International Journal of Research in Engineering and Science (IJRES)
ISSN (Online): 2320-9364, ISSN (Print): 2320-9356
www.ijres.org Volume 10 Issue 7 ǁ July 2022 ǁ PP. 901-906

Isolation and Optimization of Chlorpyrifos Pesticide Degrading Bacteria from Soil

Dr. R. D. Joshi [1], M.A. Bhate[2], S. S.Kulkarni[2], S.S. Jinturkar[1], V. P. Kulkarni[4]

1. *Asst. Professor, Department of Microbiology, Shri. Shivaji College Parbhani (MS) India.*
2. *Asso. Professor, Department of Microbiology, Shri. Shivaji College Parbhani (MS) India.*
3. *PG student, Department of Microbiology, Shri. Shivaji College Parbhani (MS) India.*

Corresponding Author: Dr. Renuka Joshi- purvarenuka@rediffmail.com

ABSTRACT
Chlorpyrifos (0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate) is an currently used insecticide all over the world. Its excessive use has led to the contamination of various soil and water systems. Microbial bioremediation is considered to be one of the most sustainable alternatives for the removal of CP from the environment. In the present study total five different bacterial strains were isolated from soil contaminated with Chlorpyrifos. Out of five bacterial strains one of the efficient pesticide degrading bacteria was isolated and identified through cultural and biochemical tests as strains of Bacillus sp. Chlorpyrifos was utilized as the sole source of carbon and phosphorus by Bacillus strain. The optimization studies on biodegradation of Chlorpyrifos were also carried out to study optimum conditions for degradations with respect to pH, temperature, incubation period & concentration of Chlorpyrifos.

Keywords: *Chlorpyrifos, bioremediation, pesticide, Bacillus sp*

Date of Submission: 15-07-2022 Date of acceptance: 29-07-2022

I. Introduction:

Chlorpyrifos is a commonly used agricultural insecticide (0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate). Chlorpyrifos is a pesticide that is made up of organo phosphorous compounds. P-O-C connections are present in Chlorpyrifos, as they are in other organo phosphorous insecticides such as parathion and diazinon. It is a broad-spectrum commercial insecticide that is used to manage pests and insects. Because of its extended residual time in soil and water, Chlorpyrifos has serious consequences for public health and the environment (Racke *et al*., 1988; Yang *et al.,* 2005; Mohan et al., 2004).

Some pesticides linger in the soil, forming contaminants that can occasionally contaminate surface and groundwater. Chlorpyrifos, a widely used organophosphate insecticide, is one of these chemicals. Pesticides are designed to be highly toxic, and they have the potential to harm ecosystem health. Several attempts have been made to isolate Chlorpyrifos degrading bacteria from agricultural soil, but not successful (Mallick *et al.,*1999). The effective measure to deal with pollution of Chlorpyrifos or bioremedy of Chlorpyrifos contaminated environment was isolation and screening of microbe strains which was able to degrade Chlorpyrifos with high performance.

Chlorpyrifos is a highly effective insecticide because it inhibits acetyl cholinesterase (AChE) enzymes permanently, causing excess acetylcholine to build up at nerve terminals, causing agitation, hyper salivation, convulsions, and eventually death in insects and mammals (Barathidasan, and Reetha. 2013). Chlorpyrifos is one of around 100 organo phosphorous pesticides now on the market. White flies, plant louses, termites, cockroaches, and ants are among the insects that Chlorpyrifos targets (Fang *et al.,* 2006). In soil, Chlorpyrifos has a half-life of 10 to 120 days, although it can last up to a year depending on abiotic conditions like temperature, moisture, pH, and so on (Singh and Walker., 2006). Chemical hydrolysis was formerly blamed for the rapid rate of Chlorpyrifos breakdown in alkaline pH soils (Aleagha *et al.*, 2011).

In this study, the research aims to isolate the bacteria which had highest ability to degrade the Chlorpyrifos pesticide by taking soil samples that have been contaminated with Chlorpyrifos pesticide. Isolation of organism carried out by enrichment culture technique, and their degrading efficiency was studied by using various concentrations of Chlorpyrifos.

