6. DISCUSSION

6.1 GENERAL OVERVIEW

Expansion of agricultural activities in recent decades has led to pollution of soil and groundwater with pesticides. Currently there are a number of possible mechanisms for the clean-up of pesticides in soil, such as chemical treatment, volatilization and incineration. The physical and chemical methods for soil clean up are very expensive, and thus it is of great interest to assess the potential use of microbes in the bioremediation of pesticide-contaminated soil. As currently soil contamination is an important environmental problem, the need to remediate contaminated soil has led to the development of new technologies that emphasize the destruction of the contaminants rather than the conventional approach of disposal. Bioremediation involves the use of microorganisms (bacteria and fungi) or microbial processes to degrade environmental contaminants. Also, bioremediation is treated as a relatively new technology for effective and efficient management of environmental pollution.

Due to environmental concerns associated with the accumulation of pesticides in food-products and water supplies there is a great need to develop safe, convenient and economically feasible methods for pesticide remediation (Zhang and Quiao, 2002). For this reason several biological techniques involving biodegradation of organic compounds by microorganisms have been developed (Schoefs et al., 2004). Microbial metabolism is probably the most important pesticide degradative process in soils (Kearney, 1998) and is the basis for bioremediation, as the degrading microorganisms obtain C, N or energy from the pesticide molecules (Gan and Koskinen, 1998).

The use of bioremediation to remove pollutants is typically less expensive than the equivalent physical-chemical methods. This technology offers the potential to treat contaminated soil and groundwater on site without the need for excavation (Balba et al., 1998; Kearney, 1998), so it requires little energy input and preserves the soil structure (Hohener et al., 1998). Perhaps the most attractive feature of bioremediation is the reduced impact on the natural ecosystems, which should be more acceptable to the public (Zhang and Quiao, 2002).
6.2 PESTICIDE USE PATTERN IN THE SELECTED STUDY SITES

The qualitative as well as quantitative surveys were performed on the use-pattern of various pesticides in the selected study sites, because it is important to ascertain the nature and amount of various pesticides being consumed in the given agro-climatic zone, before deciding upon the need to remediate the contaminated sites. Since the different formulations of pesticides differ in their potential of bioaccumulation, persistence and environmental hazards, hence it becomes important to know the formulations of pesticides being used under the current agricultural practices.

The present study showed that 31%, 26% and 36% pesticide formulations were consumed in the cultivation of cotton, groundnut and vegetables, respectively by the farmers of Rajkot taluka. The farmers of Gondal taluka utilize 33%, 22% and 25%, pesticide formulations in the cultivation of cotton, groundnut and vegetables, respectively. Out of all formulations of pesticides used, 41%, 32% and 27% of different formulations were consumed in the cultivation of cotton, groundnut and vegetables respectively, by the farmers of Jetpur taluka. It is clear that in all three selected study sites, majority of pesticide formulations are being consumed in the cultivation of cotton, groundnut and vegetables. Also, it is clear that the consumption pattern of pesticide formulations is area specific. However, the maximum formulations of pesticides are being consumed in the cultivation of cotton crop followed by vegetables, at all the three study sites. Therefore, pesticide management strategy needs to focus on the cultivation of cotton followed by vegetables.

The various formulations of pesticides which are used in the cultivation of different crops in these selected study sites need to be tested for their candidature as persistent organic pollutants as well as their environmental fate and potential hazards. The present study investigated the intricacy of biodegradation of endosulfan and chlorpyrifos using native bacterial isolates for an effective remediation of contaminated soil. This kind of study could pave the way for the development of effective bioremediation strategy for the pesticide contaminated soil, thereby reducing the environmental risk of pesticides used in crop production.
6.3 ISOLATION & MIC OF PESTICIDE-DEGRADING
BACTERIAL ISOLATES

In the present study, eleven bacteria resistant to both endosulfan (organochlorine) and chlorpyrifos (organophosphorus) were isolated. Out of eleven bacterial isolates, two were isolated from Rajkot taluka, six from Gondal taluka and remaining three from Jetpur taluka. The majority of the bacterial isolates (six out of eleven) were obtained from Gondal taluka where pesticide spraying and crop rotation were frequent. The microbial populations in this area were exposed to different formulations of pesticides, which resulted in adaptation of the microbes against the anthropogenic agrochemicals, as evident by their growth in the presence of pesticides in the culture medium.

The results showed that all isolates of Rajkot taluka showed growth in the presence of 10-20 mg/L of endosulfan and 10-60 mg/L of chlorpyrifos, within 24-96 hours for both pesticides. The MIC of bacterial isolates from Gondal taluka was found in the range of 10-30 mg/L of endosulfan and 10-100 mg/L of chlorpyrifos. The MIC of isolates from Jetpur taluka was observed in the range of 10-20 mg/L of endosulfan and 10-80 mg/L of chlorpyrifos. The most resistant isolates were obtained from Gondal taluka. These isolates could tolerate upto 30 mg/L of endosulfan and 100 mg/L of chlorpyrifos, and showed growth in 24 – 72 hours.

Insecticide alpha- endosulfan, beta-endosulfan is degraded by single bacteria like Klebsiella oxytoca, Bacillus spp., Pandoraea sp., Micrococcus sp. and by mixed bacterial co-culture. Microbial degradation of endosulfan may play important role in detoxifying the endosulfan by different groups of microorganism. The three bacterial strains, viz. Pseudomonas spinosa, P. aeruginosa, and Burkholderia cepacia, were found to be the most efficient degraders of both α- and β-endosulfan in a study by Hussain et al. (2007).

Many bacteria including Cornybacterium sp., Nocardia sp., Mycobacterium sp., Pseudomonas fluorescens, Pseudomonas aeruginosa have been reported to be endosulfan degraders and are used to develop techniques of bioremediations on endosulfan. Pseudomonas aeruginosa is able to achieve 94% degradation of endosulfan in contaminated soil (Jayashree, 2007).
**Ochrobacterum** sp, *Arthrobacter* sp, *Pseudomonas alcaligenes*, *Burkholderia* sp and *Pseudomonas* sp degraded α-endosulfan to by 50, 45, 23, 32, and 64 % respectively after 3 days of incubation which increased to 57, 74, 57, 76 and 90% respectively as compared to 9% in control after 7 days (Kumar, 2008).

