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DEGRADATION OF 2,4-DICHLOROPHENOXY ACETIC ACID IN SOIL BY BACTERIAL CONSORTIUM

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ABSTRACT

A consortium was developed of the bacterial isolates showing growth and resistance to 2,4-dichlorophenoxyacetic acid (2, 4-D) in nutrient agar and mineral salt medium with and without glucose up to concentration of 3500-6000 ppm. This consortium was used to check the degradation of 2, 4-D, when applied in soil (Sterilize and Unsterilized). The laboratory scale experiment was setup at two temperatures (30°C and 50°C) for four weeks. Effect of the addition of external carbon source (glucose) was also studied. Analysis of 2,4-D in soil (acetone extract) was done weekly for four weeks. Results showed that in sterilized soil 2,4-D was reduced up to 95.8%, in sterilized soil with glucose up to 63.0%, and in unsterilized soil up to 85.5% at 30°C in four weeks. Where as at 50°C reduction of 2,4-D was observed up to 70.0% and 50.8% in sterilized and unsterilized soil respectively after four weeks. Isolated bacterial strains have potential for bioremediation of chlorinated pesticides polluted soil.

Keywords: Biodegradation, Bioremediation, Pesticides, Herbicides, Phenoxyacetic acid, chlorinated organic pollutants, Bacteria.

INTRODUCTION

Pesticides are man-made organic compounds used to control pests of crops, besides its beneficial output, they created harmful environmental health hazards in the form of residue. Pesticides in environments are applied to protect plants from disease of weeds and insect damage, usually come into contact with soil where they undergo a variety of transformations. Pesticides disappear from soil at varying rates depending on their chemical properties, soil and weathering conditions. Their decomposition results from chemical, photochemical and microbial processes, which is usually the most significant. Some pesticides or their degrading products accumulate in soil and move downward into ground water (Doxtader and Croissant, 1992).

The chlorinated phenol and phenoxyacetic acid are group of chemical compounds, which are used as pesticides, wood preservatives and herbicides in the agriculture industry. They are also included in the U.S.EPA. list of priority pollutants. These xenobiotic compounds of environmental concern can be removed from the environment by biodegradation and biotransformation method (Haggblom, 1990) and the other group of chlorinated phenoxyacetic acid is used as herbicides to control the broad leaf weeds in both agriculture and domestic application(to control weeds of home gardens) (Shaw and Burns, 2004). The herbicides are more resistant in high concentrations and organic amendment often stimulates biodegradation. Many 2,4-D degrading bacteria have been isolated from exposed and unexposed soil (Ka *et al*., 1994 Meer *et al.*, 2004). 2,4-D has been found, to be actively degraded by several strains of bacteria individually and in the form of consortium (Pattanasupong *et. al*., 2004). It is the model compound to study the degradation of chlorinated aromatic compound in soil (Cupples *et. al.*, 2005).

There is growing concern over the wide range of xenobiotic compounds being introduced inadvertently or deliberately into soil. Such contamination can be long term and have significant impact on decomposition process and nutrient cycling and results in environmental problem in to groundwater and soil (Gadd, 2001). One strategy is the potential of using microbial bioremedial means of treating soil to enhance the breakdown of xenobiotic compounds, thus avoiding expensive excavation and removal/replacement of soil, and conserving soil structure and quality (Balba *et al.*, 1998; Hollender *et al*., 2003)

Keeping in view the significance of pesticides as environmental pollutant the present study was undertaken to isolate the chlorinated pesticides degrading bacteria using 2, 4-D, as model chlorinated compound and established the bioremediation process to clean the environment. Therefore, this study achieved the objectives of the isolation of

potential bacterial strains responsible for degradation of 2. 4-D and established the process to clean the contaminated environment.

MATRIALS AND METHODS

The samples were collected from the soil of Khairpur Mirs Sindh Pakistan exposed to pesticides for 25-30 years. Total microbial count in soil samples were made by serial dilution and spread technique on nutrient agar. Nutrient agar and mineral salt medium with and without glucose were used for screening of resistance of isolates against 2,4-D. Composition of nutrient agar, peptone from meat 5.0 gram meat extract 3.0 gram agar-agar (not present in the broth) 12.0 gram/liter and composition of mineral salt medium was KH_2PO_4,13.6 %, $(NH_3)_2\ SO_4$, 2.6 %, NAOH, 2.5 %, $MgSO_4.7H_2O$, 8.0 %, $FeSO_4.7H_2O$, 0.2 %, all weight / volume and HCL concentrated 0.4 % volume / volume glucose was 5 mM. Screening of 2,4-D degrading bacterial strains was made on the basis of their ability to utilize 2,4-D as a sole source of carbon and energy. Identification of screened strains was made on the basis of cultural, morphological and biochemical characteristics, which showed maximum tolerance up to 6000 ppm.

