; and Reetha (43.0 %) > TX-100 (13.2 %) > SDBS (9.1 %) > CTAB (0.3 %) for WWC. The results clearly indicate mixed cultures behaved differently with different surfactants. Among the surfactants studied here, one common behavior is observed that two nonionic surfactants Reetha and TX-100 are showing increase in surface tension in presence of both KC and WWC.

Table-5.4: Percentage increase in surface tension values by both mixed cultures.

Surfactant	**% increase in Surface tsension values from reference (for 60 days period)**	
	KC	**WWC**
R	16.4	43.0
T	14.3	13.2
C	0.0	0.3
S	.0.1	9.1
T + R (20:80)	19.3	25.0
T + R (80:20)	18.7	23.9
C + R (20:80)	3.7	11.8
C + R (80:20)	4.9	6.3
S + R (20:80)	3.4	14.3
S + R (80:20)	9.9	5.0

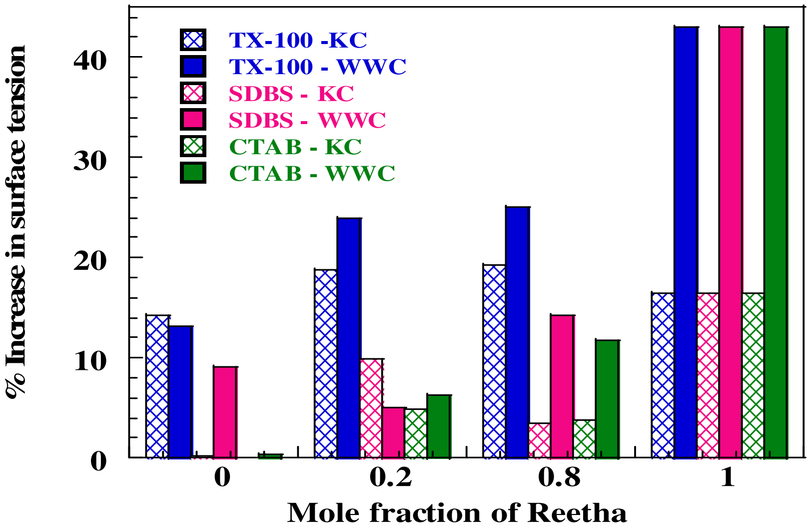

Figure-5.8: Increase in surface tension with respect to Reetha for various combinations of surfactants (for 60 day period).

Both the surfactants show highest increase in surface tension in presence of WWC. By comparing all the surfactants, pure Reetha by WWC showed rapid biodegradable property with high surface tension value. Among the synthetic surfactants a maximum of 14.3 % increase was found with TX-100 by KC.

While coming to surfactant mixtures with Reetha from Table-5.4, Figure-5.8 it is observed that % increase in surface tension for TX-100 – Reetha mixtures are more than other surfactant mixtures and are very nearer with increase in mole fraction of Reetha by both mixed cultures. Less K values with higher K_g values of TX-100/TX-100 – Reetha mixtures to increase in mole fraction of Reetha; revealed that less naphthalene degradation ability with more surfactant degradation property by mixed cultures. Like wise with the incorporation of Reetha surfactant to CTAB and SDBS increased the surfactant mixture degradation by WWC, where as exactly opposite trend followed by KC.

Although different trend followed by both cultures for CTAB and SDBS with Reetha, an increase in K_g values were seen for both as their K values differ and contributing for the growth of cultures. Here, growth may be resulted due to consumption of naphthalene while surfactant utilization was low. High and low K values with increase of Reetha fraction with low and high surfactant utilization gained increase of K_g values with increase in Reetha fraction.

5.3.5 Zeta Potential (ξ) *effect on Biodegradation*

Like the colloidal particles microorganisms also possesses certain surface charge when present in the aqueous medium. In general, microorganisms have a negative electrical surface charge which depends on carbohydrate moieties, proteins etc., which stem from phospholipid bilayer. The Zeta potential (ξ) for mixed cultures of KC and WWC in water at neutral pH observed was -23.1 mV, -30.4 mV respectively; where as in the mineral salt medium are – 14.83 mV and – 8.23 mV. The reduction of potential in MSM was more for WWC may be due to suppression of extra cellular charge and species variation. Figure-5.9 represents the zeta potential (ξ) of two cultures in presence of different surfactant medium. From the Figure it can be noticed that for the both cultures used, with increase in mole fraction of Reetha surfactant; zeta values decreased and lied in between the pure surfactants. The zeta potential (ξ) for combinations of CTAB - Reetha show less value for WWC than KC and there is a difference between the two cultures. Where as for TX-100 – Reetha and SDBS –Reetha combinations the variation in zeta potential was very small. In case of TX-100 – Reetha mixture since both are nonionic overall change is very less.

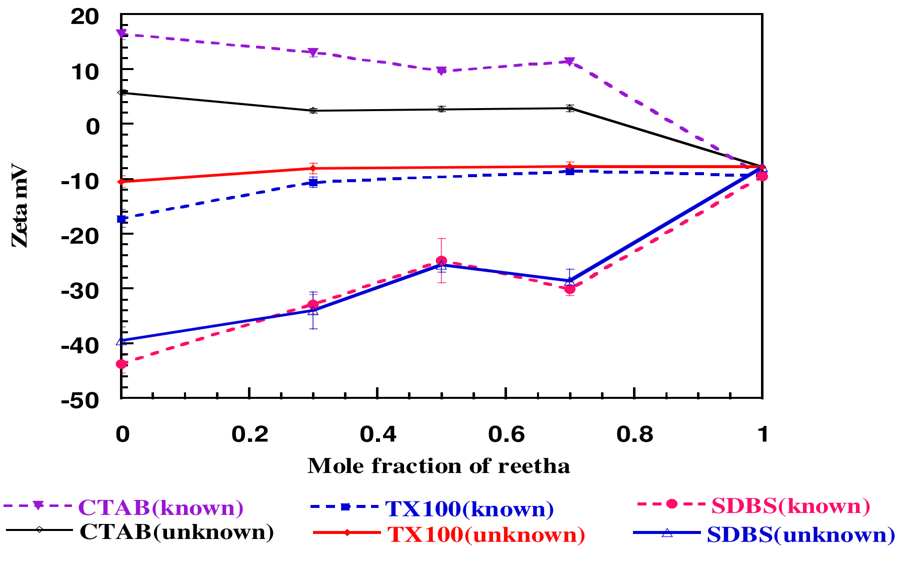

Figure-5.9: Zeta potential (ξ) comparison for KC and WWC for surfactant (TX-100, CTAB, SDBS) – Reetha mixtures.

Lower zeta values for WWC than KC for CTAB with increase in Reetha mole fraction has more *K* values with fast naphthalene depletion. Here reduction in zeta potential helped WWC to degrade naphthalene effectively than KC and variation in *K* values are significant for CTAB-Reetha mixtures by both mixed cultures used. While TX-100, SDBS with Reetha showed less zeta potential variation by both cultures and are having marginal increase in *K* values of WWC than KC, indicating the zeta effect on rate constant.

Pure surfactants TX-100 inhibited the degradation of naphthalene but enhanced after the addition of Reetha surfactant indicates the surfactant dependence on the structure although the zeta potential values are same for nonionic combinations used. Figure-5.9 depicts lowering of zeta potential value (absolute value) for ionic surfactant mixtures with increase in Reetha mole fraction increased the depletion of naphthalene, so, the plant – synthetic mixtures may increase the effectiveness of the blend when the synthetic surfactants are having inhibition tendency towards biodegradation like CTAB and TX-100.