Chlorpyrifos has been reported to be degraded co-metabolically in liquid media by *Flavobacterium* sp. and also by an *Escherichia coli* clone with an *opd* gene. *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella* sp., and *Serratia marsecens* obtained from consortia showed 84, 84, 81, and 80% degradation of chlorpyrifos (50 mg/L) in liquid medium after 20 days and 92, 60, 56, and 37% degradation of chlorpyrifos (50 mg/L) in soil after 30 days. The bacterial degradation of chlorpyrifos by *Flavobacterium* sp. ATCC 27551 and *Arthrobacter* sp., isolated from contaminated sources, which degrade chlorpyrifos cometabolically, and *Enterobacter* strain B-14, *Alcaligenes faecalis*, and *Klebsiella* sp., which degrade and utilize chlorpyrifos as sole carbon source (Lakshmi, 2009).

A *Serratia* sp. that can transform chlorpyrifos to TCP, and a Trichosporon sp. that is capable of mineralizing TCP were used for lab scale bioremediation study (Xu G., 2007). *Klebsiella* sp., *Pseudomonas putida* and *Aeromonas* sp., offered resistance upto 2mg/mL, 4mg/mL and 8mg/mL of chlorpyrifos while *Pseudomonas putida* and *Aeromonas* sp., resisted higher concentrations i.e., 10mg/mL and 20mg/mL (Ajaz, 2005).

In the present study, eleven bacterial isolates resistant to both endosulfan and chlorpyrifos were selected. For the isolates of Rajkot taluka, the MIC of *RCE-2* was 20 mg/L of endosulfan and 10 mg/L of chlorpyrifos. For the isolates obtained from Gondal taluka, the MIC of *GCC-1* was found to be highest for both endosulfan and chlorpyrifos, i.e. 25 mg/L and 100 mg/L respectively, followed by *GCC-3 & GCC-4*. The three selected isolates from Jetpur taluka included *JCE-4, JCC-2* and *JCC-3*, in which the highest MIC was observed for isolate *JCC-2* for both endosulfan (20 mg/L) and chlorpyrifos (80 mg/L).
6.4 PESTICIDE ADAPTATION AND VIABLE COUNT OF BACTERIAL ISOLATES

In the present study, the adaptation of bacterial isolates against endosulfan and chlorpyrifos has been investigated. The growth of eleven bacterial isolates (monocultures) and four bacterial mixed cultures on different media supplemented with endosulfan and/or chlorpyrifos were observed at a time interval of 24 hours and incubation at room temperature, under static as well shaking conditions.

From the present study, it was found that the bacterial isolates of Rajkot taluka were able to appear after 96 hours of incubation under both static and shaking conditions; however growth was more visible after 96 hours of incubation under shaking conditions. Therefore, it is clear that static and shaking conditions do not affect much on the initial adaptation of bacteria against pesticide, but shaking condition hastens the bacterial growth once the pesticide adaptation is secured.

The bacterial isolates of both Gondal and Jetpur talukas were able to grow after 72 hours of incubation under both static and shaking conditions, however good growth was noticed after 96 hours of incubation under shaking conditions. This indicates that although there is no noticeable effect of static and shaking conditions on the initial growth of the bacterial isolates, however once the organisms get adapted against the pesticide; their growth is found to be rapid under shaking condition.

In case of ES-treated samples, the viable count was in the order of $10^7$ per gram of soil, while in case of chlorpyrifos it was $10^8$ per gram of soil. Therefore, compare to ES-treated samples, the viable counts were found to be higher in case of CP-treated samples. When the soil samples were treated with both endosulfan and chlorpyrifos, then the viable count was found to be in the order of $10^6$ per gram of soil. This indicates that exposure of soil bacteria to multiple pesticide formulations results in the further decline in their viable counts. The proper understanding of the complexity of pesticide – microbial interactions in soil could help in the accelerated degradation of applied pesticides in the soil.
6.5 PHYSICOCHEMICAL PROPERTIES OF SOILS

In the present study, the effect of soil physical properties (bulk density, porosity, soil moisture & electrical conductivity) and soil chemical properties (soil pH, organic carbon, organic nitrogen & available phosphorus) on the abundance of pesticide tolerant bacterial diversities was investigated.

From the soils of Rajkot taluka, with average bulk density of 0.93 g/cc, porosity of 67.4%, soil moisture of 31.9%, electrical conductivity of 0.73 mS/cm, soil pH of 7.8, organic carbon of 6.7 g/kg, organic nitrogen of 0.7 g/kg and available phosphorus of 24.1 mg/kg, only two bacterial isolates, viz. RCE-2 and RCC-2 resistant to endosulfan and chlorpyrifos respectively, were isolated.

From the soils of Gondal taluka, with average bulk density of 0.96 g/cc, porosity of 63.4%, soil moisture of 47.6%, electrical conductivity of 0.93 mS/cm, soil pH of 7.5, organic carbon of 7.2 g/kg, organic nitrogen of 0.78 g/kg and available phosphorus of 30 mg/kg, six bacterial isolates viz. GCE-3, GCE-4, GCE-5, GCC-1, GCC-3 and GCC-4 resistant to varying amount of endosulfan and chlorpyrifos were isolated.

From the soils of Jetpur taluka, with average bulk density of 0.97 g/cc, porosity of 62.5%, soil moisture of 41.5%, electrical conductivity of 0.74 mS/cm, soil pH of 7.7, organic carbon of 6.7 g/kg, organic nitrogen of 0.7 g/kg and available phosphorus of 33.6 mg/kg, only three bacterial isolates viz. JCE-4, JCC-2 and JCC-3 resistant to varying amount of endosulfan and chlorpyrifos were isolated.

Therefore, it is clear that the soil physicochemical properties significantly affect the pesticide resistant bacterial diversity in the cultivated soil. The favourable soil properties are essential to ensure the presence of an active microbial population in the soil that can degrade pesticides.
6.6 CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL ISOALTES

In the present work, eleven bacterial isolates resistant to varying amount of endosulfan and/or chlorpyrifos were screened and isolated from three different talukas of Rajkot district. Two bacterial isolates viz. RCE-2 and RCC-2 resistant to endosulfan and chlorpyrifos respectively, were isolated from the soils of Rajkot taluka.

