2. REVIEW OF LITERATURE

2.1. Oil refineries

Indian refining industry has done exceedingly well in establishing itself as a major player globally. India is emerging as a refinery hub and refining capacity exceeds the demand. The last decade has seen a tremendous growth in the refining sector. The country’s refining capacity has increased from a modest 62 Million Metric Tonnes Per Annum (MMTPA) in 1998 to 215.066 MMTPA at present, comprising of 22 refineries - 17 under Public Sector, 3 under private sector and 2 in Joint Venture (JV) (Table 2.1). During 2011-12, two new JV refineries of 6 MMTPA and 15 MMTPA were commissioned in Bina, Madhya Pradesh and Bathinda, Punjab. These refineries would augment the availability of BS IV (Bharat Stage IV) compliant fuels in Central and Northern parts of the country (ICCT, 2011).

2.2. Current status of crude oil and natural gas production

The crude oil production has remained in the range from 32 to 38 MMT during this period with year to year variations. Crude oil production slightly low in 2012-13 as it was 37.862 MMT, which is about 0.60% lower than the production of 38.090 MMT during 2011-12. Natural gas production has increased from 32.202 BCM in 2005-06 to 40.679 BCM in 2012-13. However, there was a decrease in production of natural gas in 2012-13 by 14.47% over the previous. This is primarily due to increasing water cut trend, less base potential of major fields affected production, 6 well in D1, D3 and 2 wells in MA have ceased to flow due to water/sand ingress, water and sand ingress problem in wells, underperformance of newly drilled wells and some constraints were overcome. However, it is expected that higher production will be during 2013-14 (MPNG, 2013).
2.3. Crude oil

Crude oil or petroleum is simply unprocessed oil found in deep beneath the earth’s surface. The colour from clear to black and can be found as a liquid or solid. Overall properties of crude oils are dependent upon their chemical composition and structure. Crude oil is pumped and stored in barrels for future refinement. The refinement process may involve filtering, addition of additives and specialized separation techniques to create specific crude oils and crude oil products. Generally all crude oils are made up of hydrocarbon compounds. The main hydrocarbons found in crude oil are aliphatics, alicyclics and polycyclic aromatic hydrocarbons (PAH) (Wilhelm and Spitz, 2003; Ryder et al., 2004; Karpicz et al., 2005).

2.4. Crude oil pollution

As the world’s population continues to grow, the demand for petroleum and petroleum products increases vigorously, while oil spills and the improper discharge of industrial wastes contribute to pollution of the environment (Hernandez et al., 1998; Gupta and Mahapatra, 2003; Strong and Burgess, 2008). Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention. The release of contaminants, such as petroleum and petroleum derived products, into the environment is one of the main causes of global pollution (Luna et al., 2011). Large numbers of contaminants are toxic and carcinogenic, placing both human and animal health at risk (Batista et al., 2010). Through capillarity, hydrocarbons adsorb to surfaces and are trapped in a water immiscible phase, making these compounds difficult to remove from contaminated environments.

In Indian context, there have been more than 70 oil spills near the coastal area. Now it has been fairly established that the potential threat from operational or accidental oil spills from tankers and other oil related
activities could lead to large scale destruction of marine life and property of the coastal region. Oil spills in marine waters have significantly potential to damage social and economic systems as well as the natural environment of surrounding seas which support valuable fishing grounds, coastal ecosystems, protected marine national park areas, long recreational and tourist beaches (Peterson et al., 2003). Concentration of PAHs present in Indian soils has been reported to be 6.7 μg g⁻¹ in agricultural land, 9.3 μg g⁻¹ in residential, 12.9 μg g⁻¹ along roadside and 13.7 μg g⁻¹ in industrial region (Fadzil et al., 2008). Few reports revealed that the ambient PAH concentration in Delhi airport soil, bank of Yamuna river (Tyagi, 2004; Ray et al., 2008; Agarwal et al., 2009), Mumbai and Ahmadabad (Raiyani and Shah, 1993; Sahu et al., 2001). Bishnoi et al. (2009) reported 51.59-148.82 mg kg⁻¹ dry weight of PAH in the Hisar region. Malik et al. (2008) reported contamination of fish from the Gomti River with PAHs. Gomti River, a tributary of the River Ganga, is one of the most polluted rivers of India and is affected during its course by three major urban centers, viz., Lucknow, Sultanpur and Jaunpur.