Bioremediation of soil was performed using consortium of screened bacterial strains on 30ºC in sterilized soil with and without glucose and in unsterilized soil. Experiment was also conducted at 50ºC in sterilized soil and unsterilized soil. Samples of 0, 3, 7, 14, 21, and 28 days were extracted with acetone and analyzed by HPLC using column C18 Nucleosil (250 x 4.6 mm, 5µ), UV detector (210 nm) and mobile phase was acidified methanol with water as 40:60 at pH 2.0, flow rate was 1.5 ml / min. Before extraction of samples viable cell count (CFU/g) was also performed by serial dilution method to examine growth of bacterial consortium in soil samples.

RESULTS

The present study was under taken to exploit the potential of bacteria for bioremediation of chlorinated aromatic compounds using 2,4-D as model compound. The bacterial strains were isolated from agricultural soil exposed to pesticides. The colony-forming unit (CFU) of sample were $4.8x10^6$/g of soil .The bacterial colonies was picked from nutrient agar plate containing 2,4-D (100 ppm).

Table 1. Maximum tolerance limits (MTLs) of isolates to 2,4-D in nutrient agar and in mineral salt medium with and without glucose.

	Nutrient agar			PNRG			PNR		
Strain	**RG**	**GG**	**SG**	**RG**	**GG**	**SG**	**RG**	**GG**	**SG**
P_1	3500	4500	5000	1500	2500	3000	1500	2500	3000
P_2	3500	4500	5000	1500	2500	3000	1500	2500	3000
P_3	4000	5000	6000	2500	4500	5500	2000	4000	5000
P_4	4000	5000	6000	2500	4500	5000	2000	3000	4500
P_5	4000	5000	6000	2500	4500	5000	2000	3000	4500
P_6	4000	5000	6000	2500	5000	6000	1500	2000	4500
P_7	5000	5500	6000	3000	5500	6000	2000	2500	5500
P_8	3000	6000	6000	1500	5000	6000	1500	2500	3000
P_9	4000	5000	6000	1000	2500	4000	1500	2000	2500
P_{10}	3000	4500	5000	1500	2500	3500	1500	2500	3000
P_{11}	5000	6000	6000	4000	5000	6000	1500	2000	4000
P_{12}	4000	4500	6000	3000	4000	5500	3000	4000	5500
P_{13}	4000	5000	6000	2500	4500	6000	3000	4000	4500
P_{14}	4000	5000	6000	2500	4500	6000	3000	4000	4500
P_{15}	4000	4500	5000	1000	2000	45000	1500	2000	4000
P_{16}	2000	3000	4500	1000	1500	2500	1500	2000	2500
P_{17}	5000	6000	6000	2500	5000	6000	3500	5000	6000
P_{18}	4000	5000	6000	2000	4000	5500	2000	2500	4000
P_{19}	4000	5000	6000	2000	4000	5500	2000	2500	4000
P_{20}	3000	4500	5000	1500	2500	3000	1500	2000	3000

PNRG; mineral salt medium with glucose, PNR; mineral salt medium; RG; rich growth, GG; good growth, SG; slight growth