5.3.6 Correlation of growth and degradation mixed cultures

The variation in results by PAH degrading mixed cultures regarding growth may be due to utilization of surfactants as growth substrates in preference to naphthalene or naphthalene alone or both in varying rates. The contradictory results for degradation are due to the interactions among PAH degrading culture, PAH and surfactant. If surfactants are neither toxic nor growth substrates, they can either enhance degradation of PAH by forming micelles that are accessible to microorganisms or decrease degradation by preventing cells from directly contacting PAH.

K_g values of naphthalene in presence for pure surfactants showed highest for Reetha by both mixed cultures than all other synthetic surfactants conversely not giving high *K* values indicate rapid degradation potential of Reetha. Coming to surfactant mixtures with increase in mole fraction of reetha an increase in growth of mixed cultures was observed which was greater than pure synthetic surfactants and less than pure reetha. This indicates that reetha addition increases growth. It is expected that more degradation with increase in growth of cultures. But in contrast K_g values order is not matching with *K* values. This indicates naphthalene alone is not a growth substrate here but surfactants (pure/mixtures) utilization influencing the microbial growth as well as naphthalne degradation.

Figure-5.6 suggests increase or decreases of *K* values are not in the order of K_g values; especially TX-100 (pure/mixture) showed more deviation than CTAB, SDBS (pure/mixtures). This indicates varying surfactants (pure/mixtures) utilization by both mixed cultures. Mixed cultures ability to utilize the surfactants (pure/mixed) is studied qualitatively by monitoring surface tension measurements. Figure-5.8 suggests the addition of reetha in small amounts to TX-100 showed more degradation than CTAB and SDBS mixtures. While other surfactants showed increase in surfactant degradation with increase in reetha, which indicates nonionic surfactant combinations with reetha are more degradable than ionic surfactants.

When naphthalene was given as a substrate along with excess surfactants, chance of utilizing both observed in varying amounts. This may be the reason for variation in *Kg* values with respect to *K* values. Figure-5.6 suggests, for TX-100 – Reetha mixture naphthalene degradation was low with increase in surfactant-mixture degradation by both mixed cultures. While for CTAB and SDBS – Reetha mixtures, naphthalene degradation was more and surfactant-mixture degradation was less than TX-100 – Reetha mixture. Non- zero *K* values of all indicate naphthalene and surfactants (pure/mixtures) utilized as substrates with varying degradation rates. Ionic surfactants with Reetha are advisable for remediation studies as they degrade PAH like naphthalene in limited time with itself having moderate degradation rates. Zeta studies revealed that hydrophilic nature of mixed cultures decreased with addition of Reetha to pure synthetic surfactants and naphthalene degradation was favored. The reduction of hydrophilicity differed for two mixed cultures used and more naphthalene degradation seen with WWC which is having low zeta.

5.4 Conclusion

In this study, we found that the addition of plant based Reetha surfactant increased the degradation of naphthalene especially by ionic surfactants CTAB, SDBS than nonionic TX-100. KC showed better naphthalene degradation potential in the presence of pure surfactants while WWC dominated in surfactant mixtures. Synthetic surfactant degradation was enhanced by the addition of Reetha and is of the order TX-100 > SDBS > CTAB. Surfactant interference is seen with degradation of naphthalene and is varied for different surfactants used. Zeta potential (ξ) varied for two mixed cultures used and decrease of zeta potential with Reetha additon showed its enhanced effect on naphthalene degradation especially for ionic – Reetha surfactant mixtures. Use of natural surfactant Reetha will make a promising remediation tool by replacing or substituting in part or full of synthetic surfactants; ultimately making their use and easy removal from the environment with effective biodegradtion.

Chapter 6

Conclusions and suggestions for future work

The plant based surfactant Reetha was investigated primarily for its solubilization potential with synthetic nonionic, cationic and anionic surfactants and their property to enhance bioavailability and biodegradation of naphthalene also studied.

By comparing the solubility of Reetha with the synthetic surfactants the following order of MSR and log K_m values are observed: TX–100 > CTAB > Reetha > SDBS > AOT > SOS. The increasing order of CMC values of these surfactants are: TX–100 < Reetha < CTAB < SDBS < AOT < SOS. In general, lower the CMC values higher the solubilization efficiency of the surfactants. Here, except Reetha, all the synthetic surfactants studied are followed the CMC rule. Efficiency of Reetha is expected after TX–100 but practically it is just after CTAB. In general, it is reported, non ionic surfactants are better solubilizing than ionic surfactants, because of their lower CMC. Even, solubilization capacity of a nonionic surfactant of polyoxyethylene type for water insoluble materials is much greater than that of an ionic surfactant having the same hydrocarbon chain length as that of the nonionic surfactant, this fact suggests that the polyoxyethylene part in the molecule also plays an important role in solubilization. Between cationic and anionic surfactants with similar chain length cationic surfactant shows greater solubilization efficiency of PAHs due to adsorption at micellar water interface in addition to solubilization in the inner core of the micelle. Less solubilization efficiency of Reetha than expected based on the CMC value is attributed in terms of complex structure of the micellar core, hydrophobic part is consisting of five six member cyclic hydrocarbon rings resulting shorter effective chain length, presence of carboxylic group in the hydrophobic region, and low micellar aggregation no.

Solubilization in the mixed surfactant systems Reetha - TX-100 is showing ideal behavior and Reetha with all other ionic surfactants are showing nonideal behavior. The deviation in solubility from ideality is identified by the deviation ratio (R). All the ionic surfactants show negative deviation in presence of Reetha. The maximum deviation occurs for CTAB, SDBS, AOT, and SOS are – 18 %, - 39.68 %, - 53 %, and 78.4 % respectively. Now, by summarizing all the results of mixed surfactant systems we can conclude that ascending order of Δ_{MSR} (% change in MSR) are TX–100 – Reetha < CTAB – Reetha < SDBS – Reetha < AOT – Reetha < SOS – Reetha, which is the reverse order of sequence that of single surfactants for solubilization or same sequence of CMC. In the mixed surfactant systems when the synergism is there (more negative β or interaction parameter of mixed micelle) the micelle will be more densely packed and solubilization may decrease; but at the same time the mixed micellar CMC also will be low. In that case, at a particular concentration, since the number of micelle will be more the solubilization also will enhance. The value of positive or negative deviation will depends on the factor which is predominant.

Solubilization and surface tensiometry have been used to study mixtures of nonionic Reetha with nonionic, cationic and nonionic surfactants without electrolyte. Interaction parameters estimated from surface tension data suggest interactions between Reetha with other surfactants to follow an order anionic/Reetha > cationic/Reetha > nonionic/Reetha. Moderate synergy between Reetha with cationic and anionic surfactants observed with more interaction and excess enthalpy of micellization was negative, implying that the mixed micellization was exothermic. On the other hand, behavior of mixtures of Reetha - nonionic surfactant (TX-100) is close to that for ideal mixing with limited interaction. A 0.35 CMC ratio of Reetha with other surfactants showed better interaction and improved with less than 0.35. The bioavailability and biodegradation of naphthalene studied by two mixed cultures with Reetha and synthetic surfactants like nonionic (TX–100), cationic (CTAB), anionic (SDBS). The solubilization efficiency of single surfactants showed that Reetha was less effective than TX–100, CTAB and similar to SDBS, but biodegradation of naphthalene was more in the presence of surfactant mixtures indicate more bioavailability to microorganisms. Two mixed culture's naphthalene degrading ability in the presence of surfactants (pure/mixtures) tested. Naphthalene biodegradation explained with the help of growth rate constants (K_g), naphthalene degradation rate constants (K), surfactant (pure/mixtures) utilization (by % change in surface tension) and with zeta potential values of the surfactant combinations. Naphthalene degraded was more in CTAB, SDBS - Reetha mixtures and less in case of TX-100 - Reetha mixtures, while it is opposite in mixed surfactant utilization. Additon of Reetha surfactant to surfactants TX-100, CTAB and SDBS proved more beneficial to WWC than KC. Utilization of naphthalene along with surfactants (pure/mixtures) explained and mixed micellar concentrations above critical micelle concentration were not toxic to the microorganisms used. Zeta potential for mixed cultures was monitored for surfactants (pure/mixtures) and its relation to K values explained. To solubilize wide range of contaminants we are proposing a treatment scheme that employs plant-based natural surfactants along with synthetic surfactants, which in turn limits the synthetic surfactant load to soil and ground water remediation and helps easy removal after the process. The results of this study provide the usefulness of applying plant based natural surfactants with synthetic surfactants in surfactant enhanced remediation and degradation.