2.5. Health impacts and hazards of petroleum hydrocarbon

The primary effects of exposure to petroleum hydrocarbons are central nervous system depression and polyneuropathy. Studies have shown that repeated exposure of laboratory animals to high concentrations of whole gasoline vapors at 67,262 and 2,056 ppm caused kidney damage and kidney cancer in rats and liver cancer in mice. The International Agency for Research on Cancer (IARC) has listed gasoline as possibly carcinogenic (2B) to humans (Mortelmans et al., 1986; IARC, 2000). Among the components of gasoline, N-heptane and cyclohexane are known to cause narcosis and irritation of the eyes and mucous membranes. The IARC, the national toxicology program (NTP) and the occupational safety and health
administration (OSHA) all classify benzene as a human carcinogen. Studies have shown that the effects of benzene exposure can include leukemia at concentrations as low as 1 ppm.

The isomers of trimethylbenzene may cause nervousness, tension, anxiety and asthmatic bronchitis. n-Hexane is known to cause the polyneuropathy (peripheral nerve damage) after repeated and prolonged exposure, while other hexanes show narcotic effects at 1000 ppm and are not metabolized like n-hexane. Toluene can cause impairment of coordination and momentary loss of memory at exposures of 200-500 ppm and causes palpations at 500-1500 ppm. Crude oil is not considered a hazardous waste (petroleum is considered a hazardous material), but crude oil coats and kills sea life and mucks up the surrounding beaches, rocks, trees and etc (Viswanathan, 1999).

2.6. Impacts of crude oil pollution on environmental, plants and human

The gasoline and diesel stations the spills due to leakage may be small but continuous and prolonged. Because of its persistence, the chance for groundwater contamination is high. Fellenberg (1980) reported that, when in contact with water, oil and its by-products spread and form a thin layer on the surface that prevents gas exchange between air and water and blocks sunlight to phytoplankton, breaking the food chain. Confirming this theory, Asimiea and Sam-Wobo (2011) observed that the impact of hydrocarbon waste on phytoplankton communities, which suffered structural changes due to the presence of these compounds.

One litter of oil can deplete the oxygen in one million litters of water and form a thin layer of 1000 m² on the soil surface in a few days, blocking the passage of sunlight and water and thus preventing the respiration and photosynthesis of the plants present therein (Yeung et al., 2011). Plants are prevented from performing respiration and photosynthesis due to a sealing
The entrance of the stomata and plant roots are inhibited from absorbing soil nutrients (Fellenberg, 1980).

The aromatics in crude oils also have numerous adverse effects on the environment particularly to the local microbial flora. It was shown that α-pinene, limonene, camphene and isobornyl acetate were inhibitory to the microorganisms. The phenolic and quinonic naphthalene derivatives inhibited the growth of the cells (Sikkema et al., 1995). Calder and Lader (1976) demonstrated that increasing amounts of naphthalene, 2-methylnaphthalene, pyrene and others resulted in an increased lag phase and lowered the growth rates of two bacteria growing on these compounds. Uribe et al. (1990) reported the toxic effects of cyclohexane on the energy transduction in Saccharomyces cerevisiae. Cyclohexane inhibited oxygen uptake in intact cells and isolated mitochondria. Studies on isolated mitochondria showed that ATP synthesis was impaired whereas ATP hydrolysis was slightly increased. Uptake of potassium ions was impaired and dissipation of the mitochondrial membrane potential was observed. These studies indicate that the permeability barrier of the inner mitochondrial membrane was disrupted by cyclohexane.

Toxicity of crude oil includes liver necrosis, congestion of the liver, fat degeneration and dissociation of hepatocytes. Birds and animals in oil-contaminated area are found to have black emulsion in the digestive tract with a petroleum odor. This leads to decrease in the absorption of nutrients and finally leads to death of these birds and animals due to rupture of capillaries and hemorrhage, hepatocellular dissociation, hemosiderosis, renal tubular necrosis and anemia (Khan and Ryan, 1991).