Six bacterial isolates viz. GCE-3, GCE-4, GCE-5, GCC-1, GCC-3 and GCC-4 resistant to endosulfan and chlorpyrifos were isolated from the soils of Gondal taluka, while three bacterial isolates viz. JCE-4, JCC-2 and JCC-3 resistant to endosulfan and chlorpyrifos were isolated from the soils of Jetpur taluka.

The bacterial isolates were identified as *Bacillus subtilis* (RCE-2), *Pseudomonas aeruginosa* (RCC-2), *Arthrobacter sp.* (JCE-4), *Staphylococcus sp.* (JCE-2), *Streptococcus sp.* (JCC-3), *Arthrobacter sp.* (GCE-3), *Pseudomonas putida* (GCE-4), *Bacillus pumulus* (GCE-5), *Staphylococcus sp.* (GCC-1), *Flavobacterium sp.* (GCC-3) and *Azomonas sp.* (GCC-4).

Isolation, characterization and identification of bacterial isolates from diversifying soil types by different research groups provide very diverse types of microorganisms capable of pesticide degradation. A widely available insecticide alpha- endosulfan, beta-endosulfan is degraded by single bacteria like *Klebsiella oxytoca*, *Bacillus spp.*, *Pandoraea sp.*, *Micrococcus sp.* and by mixed bacterial co-culture (Bhalerao, 2007). *Flavobacterium sp.*, *Pseudomonas diminuta*, *Pseudomonas putida*, *Enterobacter Strain B-14* are isolated from chlorpyrifos contaminated sites and showed degradation capacity for chlorpyrifos (Singh, 2004).
6.7 GROWTH RESPONSE OF BACTERIAL ISOLATES IN PRESENCE OF PESTICIDES

In the present study, bacterial mono-cultures showed higher growth in presence of pesticides when cultured using salt rich LB and M9 media compared to that in N-agar media. The same trend for growth response was also observed in case of bacterial mixed-cultures. Bacteria are tending to be adapted under different environmental conditions they encounter. As time passes, bacteria get adapted to the exposed condition i.e. presence of pesticide. The initial phase of adaptation may lead to the decrease in bacterial growth as well as decrease in pesticide degradation rate.

It has been reported that in presence of chlorpyrifos, growth of Klebsiella sp., Pseudomonas putida, Aeromonas sp. increases and shows resistant to the effect of chlorpyrifos. At initial concentration of pesticide bacterial isolates increases growth till moderate range of concentration but it has been found that at high to very high concentration bacterial growth slightly decreases or it becomes somewhat sensitive (Ajaz et. al., 2005).

During the process of adaptation, it was observed that in the presence of high concentration of insecticides, the bacteria were greatly stressed and as a consequence, their growth was slowed. It also has been found that addition of external carbon source like dextrose increased the degradation efficiency of bacteria and also increases the growth of bacteria in presence of pesticide (Mathava kumar and Ligy Philip, 2008).
6.8 EFFECT OF TEMPERATURE AND pH ON THE GROWTH OF BACTERIAL ISOLATES

In the present study, the optimum growth temperature for the endosulfan-resistant isolate of Rajkot taluka (RCE-2) was 30°C and that of chlorpyrifos-resistant isolate (RCC-2) it was 37°C. For the isolates of Gondal taluka, the optimum growth temperature was 25°C (GCE-3) and 30°C for endosulfan-resistant isolates (GCE-4 and GCE-5), and 37°C (GCC-1), 25°C (GCC-3) and 30°C (GCC-4) for chlorpyrifos-resistant isolates. In case of isolates of Jetpur taluka, the optimum growth temperature was 25°C for endosulfan-resistant isolates (JCE-4) and 37°C for chlorpyrifos-resistant isolates (JCC-2 and JCC-3).

The effect of temperature on the biodegradation of pesticide depends on the molecular structure of the pesticide. Temperature affects solubility, adsorption and hydrolysis of pesticides in soil. The activity of soil microorganisms is stimulated with the rise in temperature. The maximum growth and activity of microorganisms in soils are reported at 25°C to 35°C of temperature. It has been also reported that the pesticide degradation is optimal at temperature range of 25°C to 40°C. At lower temperature, the persistence of various pesticides in the soil is found to be higher (Alexander, 1977; Jitender, 1993; Topp, Vallayes and Soulas, 1997).

In the present study, the optimum growth pH for the endosulfan-resistant isolate of Rajkot taluka (RCE-2) was 7.0 and that of chlorpyrifos-resistant isolate (RCC-2), it was 7.5. For the isolates of Gondal taluka, the optimum growth pH was 7.0 for endosulfan-resistant isolates (GCE-3, GCE-4 and GCE-5) and 7.5 (GCC-1 and GCC-4) and 7.0 (GCC-3) for chlorpyrifos-resistant isolates. For the isolates of Jetpur taluka, the optimum growth pH was 7.0 for ES-resistant isolate (JCE-4) and 8.0 (JCC-2) and 6.0 (JCC-3) for chlorpyrifos-resistant isolates.

The soil pH may affect pesticide adsorption, abiotic and biotic degradation processes. It also influences mobility and bioavailability of pesticide in the soil. The effect of soil pH on degradation of a given pesticide depends greatly on whether a compound is susceptible to alkaline or acid catalyzed hydrolysis. Soil pH influences the sorptive behaviour of pesticide molecule on clay and organic surfaces in the soil (Burns, 1975 and Hicks et. al., 1990).
6.9 RECOVERY OF PESTICIDES FROM N-BROTH AND SOIL SLURRY

In the present study, the recovery efficiency of endosulfan from N-broth and soil slurry media using chloroform extraction followed by D-TLC estimation ranged from 59.6% to 88.0% in case of former, while it was between 46.1% and 66.3% in case of latter. The average recovery efficiency of endosulfan was found to be 76.4% from N-broth and 55.9% from soil slurry. It is clear that the recovery efficiency of endosulfan is lower from soil slurry compared to that of N-broth. The $R^2$ value of calibration curve of endosulfan concentration versus spot intensity in D-TLC was found to be 0.945, which is statistically significant.

The recovery efficiency of chlorpyrifos using chloroform extraction followed by D-TLC ranged from 63.0% to 91.7% in case of N-broth, while it was between 48.2% and 70.8% in case of soil slurry. The average recovery efficiency of chlorpyrifos was found to be 77.9% from N-broth and 60.0% from soil slurry. The recovery efficiency of chlorpyrifos was found to be lower in case of soil slurry compared to that of N-broth medium. The $R^2$ value of calibration curve of chlorpyrifos concentration versus spot intensity in D-TLC was found to be 0.981, which is statistically highly significant.