Suggestions for future work

Following suggestions are made for the future work

- Plant - synthetic surfactant mixtures property to solubilize a wide range of complex organic compounds may be investigated.
- Experimenting and substituting a part or in full of synthetic surfactants with plant based surfactants for soil/water remediation studies by knowing their interaction behavior.
- Hazardous contaminants degradation enhancement by various microorganisms including innate fungal and other species in the presence of plant surfactant mixtures should be tested.

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Publications

Journal

- K. Jagajjanani Rao and Santanu Paria. "Solubilization of Naphthalene in presence of plant and plant – synthetic mixed surfactant systems", *Journal of Physical Chemistry B* 113, 474–481 (2009).

To be communicated

- K. Jagajjanani Rao and Santanu Paria. "Mixed micellar characteristic of *Sapindus mukorossi* – synthetic mixed surfactant systems".
- K. Jagajjanani Rao and Santanu Paria. "Biodegradation of naphthalene in presence of *Sapindus mukorossi* – synthetic mixed surfactant systems".

Presented at conference

- K. Jagajjanani and Rao Santanu Paria. "Mixed micellar and solubilization behavior of *Sapindus mukorossi* – synthetic mixed surfactant systems", *International conference on Environmental Research* (ICER-2008), BITS – Goa, Dec. 18-20, 2008.
- K. Jagajjanani Rao and Santanu Paria**.** "Biodegradation of Naphthalene in presence of plant, synthetic and mixed surfactant systems", *International conference on Environmental Research* (ICER-2008), BITS – Goa, Dec. 18-20, 2008.

THE JOURNAL OF
PHYSICAL CHEMISTRY B

Solubilization of Naphthalene in the Presence of Plant#Synthetic Mixed Surfactant Systems

K. Jagajjanani Rao, and Santanu Paria

J. Phys. Chem. B, **2009**, 113 (2), 474-481 • DOI: 10.1021/jp8071298 • Publication Date (Web): 19 December 2008

The Journal of Physical Chemistry B is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Solubilization of Naphthalene in the Presence of Plant−Synthetic Mixed Surfactant Systems

K. Jagajjanani Rao and Santanu Paria*

Department of Chemical Engineering, National Institute of Technology, Rourkela 769 008, Orissa, India

Received: August 10, 2008; Revised Manuscript Received: November 7, 2008

Solubilization efficiencies of naphthalene by micellar solution of a plant-based surfactant extracted from fruit of *Sapindus mukorossi* (reetha) and the synthetic surfactants like nonionic (Triton X-100 or TX-100), cationic (cetyltrimethylammonium bromide or CTAB), and anionic (sodium dodecylbenzenesulfonate or SDBS; dioctyl sodium sulfosuccinate or AOT; sodium octanesulfonate or SOS) in their single and as well as binary mixed (plant−synthetic) systems were measured and compared. The solubilization efficiency of single surfactants shows that reetha is less effective than TX-100, similar to SDBS, and more than AOT and SOS. The mixed surfactant systems show negative deviation in molar solubilization ratio (MSR) from ideality. The ascending order of percent change in MSR (Δ_{MSR}) is TX-100−reetha < CTAB−reetha < SDBS−reetha < AOT−reetha < SOS−reetha. The mixed micellar solution for a particular combination also shows that Δ_{MSR} is more negative when the interaction parameter for the mixed micelle (β) is more negative. The results of this study may be useful for the applications of natural or natural−synthetic mixed surfactants in surfactant-enhanced remediation or detergency.

1. Introduction

Hydrophobic organic contaminants (HOCs) are found in the priority list of hazardous substances listed by the U.S. EPA and the Agency for Toxic Substances and Disease Registry of USA.[1] The hydrophobicity of an organic contaminant is one of the important factors that determine the fate of the contaminant in the environment. In general, the more hydrophobic contaminant is more likely to be associated with the nonpolar organic matter such as humic substances and lipids (fats) in the soil. Polyaromatic hydrocarbons (PAHs) like naphthalene, anthracene, and phenanthrene are commonly found in petroleum fuels, coal products, and tar. Because of their persistence in nature, these are generally hazardous, sometimes above the chronic level and prevalent at many contaminated sites, such as former manufactured gas plant sites and wood treatment facilities.[2] Because of their existence in the environment, they are affecting human health by toxic, mutagenic, and carcinogenic properties; much research effort has been aimed for remediation of PAH-contaminated sites.[3] PAHs are removed from contaminated sites principally by microbial degradation. However, other possible fates of PAHs are volatilization, photooxidation, chemical oxidation, bioaccumulation, adsorption, and adhesion to the soil matrix.[4] From the remediation perspective, it is important to examine the environmental properties of these compounds. Because of their high adsorption capacity and low water solubility, these compounds are relatively immobile and are often not readily bioavailable, and they can stay for a long time in the soil.[5]

Surfactants flushing emerged as new alternative to treat PAH-contaminated soil and water systems, and many laboratory studies are reported.[6,7] Surfactants in aqueous solution solubilize the water insoluble hydrophobic compounds in the core of the micelles.[8] The effectiveness of a surfactant in solubilizing a particular solute is known as MSR, expressed by[8]

$$\mathrm{MSR} = (S - S_{\mathrm{CMC}})/(C_{S-\mathrm{CMC}}) \qquad (1)$$

where S and S_{CMC} are the apparent solubility of an organic compound at surfactant concentration C_S (C_S > CMC) and that at the CMC, respectively. Thus, when solute concentration in the solvent phase is plotted against surfactant concentration above the CMC, MSR can be determined from the slope of the linearly fitted line.

Mixed surfactant systems for the remediation of organic contaminants are gaining more and more importance over the single surfactants in recent years due to their better performance than the single surfactants.[9,10] The solubilization performances of organic compounds in mixed surfactants aiming for remediation application are mostly laboratory scale and limited than that of single surfactants.[6,9−13] However, until now there is a very little information available on their fate in subsurface. The surfactants left in subsurface after the remediation process may have a negative or positive influence on the biodegradation of the organic compounds.[14] The nonbiodegradable synthetic surfactants along with organic contaminants become hazardous to the environment. In this case, the surfactant toxicity and its potential are to be carefully considered prior to the selection of surfactants for soil and water bodies cleanup.[15] Biosurfactants are found to be promising recently due to their environmentally friendly nature; however, their use has been limited in industry due to some technical and/or economic reasons. Natural surfactants from plant origin may gain more importance in the future, but very few studies have been done employing them for hydrophobic contaminants removal from the soil.[16]

Plant-based natural surfactant derived from *Sapindus mukorossi*, commonly known as "soapnut" or reetha, is a saponin, a non-ionic glycoside containing sugars such as D-glucose, D-xylose, L-arbinose, L-rhamnose, and glucoronic acid.[17] Solubilization studies using reetha surfactant exposed its remediation potential on different hydrophobic contaminants in soil and water systems[18,19] along with efficient biodegradable property under both aerobic and anaerobic conditions.[19,20]

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* Corresponding author. E-mail: santanuparia@yahoo.com or sparia@nitrkl.ac.in; Fax +91 661 246 2999.