One of the most impacting spills occurred recently, in November 2011, in the Sedco 706 oil platform, operated by Chevron Brasil in Campos Bay (Rio de Janeiro, Brazil). The oil leakage was equivalent to 5943 L and
reached about 163 km² (ANP, 2011). The potential threat to human health posed by hydrocarbons is connected to their physical and chemical properties, which allow these compounds to be absorbed by the skin and quickly spread through the organism if ingested or inhaled (Costa et al., 2012). Exposure to BTEX hydrocarbons for a long period of time at low concentrations presents a series of chronic effects. Among them, benzene is regarded as the most toxic and may cause depression of pluripotent primitive blood cells, extending through any cell maturation stage; damage to bone marrow, such as necrosis, edema, hemorrhage and fibrosis, which also interfere with blood cell production leukemia and liver cancer. The estimated value of the minimum lethal dose (LDL) for humans is 194 mg/kg (Melo et al., 2007).

2.7. Properties of spilled oil

Crude and refined oil are complexes and their physical and chemical properties are determined by constituents and each percentage. Some compounds are carcinogenic, mutagenic or toxic to humans, animals and plants. Low ambient temperatures usually result in increased viscosity of oil, reduced evaporation of volatiles and increased water solubility and thus delayed onset of biodegradation (Atlas, 1991; Margesin and Schinner, 2001). Oil spills in winter on Taiga forests underlain with permafrost were long-term and chronic while the summer spills were relatively shorter-term and more acute (Collins et al., 1993). In addition, the volume of spilled oil, the impacted area, the duration of the spill should be the major concerns (Barnes and Filler, 2003). The susceptibility to microbial attack differs in hydrocarbons. Aliphatic compounds are more prone to degradation than polycyclic aromatic hydrocarbons (PAHs) because of the low water solubility and high sorption capacity of PAHs in cold environments (Margesin and Schinner, 1999). Isoprenoids, phytanes and pristanes are more resistant to

2.8. Treatment strategies for crude oil contamination

There are several mechanisms for the degradation and dispersion of aquatic released oil including dissolution, emulsification, absorption, mixing, evaporation, biodegradation, photodegradation and chemical reactions (Barbosa et al., 2003; Sterling et al., 2003).

2.8.1. Physical methods

The guide document of environmental protection agency, USA in the year 1999 clearly describes that the physical methods and its advantages and disadvantages of those methods. Based on this report physical removal of oil from shorelines and especially beaches, is time-consuming and requires much equipment and many personnel. Methods used to physically clean oil from shorelines include Wiping with absorbent materials, pressure washing and raking or bulldozing. Materials that are capable of absorbing many times their weight in oil can be used to wipe up oil from contaminated shorelines. These materials are often designed as large squares, much like paper towels, or shaped into “mops.” The squares or mops are used to wipe the shoreline or oily rocks during which time the absorbents are filled with as much oil as they can hold. Pressure washing has the advantage of being relatively inexpensive and simple to apply; however, it requires many people.

In case of water physical methods are commonly used to control oil spills in a water environment. They are mainly used as a barrier to control the spreading oil spill without changing its physical and chemical characteristics. A variety of barriers are used to control oil spills including: (a) booms (b) skimmers and (c) adsorbent materials (Fingas 2011; Vergetis, 2002).
2.8.1.1. Booms

Booms are a common type of oil spill response equipment which are used to prevent spreading of the oil spill by providing barrier to oil movement which can improve the recovery of oil through skimmers or other response techniques. There are three categories of booms as shown in Fig. 2: (a) fence boom (b) curtain boom and (c) fire-resistant boom (Potter and Morrison, 2008). Booms are light weight, take up minimal storage space, resist abrasion, are easy to handle, clean and store are highly reliable in calm quiet waters. However, they have several disadvantages including low stability in strong winds and currents, low flexibility for towing and low efficiency in high waves (Ventikos et al., 2004; OSS, 2010).

2.8.1.2. Skimmers

These devices can be used in conjunction with booms to recover oil from water surface without changing its properties so it can be reprocessed and reused. Skimmers consist of disks, belts, drums and brushes (Larson, 2010; Hammoud, 2001). They may be self-propelled, used from shore or operated from vessels. Skimmers are three categories as shown in Fig. 3: (a) weir, (b) oleophilic and (c) suction (Nomack and Cleveland, 2010). The success of skimming depends on the type and thickness of the oil spill, the amount of debris in the water, the location and the weather conditions. They are generally effective in calm waters and subject to clogging by floating debris. They work well with less viscous, low density oil and non-emulsion oil. However, they have significantly low efficiency with oil emulsion and are frequently jammed and clogged by floating debris (Jensen et al., 1995).