6.10 ES-DEGRADATION BY BACTERIAL MONO- & MIXED CULTURES

In the present study, the effect of treatment duration (incubation period) with the same volume of bacterial monocultures was found to be highly significant in case of endosulfan degradation. The ES degradation was found to be slightly higher in N-broth medium than in soil slurry medium, with the same volume of bacterial monocultures. The degradation of ES was found to be 12.4, 27.6, 56.8 and 64.8% in N-broth medium, and 11.6, 25.0, 52.2 and 58.7% in soil slurry medium, when each medium inoculated with 10% of bacterial monoculture $RCE-2$, after 5, 10, 15 and 30 days of incubation period respectively, at room temperature. The degradation of ES was 14.3, 23.7, 52.8 and 58.4% in N-broth, and 13.2, 21.5, 48.6 and 53.0% in soil slurry medium with isolate $JCE-4$. The ES degradation was 14.3, 23.7, 52.8 and 58.4% in N-broth, and 13.2, 21.5, 48.6 and 53.0% in soil slurry medium with isolate $GCE-4$, after 5, 10, 15 and 30 days of incubation period respectively, at room temperature. In case of isolate $GCE-5$, the ES degradation was 16.5, 27.7, 61.6 and
67.1% in N-broth and 15.1, 25.1, 56.7 and 60.9% in soil slurry medium, after 5, 10, 15 and 30 days of incubation period respectively, at room temperature.

The effect of culture volume and inoculation media on degradation of endosulfan was found to be quite significant in case of bacterial monocultures with the same period of incubation. The degradation of ES was found to be 22.4, 37.5, 66.8 and 74.8% in N-broth medium, and 21.4, 35.1, 62.3 and 68.7%, in soil slurry medium, when each medium separately inoculated with 10%, 15%, 20% and 25% of bacterial monoculture RCE-2 respectively, for an incubation period of 10 days. The degradation of ES was 27.4, 46.5, 75.2 and 80.2% in N-broth, and 26.0, 43.1, 70.0 and 74.6% in soil slurry medium with isolate JCE-4. The ES degradation was 24.3, 33.7, 62.9 and 68.4% in N-broth, and 23.2, 31.5, 58.6 and 63.0% in soil slurry medium with isolate GCE-4. In case of isolate GCE-5, the ES degradation was 26.5, 37.6, 71.7 and 77.1% in N-broth and 25.2, 35.1, 66.7 and 70.9% in soil slurry medium, when separately inoculated with 10%, 15%, 20% and 25% of bacterial monoculture respectively, for an incubation period of 10 days at room temperature.

The effect of treatment duration on endosulfan degradation was found to be highly significant with the same volume of bacterial mixed-cultures. The ES degradation was also found to be slightly higher in N-broth medium than that in soil slurry medium, with the same volume of bacterial mixed-cultures. The degradation of ES was 21.5, 34.2, 73.0 and 80.5% in N-broth, and 19.7, 31.0, 67.2 and 73.0% in soil slurry medium with mixed-culture GCE345. The ES degradation was 16.0, 24.8, 35.2 and 41.0% in N-broth, and 14.7, 22.5, 32.4 and 37.2% in soil slurry medium with mixed-culture GCC134, after incubation period of 5, 10, 15 and 30 days respectively, at room temperature.

In response to culture volume and inoculation media with the same period of incubation, the degradation of ES was found to be fairly significant with bacterial mixed-cultures. The degradation of ES was 31.5, 44.2, 63.1 and 73.5% in N-broth, and 29.7, 41.1, 47.2 and 63.0% in soil slurry medium with mixed-culture GCE345. The ES degradation was 36.1, 34.8, 45.2 and 51.0% in N-broth, and 24.7, 32.5, 42.4 and 47.2% in soil slurry medium with mixed-culture GCC134, when separately inoculated with 10%, 15%, 20% and 25% of bacterial mixed-culture respectively, for an incubation period of 10 days, at room temperature.
In the present study, the degradation of CP was found to be only 18.3% after 5 days of treatment duration. However, the degradation was 57.0% after 30 days of treatment duration in N-broth medium using 10% culture volume of bacterial mono-culture RCC-2, for incubation at room temperature. In soil slurry medium, the CP degradation was 16.6% and 51.7% after treatment duration of 5 days and 30 days respectively, using 10% culture volume of bacterial mono-culture RCC-2. In case of bacterial isolate GCC-1, the CP degradation was 10.5% and 37.6% in N-broth, and 9.6% and 34.2% in soil slurry media, after 5 days and 30 days of treatment duration respectively, at room temperature. In case of isolate GCC-3, the degradation of CP was 11.2% and 42.2% in N-broth, and 10.2% and 38.3% in soil slurry media, after 5 days and 30 days of treatment duration respectively, at room temperature. In case of isolate JCC-2, the degradation of CP was 20.2% and 74.6% in N-broth, and 18.3% and 69.5% in soil slurry medium, after 5 days and 30 days of treatment duration respectively, at room temperature. Therefore, it is clear that the effect of treatment duration with the same volume of bacterial monocultures was very significant in case of degradation of chlorypyrifos. The degradation of CP was also found to be slightly higher in case of N-broth than that in soil slurry medium. Also, the degradation of CP varied significantly with the type of bacterial mono-cultures used.

The effect of culture volume and inoculation media with the same period of incubation, on the degradation of chlorpyrifos was found to be quite significant in case of bacterial monocultures. The CP degradation was found to be 28.3% and 67.0% in N-broth, and 26.6% and 61.7% in soil slurry media with isolate RCC-2, using 10% and 25% of bacterial monoculture (v/v) respectively, for an incubation period of 10 days at room temperature. The CP degradation was 20.5% and 47.6% in N-broth, and 19.6 and 44.2% in soil slurry medium with isolate GCC-1, using 10% and 25% of bacterial monoculture (v/v) respectively, for an incubation period of 10 days at room temperature. In case of isolate GCC-3, the CP degradation was 21.2% and 52.2% in N-broth, and 20.2% and 48.3% in soil slurry media, using 10% and 25% of bacterial monoculture (v/v) respectively, for an incubation period of 10 days at room temperature. In case of isolate JCC-2, the degradation of CP was 30.2% and 74.6% in N-broth, and 28.4% and 69.5% in soil slurry media, using 10% and 25% of bacterial monoculture (v/v) respectively, for an incubation period of 10 days at room temperature.
temperature. Therefore, it is clear that the degradation of CP varied significantly with the culture volume. The CP degradation was higher with the increased culture volume of bacterial mono-cultures. Also, the degradation of CP was slightly higher in case of N-broth than that in soil slurry medium.