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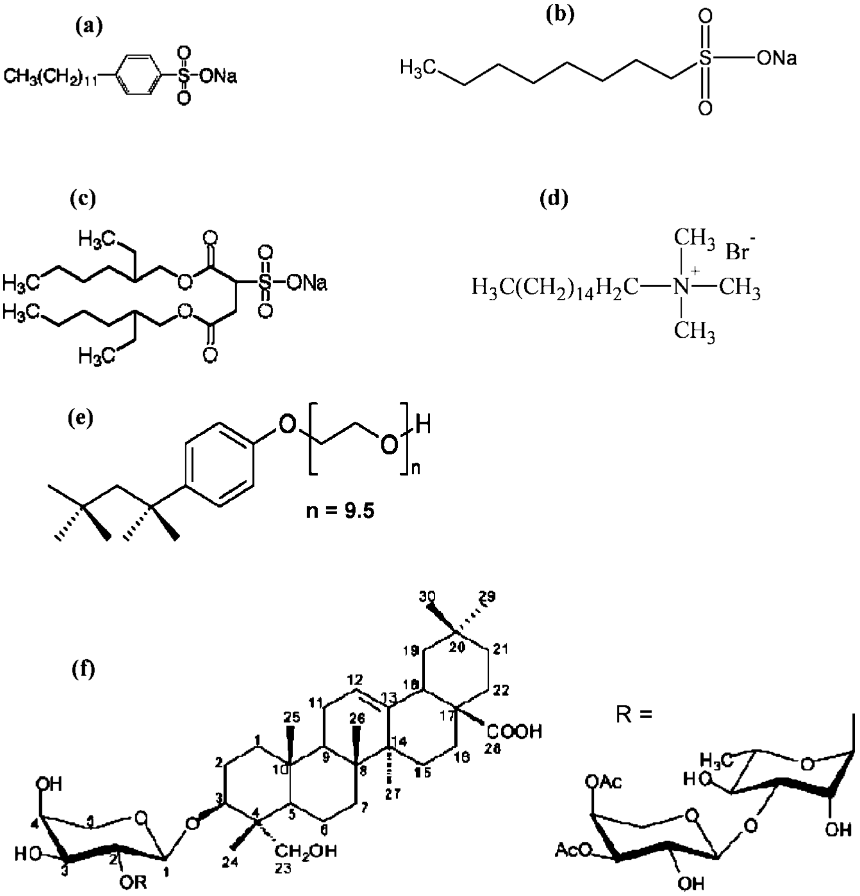

Figure 1. Structures of surfactant molecules used in this study: (a) SDBS, (b) SOS, (c) AOT, (d) CTAB, (e) TX-100, and (f) reetha.

In this paper, we have studied the solubilization of naphthalene in the presence of reetha in different proportions with cationic, nonionic, and anionic synthetic surfactants, which has not been reported to the best of our knowledge. The objective of this paper is to evaluate the efficiency of reetha surfactant and synthetic−reetha surfactant combinations to solubilize naphthalene in aqueous systems using batch studies for the application of surfactant-enhanced remediation soil and other applications like detergency and industrial cleaning, etc. As the synthetic surfactants hinders biodegradation process,[21,22] that used in remediation process or in laundry cleaning creates lots of environmental problem. The use of only natural surfactant or synthetic−natural surfactant mixture enables easy degradation. Nowadays, many countries are trying to focus on the biosurfactants from microorganism as a substitute of synthetic surfactants, but in our view, production of plant-based surfactant is very easy in comparison to biosurfactants, and a huge amount of plant surfactant production is possible to substitute synthetic surfactants. In that respect, it is worthy to give more insight in this area.

2. Experimental Section

2.1. Materials. The surfactants TX-100, CTAB, AOT, and SOS were obtained from Loba chemie Pvt Ltd., India, sodium dodecylbenzenesulfonate (SDBS) was from Sigma Aldrich, Germany, and naphthalene was from Spectrochem Pvt. Ltd., India. All the chemicals were used as received. Dry fruit (reetha or *S. mukorossi*) was purchased from the local market, Rourkela, Orissa, India. The structures of surfactants used in this study are shown in Figure 1. Ultrapure water (Sartorius AG Gottingen, Germany) was used for the experiments of 18.2 mΩ resistivity and pH 6.8−7. Surface tensions of surfactant solutions were determined by the Whilmey plate method using a surface tensiometer, Data Physics, Germany (DCAT-11EC). Naphthalene concentrations were measured by a UV−vis spectrophotometer (Jasco, Japan, V-530). The organic content of the plant surfactant solution was measured by total organic carbon analyzer (Shimadzu, Japan, TOC-VCPN).

2.2. Extraction of Reetha Surfactant from the Fruit. After removing seed from fruit, the pericarp was dried in an oven at 50 °C for about 48 h and then powdered in a grinder. The powdered sample was extracted with methanol gave a brown syrup. The suspended particles were separated by centrifugation at 8000 rpm for 15−20 min and followed by vacuum evaporation and drying of the extract at 60−80 °C, which gave a light yellowish white powder.

2.3. Methods. All the solubility experiments were done in the polypropylene falcon tubes of capacity 15 mL. A stock solution of naphthalene (400 mM) was prepared by dissolving naphthalene in pure methanol is preferred over higher alcohols because of its negligible effect on solubilization and CMC values.[23] For experiment 0.1 mL of naphthalene from the stock was added to 10 mL of surfactant solution of a specified strength in falcon tube, as 1% methanol will not affect the solubility of PAH.[8,9] The concentration and volume of the stock naphthalene solution used in each experiment was selected to (i) keep the methanol concentration in the test solution to below 1 vol % and (ii) to give an excess of naphthalene in the test solution. The tube was sealed with a screw cap to prevent volatilization loss of naphthalene from water. For the study 24 h equilibration time was taken as kinetics of solubilization show no change in solubility of naphthalene;[9] after 24 h equilibration period and

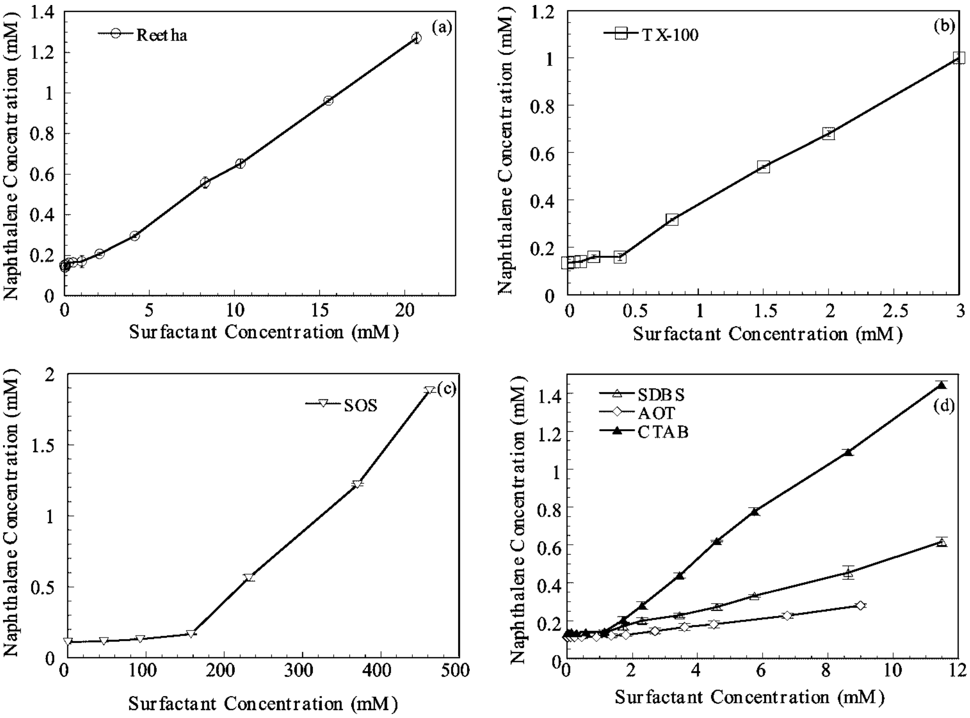

Figure 2. Solubilization of naphthalene by using surfactants (a) Reetha, (b) TX-100, (c) SOS, and (d) SDBS, CTAB, AOT.