2.8.2. Chemical methods

Chemical methods are used in combination with physical methods for marine oil spill remediation as they restrict the spreading of oil spill and help to protect the shorelines and sensitive marine habitats. Various
chemicals are used to treat the oil spills as they have capabilities to change the physical and chemical properties of oil (Vergetis, 2002). The chemicals used to control oil spills include: (a) dispersants and (b) solidifiers.

2.8.2.1. Dispersants

Dispersants consist of surfactants (surface active agents) dissolved in one or more solvents and stabilizer Table 2. Dispersants have capabilities to break down the slick of oil into smaller droplets and transfer it into the water column where it undergoes rapid dilution and can be easily degraded (Lessard and Demarco, 2000). Dispersants are usually applied by spraying the water with the chemical and ensuring that it is well mixed either by wind or the propeller of a boat (Sitting, 1974). The dispersants available today are less toxic and more effective compared to the compounds that were previously used (Lessard and Demarco, 2000). These concentrated types of dispersants include: Slickgone NS, Neos AB3000, Corexit 9500, Corexit 8667, Corexit 9600, SPC 1000™, Finasol OSR 52, Nokomis 3-AA, Nokomis 3-F4, Saf-Ron Gold, ZI-400 and Finasol OSR 52 (USEPA, 2011b). Davies et al. (1998) reported that 50-75% of No. 5 bunker oil slick (20 ton) was dispersed with the application of Corexit 9500 dispersant. Siang (1998) reported that the target oil spill in the history of Singapore (October, 1997) was cleaned up in a record of 3 weeks with the application of Corexit 9500 dispersant. Dispersants proved their capabilities to treat up to 90% of spilled oil and are less costly than the physical methods (Holakoo, 2001). They can be used on rough seas where there are high winds and the mechanical recovery is not possible. They also allow for rapid treatment, slow down the formation of oil-water emulsions make the oil less likely to stick to surfaces (including animals) and accelerate the rate of natural biodegradation by increasing the surface area of the oil droplets. Applicability of dispersants depends on type of oil, temperature, wind speed
and sea conditions (Nomack and Cleveland, 2010). However, the inflammable nature of most dispersants can cause human health hazards during applications and potential damage to marine life. They are also responsible for fouling of shorelines and contamination of drinking water sources (NRC, 1989).

2.8.3. Biological methods

Oil contamination with petroleum and petroleum-based hydrocarbons has caused critical environmental and health defects and increasing attention has been paid for developing and implementing innovative technology for cleaning up this contaminant. Bioremediation methods are currently receiving favourable publicity as promising environmental friendly treatment technologies for the remediation of hydrocarbons. Moreover, biological methods can have an edge over the physico-chemical treatment regimes in removing spills as they offer cost effective in situ biodegradation of oil fractions by the microorganisms.

Bioremediation can be described as the conversion of chemical compounds by living organisms, especially microorganisms, into energy, cell mass and biological waste products. Different types of methods of restoration of oil-polluted sites are exists. According to Baker (1970), Stebbings (1970), Odu (1972), natural vegetation of the area affected by light spillages of crude oil have occurred without any special treatment. At low levels of contamination of crude, cultivation of soil without nutrient amendment is possible since reclamation of the minerals in the soil can take place in a very short time (Plice, 1948; Toogood, 1974). Naturally occurring microbial communities that respond to the presence of contaminating hydrocarbons normally have more than one type of hydrocarbon utilizing microorganisms. For seeding oil slicks therefore, mixture of hydrocarbon
utilizing microorganisms or a genetically engineered microorganism have been suggested (Horowitz and Atlas, 1978).

