Chapter 5

DISCUSSION
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Biodegradation plays the most important role in the natural decontamination of sites polluted with hydrocarbons. These hydrocarbons can be from petroleum or non-petroleum sources and can be aliphatic, aromatic, resin or asphaltenes. It is important therefore to study the effect of such contamination and its bioremediation. Moreover, Gujarat with its high number of oil fields, refineries and a big network of oil carrying pipelines is a suitable and important place for studies on microbial hydrocarbon degradation.

We have isolated and studied bacterial strains not only capable of degrading crude oil and its various components but which possess a high capacity of degrading other oils such as castor oil and 2T oil. Castor oil is representative of vegetable oils, which once spilled has similar properties and effects on the environment as crude oil.

Effect of hydrocarbons on the population density and microbial diversity

Soil and oil samples were collected from the contaminated sites of the Navagam field area, situated in Gujarat. The number of heterotrophs in the contaminated soil samples ranged from $1.5 \times 10^7$ to $2 \times 10^8$ g of soil whereas in oil samples it ranged from $3 \times 10^6$ to $6 \times 10^7$ g of soil. Depending on the location, 0.015-0.14% of soil heterotrophic bacteria determined by the MPN method, could degrade crude oil as sole carbon source and this ranged from 0.058%-0.85% in case of oil samples. Similar results were obtained by Roy et al (2002) in their study on crude oil-degrading bacteria in the Sunderban Biosphere at the Haldia Port.
Crude oil-degrading bacteria accounted for 0.08-2% of cultivable heterotrophs depending on the site of collection of sample with the highest numbers being obtained in highly polluted waters. It was observed during our studies that the number of heterotrophs in the soil and oil samples was two to three orders higher than the number of hydrocarbon utilizers indicating that only a small population of bacteria could degrade whole crude oil. Hydrocarbon degrading bacteria is present in almost all environments (Atlas, 1981) but contaminated sites are more likely to harbour such bacteria in greater numbers, having the capability to degrade hydrocarbons as compared to uncontaminated sites as they undergo a process of adaptation. Acclimatization of the organisms after prolonged exposure to hydrocarbons eventually results in its degradation.

Thus population size data can be used as a useful indicator for pollution monitoring.

Bacteria obtained from a contaminated site are thought to be adapted to the contaminant. The soil at Navagam field was exposed to oil for a long time and so bacteria originating from soil at various sites on the field were isolated and studied for their biodegradation potential. Thirty strains capable of crude oil biodegradation were obtained from the crude oil and the crude oil-contaminated soil samples from the Navagam project area of O.N.G.C. Higher number of strains having a high potential of hydrocarbon degradation was obtained from the soil samples as compared to oil samples. In their study, Venosa et al (1992) have found indigenous microorganisms to be more efficient in degrading the Alaska North Slope oil under flask condition than those obtained from pristine site.

Biological changes produced by hydrocarbon contamination are useful monitors of environmental pollution. Bacteria which live in complex communities adapt rapidly to environmental changes and thus only those communities, which have the capacity to withstand these changes become dominant, thus increasing the population of such communities and decreasing diversity.
Gram reaction and morphological studies of the thirty strains isolated from the spillage soil and crude oil of Navagam field suggested that 24 of the 30 strains that is 80% were Gram negative rods whereas 20% were Gram positive cocci. The five strains of Class I were identified based on their Gram reaction, morphological and colony characteristic and biochemical tests. Three of the five strains XRF8, 10 and 15 were found to be belonging to genus *Pseudomonas* and XRF18 was found to be belonging to genus *Micrococcus* while XRF19 belonged to genus *Flavobacterium*. Ridway *et al* (1990) and Radwan *et al* (1995) have found that *Pseudomonas* is the prominent species among the hydrocarbon degrading bacteria. Studies by Patel and Ghosh (2003) on petroleum biodegrading organisms present in the soil and oil of Mehsana and Ahmedabad oil field areas of Gujarat have shown that all the 47 strains isolated belonged to genus *Pseudomonas*. *Micrococcus* and *Flavobacterium* are also reported to be hydrocarbon degrading (Okerentugba and Ezeronye, 2003 and Leahy and Colwell, 1990). Though the presence of hydrocarbons in the environment increases the number of hydrocarbon degraders, it also decreases microbial diversity. Only those species which are able to adapt to high concentration of hydrocarbons survive thus decreasing the diversity of the site. Studies by Macnaughton *et al* (1999) on microbial population changes during bioremediation of an experimental oil spill indicates a community shift towards gram negative biomass and adaptation to metabolic stress as compared to the unoiled plots under study. Predominance of *Pseudomonas aeruginosa* in crude oil-degrading mixture has been reported by Frontera–Suau *et al* (2002). Study of microbial diversity is a way of evaluating pollution as stressed environments are less diverse compared to non stressed environments. Only five of the thirty strains isolated, have been identified and therefore the presence of many other genera of bacteria from the same site and possessing a high capacity of hydrocarbon degradation cannot be ruled out.