In the present study, when bacterial mixed-cultures, viz. GCE345 and GCC134 were used, a trend similar to that of bacterial mono-cultures was observed. The effect of treatment duration with the same volume of bacterial mixed-cultures was found to be highly significant in case of chlorpyrifos degradation. Also, the CP degradation was also found to be slightly higher in N-broth medium than soil slurry medium, with the same volume of bacterial mixed-cultures.

In response to culture volume and inoculation media with the same period of incubation, the degradation of chlorpyrifos was found to be significant in case of bacterial mixed-cultures. The degradation of CP was 20.8% and 46.7% in N-broth, and 19.8% and 37.4% in soil slurry media, by mixed-culture GCE345, using 10% and 25% of culture volume (v/v) respectively, for the treatment duration of 10 days at room temperature. In case of bacterial mixed-culture GCC134, the CP degradation was 38.8% and 75.2% in N-broth, and 27.1% and 61.3% in soil slurry medium, using 10% and 25% of culture volume (v/v) respectively, for the treatment duration of 10 days at room temperature. Therefore, it is clear that the degradation of CP using bacterial mixed-cultures is significantly affected by the culture volume and inoculation medium used for the purpose of bioremediation.
6.12 GC-MS ANALYSIS OF ES AND CP DEGRADATION

The GC-MS analysis of soil slurry samples containing endosulfan and treated separately with bacterial mono-culture and mixed-culture for 10 days showed the presence of endosulfan at R.T. of 11.325 minutes. The comparison with standard library of Wiley Registry of Mass Spectral Data version-7 confirmed the matching of mass/charge ratio v/s relative intensity at R.T. 11.325 for both the samples to standard spectra of endosulfan. The results of GC obtained in this work matched with that of Kumar and Philip (2006) and Leung et. al. (1996). The mass spectra of endosulfan containing samples treated with bacterial mono- and mixed-culture showed no any known toxic intermediates. The results suggested that our bacterial isolates were not forming any toxic intermediates during the degradation of endosulfan and thus could be utilized for the bioremediation process of endosulfan contaminated soil. Similar kind of results were also reported by Kumar & Philip (2006) for anaerobic culture, while our isolates are aerobic and can be easily applied for traditional bioremediation process of pesticide-contaminated soil.

In case of soil slurry containing chlorpyrifos and treated separately with bacterial mono- and mixed-culture for 10 days, the GC-MS analysis showed the presence of chlorpyrifos at R.T. of 9.558 minutes. The comparison with standard library of NIST-07 mass spectral database confirmed the matching of mass/charge ratio v/s relative intensity at R.T. 9.558 for both the samples to standard spectra of chlorpyrifos. The mass spectra obtained in our study showed that the chlorpyrifos was degraded to some small metabolites which could not be identified using the available library database. The results of mass spectra matched with observations of Geetha and Fulekar (2008). The presence of chlorpyrifos was observed at R.T. 9.558 minutes but no any intermediate was identified till R.T. 22 minutes. This indicated that chlorpyrifos is probably completely metabolized by the bacterial isolates into smaller intermediates.
6.13 EFFECT OF TEMPERATURE ON BIOREMEDIATION OF ES AND CP

In the present study, in case of bioremediation using bacterial monocultures in the soil slurry medium, the degradation of ES (23.7%) was found to be highest at 37°C and that of CP (25.9%) at 45°C by the isolate RCE-2, after 10 days of incubation. By using isolate GCE-4, the degradation of ES (44.1%) and that of CP (25.2%) was highest at 37°C of incubation temperature. In case of isolate RCC-2, the degradation of ES (32.1%) and that of CP (40.0%) was highest at 37°C. By using isolate GCC-3, the degradation of ES (27.0%) was highest at 45°C and that of CP (41.2%) was highest at 37°C, after 10 days of incubation.

In case of bioremediation using bacterial mixed-cultures in soil slurry medium, with culture GCE345, the degradation of both ES (45.8%) and CP (30.9%) was highest at incubation temperature of 37°C, after 10 days of incubation. By using culture GCC134 too, the degradation of both ES (36.0%) and CP (44.6%) was highest at 37°C, after 10 days of incubation.

Bioremediation of chlorpyrifos is much affected by the change in temperature and pH. As pH and temperature increases degradation rate of chlorpyrifos also increases. It has been reported that as temperature increases from 8°C to 28°C, the degradation of chlorpyrifos by Aspergillus sp. is enhanced (Liu et. al., 2003).

Wang et. al. (2006) reported that the degradation rate of chlorpyrifos by B. latersprorus DSP in pure cultures was affected by temperature showing an order of 35°C > 25°C > 15°C. The results showed that the increase in temperature enhances the degradation rate of chlorpyrifos (Fang et. al., 2008).

In the present study, the degradation of endosulfan as well as chlorpyrifos was found to be higher in the temperature range of 30 to 37°C for majority of the bacterial isolates. The variation in the degradation of endosulfan and chlorpyrifos with changing temperature was quite significant in both the cases of treatments, viz. treatment with bacterial mono-culture and treatment with bacterial mixed-culture.
Arshad et. al. (2007) reported that the biodegradation of endosulfan by *Pseudomonas aeruginosa* in loam soil slurry varied significantly with temperature. The degradation of α-endosulfan ranged from 48% to 84%, while of β-endosulfan ranged from 46% to 82% as the incubation temperature increased from 20 to 30°C and decreased at temperatures greater than 30°C. The slowest biodegradation in inoculated flasks (43%) occurred at 45°C.

The effect of temperature on pesticide bioremediation depends on the molecular structure of the pesticide. It is expected that the solubility of pesticide increases with the rise in temperature. It is also expected that the rise in temperature results in the stimulation of microbial activities. It is also found that the maximum growth and activity of microorganisms in soils occur between 25 to 35°C of temperature (Burns; 1975, Racke et. al.; 1997 & Topp et. al.; 1997).