2.8.3.1. Phyto remediation of petroleum wastes

The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites. These techniques not only have some adverse effects on the environment and are also expensive (Frick et al., 1999). Recently biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum (Njoku et al., 2009). Phytoremediation is a viable remediation method for petroleum-contaminated soil. The use of vegetation for remediation of contaminated sites is attractive and eco-friendly (Deka et al., 2009). It is an emerging technology which uses various plants to extract, contain, degrade and/or immobilize contaminants. There are various phytoremediation processes, including phytoextraction, rhizofiltration, phytostabilization, rhizodegradation, phytodegradation and phytovolatilization. Rhizodegradation is the breakdown of organic contaminants in soil through microbial activity which is enhanced by the presence of the root zone (EPA-report, 2000). Thus, rhizo degradation of pollutants in soil becomes promising for the areas when plants can grow (Korade and Fulekar, 2009).

2.8.3.2. Microbial treatment

In nature, microorganisms which can degrade oil exist widely in soil, ground water, oceans and lakes. Many of them can live with oil as their only carbon source. Now more than 70 genera and 200 strains have been found that can oxidatively biodegrade one or more types of petroleum hydrocarbons (Feng, 1991; Englert et al., 1992; Li et al., 2001a; Li and; Liang et al., 2005; Feng et al., 2007). Some of bacterial and fungal genera (Korda et al., 1997; Chaineau et al., 1999; Pointing, 2001) reported in the
literature are listed in Table 2.2. In addition, some algae can biodegrade oils as well (Walker et al., 1975; Semple et al., 1999). It was pointed that enhanced bioremediation through rhizosphere interactions (Su and Yang, 2009; Toyama et al., 2011) is also very important but this is at its early stages and needs further investigation (Robertson et al., 2007; Wan, 2011a). Bacteria degrade PAH compounds by an assimilative process where they gain carbon and energy for the growth, which typically leads to mineralization of the compound (Kastner et al., 1994; Haderlein et al., 2006). Bacteria generally use intracellular dioxygenase enzymes for the degradation of PAHs (Johnsen et al., 2005).

Algae are a diverse group of eukaryotic organisms that contain chlorophyll and carry out photosynthesis. They are mainly aquatic organisms, although some are found in soil or vegetation where there is enough moisture (David et al., 2003). Some species of micro-algae have the ability to degrade hydrocarbons, example is, *Onchromonas danica* that can degrade polycyclic aromatic hydrocarbons. Crude oil is a dark viscous liquid and a mixture of organic compounds. It varies in composition and color, which depends on the area from which it is obtained (Bajah and Godman, 1975). Since hydrocarbons are natural products, it should be no surprise to find hydrocarbon degrading microorganisms in many environments irrespective of whether they have been contaminated or not (Sayles, 1993). This research aimed at determining the ex-situ bioremediation of polluted soil using *Aphanocapsa elachista* algal species.

**2.8.3.3. Bioaugmentation and biostimulation**

Bioaugmentation of oil-contaminated soil involves the introduction of microorganisms, usually heterotrophic bacteria, to increase the rate and extent of oil degradation. The rationale behind bioaugmentation is that native microbial populations may not be capable of degrading the wide
variety of chemical compounds present in crude oil, or are not present at sufficient population sizes. The impacts of bioaugmentation on oil degradation in marine ecosystems have shown mixed results. No increase in oil degradation was observed in seawater inoculated with mixed bacterial cultures (Tagger et al., 1982). Fuel oil degradation was not enhanced by addition of commercial bacterial cultures (Dott et al., 1989). Oil degradation increased in saline Arctic ponds after inoculation with Pseudomonas sp., but no increase in degradation was observed in freshwater ponds (Atlas and Busdosh, 1976). Bioaugmentation has been shown to increase population sizes of hydrocarbon degrading microorganisms in some studies but not in others (Venosa et al., 1992; Bragg et al., 1994; Aldrett et al., 1997; Neralla and Weaver, 1997).

Some microorganisms are present in the contaminated site, but for effective remediation, growth of microorganism was stimulated. Biostimulation is the process of adding nutrient, electron acceptor and oxygen to stimulate existing bacteria involved in bioremediation. This is the process of optimizing the environment condition of the remediation site. Additives are usually added to the subsurface through injection wells. Subsurface characteristics such as groundwater velocity, hydraulic conductivity of the subsurface and lithology of the subsurface are important in developing a biostimulation system (Vidali, 2001). The indigenous microorganism present in the soil is responsible for degradation of the pollutant, but biostimulation can be improved by bioaugmentation.