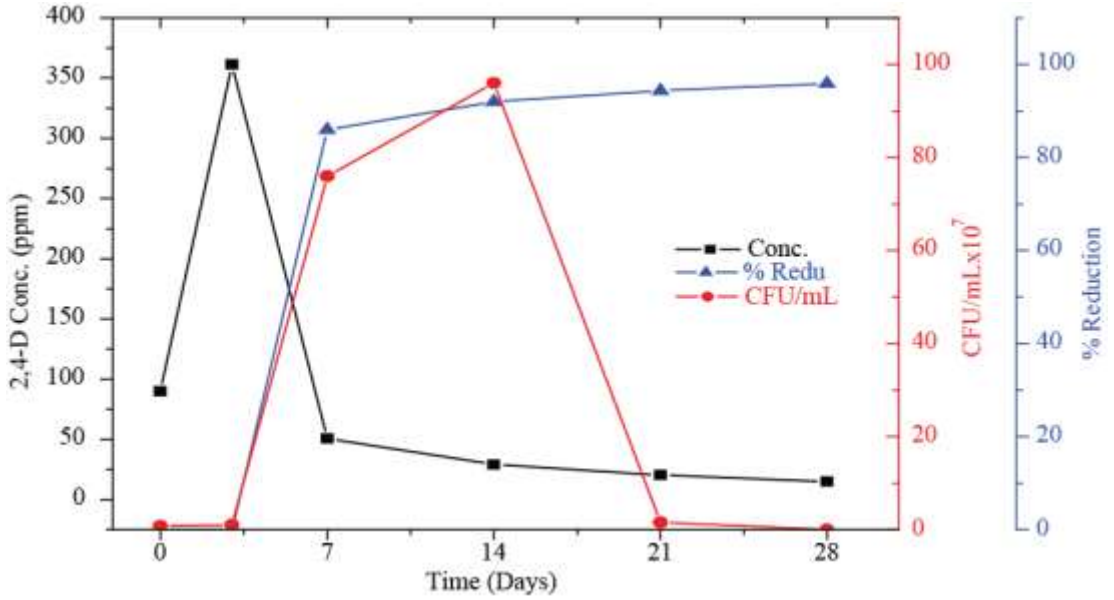

Fig. 1. Bioremediation of 2, 4-D in sterilized soil by bacterial consortium at 30°C.

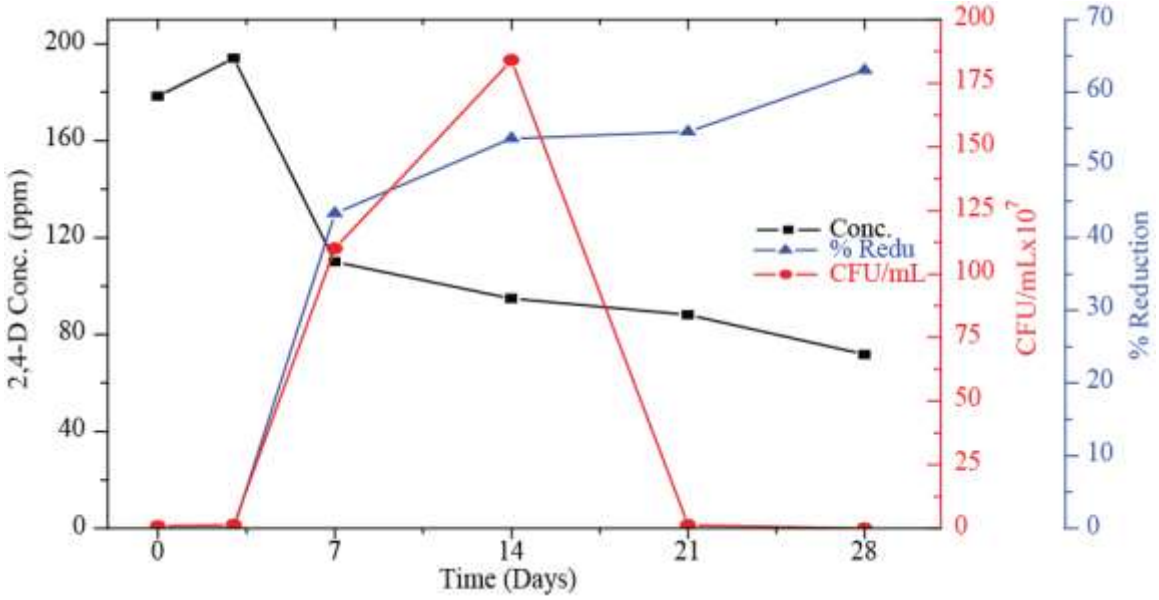

Fig. 2. Bioremediation of 2, 4-D in sterilized soil with glucose by bacterial consortium at 30°C.

Screening of Selected Strains on 2,4-D for MTLs

The selected bacterial, strains (52) were subjected to check the growth on nutrient agar and mineral salt medium with and without glucose containing different concentration of 2,4-D (100-6000 ppm). Different levels of growth were observed with 2,4-D when tolerance was tested on nutrient agar and mineral salt medium. Selected strains showed maximum tolerance on nutrient agar (2000-6000 ppm) as compared to mineral salt medium (1500-5000 ppm) (Table 1). From the results it is clear that the selected strain utilizes 2,4-D as a sole source of carbon and energy.