**Effect of oil composition on biodegradation**

Increase in the world population has resulted in an enormous increase in the consumption of both castor oil and 2T oil apart from crude oil, which is the main
source of fuel energy. Castor oil has the highest and the most stable viscosity index among all vegetable oils combined with high lubricity, especially under low temperatures. It finds use in different industrial sectors like paintings and coatings, polyurethane coating, plastics, transport, cosmetics, textiles and leathers. World annual production of castor oil is 460,000 tonnes with India and Brazil being the chief producers. Vegetable oil spills have similar effect on the environment as crude oil and their deleterious effect have been documented (Fingas et al, 2001 and Mc Kelvey, 1980).

Though the biodegradation of individual oils has been reported, a comparative study of the biodegradation of crude oil, vegetable oil like castor oil and lubricating oil like 2T oil has not been reported. Our study aimed at obtaining organisms which could degrade oils of different categories and therefore be useful tools for bioremediation.

Each of the 30 isolates was grown in presence of different oils like crude oil, castor oil and 2T oil and growth was measured spectrophotometrically. Depending on their capacity to degrade various oils, the isolates were divided into three classes with Class I containing the isolates which had the highest potential to degrade all the three oils efficiently followed by Class II which had an overall good growth and Class III which had average growth in one or more oils. Following strains were placed in different classes:

Class I - which constituted isolates having the highest potential to degrade all the three oils viz. 8, 10, 15, 18 and 19.
Class II - which constituted isolates having good growth in all the three oils viz 2, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 16, 20, 22, 24, 25, 26, 27, 29 and 30
Class III - 1, 17, 20, 21, 23 and 28 had average growth in one or more oils

17% of the strains were very efficient degraders and were placed in Class I and 20% had low hydrocarbon degrading property (Class III) whereas the remaining 63% had an average capacity (Class II) to degrade different oils. Class I strains were more versatile in their degradation and therefore selected for further experimentation.
Studies on the growth and degradation of various oils indicate that castor oil which is composed mainly of straight chain hydrocarbons was degraded maximum. Growth of all the 30 isolates was the highest in castor oil as shown by culture densities. Our studies on the biodegradation using *Pseudomonas sp.* XRF8, *Pseudomonas sp.* XRF10, *Pseudomonas sp.* XRF15, *Micrococcus sp.* XRF18 and *Flavobacterium sp.* XRF19 have indicated a high biodegradation of castor oil under aerobic conditions. Around 70-80% of castor oil was degraded aerobically within 72 hours. Li *et al.* (2001) and Li and Wrenn (2003) have shown anaerobic biodegradation of vegetable oil. A study by Li and Wrenn (2003) on the biodegradation of canola oil showed that complete biodegradation of the oil occurred anaerobically in 120 days. Our study has found that the biodegradation of castor oil was fast under aerobic conditions and 75% of the oil was lost after 72 hours. Our study supports the findings of Periera *et al.* (2002) on the biodegradation of linseed oil which indicated that biodegradation is higher under aerobic conditions than anaerobic conditions. High growth and complete emulsification of oil during incubation could be due to the greater production of lipase by the oil-degrading bacteria especially under aerobic shake flask conditions.

Lubricating oils are obtained from the distillation of crude oil (Kalichevsky, 1960) and apart from its petroleum base contain additives like amines, phenols and metals like Ba, Mg, Pb and Ca (Obideki, 1985). Biodegradation of Servo 2T oil which is a semi-synthetic oil with petroleum base was the lowest. Studies on the biodegradation of Servo 2T engine oil by the five strains showed that around 35-45% degradation occurred in 10 days of incubation under aerobic conditions. Emulsification of oil occurred during the shake flask conditions of incubation which made the substrate available as a microdroplet thus facilitating hydrocarbon uptake by the bacteria. Presence of additives like heavy metals may be inhibitory to some microorganisms or certain enzymes and thus a low growth and degradation as compared to other oils was observed when 2T oil was used as sole carbon source for the growth of organisms.
Few studies have been carried out on the biodegradation of different lubricating oils. Xie *et al.* (1995) have reported very low (23%) degradation of lubricating oil in aquatic environment and Jirasripongpun (2002) has reported biodegradation ranging from 0 to 50% after 7 days of incubation depending on the strain used.