2.8.3.4. Biosurfactant

Biosurfactants are surfactants synthesized as metabolic products of different microorganisms, mainly aerobic ones, such as bacteria, yeasts and filamentous fungi using several substrates such as carbohydrates, hydrocarbons, oils and fats, industrial and agricultural residues or a mix of
them (Pirollo et al., 2008). A property of great importance to industrial and biotechnological applications is the stability of most biosurfactants in different conditions, enduring high autoclaving temperatures (121°C for 20 min) and also low temperatures (-18°C for 6 months) and pH (5-11) found in the environment (Muthusamy et al., 2008). According to Pirollo (2006), the main biosurfactant classes currently known are glycolipids (rhamnolipids, sophorolipids and trehalose lipids), lipopeptides and lipoproteins (peptide, viscosin, serrawettin, surfactin, subtilisin, gramicidin and polymyxin), fatty acids, neutral lipids and phospholipids (fatty acids, neutral lipids and phospholipids), polymeric surfactants (emulsan, biodispersan, liposan, carbohydrate-lipid-protein and mannan-lipid-protein) and particulate surfactants (Table 2.3). Biosurfactants present many advantages in comparison with synthetic surfactants, such as high biodegradability, low toxicity, biocompatibility, biodigestibility (which allows their application in cosmetic and pharmaceutical products and as food additives), possibility to be produced from low-cost sources and industrial waste, use in bioremediation of oil-affected sites, biodegradation and detoxification of industrial effluents, in addition to efficacy in extreme temperature, pH and salinity conditions (Pirollo, 2006).

2.8.3.4.1. Mechanism of biosurfactant

Biosurfactants are released, their monomers organize spherically (micelles), in a way that the hydrophobic portion is turned to the centre, composing the nucleus and the hydrophilic part is turned to the sphere surface, making an interface with water. Thus, the surfactant reduces the surface tension between water and oil and contributes to micelle formation, increasing hydrocarbon exposure to bacteria and oxygen and favouring hydrocarbon biodegradation (Chavez and Maier, 2010). The modifications in the membrane, such as change in proteins composition or increase of the
cell wall hydrophobicity by the reduction of lipopolysaccharides, caused by the biosurfactant, promote higher accessibility to hydrocarbons by microbial cells. This occurs due to the dispersion of the hydrocarbon through its encapsulation in micelles, spherical or irregular vesicles and lamellar structures (Aparna et al., 2012).

The representation of micelle formation follows the Stigter’s scheme, on which surfactant monomers organize spherically with the hydrophobic part composing the nucleus and the hydrophilic portion on the external part of the grouping, making an interface with water. In a micelle, on the hydrophilic end of the biosurfactant, which makes an interface with water, a double, compact, electric layer that surrounds the external surface of the micelle sphere is formed and is called Stern Layer (Tondo et al., 2010). The biosurfactant efficiency is determined by critical micelle concentration, the point in which micelles start to form (Soberon-Chávez and Maier, 2010). These aggregates are produced as a result of several weak chemical interactions, such as Van der Waals and hydrogen bridges. Micelle formation leads to the reduction in surface and interfacial tension, due to the decrease in the force of repulsion between immiscible liquid phases (Aparna et al., 2012).

2.8.3.4.2. Manufacture of biosurfactants

Several companies in different countries are now manufacturing biosurfactants on various scales. Rhamnolipids are produced by at least two companies in the USA using strains of *P. aeruginosa*. AGAE Technologies (www.agae.com) is producing small quantities of highly purified rhamnolipids using strain NY3 and although full details of the process are not declared on their website, it appears that glycerol is the probable major carbon substrate and yields of about 12 g/l are achieved. The final product is stated to be 95% pure. Larger production is being carried out by Jeneil Biotech (www.jenielbiotech.com) which is a general food additive company.
Rhamnolipid products offered by Jeneil range from the crudest preparation comprising fermentation broth with approximately 2% rhamnolipids of partially purified products with up to 99% rhamnolipids.