II. Materials and Methods:

Soil Sample Collection :-

Soil sample was collected from Pimplegaon, Dist. Parbhani, and Maharashtra which had an almost 4-5 year history of Chlorpyrifos pesticide use in pest control activities.

Isolation of Chlorpyrifos degrading bacteria :

MSM media (KH_2PO_4 - 4.8 gm, K_2HPO_4 -1.2 gm, NH_4NO_3 - 1.0 gm, $MGSO_4$. $7H_2O$ -0.2 gm, $Ca(NO_3)_2$ $4H_2O$ - 0.04 gm, $Fe(SO_4)_3$- 0.001 gm) was prepared to assess Chlorpyrifos as a sole carbon source for micro-organism. 0.5 gm of soil sample was added into 10 ml saline water. 1 ml of saline water from soil containing saline water was added into MSM media. 0.05 gm pesticide was also added into MSM media aseptically. The flask was incubated in a Rotary Shaker at 250 rpm for 7 days at 30ºC. After 7 days, a loopfull of bacterial suspension from flask was streaked onto Nutrient Agar plates and plates were incubated for 24 hours at 37 ºC. The colonies that grew on Nutrient Agar plates were subcultured onto Nutrient Agar slants until pure cultures were obtained.

Identification of bacterial Isolates: The pesticide degrading bacterial strain was identified on the basis of morphological & biochemical characterization.

III. RESULT AND DISCUSSION

In the present study, a total of 05 bacterial isolates were isolated from collected soil sample S1 able to utilize Chlorpyrifos for its growth was isolated from the pesticide contaminated soil of agricultural field of Pimplegaon, district Parbhani. As Chlorpyrifos is the only carbon source is the medium, it was found that, the isolated bacterial strain is having ability to degrade Chlorpyrifos.

Five samples were processed by the enrichment culture technique for the isolation of bacterial strains. Soil enrichment was carried out in minimal salt medium with the Chlorpyrifos (0. 5%), which are capable of utilizing it as a sole source of carbon and energy. The microbial cultures S1, S2, S3,S4 and S5 showed maximum absorbance of 0.49, 0.32, 0.35, 0.36 and 0.33 respectively, in enrichment culture technique after 7 days of incubation . Among all the isolates, the organism with maximum OD value S1 was processed for identification. Both the organisms were identified by cultural and biochemical tests.

Test	**Result**
Indole	Positive
Catalase	Negative
Amylase Production	Positive
Citrate Utilization	Negative
Gelatin Hydrolysis	Negative
Methyl Red	Negative
Voges-Proskauer	Negative

Bacterial colonies were observed on Mineral Salt Agar medium enriched with Chlorpyrifos pesticide. Biochemical studies were carried out. By studying biochemical tests, the enriched and isolated bacterial species was identified as *Bacillus* species.

Isolate S1 was identified as *Bacillus sp*. The growth response of the isolates in MSM supplemented with Chlorpyrifos (0.5%) showed that the S1 isolate utilized the pesticide as the only carbon source. The OD values were observed at 600 nm at every 48-72 hours.The degradation efficiency of the strain was determined and estimated by the removal percentage of Chlorpyrifos from the liquid culture. Both the isolate showed the degrading capability of Chlorpyrifos in MSM. The isolate, *Bacillus sp.* was more potent in degrading the 80% of the total compound from the media in 2 weeks of incubation.