Navagam crude oil was paraffinic based and contained 80% saturate fraction, 13% aromatic fraction and equal amount of resin and asphaltenes. The biodegradation of Navagam crude oil by the five strains *Pseudomonas sp.* XRF8, 10 and 15, *Micrococcus sp.* XRF18 and *Flavobacterium sp.* XRF19 ranges from 65%-75%. As the percentage of saturate fraction is higher in this oil, it is easily susceptible to microbial attack and therefore 65-75% of the oil is lost within 120 hours, out of which 10% is due to weathering. The crude oil is paraffinic base with an API gravity of 31. McMillen (1995) has observed that biodegradability of crude oil was proportional to the API gravity. Studies by Frontera-Suau (2002) on the biodegradation of crude oils of different API gravities indicate that the Bonny light crude oil with an API of 35.5 was most biodegradable followed by Alaskan North Slope oil (API 27.8) and Venezuelan oil (API 11.8) was the least biodegradable. Similar results have been obtained by McMillen *et al.* who reported 60% loss of oil having API 46 in four weeks as compared to 10% loss of oil having API 15. Okerntugba and Ezeronye (2003) have also shown better degradation of Bonny light crude oil as compared to Bonny medium which had a lower API gravity.

As mentioned earlier, aerobic conditions are conducive for bacterial growth and emulsification leads to a larger surface area of the oil being available to the bacteria for attack. Production of surfactants may also facilitate degradation. Thus a preliminary study on the biodegradation of various oils allows for the screening of potential strains which can be used for bioremediation purpose.
Effect of Salinity and Hydrocarbon concentration on the growth of the bacterial isolates:

Salinity plays an important role in marine oil spill bioremediation as the microorganisms used for such purpose should be halotolerant. Few studies have been carried out on the effect of salinity on the biodegradation of oils. Effect of salinity on the growth of the five isolates indicated that none of the isolates could tolerate NaCl concentration over 0.5 M (around 3% NaCl). Thus these isolates cannot be used for high salinity conditions. Our studies confirm the results of Ward and Brock (1978) who observed that as salinity increases the rate of metabolism of compounds decreases and vice versa. During their study on the effect of inoculation protocols and salinity on the degradation of PAH, Kastner et al. (1998) observed that increase in salinity inhibited PAH biodegradation. Increased concentration of salt proves to be inhibitory to these soil organisms as they have not been exposed to high concentration of salt in the soil and are hence not adapted to it. Use of organisms isolated from salt marshes or slow acclimatization of these organisms may be tried for use under high salt concentrations.

Environmental contamination of crude oil can often be localized as in the case of terrestrial spills and the concentration of hydrocarbons in such environment is very high. All the five isolates could grow efficiently in high concentration of crude oil. Crude oil concentration was varied between 1-20% and it was found that for most strains maximum growth occurred between 5-7.5%. Though growth was less at 20% v/v concentration of crude oil, a complete inhibition was not observed. All the five strains could tolerate and degrade a high concentration of crude oil. Dibble and Bartha (1979) have reported an increase in CO$_2$ evolution over 1.25-5% hydrocarbon mass/dry weight of soil but no increase thereafter at levels of 10% and 15%. Decrease in activity was attributed to inhibition of microbial activity by the toxic components at high concentration of oil. Fusey and Oudot (1984) have also reported that contamination of seashore sediments with crude oil above threshold concentration prevented biodegradation. Our results are in contrast to those observed by Frontera-Suau who reported a drastic decrease
in degradation with increase in hydrocarbon concentration. Okoh et al (2001) have also observed an inhibition in growth of *Burkholderia cepacia* strain RQ1 at crude oil concentrations above 6% (w/v). No such inhibition in growth was observed in our experiments till 20% v/v concentration aerobically, over a period of 120 hours. This could be attributed to the high aerobic conditions prevailing due to shake flask incubation and easy availability of nutrients like N and P and increased contact area which is not the case with soil sediments. Also, Navagam crude is paraffinic base with 80% saturate fraction which are easily susceptible to microbial attack and an increase in the concentration of crude oil increases the saturate fraction. The aromatics which are thought to cause the inhibition in growth of various microorganisms accounts for 12-13% and any loss due to weathering may result in the decrease in the more toxic aromatic hydrocarbons thus making the organisms more tolerant to high concentration of crude oil. Acclimatization of the isolates in lower concentration of crude oil prior to their exposure to high concentrations can allow for higher tolerance and biodegradation of high concentration of hydrocarbons. It will also lead to a shorter lag phase and such acclimatized organisms can be used for seeding purpose.

