SUMMARY

Industrial development is considered as necessary evil which is essential for the rapid growth of the country and people, but evil from the point of environment. Large amounts of hydrocarbon contaminants are released into the environment as a result of human activities. While releases like industrial emission can be controlled and carefully regulated, catastrophic releases like major spillage from tankers, pipelines and storage tanks are largely accidental, unavoidable and occur frequently. Such releases often pose severe, immediate, as well as long-term ecological and environmental repercussions, since a lot of hydrocarbon compounds are toxic and persistent in terrestrial and aquatic environments. The hydrocarbons are eaten by some bacteria which are called oleophilic bacteria or Oil Eating Microbes (OEMs). They are the bacteria that naturally use oils in the environment as their food source.

Bioremediation is recommended for treating contaminated sites because of its low cost and ability to convert contaminants to harmless end products which is the conversion of chemical compounds by living organisms, especially microorganisms, into energy, cell mass and biological waste products. One important characteristic of bioremediation is that it is carried out in non-sterile open environments that contain a variety of organisms of these bacteria, such as those capable of degrading pollutants, usually have central roles in bioremediation, whereas other
organisms also affect the process. A deeper understanding of the microbial ecology of contaminated sites is therefore necessary to further improve bioremediation processes.

Microbiologists have now realized that natural microbial populations are much more diverse than those expected from the catalogue of isolated and available microorganisms. This is also the case for pollutant-degrading microorganisms, implying that the natural environment harbors a wide range of unidentified pollutant-degrading microorganisms that have crucial roles in bioremediation. This research promotes the influence for the treatment of Refinery waste water using EM Technology.

For isolation and purification of different Oil degrading bacteria, initially six samples were collected from refinery unit monthly which were further being used for the experimental analysis. After collection of water samples were employed for the bacterial isolation. A total of 19 bacterial strains were obtained from the different samples from (July-December, 2010), which were isolated and further purified at 37º C in sterilized condition in NAM medium. After isolation, to screen the bacteria which have ability to degrade oil, all 19 isolates were screened on the basis of oil degrading capability. The isolates which showed oil degradation characteristics were B1, B5, B9, B10, B15 and B18. So these bacterial strains were considered for the further treatment studies of the waste water. After screening, optimization of six
oil degrading bacteria was done in three different parameter (pH, temperature and different media) for the growth of bacteria. To screen the better media for growth, six media were selected in present study (i.e., Nutrient agar, Czapek-Dox agar, Trypticase soya agar, Yeast extract medium and Sucrose media). All six selected media were prepared in different pH (5, 6, 7, 8, 9) and growth was observed at different incubation temperature after 24hr for each (25ºC, 30ºC, 35 ºC, 40ºC and 45ºC).

Overall it was observed that the best medium for the growth of all the oil degrading bacteria was NAM at pH 7. The optimum temperature for all the bacteria was 35 ºC. After optimization growth profile analysis of isolated bacteria were done in six different broth i.e., Nutrient broth (NB), Sucrose broth (SB), Trypticase Dextrose broth (TDB), Trypticase Soya broth (TSB), Czapek-Dox broth (CDB), Yeast Extract broth (YEB).

After optimization these bacterial isolates were identified on the basis of morphology, physiology and by performing different biochemical experiments and at the last these isolates were further confirmed by BD-BBL Auto reader software for rapid identification of bacteria. After biochemical test for confirmation of the identified bacterial, a new technique of BD-BBL auto reader was also used for rapid identification of bacteria. It showed results between 95 to 99% purity and identified the bacteria.
The bacteria were identified as:

B2: *Bacillus subtilis*

B5: *Pseudomonas aeruginosa*

B9: *Acinetobacter baumanii*

B10: *Alcaligenes faecalis*

B15: *Corynebacterium propinquum*

B18: *Bacillus licheniformis*

The bacterial consortium was used as the EM solution for the bioremediation or treatment of the refinery waste water. After treatment of the waste water samples a vast variation in the physico-chemical parameters were observed. Principally major characteristics of the waste water studied were colour, pH, TDS, BOD, COD, Sulphide, nitrogen, oil, phenol, heavy metals (zinc, lead, chromium and cadmium), and Total Petroleum Hydrocarbon (TPH). After treatment with the consortium of isolated bacteria (EM solution), colour was reduced to much extent, nearly colourless.

Physical parameters like TDS, BOD and COD showed good reduction after treatment with bacterial consortium. The maximum reduction in TDS, BOD and COD was recorded as 79.50, 87.45 and 83.20% respectively. Chemical
parameters; sulphide, nitrogen and nitrate showed reduction upto 53.27, 82.57 and 66.84% respectively. Oil contents of the waste water also showed good reduction upto 89.80%. Phenol in waste water was observed reduced upto 96.60% after treatment with the bacterial consortium.

In case of heavy metals, different heavy metal showed reduction in different manner. The maximum reduction of cadmium was recorded 98% and minimum was 47.83%. The maximum and minimum reduction of chromium was recorded as 98.23% and 92.71% respectively. In case of lead, maximum reduction was noticed as 93.78% and minimum as 58.93%. The maximum concentration reduction of zinc was calculated 72.33% while minimum was 34.13%.

The chemical analysis of GC-FID for TPH (Total Petroleum Hydrocarbons) estimation in the refinery waste water revealed a remarkable decrease in the concentration after treatment with the microbes (bacteria). The samples of refinery waste water collected from January-December, 2011 were treated with the bacterial consortium for TPH removal. The overall reduction in TPH was recorded as 57.29%, 22.61%, 55.27%, 57.32%, 54.44%, 43.52%, 44.05%, 67%, 58.52%, 62.71%, 57.17% and 66.06% during the course of study from January to December, 2011 respectively. The highest and lowest percentage of TPH removal was observed in August (67%) and February (22.61%) after 20 days of bioremediation setup.
Findings of the Study

➢ From the present study, it can be concluded that the degradation of total petroleum hydrocarbons using indigenous microorganisms is possible.

➢ The incorporation of biological treatment of waste water by EM Technology may solve the problem upto remarkable extent.

➢ The bacterial community in crude oil contaminated sites response to biodegradation treatment, which results as these begins to actively degrade the pollutants and detoxify the environment allowing other starving populations to increase their community.

➢ Microbial communities adapt to the hydrocarbons and tend to be more capable of oxidizing hydrocarbons rather than other communities.

➢ Microbial communities capable of degrading crude oil components can lead to decontaminate the polluted site from heavy metals too.
Petroleum removal efficiencies in terms of TPH removal can reach up to 65% over a period of three weeks in *in situ* condition within the range of experimental conditions investigated in this study.