6.14 EFFECT OF pH ON BIOREMEDIATION OF ES AND CP

In the present study, the degradation of ES (26.6%) and CP (20.4%) using bacterial monoculture in soil slurry medium was highest at pH 8.0 and pH 7.5 respectively, by the isolate *RCE-2*, with 10% of actively growing culture and 10 days of incubation period at room temperature. In case of isolate *GCE-4*, the degradation of ES (23.8%) was highest at pH 8.0 and the degradation of CP (20.0%) was maximum at pH 7.5. With isolate *RCC-2*, the degradation of ES (21.3%) was maximum at pH 8.0 and that of CP (29.4%) was highest at pH 7.0. In case of isolate *GCC-3*, the degradation of ES (24.0%) was highest at pH 8.0 and that of CP (39.2%) at pH 7.5, with 10% of actively growing culture and 10 days of incubation period at room temperature.

In case of bioremediation using bacterial mixed-culture *GCE345* in soil slurry medium, the degradation of ES (36.9%) and CP (23.6%) was highest at pH 8.0 and pH 7.5 respectively, with 10% of actively growing culture and 10 days of incubation period at room temperature. By using bacterial mixed-culture *GCC134*, the degradation of ES (30.1%) was highest at pH 8.0 and that of CP (33.7%) was maximum at pH 7.5, with 10% of actively growing culture and 10 days of incubation period at room temperature.
It is known that the rates of base-catalyzed hydrolysis for many organophosphorus insecticides are often greatly accelerated in water at pH values above 7.5 (Greenhalgh et. al., 1980). Wang et. al. (2005) reported that the biodegradation rates of chlorpyrifos by *Fusarium LK* were higher at pH 6.5–9.0 in pure cultures. Degradation of chlorpyrifos in the two acidic soils was slow, especially in the soil with pH 4.7, where the half-life was 256 days. Chlorpyrifos degradation at pH 5.7 was somewhat faster, with the half-life being 58 days. Formation of the metabolite TCP was more pronounced at pH 5.7 than at pH 4.7. Chlorpyrifos degradation in the more neutral pH 6.7 soil was quicker than in the two acidic soils, with a half-life of 35 days. Chlorpyrifos degradation was rapid in the two alkaline soils (pH 7.7 and 8.4), with a half-life of 16 days in both of them (Singh et. al., 2003 and Fang et. al., 2008).

The pH of soil may affect pesticide degradation by altering the pesticide adsorption and also by influencing the microbial activity in the soil. Soil pH may also affect the mobility and bioavailability of pesticides. The effect of soil pH on degradation of a given pesticide depends greatly on whether the pesticide is susceptible to alkaline or acid catalyzed hydrolysis (Burns, 1975, Hicks et. al., 1990 and Racke et. al., 1997).
6.15 EFFECT OF AERATION ON BIOREMEDIATION OF ES AND CP

In the present study, the effect of aeration on bioremediation of ES and CP using bacterial mono- and mixed-cultures was investigated in soil slurry medium. The degradation of ES was found to be 21.8% and 27.5%, and that of CP was 12.8% and 16.6% under static and shaking conditions respectively, by the isolate RCE-2, after 10 days of incubation at room temperature.

The degradation of ES was 23.5% and 35.6%, and that of CP was 9.9% and 14.3% under static and shaking conditions respectively, by isolate GCE-4, after 10 days of incubation at room temperature. The degradation of ES was found to be 11.1% and 19.8%, and that of CP was 17.1% and 31.5% under static and shaking conditions respectively, by isolate RCC-2, after 10 days of incubation at room temperature. In case of isolate GCC-3, the degradation of ES was 13.7% and 20.7%, and that of CP was 18.9% and 34.6% under static and shaking conditions respectively, after 10 days of incubation at room temperature. Therefore, it is clear that the degradation of both ES and CP by bacterial mono-cultures was significantly higher under shaking condition compared to that of static condition.

In case of bioremediation using bacterial mixed-cultures in soil slurry medium, the degradation of ES was 33.3% and 42.6%, and that of CP was 22.0% and 29.6% under static and shaking conditions respectively, with culture GCE345, after 10 days of incubation at room temperature.

The degradation of ES was 21.3% and 29.5%, and that of CP was 30.0% and 42.9% under static and shaking conditions respectively, by mixed-culture GCC134, after 10 days of incubation at room temperature. Therefore, it is clear that the degradation of ES as well as CP by bacterial mixed-cultures was significantly higher under shaking condition compared to that of static condition.

Arshad (2007) reported that with aeration, biodegradation of α- and β-endosulfan was 86% and 82%, when compared with 69% and 67% under static conditions, respectively.
In the present study, the effect of organic amendments viz. compost, cow dung, leaf litter and crop residues, on bioremediation capability of bacterial mono- and mixed-cultures in soil slurry medium was investigated. In case of bacterial mono-cultures, the degradation of ES was 26.6% and 29.6% by the isolates RCE-2 and GCE-4 respectively, when amended with compost in soil slurry medium, after the incubation period of 10 days at room temperature. When amended with cow dung, the degradation of ES was 22.5% and 26.8% by the isolates RCE-2 and GCE-4 respectively. When amended with leaf-litter, the degradation of ES was 19.4% and 19.6% by the isolates RCE-2 and GCE-4 respectively. When amended with crop residues, the degradation of ES was 13.6% and 11.8% by isolates RCE-2 and GCE-4 respectively, in soil slurry medium, after the incubation period of 10 days at room temperature. Therefore, it is clear that the biodegradation of ES by bacterial mono-cultures was higher when soil slurry medium amended with compost.

In case of bacterial mono-cultures, the degradation of CP was 32.4% and 41.5% by the isolates RCC-2 and GCC-3 respectively, when amended with compost in soil slurry medium, after the incubation period of 10 days at room temperature. When amended with cow dung, the degradation of CP was 28.0% and 43.9% by the isolates RCC-2 and GCC-3 respectively. When amended with leaf-litter, the degradation of CP was 19.3% and 24.0% by the isolates RCC-2 and GCC-3 respectively. When amended with crop residues, the degradation of CP was 15.0% and 16.2% by isolates RCC-2 and GCC-3 respectively, in soil slurry medium, after the incubation period of 10 days at room temperature. Therefore, it is clear that the biodegradation of CP by bacterial mono-cultures was higher when soil slurry medium amended with compost. Also, it is clear that the effect of organic amendments on bioremediation of ES and CP varied with type of bacterial mono-cultures used.