**Biodegradation of various hydrocarbon fractions**

Biodegradation of crude oil depends not only on the microorganisms but various other biotic and abiotic factors including the composition of crude oil and its concentration. To determine the extent of degradation of various hydrocarbons, crude oil was fractionated into saturates, aromatics, resins and asphaltenes. It was found that all the five strains degraded the saturate fraction maximally followed by the aromatic fraction. Resins and asphaltenes had low to negligible degradation and thus can be said to be recalcitrant. The recalcitrance can be attributed to lack of bioavailability of these compounds. Lack of solubility may also influence biodegradability as solubility decreases as its carbon number increases. Steric hindrance to the catabolizing enzymes or toxicity of certain components also decreases the biodegradability. These organisms have been isolated from soil and any prior exposure to plant hydrocarbons, which are predominantly saturates can also result in a higher biodegradation of alkane fraction. Outer membrane permeability may be one of the major factors which determines biodegradability.
Efficient degradation of saturate fraction by microorganisms has been reported earlier (Perry, 1984, Leahy and Colwell, 1990 and Dutta and Harayama, 2000). Studies on the n-alkane degradation by *Pseudomonas aeruginosa* conducted by Norman *et al.* (2002) indicated that increased degradation of n-alkane resulted in increased cell surface hydrophobicity. Asphaltenes being high molecular weight were not efficiently biodegraded within the time period tested. Few microorganisms have been shown to degrade all classes of compounds and the degrading capacity of all the five isolates is limited to saturated and aromatic fraction with all the five isolates showing a better capacity to degrade only these class of compounds. With the crude oil obtained from Navagam being paraffinic in nature the overall degradation is reported to be good.

**Screening for plasmids**

All the five isolates were screened for the presence of plasmids and it was found that all the five strains possessed high molecular weight plasmids. Higher incidence of plasmids from contaminated environment has been reported earlier (Burton *et al.*, 1982, Hada and Sizemore, 1982, Day *et al.*, 1988 and Barkay, 1988). By contrast Leahy *et al.* (1990) in a study of bacteria isolated from sediments of Campeche Bank region of the Gulf of Mexico, concluded that there was no clear association between plasmid frequency and proximity to an oil field.

Biodegradation of hydrocarbons takes place by the induction or derepression of enzymes involved in the catabolism of particular hydrocarbons or by changes in the genetic makeup of organisms. Biodegradation of hydrocarbons is often reported to be plasmid mediated as plasmids encode enzymes for the degradation of hydrocarbons thereby allowing strains to adapt to the contamination. The pathways for the metabolism of naphthalene, salicylate, camphor, xylene and toluene have been shown to be encoded on plasmids in *Pseudomonas* (Chakravarty, 1976). Three of the five strains belong to genus *Pseudomonas* and the possibility of the biodegradability activity of all the five strains being encoded by plasmids does exist and further studies on this aspect are suggested.
Only five of the thirty strains isolated from the Navagam field area have been studied for plasmid incidence and studies on other strains is likely to reveal a higher incidence of plasmids in other strains isolated from that site.

**Bioremediation using seeding and fertilization**

Bioremediation is a versatile process which can be used in a site specific manner. Biostimulation or addition of fertilizers can be used when nutrient deficient conditions are expected whereas bioaugmentation is used when the proportion of degradative microflora is small as compared to the contaminant and needs to be increased. Bioaugmentation (seeding) of a crude oil-contaminated soil with bacteria having high hydrocarbon degrading capacity has been used for amending oil-contaminated soil. It is also a low cost treatment and according to Alper (1993), it is at least six times cheaper than incineration.