Bioremediation Experiment

When bioremediation of 2,4-D in sterilized soil with and without glucose and in unsterilized soil samples was checked with bacterial consortium at 30°C, the observations were, in sterilized soil degradation was 95.89%, in sterilized soil with glucose reduction was 63% and in unsterilized soil there was 85.5% reduction of 2,4-D after 28

days. Viable cell counts of samples were increased in CFU/g of soil up to 14 days of incubation, which were 96×10^7/g soil in the case of sterilized soil, 184×10^7/g in the case sterilize soil with glucose and 182×10^7/g soil in unsterilized soil there after decrease in cell number was observed upto 28 days of incubation (Fig. 1, 2, and 3). In the experiment conducted at 50°C, in sterilized soil 70% reduction and 50.84% degradation was observed in unsterilized soil after 28 days of incubation. Viable cell counts in this experiments also showed same pattern as above showing, increase in cell number upto 14 days of incubation, then there was a decrease (Fig. 4, and 5).

Overall degradation of 2,4-D results showed that, their was higher rate of degradation in sterilized soil, 95.89%. Unsterilized soil augmented with selected strains showed less degradation 85.5%, while biostimulated (sterilized soil containing glucose) showed only 63% degradation, which was lower as compared to sterilized soil without glucose. At high temperature (50°C) though there was degradation, but it was lower as compared to low temperature (30°C). Enhanced degradation was observed at 30°C in sterilized soil where it shows upto 85% reduction in 2,4-D just after 7days incubation. The growth was reported progressively increased upto 14 days of incubation and then declined. Unsterlized soil, and soil enriched with glucose showed much higher count than sterilized soil at 30°C. Growth was also lower at 50°C as compare to soil at 30°C (Table 2, and Fig. 1-6).

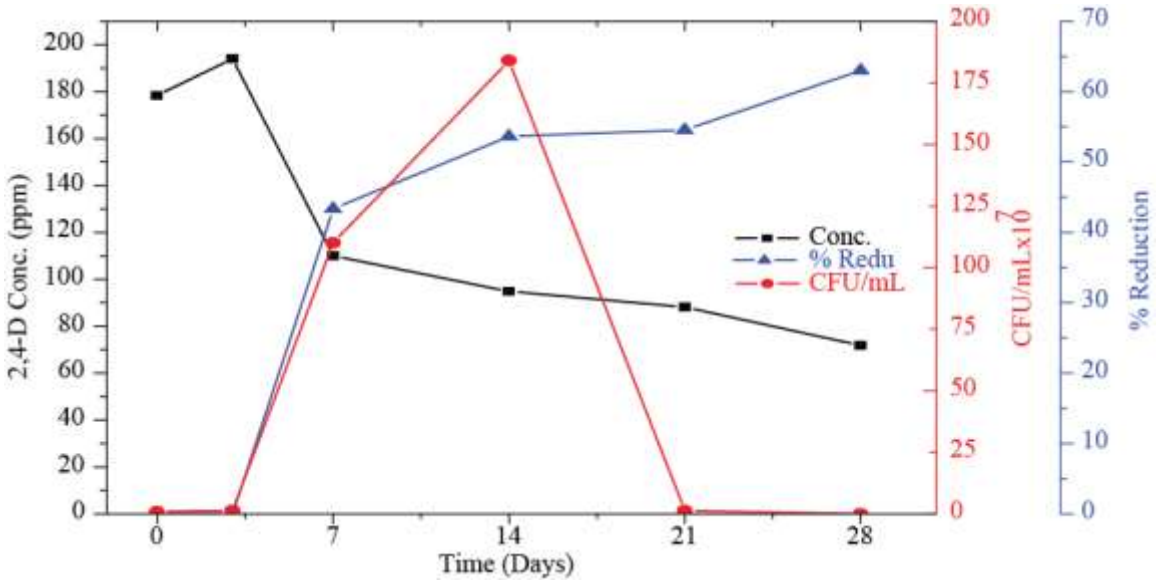

Fig. 3. Bioremediation of 2, 4-D in unsterilized soil bybBacterial consortium at 30°C.

