6. SUMMARY AND CONCLUSION

Oily wastewater, especially from oil field, has posed a great hazard for terrestrial and marine ecosystems. Oil spills causes great damage to ecosystem. The damage sometimes cannot even be repaired and that is what makes it worse. The unfortunate thing is that oil spill mostly happens because of people’s lack of care and attention. The traditional treatment of oily wastewater, such as containment and collection using floating booms, adsorption by natural or synthetic materials etc. cannot degrade the crude oil thoroughly. So far, biodegradation suggests an effective method. During biodegradation, crude oil is used as an organic carbon source by a microbial process, resulting in the breakdown of crude oil components to low molecular weight compounds.

The crude oil contaminated water and soil samples from Namakkal, Salem and Erode districts of Tamil Nadu were collected and processed for isolation of microorganisms and analysis of physic-chemical parameters. Since the maximum crude oil content (7000 ppm) in the samples collected from Tiruchengode was observed, this sample alone processed in further studies.

The parameters such as TDS, TSS, TS, BOD, DO, COD, alkalinity, total hardness, chloride and total crude oil content were high in the contaminated water samples collected from Tiruchengode. It was drastically reduced after bioreactor treatment with bacterial consortium. The total heterotrophic bacterial (THB) population was found to be higher in all soil samples when compared to water. There are 131 bacterial strains were isolated from water and soil samples and they were identified up to generic level. The generic composition of the heterotrophic bacterial species found in both water and soil
samples were *Micrococcus* spp., *Bacillus* spp., members of Enterobacteriaceae, *Pseudomonas* spp., *Alcaligenes* spp., *Moraxella* spp., *Acinetobacter* spp., *Vibrio* spp. and *Actinobacteria* spp. Among the bacterial species *Bacillus* spp. was recorded as predominant (48%). The fungal strains were identified as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus* spp. and *Penicillium* spp. among 50 strains isolated.

Among the 131 isolates, 5 isolates (EW4, EW6, SS11, SSM2 and SSM10) showed higher crude oil degradation ability selected based on the primary and secondary screening. For fungi, *Aspergillus fumigatus* (PN1) showed higher crude oil degrading ability when compared to the bacterial strains. This strain alone degraded the crude oil content in aqueous medium about 77.9% within 60 hrs of incubation period.

Antagonistic activity was carried out to find out the communal relationship between the potential bacterial strains (EW4, EW6, SS11, SSM2 and SSM10). Based on this assay, 7 different consortia were formed. From this the EW4, EW6 and SSM2 showed the maximum degradation of crude oil in aqueous medium. These three efficient strains were processed for molecular identification and they were identified as *Bacillus* sp. EW4 (KJ600629), *Bacillus* sp. EW6 (KJ600630) and *Bacillus* sp. SSM2 (KJ600631).

The bacterial consortium with coir waste as amendment removed oil content significantly in aqueous media up to 95.5%. Here, coir waste absorbed crude oil content which floating on the surface of the medium. This result clearly indicates that very significant improvement of coconut coir pith to absorb oil in petroleum spill situations.

The influences of carbon sources, nitrogen source, pH and temperature were also carried out to find out the efficiency of bacterial isolate in the
degradation of crude oil. Dextrose and yeast extract were selected as carbon and nitrogen source respectively since they showed higher crude oil degradation. In this optimization study about 72.22% of crude degradation was achieved using yeast extract as a nitrogen source. The pH also plays a crucial role for the physiological performances of the bacterial cell. It helps to transport various micro nutrients and minerals across the cell membrane aiming at maximizing the degradation process. The maximum degradation activity of 72.22% was observed at pH 7. For the temperature, 35°C shows higher (78.8%) degradation activity. The temperature 35°C and pH 7 was found to be optimum selected for further crude oil degradation process. In the optimization of environmental conditions, each and every parameter depend other parameters. This study shows that 1% of the yeast extracts and 1% dextrose produced maximum crude oil degradation (72.22%) at pH7 and 35°C. But the degradation percentage slightly decreased when the pH and temperature changed to below or above.

The bioreactor study was conducted to evaluate the degradation ability of the bacterial consortium in contaminated water under a lab scale setup. In these study four different sets of self-designed bioreactor was continuously operated for a period of 10 days and the amount of total crude oil content was analyzed at every 12 hrs. The maximum oil content was removed up to 95.22% in bioreactor: A set up. All the physico-chemical parameters in the treated water found to be lower when compared to untreated water.

The contaminated soil was treated through soil column study using individual and bacterial consortium. Maximum crude oil degradation (93%) was recorded in the study carried out with bacterial consortium as inoculum.
The results demonstrated that compared to the individual strains, the bacterial consortia showed a superior crude oil degrading ability in soil.

The bacterial strains *Bacillus* sp. (EW4), *Bacillus* sp. (EW6) and *Bacillus* sp. (SSM2) and a fungi *A. fumigates* (PN1) were used as efficient strains to produce biosurfactant in aqueous media enriched with dextrose as potential carbon source. After extraction of biosurfactant the emulsification index, foaming index and oil spread assay was carried out. Here, the biosurfactants emulsifies 50% of petrol and fresh engine oil, 23% of kerosene and used engine oil and 16 % in diesel. The synthetic surfactants gave a very significant emulsification rate (63%) compared to the biosurfactant.