subsequent centrifugation at 5000 rpm for 30 min to separate the suspended solids, if any, the concentration of naphthalene was determined by measuring the absorbance at 274 nm wavelength with a quartz cuvettes of 10 mm path length using a UV−vis spectrophotometer. Note that subsequent dilutions were made whereever needed, and surfactant concentration was kept the same in both the reference and the measurement cells to minimize the effect of surfactant on UV absorbance. All experiments were performed at room temperature, 25−28 °C. Experiments were done in triplicate, and the average values are reported.

3. Results and Discussion

3.1. Structure and Physical Properties of Reetha Solution. The structure of reetha is most important to know before doing the experiment. Huang et al.[24] have proposed the structure of saponin isolated from *S. Mukorossi* is shown in Figure 1. They have mentioned that depending on the R group seven different structures are possible. In our sample total carbon content was analyzed using TOC analyzer and found the value of 152.5 ppm for 0.03 wt % solution. Back-calculating the TOC of all the probable structures proposed by Huang et al.,[24] our value shows less error when we consider the R group given in Figure 1 and with a molecular weight of 966. We have also compared our TOC value with the reported value by Kommalapati et al.[19] which was 41 g/L for 10% solution, which is close to our value 49.24 g/L. The CMC of reetha solution found was around 0.05 wt % (0.510 mM) as measured by a surface tensiometer, which is comparable with the reported value 0.045 wt %.[18] The CMC value obtained from solubilization curve (0.517 mM) is also shows good agreement with the surface tension data. pH of 1% solution was 4.34 close to the reported value of 4.5.[19]

3.2. Solubilization by Single Surfactants. In this paper we have mainly focused on the solubilization behavior in presence of mixture of reetha and different synthetic surfactants. Before studying the mixed systems, single surfactants were studied to get an idea about the effectiveness of different synthetic surfactants (TX-100, CTAB, SDBS, SOS, and AOT) with respect to reetha. The solubility of naphthalene in presence of synthetic surfactants and reetha is shown in Figure 2a−d. MSR values of the single surfactant systems are calculated from the slope of the linear portion of the experimental data (surfactant concentration in mM vs naphthalene concentration in mM) given in Table 1. The effectiveness of solubilization can also be expressed in terms of the other parameter like partition coefficient (K_m). The partition coefficient is defined as partition of the organic compound between micelles and the aqueous phase, which can be written as

$$K_m = X_m/X_a \tag{2}$$

where X_m and X_a are the mole fractions of solute in micelles and the aqueous phase, respectively. The value of X_m can be calculated as $X_m = \mathrm{MSR}/(1 + \mathrm{MSR})$, and X_a can be expressed as $X_a = S_{CMC}V_w$, where $V_w = 0.018\,05$ L/mol is the molar volume of water. Consequently, eq 2 can be rearranged to yield

$$K_m = \frac{\mathrm{MSR}}{S_{CMC}V_w(1 + \mathrm{MSR})} \tag{3}$$

The following order of MSR and log K_m values is observed: TX-100 > CTAB > reetha > SDBS > AOT > SOS, shown in Table 1. The difference between reetha and SDBS is found very close. The increasing order of CMC values of these surfactants is TX-100 < reetha < CTAB < SDBS < AOT < SOS. In general, the lower the CMC values, the higher the solubilization efficiency of the surfactants. Here, except for reetha, all the synthetic surfactants studied followed the CMC rule. Efficiency of reetha is expected after TX-100, but practically it is just after CTAB. In general, it is reported that nonionic surfactants are better solubilizing than ionic surfactants because of their lower

CMC. Even the solubilization capacity of a nonionic surfactant of polyoxyethylene type for water-insoluble materials is much greater than that of an ionic surfactant having the same hydrocarbon chain length as that of the nonionic surfactant; this fact suggests that the polyoxyethylene part in the molecule also plays an important role in solubilization.[25] Between cationic and anionic surfactants with similar chain length cationic surfactant shows greater solubilization efficiency of PAHs due to adsorption at micellar water interface in addition to solubilization in the inner core of the micelle.[9] Here, though the CMCs of CTAB and SDBS are very close, due to chain length difference and cationic nature, CTAB shows a higher solubilization efficiency. Less solubilization efficiency of reetha than expected based on the CMC value is attributed in terms of complex structure of the micellar core due to bulkiness of both the hydrophobic (tail) and hydrophilic (head) group. The hydrophobic part is consists of five six-member cyclic hydrocarbon rings, resulting in shorter effective chain length and less hydrophobic in nature. As a general rule, incorporation of a phenyl group in the alkyl group increases its hydrophobicity to a much smaller extent than increasing its chain length with the same number of carbon atoms.[26] Another reason may be the presence of carboxylic group in the hydrophobic region reduces the solubilization of naphthalene molecule in the inner core due to repulsion between the carboxylic group and π-electron cloud of naphthalene ring. Among the synthetic surfactants, TX-100 and AOT have branched chain hydrocarbon tail and others have straight chains; as a result, their core region is not as complicated as reetha and more hydrophobic in nature due to the presence straight or branched chain hydrocarbon tail. Finally, the micellar aggregation number also plays an important role in the solubilization. The decreased aggregation number in the micelles causes reduced solubilization of nonpolar substances.[23] The reported values of micellar aggregation number of the surfactants TX-100, CTAB, SDBS, AOT, SOS, and reetha are ~145,[27] ~60,[28] ~28,[29] ~22,[30] ~25,[31] and ~21,[18] respectively. All the synthetic surfactants show the increasing solubility order according the order of micellar aggregation numbers. However, reetha has the lowest micellar aggregation number but shows higher solubility than SDBS, AOT, and SOS due to its low CMC value. A lower micellar aggregation number indicates lower solubilization capacity per micelle, but at the same time a lower CMC indicates more number of micelles present at a particular concentration. Since SDBS, AOT, and SOS show higher CMC than reetha, they have lower solubilization efficiency.