In case bacterial mixed-cultures, the degradation of ES was 31.2, 27.2, 25.4 and 14.0%, and that of CP was 24.2, 22.0, 19.9 and 14.8% when amended with compost, cow dung, leaf litter and crop residues respectively, by culture GCE345, after the incubation period of 10 days at room temperature. The degradation of ES was 27.7, 24.4, 21.1 and 12.9%, and that of CP was 41.0, 38.8, 35.8 and 31.9% when
amended with compost, cow dung, leaf litter and crop residues respectively, by culture GCC134, after the incubation period of 10 days at room temperature. Therefore, it is clear that out of four organic amendments, viz. compost, cow dung, leaf litter and crop residues, the maximum degradation of ES and CP was observed when soil slurry medium inoculated with bacterial mixed-culture was amended with compost.

Microbial activity is often stimulated by the addition of organic material to soil. Organic matter also improves many of the physical and chemical properties of soil such as the water holding capacity, aeration, pH, and ion exchange capacity (Brady and Weil, 1998). These properties influence the indigenous microbial populations and may enhance their ability to degrade hydrocarbons and other C-based contaminants (Wellman et. al., 2001). The incorporation of organic amendments affects soil enzyme activities because the added material may contain indoor extra-cellular enzymes and may also stimulate soil microbial activity (Goyal et. al., 1993). Pesticides added to soil can persist for a year or more. Sometimes, the lack of sufficient readily decomposable organic matter in soil gives inadequate substrate to stimulate microorganisms in the decomposition of pesticides. The vigorous biological activity during composting can be used to accelerate or enhance the decomposition of pesticides in soil or deliberately to treat pesticide-contaminated materials.

6.17 EFFECT OF SOIL MOISTURE ON BIOREMEDIATION OF ES AND CP

In the present study, in case of bioremediation by bacterial mono-cultures, the degradation of ES was found to be 27.8% and 14.6%, and that of CP was 13.9% and 8.9%, with and without sprinkling of sterile distilled water (SDW) respectively, by the isolate RCE-2, during incubation period of 10 days.

The ES degradation was 19.7% and 13.2%, and that of CP was 15.0% and 10.2%, with and without sprinkling of SDW respectively, by isolate GCE-4, during incubation period of 10 days. The degradation of ES was found to be 18.5% and 13.5%, and that of CP was 35.2 and 20.5%, with and without sprinkling of SDW respectively, by isolate RCC-2, during incubation period of 10 days.
In case of isolate GCC-3, the degradation of ES was 21.4% and 12.7%, and that of CP was 36.5% and 23.0%, with and without sprinkling of SDW respectively, during incubation period of 10 days. Therefore, it is clear that the presence of moisture has positive effect on bioremediation of ES and CP by bacterial mono-cultures.

In case of bacterial mixed-cultures, the degradation of ES was found to be 37.5% and 22.5%, and that of CP was 18.6% and 13.1%, with and without sprinkling of SDW respectively, by culture GCE345, during incubation period of 10 days. The degradation of ES was 20.5% and 16.8%, and that of CP was 38.8% and 19.4%, with and without sprinkling of SDW respectively, by culture GCC134, during incubation period of 10 days.

In many previous studies of water stress effects on physiology, salts were added to soil slurries to show that the nitrification rates (Stark and Firestone, 1995) and 2,4-D degradation rates (Han and New, 1994) decreased with decreasing water potential. Adjusting the salt concentration of soil slurries or pure liquid cultures alters the solute component of water potential and may or may not also result in specific ion toxicity. Schnell and King (1996) reported that methane oxidation rates decreased with decreasing water potential when either salts (KCl or NaCl) or sugar (sucrose) were used to lower the solute or water potentials, respectively, of soil slurries.

In whole soil, nutrient limitations at low water content will restrict microbial processes (Stark and Firestone, 1995; Schnell and King, 1996). The studies of methanotrophs showed that water stress affected methane oxidation rates similarly in liquid cultures and in whole soils (Schnell and King, 1996). Through its relationship to water film thickness (Taylor and Ashcroft, 1972), matric water potential affects the supply of gas and solution phase nutrients to microorganisms (Papendick and Campbell, 1981). Thus, matric water potential can serve as a unifying environmental determinant of unsaturated zone biodegradation.
6.18 ENZYMATIC CHANGES DURING PESTICIDE BIOREMEDIATION

The enzymatic changes triggered during bioremediation of ES and CP was investigated by measuring the activity of three enzymes, viz. cellulase, dehydrogenase and protease. The activity of cellulase in soil inoculated with ES-specific bacterial mixed-culture (GCE345) was found to be reasonably higher in presence of higher concentration of ES compared to that of control, after 7 days of treatment duration. The significant increase in cellulase activity in presence of ES was found between the treatment duration of 7 to 14 days, and after that cellulase activity was found to be declining. It was also observed that the cellulase activity was highest in presence of ES (20 mg/10g soil), after 14 days of treatment duration, with bacterial mixed-culture GCE345.

In case of soil inoculation with CP-specific bacterial mixed-culture (GCC134), cellulase activity was found to be slightly lowered in presence of CP during the course of treatment duration of three weeks. The increase in CP concentration as well as treatment duration lowered the cellulase activity; however, the most significant decline in cellulase activity was observed in presence of CP (20 mg/10g soil), after 21 days of treatment duration with bacterial mixed-culture GCC134.

When the experimental soil was inoculated with bacterial mixed-cultures (GCE345 and GCC134), cellulase activity was found to be slightly increasing till 14 days of treatment in presence of both ES and CP. However, the increase in cellulase activity in presence of both ES and CP was found to be reasonably higher from 7th day of treatment to 14th day and following that it showed a declining trend till 21st day of treatment.