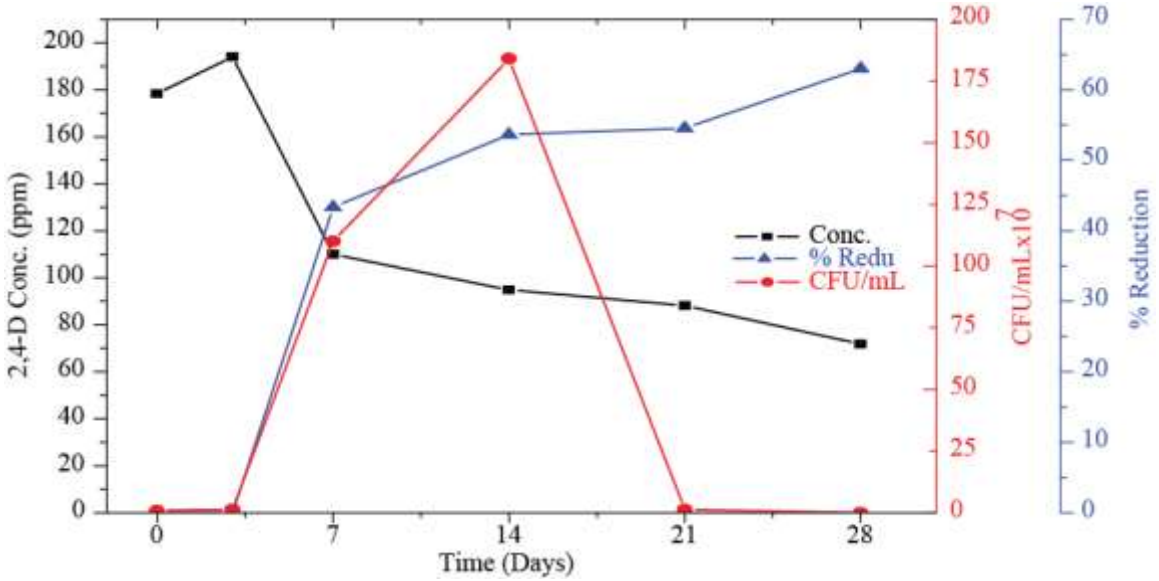

Fig. 4. Bioremediation of 2, 4-D in sterilized soil by bacterial consortium at 50°C.

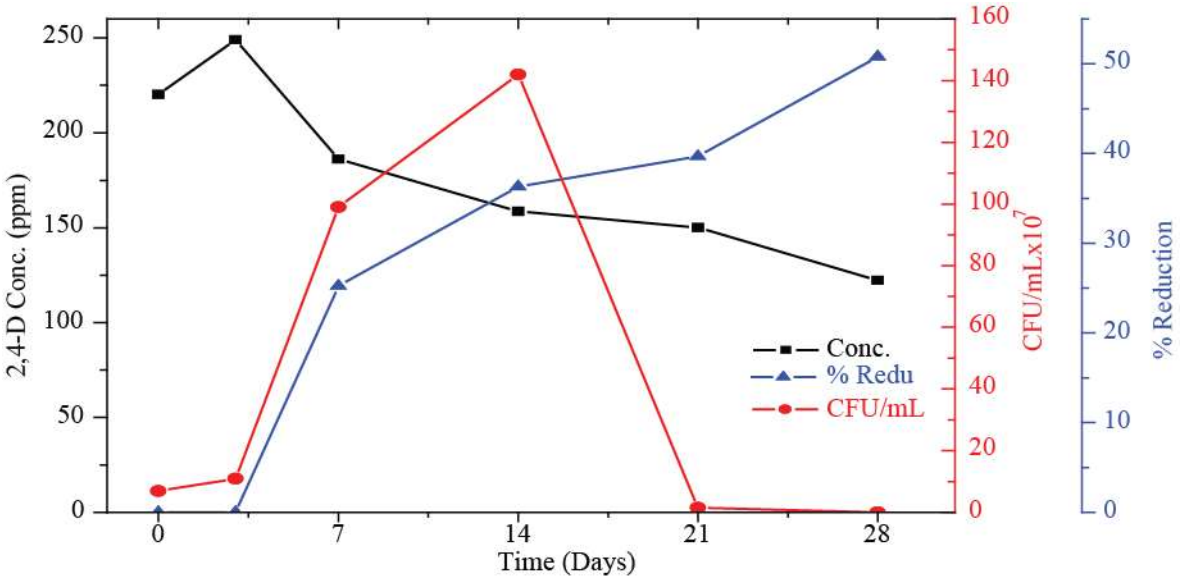

Fig. 5. Bioremediation of 2, 4-D in unsterilized soil by bacterial consortium at 50°C.