Ecotoxicity data plays an important role in the proper assessment of ecological risk and for demonstration of effectiveness of a bioremediation technique. The usage of cultures adapted to oil enhances the productivity and efficiency of the bioremediation process (Del'Arco and De Franca, 1999). To check the effectiveness of bioremediation of crude oil contaminated soil by the five isolated strains, garden soil was artificially contaminated with crude oil and sterilized. Oily soil and oil-free controls were maintained along with bioremediated experimental pots. Experimental pots contained crude oil-contaminated soil treated with the various cultures for 10 days. The results on two dicot and one monocot plant indicate that germination and growth were inhibited by 5% v/w of crude oil as only 44% of mung and 40% of mustard and rice germinated in the untreated pots. According to Dejong (1980), crude oil spillage makes soil unsatisfactory for plant growth. This is due to insufficient aeration of the soil which is caused when air spaces between soil particles are displaced by crude oil (Rowell, 1977).

After treatment with the various isolates, there was significant improvement in the rate of germination as well as the growth. The better germination rate and
growth in the treated pots can be attributed to the decrease in the toxicity of crude oil after degradation. Currier and Peoples (1954) have indicated a high concentration of light hydrocarbons, aromatics, naphtha and phenolic compounds reduce respiration, transpiration and photosynthesis in plants like barley, mustard and carrot. Significant improvement in the growth of Mung seeds was observed after treatment of oil contaminated soil with *Acinetobacter* A3 by Hanson *et al* (1997). Reduced seed germination and plant growth in untreated soil containing crude oil have been observed by Gill and Nyawuame (1996) and Spaires *et al* (2001). These results are in contrast to those obtained by Huddleston and Myers (1979) who observed that soils which contained 17,000-20,000 mg/kg of residual hydrocarbons had no adverse effects on wheat and bermuda grass germination and growth.

Soils inherently contain organisms capable of hydrocarbon degradation and as mentioned earlier, biostimulation and bioaugmentation are both techniques for bioremediation of a contaminated soil. Though, lot of studies have been carried out on the effect of fertilizers on hydrocarbon removal in soil and aquatic conditions, few studies have focused on the ecotoxicity aspect with respect to the germination and growth of plants.

Three separate experiments were conducted

- To study the effect of enhanced bioremediation through biostimulation (fertilization) of contaminated soil thereby helping the indigenous soil microbes to act.

- To study the effect of addition of fertilizer and seeding to enhance the effect of indigenous microorganisms.

- To study the effect of exogenous seeding to sterilized soil, accompanied by fertilization.

In all the three experiments, soil artificially contaminated by oil in the laboratory inhibited seed germination and plant growth. Control soil free of oil showed the highest rate of germination and a good growth, as was expected. Addition of
Pseudomonas sp. XRF15 to oil free control soils did not have any negative effect on either the germination or the growth of seeds. Pseudomonas forms a part of the normal microflora of the soil and was therefore not expected to cause inhibition in the germination or growth of the seeds. An improvement in the germination rate and growth of seeds was observed on addition of an optimum concentration of fertilizer. Contamination leads to a high amount of carbon being present in the environment. For oil-degrading organisms to grow and multiply, other molecules like nitrogen and phosphorous are required. Thus addition of nutrients is likely to cause an increase in the number of hydrocarbon degraders thereby reducing the amount of hydrocarbons in the soil. High amount of fertilizer proved to be inhibitory. No significant difference in the protein levels in the seeds sown in untreated and treated pots was observed indicating that oil stress did not significantly affect protein levels in the seedlings.

In case of non-sterile oil-contaminated soil, though an improvement in germination and growth was observed when biostimulation and bioaugmentation were used together, the difference was not pronounced as compared to biostimulation alone. Addition of nutrients (N plus P) is essential for maximizing hydrocarbon removal and inoculation with oil-degrading bacteria can increase the initial hydrocarbon removal rate. Inoculation of oily soil with Pseudomonas sp. XRF15 did support good germination and growth of Mung seedlings but this effect was smaller than the addition of nutrients. Our own results indicate that addition of oil-degrading microbes isolated from the Navagam site to autoclaved oily soil (where indigenous bacteria has been got rid of) significantly improved germination rate and growth of Mung seeds.