The activity of dehydrogenase enzyme in the soil treated with ES and inoculated with bacterial mixed-culture GCE345 was found to be declining after 7th day of treatment and continued up to 21st day of treatment. However, the decline in the activity of dehydrogenase was slightly more rapid after 14th day of treatment in presence of ES. When soil was treated with different concentrations of CP and inoculated with bacterial mixed-culture GCC134, then the activity of dehydrogenase was found to be relatively lower in presence of CP (10mg/10g soil) after 7 days of treatment duration.
The activity of dehydrogenase in soil inoculated with bacterial mixed cultures (GCE345 and GCC134) was found to be declining in presence of both ES and CP, from 7th day of treatment to till 21st day. However, the decrease in dehydrogenase activity in presence of both ES and CP (20mg of each/10g soil) was found to be most rapid on 21st day of treatment.

The activity of protease in soil inoculated with ES-specific bacterial mixed-culture GCE345 was found to be reasonably higher in presence of lower concentration of ES (5mg/10g soil) compared to that at higher concentration of ES (20mg/10g soil), after the treatment duration of 14 days. The significant increase in protease activity in presence of lower concentration of ES was found between the treatment duration of 14 to 21 days. It was also observed that the protease activity was highest in presence of 5mg ES/10g soil, after 21 days of treatment duration with bacterial mixed-culture GCE345.

In case of soil inoculation with CP-specific bacterial mixed-culture GCC134, protease activity was found to be slightly lowered in presence of CP from day 1 to till 21st day of treatment duration. The increase in CP concentration as well as treatment duration did not have much significant effect on the protease activity, however, the most significant reduction in protease activity was reported in presence of CP concentration of 20mg/10g soil, after 1 day of treatment duration compared to that of the control.

When the experimental soil inoculated with bacterial mixed-cultures (GCE345 and GCC134), protease activity was found to be increasing in presence of 5mg each of ES and CP per 10g of soil compared to control, from day 1 to till 21 days of treatment duration. However, in presence of 10mg each of ES and CP per 10g of soil, the decline in protease activity was less prominent compared to that with 20mg each of ES and CP per 10g of soil. Therefore, the reduction in protease activity in soil is reasonably significant at higher concentration of ES plus CP.
6.19 RELATIONSHIP AMONG EXAMINED PARAMETERS

The scatter plot of ES degradation by bacterial mono-culture (JCE-4) in response to treatment duration in both N-broth and soil slurry showed a positive linear relationship. The higher values of correlation coefficient of pesticide degradation and treatment duration showed that the treatment duration of ES contaminated soil could significantly contribute towards an effective bioremediation process. However, the slightly lower value of correlation coefficient in case of treatment in soil slurry compared to that of N-broth indicated that presence of some nutrients in the remediation medium could enhance the remediation process.

The scatter plot of CP degradation by bacterial mono-culture (GCC-3) in response to treatment duration in both N-broth and soil slurry showed a positive linear relationship. The values of correlation coefficient are highly significant and indicate that optimum treatment duration with bacterial monoculture could contribute significantly towards an effective bioremediation of CP. It is very clear from the values of correlation coefficient and scatter plot that the treatment duration has a significant role in bioremediation of both ES and CP. Notwithstanding, the effect of treatment duration was found to be slightly higher in for CP than for ES.

The scatter plot of ES degradation by bacterial mono-culture (JCE-4) in response to culture volume in both N-broth and soil slurry showed a positive linear relationship. The higher values of correlation coefficient of pesticide degradation and culture volume showed that the optimum culture volume could significantly contribute towards an effective bioremediation of ES.

The scatter plot of CP degradation by bacterial mono-culture (GCC-3) in response to culture volume in both N-broth and soil slurry showed a positive linear relationship. The values of correlation coefficient were highly significant and indicated a strong relationship between degradation of CP and culture volume. Thus, the optimization of culture volume could significantly contribute towards the development of an effective biotreatment process for CP bioremediation.
The scatter plot of ES degradation by bacterial mono-culture (\textit{JCE-4}) in response to temperature in both N-broth and soil slurry showed a positive linear relationship without any outlier till the temperature reaches 35°C and thereafter two outliers were observed. Thus, the optimization of temperature near 35°C for the given bacterial monoculture could be an important factor for the effective bioremediation of ES. Also, the moderately high values of correlation coefficient of pesticide degradation and temperature showed that optimum temperature could significantly contribute towards an effective bioremediation of ES.

The scatter plot of CP degradation by bacterial mono-culture (\textit{GCC-3}) in response to temperature in both N-broth and soil slurry showed a positive linear relationship. The medium values of correlation coefficient showed slightly poor relationship between biodegradation of CP and temperature. Therefore, a very careful optimization of temperature would be required for an effective bioremediation of CP using the given bacterial monoculture.

The scatter plot of ES degradation by bacterial mono-culture (\textit{JCE-4}) in response to pH in both N-broth and soil slurry showed a positive linear relationship. The higher values of correlation coefficient for pesticide degradation and pH showed that the selection of organism specific pH value could significantly contribute towards an effective bioremediation of ES.

The scatter plot of CP degradation by bacterial mono-culture (\textit{GCC-3}) in response to pH showed a positive linear relationship in both N-broth and soil slurry media. The higher values of correlation coefficient for degradation of CP and pH showed that the selection of organism specific pH value would be very important factor for the effective bioremediation process of CP by employing such selected bacterial isolates.
6.20 STRATEGIES FOR EFFECTIVE BIOREMEDIATION OF PESTICIDE-CONTAMINATED SOIL

In the present study, the effect of biotic and abiotic factors for accelerated biodegradation of endosulfan as well as chlorpyrifos in soil slurry medium inoculated with different bacterial mono-cultures and mixed-cultures were investigated. An evaluation of bioremediation requires appraising the biodegradability of the target compound by referring to prior literature, demonstrating bio-treatability using actual site samples and/or demonstrating the bioremediation during pilot testing or full-scale remediation. The possible evaluation steps can be considered in a formal step-by-step evaluation process.

The development of an effective bioremediation process for pesticides such as endosulfan and chlorpyrifos requires understanding of various parameters which affect the ultimate outcome of the process, in one way or other. The fate of pesticides in the soil depends upon many factors viz. pesticide types, pesticide structure, pesticide concentration, soil types, soil organic matter, soil moisture, soil pH, temperature, soil microbial biomass, interaction between soil microbes and pesticides, and so on. Considering these factors into account, an economical and eco-friendly design for an effective bioremediation of pesticide contaminated soil could be developed. The development of such bioremediation process could help to manage the menace of pesticide contamination of cultivated soil and thereby help to reduce and/or eliminate the entry of pesticides into the food chains.