Summary and Conclusion
Synthetic surfactants are petroleum derived products having ability to lower surface and interfacial tension of liquids. They are known to be toxic to animals, ecosystem and humans, and increase the diffusion of other environmental pollutants and are non-biodegradable. Despite this, they are routinely deposited in numerous ways on land and into water systems, as a part of industrial waste or household waste. Thus, biosurfactants can be used as a green alternative for synthetic surfactant. Biosurfactants are biological compounds having surface activity which as compared to their synthetic counterpart are eco-friendly, less toxic and highly biodegradable. Biosurfactants are beginning to acquire a status as potential performance effective molecules in various fields. Interest in research in characterization and applications of biosurfactant is gaining increased momentum in past few years and are expected to have many potential future industrial, environmental and medical applications.

Several authors have described the biosurfactant production and its applications in various fields. Different kinds of bacterial strains have been isolated by many researchers from soils contaminated with petroleum hydrocarbon products and/or industrial wastes. The commercial success of biosurfactants is currently limited by high cost production. Optimized growth and production conditions using cheaper renewable substrates and novel and efficient multi-step downstream processing methods could make biosurfactant production more profitable and economically feasible.

This thesis focuses on the production and characterization of biosurfactant produced by *Pseudomonas desmolyticum* NCIM 2112 and its application in decolorization of textile dye Brown 3REL. The present work also deals with isolation and identification of biosurfactant producing microorganisms from diesel contaminated soil. This includes identification of isolated strains at molecular level, optimization of biosurfactant production, elucidation of various physicochemical and structural characteristics of produced biosurfactant and their applications.

*Pseudomonas desmolyticum* NCIM 2112 is a very efficient biosurfactant producer. The produced biosurfactant was confirmed as the glycolipid type, which was verified on the basis of various techniques like HPTLC, FTIR, NMR and GCMS. The biosurfactant was structurally and physicochemically characterized as mono-rhamnolipid consisting of one rhamnose molecule and fatty acid chain of C₆–C₉. The rhamnolipid was
able to decrease the surface tension of medium to 33 dynes/cm and the yield obtained on
MSM-hexadecane medium was 2.79 g/l. Glucose-hexadecane served as the best carbon
source for rhamnolipid production and NH$_4$NO$_3$ and NaNO$_3$ were the best nitrogen
sources. C/N ratio of 10 was found to be optimum. Of the unconventional substrates
screened for rhamnolipid production, molasses followed by fried oil were found to be the
best. The emulsification potential associated with cell free broth was higher for diesel and
least for toluene. The obtained rhamnolipid was applied for the efficient decolorization of
textile dyes using *Bacillus* sp VUS NCIM 5342. *Bacillus* cells permeabilized with mono-
rhamnolipid (1 mg/ml) had doubled the rate of dye decolorization as compared to the
untreated cells. The kinetic constants estimated for decolorization at different dye
concentrations in the presence of 1 mg/ml of rhamnolipid were 1.052 mg/g cell/h for $V_{\text{dye, max}}$
and 50 mg/l for $K_m$. Studies on the effect of mono-rhamnolipid on the enzymes
involved in decolorization of dyes revealed the stimulatory effect on the extracellular
enzymes which was due to increased permeabilization of cells due to treatment with
rhamnolipid. In this study, induction of extracellular LiP, intracellular LiP and laccase
during decolorization were responsible for complete decolorization of Brown 3REL. The
difference in HPLC and FTIR spectrum of control dye and extracted metabolites
indicated biodegradation of Brown 3REL. SEM analysis of control and biosurfactant
treated cells revealed the morphological changes in the treated samples. Treatment of
*Bacillus* cells with rhamnolipids has increased its potential for dye decolorization thus
making it more competent and suitable for its use in the bioremediation of textile dye
contaminated sites. Thus, the study indicates the effectiveness of rhamnolipid on micro-
oorganisms involved in dye degradation and the enzymes involved in the process.
Rhamnolipid biosurfactant does not have any direct effect on the purified laccase enzyme
involved in the dye decolorization process.

Isolation of biosurfactant producing microorganisms was done from diesel
contaminated soil using enrichment culture technique. Among the obtained isolates, two
were selected for further studies on the basis of their ability to reduce the surface tension
profusely as compared to other strains. One of the selected isolates was identified as
*Bacillus cereus* NCIM 5391 on the basis of biochemical characteristics and phylogenetic
analysis. Of the various unconventional substrates used as carbon source for biosurfactant
production, groundnut oil cake served as the best carbon source, reducing the surface tension of by 66% and yield of 1.72 g/l. The emulsification index was higher for diesel and lowest for xylene. The produced biosurfactant was identified as lipopeptide type using TLC, FTIR, fatty acid and amino acid analysis and MALDI-TOF analysis. Lipopeptide was named “cerefactin”, that showed the m/z of 1242 and 1266, having a peptide part as Phe-Ala-Tyr-Ile-Ala-Tyr-Pro-Arg and side chains of C\textsubscript{15} and C\textsubscript{17} respectively. Cerefactin showed intense antimicrobial activity against both gram positive and negative microorganisms and fungal strains. It also showed anti-adhesive activity and inhibited the biofilm formation in urethral catheters against many pathogenic organisms. SEM analysis revealed morphological changes in tested microorganisms and the results of fluorescent microscopy indicated that the lysis of cells due to pore formation in the membrane is the plausible mode of action of the lipopeptide.

The strain identified as Enterobacter sp. NCIM 5392 on the basis of biochemical and phylogenetic analysis was also used for the biosurfactant production. Sunflower oil cake served as the best carbon source as compared to all the substrates used for biosurfactant production, generating 1.5 g/l of biosurfactant concentration and reducing the surface tension of the medium to 34 dynes/cm. the biosurfactant efficiently emulsified the hydrocarbons tested, with the highest emulsification index for diesel and lowest for xylene. On the basis of TLC, FTIR, fatty acid and sugar analysis the biosurfactant was found to be of glycolipid type having sugar composition of glucose, galactose and arabinose and fatty acid moiety comprising C\textsubscript{16} and C\textsubscript{18} fatty acid chain. The glycolipid showed strong antifungal activity against the strains tested. It was also found to inhibit the germination of fungal spores which is the starting stage of fungal life cycle. Thus, produced glycolipid can be used as an effective antifungal agent.

The present study indicates the potential of Pseudomonas desmolyticum NCIM 2112, Bacillus cereus NCIM 5391 and Enterobacter sp. NCIM 5392 to produce the biosurfactant by profusely reducing the surface tension of the medium. Also, they showed the significant production of biosurfactant on various unconventional substrates which may be useful for making the process of biosurfactant production cost effective. All the strains used in the study produced different types of biosurfactant. Pseudomonas desmolyticum NCIM 2112 produced mono-rhamnolipid which was applied in the dye
degradation and reduced the time of decolorization of Brown 3REL by *Bacillus* sp. VUS NCIM 5342 by 50%. *Bacillus cereus* NCIM 5391 produced lipopeptide cerefactin which exhibited high antimicrobial, antifungal, anti-adhesive, and anti-biofilm formation activities, attractive to biotechnological and biopharmaceutical applications. Cell lysis due to pore formation in the membranes was assessed as the probable mode of action of the lipopeptide. *Enterobacter* sp. NCIM 5392 produced glycolipid which was found to have high antifungal activity and inhibited the fungal spore germination. The scale-up of biosurfactants for industrial production is still challenging. Still we need further understanding of the microbial physiology and genetics of these microorganisms to harness them for efficient industrial applications.