From this information, we can assume that the yields are again in the 10-20 g/l range and that the organism being used may not be a hyper producer. Sophorolipids are already produced by several companies in, for example, France, Japan and Korea, with the material being used in products such as dishwasher formulations and Yashinomi vegetable wash. Saraya Co. Ltd. (worldwide.saraya.com) in Japan manufactures sophorolipids using Pseuodozyma with palm oil as the main fermentation substrate. Yields for the sophorolipids are not declared but can be expected to be in the 30-100 g/l range. Ecover (www. cover.com) also markets some products that contain ‘Candida Bombicola/ Glucose/ Methyl Rapeseedate Ferment’, that is, sophorolipids, whereas MGIntobio (http://mgintobio.en.makepolo.com) in Korea markets soaps containing sophorolipids specifically for acne treatment. The French company Soliance (www. soliance.com) also produces sophorolipids from rapeseed fermentation for cosmetic applications in skin care through antibacterial and sebo regulator activity.

2.8.3.4.3. Application of biosurfactant

Biosurfactants are potentially replacements for synthetic surfactants in several industrial processes, such as lubrication, wetting, softening, fixing dyes, making emulsions, stabilizing dispersions, foaming, preventing foaming, as well as in food, biomedical and pharmaceutical industry and bioremediation of organic- or inorganic-contaminated sites. Shete et al. (2006) mapped the patents on biosurfactants and bioemulsifiers (255 patents issued worldwide) showing high number of patents in the petroleum industry (33%), cosmetics (15%), antimicrobial agent and medicine (12%) and bioremediation (11%). Sophorolipids (24%), surfactin (13%) and
rhamnolipids (12%) represent a large portion of the patents; however, this may be underestimated since many patents do not specify the producer organism restricting to the specific use of the BS only. The presence of surfactants may increase microbial degradation of pollutants. Use of biosurfactants for degradation of oil in soil and water environment has gained importance only recently (Karanth et al., 2008).

2.8.3.5. Composting of petroleum wastes

In past decades, bioremediation has been developed and improved to repair soils polluted by petroleum hydrocarbons (Kirk et al., 2005; Aislabie et al., 2006). A number of bioremediation technologies such as composting, land farming, bioventing and bioreactor treatment have been used (Boopathy, 2000). Oil-contaminated soil can efficiently be remediated by composting (Jørgensen et al., 2000). Often these soils also contain PAH compounds. Small molecular weight PAHs containing two or three benzene rings are degraded during a well optimized composting process (Antizar-Ladislao et al., 2004). Unlike petroleum oil, creosote oil mainly consists of PAHs. Various strategies for bioremediation of PAH-contaminated soil have been investigated, such as amendment with various types of compost and bioaugmentation with various fungal species (Semple et al., 2001; Antizar-Ladislao et al., 2004; Pozdnyakova, 2012). Composting has been confirmed to be one of the feasible methods to clean up petroleum hydrocarbon contaminated soils (Jørgensen et al., 2000). During composting, the addition of nutrients and amendments enhances the hydrocarbon degradation and ameliorates the structure of the soil (Gestel et al., 2003). However, some petroleum hydrocarbons and their metabolites remained in the soil after composting treatment and the residual pollution levels still exceed stringent clean up standard (Lu et al., 2010).
2.9. Factors influencing biodegradation process

Knowledge of the characters and parameters that affect the microbial biodegradation of pollutants is required for the successful application of bioremediation technology. A number of limiting factors have been recognized that affect the biodegradation of petroleum hydrocarbons, many of which have been discussed by Brusseau (1998).

2.9.1. Effect of temperature in crude oil degradation

Among physical factors, temperature plays an important role in the biodegradation of hydrocarbons by directly influencing the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora. Atlas (1975) found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation. Temperature also affects the solubility of hydrocarbons (Foght et al., 1996). At low temperature, hydrocarbon water solubility increases (Bahram, 1969). Although hydrocarbon biodegradation can occur over a wide range of temperatures, rates of degradation are generally observed to decrease with decreasing temperature.