Thought most studies suggest the use of high N/P, in 1930, Schollenberger observed that soil exposed to hydrocarbons contained more N than unexposed soil. Also most soils contain high numbers of N₂ fixers and given the fact that Mung is a pulse and supports nitrogen fixation, a low ratio of N/P was also used which proved to be inhibitory to the germination and growth of plants. Thus optimization of N : P ratio after thorough study of the soil levels of the nutrients is suggested and this may vary with the contamination levels and the type of soil.
Addition of fertilizers has been found to increase biodegradation rates (Oh et al., 2001, Lindstrom et al., 1991). Horowitz and Atlas (1980) have found greater losses of oil when seeding and fertilization was used that with fertilizer itself. Fayad et al. (1992) have also shown that nutrients and bacteria had more pronounced effect than nutrients alone.

The soil losses its texture as oil binds to the soil (Atuanya, 1987) and decreases the water percolation and oxygen diffusion. Such a soil can be improved through bioremediation treatments like seeding and fertilization. In situ bioremediation experiments on the oil-contaminated site on the field will yield a better insight on the effect of bioremediation techniques like biostimulation and biodegradation. The oil degrading capacity of the isolated strains along with fertilizer in optimum concentration can be used as a potential tool for bioremediation.

Though studies have been carried out on different crude oils (Sugiura et al., 1997, Frontera-Suau, 2000), vegetable oil such as canola oil (Li et al., 2003) and lubricating oil (Jirasripongpun, 2002) separately, few studies have compared the biodegradability of the different oils. Also these studies have been carried out in western countries and little data regarding these is available for tropical countries like India where the climatic conditions as well as microbial diversity may be different.

Analysis of our results suggests that though vegetable oils are reported to be as toxic to the environment (Don Rigger, 1997) as crude oil, the biodegradation of castor oil, in our studies was found to be very high due to the presence of high amount of straight chain hydrocarbons. Lubricating oil on the other hand had low biodegradability owing to its complex constitution and the presence of heavy metals. Thus vegetable oils which have higher biodegradability and lubricity compared to 2T oil and other lubricating oils can be thought of for biolubrication purpose after proper formulation. Different countries have set different standards for biodiesel. Though the complete set of properties of castor oil is not available, it is known to have a flash point of 250°C and Iodine no. 82-88, and does match certain standards set by USA (ASTM-D-6751) and Europe, which have set the
standard of flash point of $> 130^\circ$C and iodine value of $< 120$ (journeytoforever.org).

After due research, and formulation castor oil which has high biodegradability may become a candidate for biodiesel.

The composition of each accumulation of oil is unique and varies with the producing region. The oil collected from the Navagam field was found to be paraffinic with a high saturate fraction (81.1%), thus the overall degradability of this oil was higher as all the five isolates had a high capacity to degrade aliphatic hydrocarbons. The biodegradation of the resin and the asphaltene fraction underwent negligible biodegradation which could be enhanced by the addition of easily utilizable substrates like glucose through a process of cooxidation.

The quantity of oil spilled also influences the biodegradation process with increased concentration inhibiting growth and slowing the biodegradation process (Fusey and Oudot, 1984). Oil spills can sometimes be localized and the concentration of hydrocarbons in such areas is high. All the bacterial cultures isolated by us had a high tolerance to increased hydrocarbon concentration (20%) and a good biodegradation capacity. These isolates can therefore be of importance in bioremediation studies involving high concentration of crude oil.

Study of large number of bacterial isolates has indicated that most of the strains were capable of mineralizing either aromatic or aliphatic hydrocarbon compounds. Foght et al have postulated that bacteria having multidegradative capacity might exist but few strains possess such capacities (Foght et al, 1990). All the five strains studied by us had a very good capacity to degrade aliphatic hydrocarbons as well as a good capacity of degrading aromatic hydrocarbons within the period of study indicating the occurrence of both aromatic and aliphatic hydrocarbon degradative capacity within the same strain. Further study may indicate the presence of genes coding for both catabolic pathways. Earlier studies with a consortium of all the five isolates was not very encouraging and so the consortium prepared in our laboratory was not used for further experiments.
Studies on the degradation capacity allowed for the screening of bacteria that were versatile in its degradation and thus could form an important part in bioremediation of such contaminated environments. Looking at their versatility, biodegradation capacity and preliminary study on the hydrocarbon tolerance, the isolated organisms can be used in oil and grease removing operations in effluent treatments, refinery waste management and as possible tools for the bioremediation of contaminated soils.