Conclusion

Currently, India has 18 oil refineries in the public and private sectors, with a gross refining capacity of over 112 million tones approximately. Every year tones of oil are spilled in marine and terrestrial area. The spilled petroleum hydrocarbons are one of the main environmental pollutants. Their abundance and persistence in several polluted environmental areas have been a matter of concern over years. A number of approaches have been developed for cleaning up the oil spills in marine shorelines and land. Researchers have often preferred bioremediation to clean up the toxic wastes from the environments over the conventional physical and chemical methods. Biodegradation of organic wastes is a useful side effect of microbial metabolism, thus the fundamental principles of biodegradation are integrally linked to microbial physiology. Degradation may be enhanced at some sites because of the adaptation of microorganisms to chronic exposure to chemicals.

Therefore, this study was taken up to isolate a bacterial strain that could efficiently degrade petroleum hydrocarbon along with other organic solvent wastes. The underlying mechanism of degradation and the simultaneous effect of organic wastes on the bacterial cell were also part of the study. Efforts were given to study the application of the isolated strain in biodiesel production.

Thus, it can concluded from the study that

- Among the thirty two isolated petroleum hydrocarbon degrading bacteria, PS11 strain was selected for further work based on highest zone of crude oil utilization. It was identified as *Geobacillus stearothermophilus* on the basis of phenotypic characteristics and phylogenetic analysis.

- The isolated PS11 strain could utilize crude oil. However, presence of crude oil decreased its growth rate compared to cells grown in absence of crude oil.

- PS11 cells also exhibited growth in presence of various other toxic solvents having a wide range of log *P* (2-4) value. Solvents having log P value less than 1 inhibited its growth.

- Transmission electron microimages showed that presence of organic solvent initially affected membrane system of PS11 cells. Continuous exposure of

solvents resulted in reorganization of cell membrane indicating solvent tolerant characteristic of PS11 cells.

- Gas chromatography analysis showed complete degradation of aromatic compounds and partial metabolic transformation of alkanes in TPH by PS11 strain.

- Complete degradation of benzene and toluene with partial degradation of xylene and ethylbenzene by PS11 cells was also noted in gas chromatograph.

- Membrane adaptation profile of PS11 cells in presence of petroleum hydrocarbon was noted using gas chromatography. Increase in membrane glycolipid and decrease in membrane straight chain fatty acid with simultaneous increase in loosely packed branched chain iso-fatty acid indicated increased membrane stability.

- PS11 cells harbored a mega plasmid of 20 kb as confirmed by agarose gel electrophoresis followed by restriction digestion profile analysis. The role of mega-plasmid in petroleum hydrocarbon degradation was studied by plasmid curing assay. Plasmid cured cells of PS11 could not grow in presence of crude oil, thus, confirming mega plasmid mediated petroleum hydrocarbon degradation.

- *E.coli* JM109 cells were transformed with mega plasmid of PS11. Growth of the transformed JM109 cells in presence of crude oil provided a conclusive proof for the involvement of plasmid DNA in the degradation of petroleum hydrocarbon.

- TPH and BTEX degradation by transformed *E. coli* JM109 generated similar chromatograph as wild PS11 in the same time period.

- PCR amplification of gene encoding catechol 2, 3 dioxygenase, an enzyme involved in catechol metabolism, a common intermediate of aromatic petroleum hydrocarbon meta degradation pathway, using plasmid as template successfully yielded a PCR product of about 900 bp.

- Sequence analysis of the PCR product (916 bp) showed 100% nucleotide homology and amino acid with catechol 2, 3 dioxygenase.

- PS11 cells produced an extracellular, organic solvent tolerant, alkaline lipase. Optimization of production conditions by OVAT approach enhanced the enzyme production by 2.46 folds.

- Lipase was purified from the extracellular medium by ammonium sulfate precipitation followed by anion exchange chromatography and gel filtration. PS11 lipase was purified by 8.04 fold with 22.6% yield.

- SDS-PAGE analysis of purified lipase indicated that the enzyme was probably a single chain protein or a homomultimeric protein of 27 kD subunits.

- Optimum pH and temperature for lipase was 10 and 50 °C, respectively. It showed 100% stability in the pH range 8 to 11 for 2h. The enzyme retained 50 % activity at 70 °C for 2h.

- Organic solvent tolerance property of lipase from PS11 cells was confirmed when the enzyme restored 90% of its activity in presence of p-xylene, benzene, toluene, hexane and methanol.

- High stability of PS11 lipase even in presence of higher concentration of methanol (30- 50% v/v) makes it a promising tool in the field of biodiesel production.

- Gas chromatography analysis of the enzymatic trans-esterification of sunflower oil in presence of methanol confirmed the catalytic activity of lipase from PS11 cells in biodiesel production

Thus, the isolated strain *Geobacillus stearothermophilus* PS11 could serve a dual purpose towards "GREEN ECOSYSTEM". Firstly, it can be employed for cleaning up of petroleum hydrocarbon wastes and secondly, for ecofriendly biofuel production.