3.3. Solubilization by Surfactant Mixtures. Solubilization of naphthalene by aqueous surfactant mixtures composed of reetha with TX-100, CTAB, SDBS, AOT, and SOS is done by taking different mole proportions of surfactants and compared with those by single surfactants (The plots of change of naphthalene solubilization in the presence of mixed surfactant systems are shown in the Supporting Information.) Table 1 summarizes different parameter values of mixed surfactant systems like mixed CMC, MSR, and log K_m. Mixed CMC values reported here are obtained from the surface tension data; there is also a good agreement with the values obtained from solubilization plot. It is observed from Table 1 that mostly the MSR values of mixed systems are in between that of the single surfactants. Figure 3a depicts the effect of change in MSR values of different synthetic surfactants in presence of reetha. The figure clearly indicates the MSR values are changing nonlinearly with the change of mole fraction of reetha (X_A); in addition, that is an indication of nonideal behavior of mixed surfactant systems. To measure the nonideality and also to see the nature of deviation (positive or negative), we have calculated the deviation ratio. The deviation ratio is calculated by

$$R = \mathrm{MSR}_{\mathrm{exp}}/\mathrm{MSR}_{\mathrm{ideal}} \qquad (4)$$

where $\mathrm{MSR}_{\mathrm{exp}}$ is the experimentally determined MSR and $\mathrm{MSR}_{\mathrm{ideal}}$ is the MSR of naphthalene in a binary surfactant mixture based on ideal mixing, i.e.

$$\mathrm{MSR}_{\mathrm{ideal}} = \mathrm{MSR}_1 X_1 + \mathrm{MSR}_2 X_2 \qquad (5)$$

where X_1 and X_2 are mole fraction of surfactant 1 and 2, respectively; MSR_1 and MSR_2 are MSR values of pure surfactants 1 and 2, respectively. The values of $R > 1$ indicate positive deviation, and $R < 1$ indicate negative deviation. The change in R with the mole fraction of reetha is shown in Figure 3b.

3.3.1. Mixing Effect of Nonionic−Reetha Surfactants. The mixing behavior of TX-100−reetha systems are depicted in Figure 3a,b and Table 1. The MSR and log K_m values with different mole fractions of reetha show the values are decreasing with the increase in mole fraction of reetha (X_R). The results indicate solubilization follow almost ideal behavior with small deviation for this combination of surfactants. From the table it is clear the deviation ratio (R) is close to 1 for most of the compositions and percent change in MSR (Δ_{MSR}, $(\mathrm{MSR}_{\mathrm{exp}} - \mathrm{MSR}_{\mathrm{ideal}}) \times 100/\mathrm{MSR}_{\mathrm{ideal}}$ or $(R - 1) \times 100$) is less than ~−14%. The maximum Δ_{MSR} is observed at $X_R = 0.07$. It is also observed that MSR values are changing linearly (with correlation coefficient of linear fit above 0.97 and above 0.99 if the point $X_R = 0.07$ is excluded) for this combination with the change of reetha mole fraction, an indication of ideal behavior.

3.3.2. Mixing Effect of Cationic−Reetha Surfactants. Similar to the previous combination, the MSR and K_m values for CTAB−reetha combination also mostly decreased with the increase in mole fraction of reetha (see Figure 3a,b and Table 1). Initially, with very low concentration of reetha Δ_{MSR} is positive, and above the reetha mole fraction of 0.14 the value becomes negative. The maximum values of positive and negative change are ~2% ($X_R = 0.14$) and ~−18% ($X_R = 0.77$), respectively. A similar trend is also observed for log K_m.

3.3.3. Mixing Effect of Anionic−Reetha Surfactants. We have studied three anionic surfactants SDBS, AOT, and SOS with a variation of chain length and structure. The change in MSR values with X_R does not follow a linear relationship, indicating the nonideal behavior for all the cases. It is observed from Figure 3b that the nature of change in R for SDBS is in reverse order to that of CTAB with respect to mole fraction of reetha. We will explain this in detail in the section 3.5. The negative deviation for SDBS is maximum (−39.68%) at $X_R = 0.04$. Negative deviation is observed up to ~0.5 mole fraction; after that the deviation is positive with a very low value of maximum 1.48%, i.e., close to ideal behavior. In contrast to previous surfactants (TX-100, CTAB, and SDBS) AOT shows overall negative deviation for all the compositions with more negative values and a maximum of −53% at $X_R = 0.16$. Similar to AOT, SOS also shows over all negative deviation. Among all the surfactants studied here SOS shows highest negative deviation of −78.43% at $X_R = 0.09$.

Now, by comparing all the results of mixed surfactant systems, we can conclude that ascending order of Δ_{MSR} is TX-100−reetha < CTAB−reetha < SDBS−reetha < AOT−reetha < SOS−reetha, which is the reverse order of sequence that of

TABLE 1: Values of X_R, MSR, R, Δ_{MSR}, log K_m, B, β, CMC, and x_1 for Mixed Surfactant Systems

surfactant	X_R or α_1	MSR_{ideal}	MSR_{exp}	log K_{mExp}	R	Δ_{MSR} (%)	B^b	β^a	CMC_{ideal} (mM)	CMC_{exp} (mM)	x_1
TX-100	0.00	0.312	0.312	4.937	1.000	0.000	0.000	0.00	0.309	0.309	0.000
	0.07	0.294	0.253	4.866	0.859	−14.09	0.878	−1.009	0.318	0.239	0.197
	0.22	0.255	0.251	4.863	0.985	−1.46	1.018	−0.984	0.339	0.292	0.227
	0.40	0.209	0.219	4.815	1.049	4.95	1.029	−0.625	0.368	0.322	0.331
	0.61	0.155	0.159	4.698	1.028	2.78	0.779	−0.179	0.409	0.392	0.485
	0.86	0.091	0.081	4.437	0.897	−10.26	0.128	−0.194	0.437	0.456	0.765
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.472	0.517	1.000
CTAB	0.00	0.124	0.124	4.630	1.000	0.00	0.000	0.000	1.097	1.097	0.000
	0.04	0.121	0.133	4.629	1.096	9.59	1.552	−0.106	1.050	1.029	0.097
	0.14	0.114	0.117	4.579	1.021	2.08	0.802	−0.318	0.949	0.897	0.283
	0.27	0.105	0.086	4.459	0.821	−17.92	0.072	−0.430	0.839	0.755	0.455
	0.47	0.091	0.080	4.430	0.878	−12.22	0.319	−0.653	0.719	0.619	0.617
	0.77	0.070	0.057	4.292	0.817	−18.30	−0.705	−1.247	0.588	0.480	0.770
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000
SDBS	0.00	0.051	0.051	4.249	1.000	0.00	0.000	0	1.148	1.148	0.000
	0.04	0.051	0.031	4.038	0.699	−39.68	−5.339	−0.124	1.096	1.069	0.102
	0.13	0.052	0.032	4.045	0.695	−38.99	−2.377	−0.318	0.987	0.927	0.283
	0.27	0.052	0.034	4.077	0.730	−34.57	−1.681	−0.429	0.867	0.781	0.455
	0.46	0.052	0.053	4.262	1.093	1.06	0.004	−0.652	0.737	0.634	0.617
	0.76	0.053	0.054	4.270	1.049	1.48	0.060	−1.036	0.594	0.544	0.820
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0	0.517	0.517	1.000
AOT	0.00	0.020	0.020	3.853	1.000	0.00	0.000	0	2.700	2.700	0.000
	0.05	0.022	0.016	3.758	0.739	−26.08	−2.415	−0.591	2.240	1.724	0.310
	0.16	0.026	0.012	3.634	0.469	−53.07	−3.955	−0.758	1.593	1.319	0.506
	0.32	0.031	0.019	3.831	0.620	−38.02	−2.848	−1.207	1.159	0.886	0.635
	0.52	0.038	0.023	3.912	0.613	−38.67	−3.166	−1.208	0.848	0.699	0.754
	0.81	0.047	0.034	4.077	0.720	−28.01	−3.686	−1.347	0.614	0.566	0.896
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000
SOS	0.00	0.005	0.005	3.257	1.000	0.00	0.000	0.000	157.203	157.2	0.000
	0.02	0.006	0.002	2.838	0.307	−69.28	−10.598	−2.256	18.831	6.849	0.637
	0.09	0.009	0.002	2.861	0.216	−78.43	−14.849	−1.930	5.715	3.524	0.762
	0.18	0.014	0.009	3.511	0.646	−35.38	−9.403	−4.080	2.788	2.069	0.827
	0.34	0.022	0.011	3.597	0.506	−49.42	−7.955	−5.370	1.498	1.061	0.826
	0.67							−7.255	0.773	0.525	0.832
	1.00	0.054	0.054	4.270	1.000	0.00	0.000	0.000	0.517	0.517	1.000