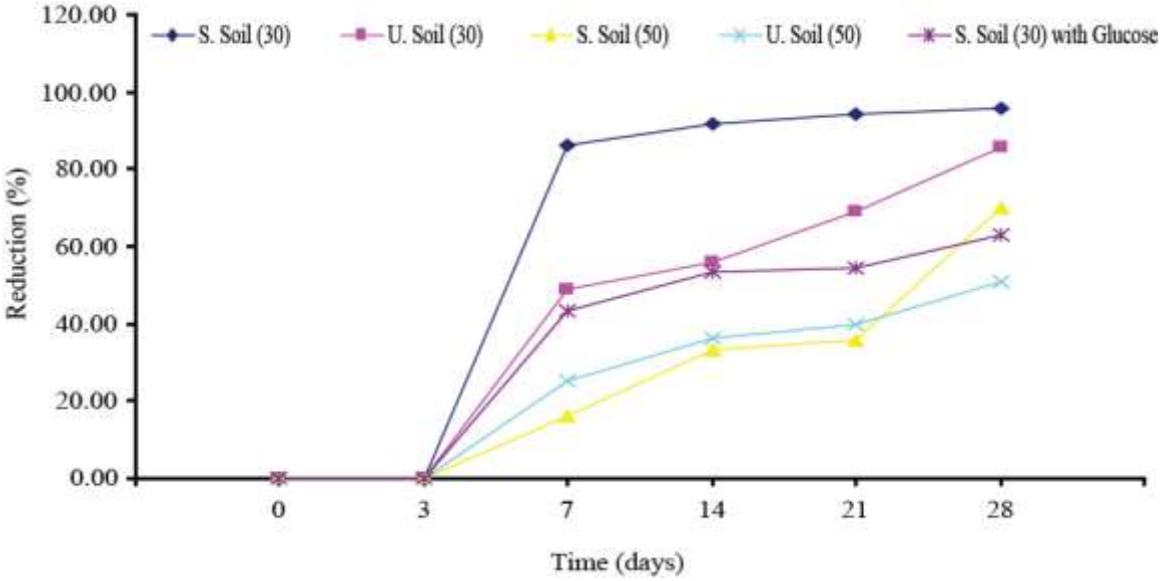

Fig. 6. Bioremediation of 2,4-dichlorophenoxyacetic acids using bacterial consortium, comparison percentage reduction.S-Sterile, U- Unsterile

DISCUSSION

Biological treatment is an important means by which toxic hazardous compounds can be converted into less toxic materials. Composting is a dynamic process in which organic matter is converted to CO_2, water, biomass and humus by microbial action. Large amount of chlorinated organic chemicals have been used in agriculture as pesticides, among these 2,4-D has received widespread use as an herbicide for more than forty years (Ka *et al*., 1994).

In the present study, we have isolated bacteria from agricultural soil exposed to pesticides, and checked their ability to grow at high concentrations of 2,4-D and degrade it efficiently. The population metabolize 2,4-D using as a sole source of carbon, were isolated from pesticides exposed soil. The mineralization of 2,4-D has been shown to occur in agriculture soil in the form of consortium as well as pure culture of bacteria (Meer *et al*., 2004). Pattanasupong *et al*., (2004) reported that a fungicide, carbendazim (methyl-2-benzimidazole carbamate; MBC), and a herbicide, 2,4-D, could be simultaneously degraded by a microbial consortium obtained from several soil samples

in Japanese paddy fields with enrichment continuous culture. Tuxen *et al*., (2005) reported that addition of oxygen enhanced degradation of the six original phenoxy acids and six original chlorophenols. Almost same results were reproduced by Hermosin *et al*., (2006).

Results showed that many bacterial strains are able to grow using 2,4-D as a carbon source. Our observations were analysis of the diversity of predominant 2,4-D degrading bacteria, isolated and characterized them from which majority were Gram positive bacilli belong to genus *Bacillus* while one strain was Gram positive cocci i.e. *Staphylococcus aureus* and seven strains were Grams negative belong to genera *Pseudomonas* and *Acinetobacter*, same results reported by Ka *et al*., (1994). Abundance of 2,4-D degrading microbes were found in agricultural soil which is indicated from colony forming units (CFU) i.e. $4.8x10^6$/g, according to the results of Pallud *et al*. (2004). Isolates have great potential as observed in the results to clean the environment contaminated with chlorinated aromatics because 2,4-D is a model chlorinated compound as stated by Pallud *et al.* (2004).

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