In case of oxygen, being highly reduced substrates, require an electron acceptor, with molecular oxygen being most common. Though most studies have shown the biodegradation of hydrocarbons to be an aerobic process, anaerobic biodegradation of hydrocarbons has also been reported. In the absence of molecular oxygen, nitrate, iron, bicarbonate, nitrous oxide and sulfate have been shown to act as alternate electron acceptors during hydrocarbon degradation. Oxygen is typically the limiting factor in aerobic bioremediation at many sites. The degradation of petroleum hydrocarbons occurs much faster under aerobic conditions compared to anaerobic conditions. Therefore, the addition of oxygen can significantly increase the
remediation rate. The initial steps in the catabolism of aliphatic (Singer and Finnerty, 1984a), cyclic (Perry, 1984) and aromatic (Cerniglia, 1984a) hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases. The concentration of oxygen has been identified as the rate limiting variable in the biodegradation of petroleum in soil and of gasoline in groundwater (Jamison et al., 1975; Wedel et al., 1988).

2.9.2. Effect of nutrients in crude oil degradation

The nutrients are also important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen, phosphorus and in some cases iron (Cooney, 1984). Some of these nutrients could become limiting factors, thus affecting the biodegradation processes. Atlas (1985) reported that when a major oil spill occurred in marine and freshwater environments, the supply of carbon was significantly increased and the availability of nitrogen and phosphorus generally became the limiting factor for oil degradation.

2.9.3. Effect of Carbon source in crude oil degradation

Most of the results showed that the microbial strains degradation efficiency was enhanced by the use of carbon sources like glucose and starch. The explanation is that glucose and starch can be used as co-metabolism in the medium during the petroleum degradation process (Yiyong et al., 2005). Thus, bacteria activity can be improved by adding the appropriate carbon sources, in this case glucose and starch. It has been previously reported that using glucose as carbon source improves the degradation of PAH and if glucose is fed intermittently the abilities of bacteria to degrade petroleum hydrocarbon could be maximized (Kishore et al., 2007).

2.9.4. Effect of pH in crude oil degradation

The acidity and alkalinity (PH) is also a very important factor in the biodegradation of petroleum hydrocarbon. In contrast to most aquatic
ecosystems, soil pH can be highly variable, ranging from 2.5 in mine spoils to 11.0 in alkaline deserts (Bossert and Bartha 1984). Most heterotrophic bacteria and fungi prefer a pH near neutral, with fungi being more tolerant of acidic conditions (Atlas 1988). Extremes in pH, as can be observed in some soils, would therefore be expected to have a negative influence on the ability of microbial populations to degrade hydrocarbons. Verstraete et al. (1976) reported a near doubling of biodegradation rates of gasoline in an acidic (pH 4.5) soil by adjusting the pH to 7.4. Rates dropped significantly, however, when the pH was further raised to 8.5.

**2.9.5. Effect of moisture in crude oil degradation**

Soil moisture is another important parameter in determining the rate of biodegradation of petroleum compounds. Microbes live in the interstitial water of soil pores and thus, a lower amount of water means a smaller number of microbes are present and the removal rate through biodegradation is slower (Dibble and Bartha 1976). In terrestrial ecosystems, hydrocarbon biodegradation may be limited by the availability of water for microbial growth and metabolism. Water potential ($a_w$) of soils can range from 0 to 0.99, in contrast to aquatic environments, in which water activity is stable at a value near 0.98 (Bossert and Bartha, 1984).

Bioavailability is also a key factor in the efficient biodegradation of pollutants. It is the amount of a substance that is physiochemical accessible to microorganisms. The application of external non-ionic surfactant, e.g., the main components of oil spill dispersants, influences the alkane degradation rate (Bruheim and Eimhjelle, 1998; Rahman et al., 2003). Physical disruption of soil aggregates using sonication has been reported to increase biodegradation rates effectively in a land farm experiment. Weissenfel et al., (1992) demonstrated that soil constituents have significant impact on the bioavailability of contaminants.
From the overall review physical, chemical and biological processes have been developed for crude oil degradation in aquatic and terrestrial system. Although physical and chemical process like booming, skimming, evaporation, absorption, dispersing and solidification are very effective in adopting to remove crude oil from contaminated water and soil, but currently they have limited potential for full scale operation and / or their complex process on plant scale. Due to these limitations for crude oil removal/ remediation from contaminated water and soil, the most versatile and widely used technology is microbial degradation. Hence the work was planned to remove the crude oil from contaminated water and soil using aerobic microorganisms and microbial biosurfactants.