[a] β_{av}: TX-100 = −0.560; CTAB = −0.458; SDBS = −0.511; AOT = −1.022; SOS = −4.178. [b] B_{av}: TX-100 = 0.766; CTAB = 0.408; SDBS = −1.866; AOT = −3.214; SOS = −10.701

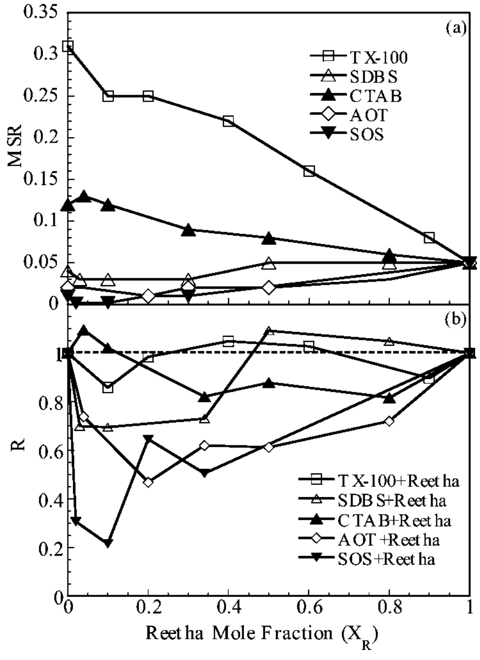

Figure 3. Comparison of (a) MSR and (b) deviation ratio, R, of naphthalene as a function of mole fraction of reetha for different mixed surfactant systems.

single surfactants for solubilization or same sequence of CMC. From Table 1 it is worthy to note that when the synthetic surfactants are having higher MSR than reetha, the mixed systems are showing lower MSR than only synthetic surfactant. Similarly, when the synthetic surfactants are having lower MSR than reetha, then the mixed systems are showing higher MSR than only synthetic, but the negative deviations indicate lower MSR than expected or calculated considering ideal mixing.

3.4. Effect of Surfactant Mixtures on Micelle−Water Partition Coefficient. Treiner et al.[32] have suggested the following relationship for the partitioning of a neutral nonpolar organic solute between micelle and aqueous phase in a mixed surfactant systems as

$$\ln K_{m1,2} = X_{m1} \ln K_{m1} + (1 - X_{m1}) \ln K_{m2} + BX_{m1}(1 - X_{m1})$$

where K_{m1}, K_{m2}, and $K_{m1,2}$ are the micelle−water partition coefficients of a solute for the single surfactants constituting the mixed micelle and mixed systems, respectively, and X_{m1} is the micellar mole fraction of surfactant 1. B is an empirical parameter including surfactant−surfactant (like β) and surfactant−solute interactions. The value $B = 0$ indicates there is no mixing effect of surfactants on the partition of the solute;[33] $B > 0$ or $B < 0$ implies that K_m in the mixed surfactant system is larger or smaller, respectively, than predicted by ideal mixing rule.[11] X_1 can be calculated using mixed micellar theory discussed in the next section.

The B values are calculated from the above relation for nonionic–reetha, cationic–reetha, and anionic–reetha mixtures and presented in Table 1. The average B values obtained for napthalene in TX-100–reetha, CTAB–reetha, SDBS–reetha, AOT–reetha, and SOS–reetha mixed surfactant systems are 0.77, 0.40, −1.86, −3.21, and −10.701, respectively. The B values are found to be negative except TX-100 and CTAB–reetha mixtures, and the positive values are also less than one. According to the values of B, the mixing effect of anionic–reetha surfactants on the partition of naphthalene is negative in SDBS–reetha, AOT–reetha, and SOS–reetha mixed systems and consistent with the result of negative deviation of MSRs from ideal, which can be used to explain the mixing effect of anionic–nonionic surfactants on the solubilization of naphthalene. Similarly, that of solubilization of pyrene reported by Zhou et al.[33] for anionic–nonionic systems as they got positive B value and positive deviation of MSR. However, mixtures of TX-100–reetha and CTAB–reetha show the partition of naphthalene is positive, which are contradictory with the some negative deviation of MSRs for some combinations as shown in Table 1 with different mole fractions of reetha from the ideal mixture. Similar contradictory results were also found before in other studies.[33−35] Hence, these results indicate sometimes the mixing effect of surfactants on the $K_{\mathrm{m}1,2}$ is contrary to that on the solubilization of organic compounds in mixed surfactants.

3.5. Solution Properties of Mixed Surfactant Systems. The results of solubilization in the mixed surfactant systems indicate the behavior is complex in nature and mostly follow nonideal path. Since solubilization is closely associated with the solution properties of the surfactant micelles, to explain the above results we have studied mixed micellar properties of all the combinations. The mixed CMC values obtained from the experiments are reported in Table 1. To know whether the mixed solution follows ideal or nonideal behavior mixed CMC values were calculated using ideal solution theory.[36]

$$\frac{1}{\mathrm{CMC}_{12}} = \frac{\alpha_1}{\mathrm{CMC}_1} + \frac{1-\alpha_1}{\mathrm{CMC}_2} \tag{6}$$

where CMC_1, CMC_2, and CMC_{12} are the critical micelle concentrations of reetha, pure synthetic surfactant, and mixed surfactant. α_1 is the mole fraction of component 1 in mixed surfactant solutions. Table 1 clearly indicates that there is a deviation between the ideal and experimental CMC values. The mixed CMCs of TX-100, CTAB, SDBS, and AOT with reetha surfactant are intermediate between the respective individual surfactants used in the combination. In the case of the TX-100–reetha mixture the experimental values are close to ideal, and in the other cases experimental CMC values are lower than the ideal values, indicating negative deviation from the ideal behavior for mixed micelle formation. The data for the other surfactants show there is a difference between the experimental and theoretical CMC values, and the deviation is highest for the SOS–reetha mixture. In further, for nonideal binary mixture of surfactants Rubingh's equation of regular solution theory can be used to calculate the micellar mole fraction.[37]

$$\frac{x_1^{\,2}\ln\left(\dfrac{\alpha_1 C_{12}}{x_1 C_1}\right)}{(1-x_1)^2\ln\left(\dfrac{(1-\alpha_1)C_{12}}{(1-x_1)C_2}\right)} = 1 \tag{7}$$

The micellar mole fraction (x_1) can be calculated from eq 7 by solving iteratively. To show the interaction between two surfactants, β is an interaction parameter which indicates the interaction between two surfactant molecules in the mixed micelle and is a measure of deviation from the ideal behavior. The parameter β can be now calculated by substituting the value of x_1 in the equation

$$\beta = \frac{\ln\left(\dfrac{\alpha_1 C_{12}}{x_1 C_1}\right)}{(1-x_1)^2} \tag{8}$$