The preliminary analysis demonstrated the composition of biosurfactant which was isolated from *A. fumigates* (PN1) as a glycoprotein, consisting of 70% protein and 15% of carbohydrates. In the case of bacterial consortia based biosurfactant contains 85% of protein and 20% of carbohydrates. Based on the ionic study, this simple test demonstrated the non-ionic character of the fungal biosurfactant and anionic character of the bacterial biosurfactant. The results of well diffusion method clearly reveals that, the biosurfactant produced from *A. fumigates* (PN1) and bacterial consortium had there is no antibacterial properties.

The stability of the biosurfactant in various temperature, pH and different NaCl concentrations were carried out. In this study the observations clearly proves that the biosurfactants were stable in all the above parameters and there is no notable changes on biosurfactant activity was obtained in emulsification index of all the above parameters.

In the column study carried out with various concentrations of biosurfactants and synthetic surfactants, bacterial surfactant is a very good competitor with SDS. After final day of the study, toluene extraction method
was carried out to find out the presence of crude oil content in treated soil. In the column treated, the crude oil degradation was recorded up to 57, 68 and 43% by the application of 0.5, 1.0 and 1.5% of the bacterial biosurfactant respectively. In the case of fungal biosurfactants at 0.5, 1.0 and 1.5% concentrations, the crude oil removal were 52, 53 and 29% respectively. The chemical surfactants SDS and triton X100 were plays a higher emulsifying activity showed up to 75% of crude oil removal in treated soil.

Instrumental analysis of biologically treated water and soil samples was also proved the crude oil degradation ability of this study. The GC-FID results clearly revealed that the four sets of bioreactor treatment, the sterilized contaminated water and bacterial consortia with 1% carbon source shows 99.97 % degradation. In the treatment of soil, the fungal biosurfactents (98.96%) showed a higher efficiency on crude oil removal when compared with synthetic surfactant. Bacterial biosurfactant also emulsified in greater percentage which was 95.98%.

The results of GC/MS analysis confirmed the capability of the bacterial consortium to degrade crude oil. Lower cases peaks from decane \((C_{10})\) to pentadecane \((C_{15})\) were detected in the crude oil at the beginning of the incubation time and low-molecular alkanes \(<C_{10}\) were not detected, either because these components were rapidly volatilized or biodegraded. After treatment the residual oil showed a sharp decrease of component abundance in the range from decane \((C_{10})\) to pentadecane \((C_{15})\), thus confirming that the bacterial consortia was able of degrading a broad range of petroleum hydrocarbons within 30 days, including by-products. The GC-MS spectrum for control (distilled \(H_2O\)) with biosurfactant, which were left stagnant after 10 days, 32 peaks were shown in the treated soil sample (control) spectrum while the
treated with biosurfactant showed only 14 peaks. That is branched alkanes, alkene, carotane and alkynaphthalenes and was thoroughly degraded.

The toxicity test, results clearly shows that the green gram leaf growth and the elongation of secondary roots occurred under all biosurfactant treated soil samples. The 90% of the plant growth was inhibited by synthetic surfactants. This study clearly proved that the chemical surfactants were highly toxic to the plants and natural ecosystem.

The potential cytotoxic and genotoxic effects of oil column extracts, treated soil and bioreactor treated water on onion (*Allium oschyaninii*) were evaluated. In macroscopic observation found that onion root growth was decreased. It indicates that the samples were toxic; our results showed other aberrations, also induction of sticky chromosomes, bridges and disturbance of spindle fibers at different stages of mitotic division in the root cells.

From the present study, as summarized above, the following conclusions are drawn.

- The physico-chemical and bacteriological characteristics gave the basic information about the pollution load of the contaminated water and soil. The bacterial consortium and *Aspergillus fumigatus* (PN1) were proved its efficiency in crude oil toxic resistant character and degradation potency by primary and secondary screening.

- Based on the bioreactor and soil column study concludes the bacterial consortium were efficient compare to individual bacterial strain on crude oil degradation. The optimization study showed the influence of the environmental parameters on degradation process.

- The preliminary analysis was proved the surfactant production character of the potent strains. The efficiency of emulsifying activity on various
hydrocarbon sources and oil spreading activity can show the biosurfactant producing ability of the strain.

- Biosurfactant ability in extreme condition was done by stability studies, consisting of various pH, temperature and NaCl concentrations. The parameters revealed there is no notable change in biosurfactant activity.

- The sand absorption method also displays the crude oil removal in artificially contaminated sand. In the soil column study bacterial biosurfactant, bungal biosurfactant and synthetic surfactants competits with each other on emulsification of crude oil. However, chemical surfactant showed their toxicity in the green gram plant growth assay.

- There is no germination occurred in the growth study carried out with sodium dodecyl sulphate extracts. Irrigation by the extracts of Triton X100 produce shoots in very slowly. The soil column extracts by fungal biosurfactant shows the germination of green gram in more or less equal to the control plants using distilled water.

- The onion root tip assay also proved the geno toxicity of the extracts. The biosurfactants shows normal chromosome aberrations; however, the chemical treated extracts show some deviations in anaphase of the onion root chromosomes.

Based on the observation, it could be concluded that the bacterial consortium and biosufactant produced by bacterium and *Aspergillus fumigatus* (PN1) is a potential biological resource and can be used for remediation of water and soil contaminated with crude oil pollution.