The β parameter quantitatively captures the extent of nonideality for a mixed surfactant system. The negative value of β indicates synergism in mixed micelle formation, a positive value indicates antagonism, and, if zero, then mixed micelle formation is ideal. The larger the absolute value of β, the stronger the mixing nonideality. The negative deviation indicates a reduction in free energy of micellization over that predicted by ideal solution theory. The larger negative value of β denotes the greater negative deviation of CMCs from ideal mixture. All the surfactants used here show a synergistic effect in the mixture as it satisfied the conditions (i) β must be negative and (ii) $\ln(\mathrm{CMC}_1/\mathrm{CMC}_2) < \beta$.[38] The extent of deviation from ideality is different for various surfactant combination used as mentioned in Table 1. From the table it is very clear that β values are not constant for all the compositions in a particular binary mixture, and for some compositions the variation is more. In addition, it is also observed that β values are very much sensitive to x_1; even a small change in second or third decimal level in x_1 can give a very significant change in the β parameter. The average β values for TX-100, CTAB, SDBS, AOT, and SOS are −0.560, −0.458, −0.511, −1.022, and −4.178, respectively, indicating the ascending deviation order as CTAB < SDBS < TX-100 < AOT < SOS. These sequences are almost following the same as that of Δ_{MSR} except TX-100. Actually, the values are very close for TX-100, CTAB, and SDBS and since the β parameter is very sensitive to x_1 value; the change in sequence from the expected may be due to that. In summary, for the combinations studied here indicate if the β parameter is more negative, Δ_{MSR} is also more negative. From the β parameter values it can be attributed that AOT and SOS are having more interaction with reetha in the mixed micelle; as a result, surfactant molecules are more closely packed in the mixed micelle than the pure micelle. Since it reported that looser packing of surfactant molecules in the mixed micelle may increase the solubilization of organics in the micellar phase,[39−42] in our study we have also found a similar observation. More closely packed surfactant micelles resist entering the naphthalene molecule from aqueous phase to micellar phase by diffusion which ultimately may reduce the solubilization efficiency. When the interaction parameter is more, micelles are closely packed and lead to less solubilization and greater deviation from the predicted values. It is also observed for the ionic surfactants deviation is more than nonionic and may be due to closer packing of ionic micelle in the presence of nonionic reetha due to reduction in headgroup repulsion.

To explain the results of Figure 3a,b, we have plotted β and Δ_{MSR} vs mole fraction of reetha in Figure 4a,b. For the CTAB–reetha mixture decreasing Δ_{MSR} at high reetha mole fraction is attributed in terms of the following reasons. (i) With increasing the reetha mole fraction as interaction parameter value (β) increases, micelles are closely packed, (ii) the presence of

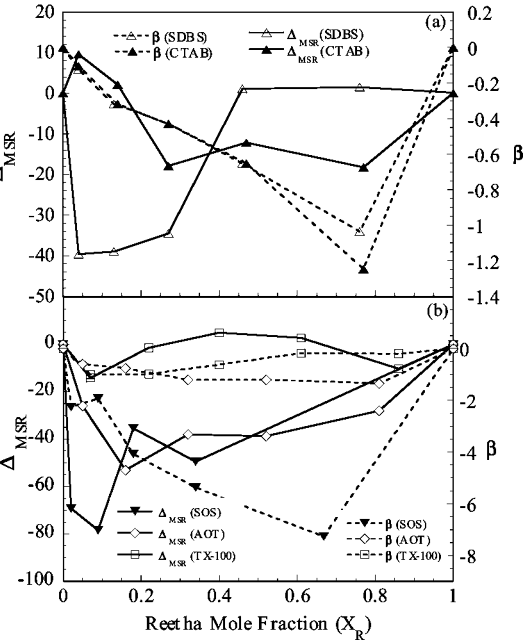

Figure 4. Comparison of β and Δ_{MSR} vs mole fraction of reetha for (a) SDBS, CTAB and (b) SOS, AOT, TX-100.

more nonionic group in the micelle shields the cationic charge of the micelle at water micelle interface and, in turn, reduces the solubilization by adsorption at the micellar surface, (iii) micelle consists of more reetha molecules which has lower solubilization capacity. For SDBS−reetha composition we have observed the opposite trend that of CTAB is also attributed: (i) The MSR values of SDBS and reetha are very close, so ideally there should not be much deviation of solubilization in the mixture. However, at low concentration of reetha repulsion between the headgroups of SDBS molecules in the micelle decreases, which may cause close packing of the molecules in the micelle and ultimately reduce the diffusion of naphthalene molecules inside the micellar core. (ii) With increasing reetha mole fraction when the micelle is dominating by reetha (x_1 ∼0.5), the deviation is less and becomes close to ideal. When the micelle is dominating by reetha, solubilization is close to that of pure reetha and the presence of more reetha molecule in the micelle may shield the negative charge of the SDBS; as a result repulsion between the mixed micelle and π-electrons of naphthalene molecule also reduced. For the other two anionic surfactants probably due to a similar reason initially there is a deep minimum, and with increasing reetha concentration as x_1 increases, the solubilization of the mixture is close to reetha and ultimately deviation decreases.

Previously, some researchers[11,12,43] have reported the opposite trend in MSR in the mixed surfactant systems, like higher MSR in the mixed systems than the individual surfactants. The solubilization efficiency depends on many factors like type and structure of surfactants, type of solubilizates, mixing ratio of surfactants, and so on.[23] For those reported studies the nonionic surfactants mixed with the ionic surfactants were higher MSR values than the ionic surfactants, and different PAHs were used. Whereas in contrast to that, for our study MSR values of TX-100 and CTAB are higher than reetha, SDBS is close to that, and SOS and AOT are lower than that; as a result we got little positive deviation in MSR for last two surfactants. In addition to that it is noteworthy in the mixed surfactant systems when the synergism is there (more negative β), the micelle will be more densely packed and solubilization may decrease; but at the same time the mixed micellar CMC also will be low. In that case, at a particular concentration, since the number of micelles will be more, the solubilization also will enhance. The amounts of positive or negative deviation will depend on the factor which is predominant.

4. Conclusion

The results of this study can be summarized as follows:

The solubilization efficiency of naphthalene by reetha in terms of MSR is less than TX-100 and CTAB, close to SBDS, and higher than AOT and SOS. The MSR values of all the mixed systems are in between that of single surfactants and are nonlinearly changing with mole fraction of reetha; the negative change in MSR than expected (Δ_{MSR}) is in the sequence of TX-100−reetha < CTAB−reetha < SDBS−reetha < AOT−reetha < SOS−reetha, which is the reverse order of the sequence that of single surfactants for solubilization.. The interaction parameter for mixed micellization (β) is negative for all the mixed surfactant systems, indicating the synergistic behavior. The average interaction parameter for mixed micellization, β_{av}, for the mixtures of TX-100, CTAB, SDBS, AOT, and SOS with reetha is −0.560, −0.458, −0.511, −1.022, and −4.178, respectively, indicating the same order as of Δ_{MSR}, except the TX-100 mixture. When the value of interaction parameter is more negative, micelles are densely packed and solubilization is less. From the application point of view, although the plant surfactant is environmentally friendly due to its fast biodegradable nature, but in terms of solubilization efficiency, commonly used synthetic surfactants like TX-100, SDBS, CTAB are better than reetha. So, use of plant−synthetic mixed surfactant systems may increase the solubility than only plant surfactant and reduce the environmental problem by replacing a certain fraction.

Acknowledgment. The financial support from University Grants Commission (U.G.C), New Delhi, India, for this project is gratefully acknowledged.

Supporting Information Available: Figure showing solubilization of naphthalene in the presence of TX-100 + reetha, CTAB + reetha, SDBS + reetha, AOT + reetha, and SOS + reetha. This material is available free of charge via the Internet at http://pubs.acs.org.

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JP8071298