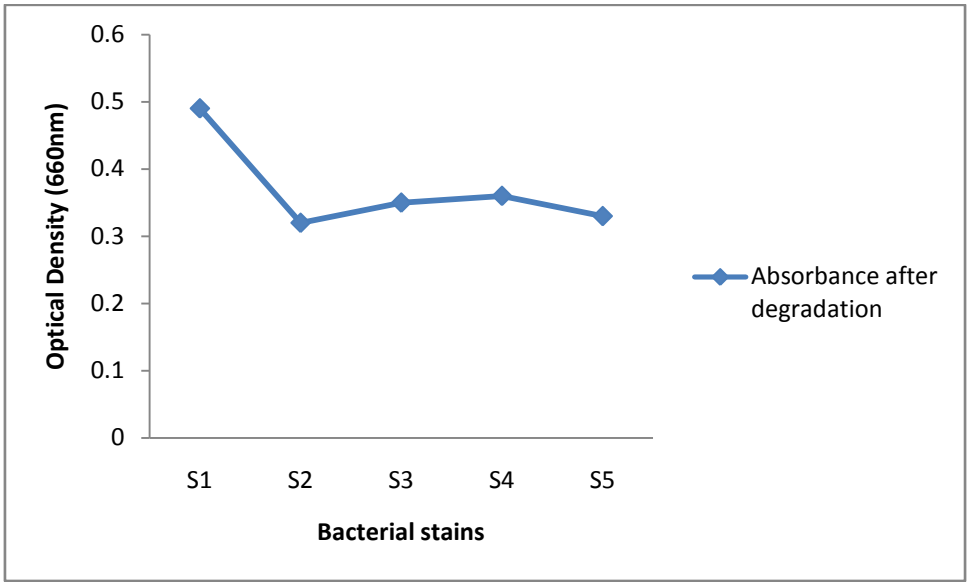

Fig 1: Efficiency of bacterial strains to degrade Chlorpyrifos

The difference is the degradation percentage at the same concentration (0.5% v/v) of Chlorpyrifos is due to the ability of the microbe to utilize available Chlorpyrifos. The substrate availability is a key factor determining the rate of degradation of the pesticide by the bacterial agents. The effect of different physicochemical parameters on pesticide degradation was studied with reference to effect of pH, temperature, incubation time & pesticide degradation.

Effect of pH on Pesticide degradation

The effect of pH showed that pH-6 was optimum pH for pesticide degradation. Along with increase in pH there was decrease in pesticide degradation. As Chlorpyrifos could be hydrolyzed and lost efficacy under alkaline condition, we investigated the effect of pH 5.0–8.0 at a temperature of 30°C, initial bacteria concentration of 0.25%, rotation speed of 150 r/min. Results were shown in Fig. 2. As revealed in Fig. 2, *Bacillus* sp. could degrade Chlorpyrifos at pH of 5–9. The best degradation effectivity was obtained at pH 6.0, under which the maximum growth of *Bacillus* sp was observed, indicating the pesticide degradation. Both the acid and alkaline conditions could restrain the growth of *Bacillus* sp.

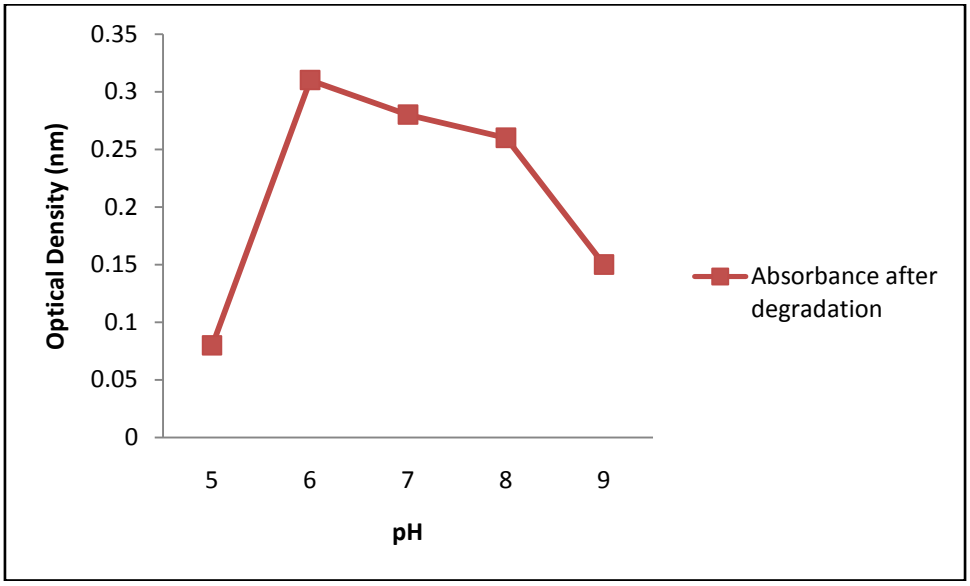

Fig 2: Effect of pH on Chlorpyrifos degradation by *Bacillus* Sp.

Effect of Temperature on Pesticide degradation

The effects of temperature were investigated at a pH of 6.0, rotation speed of 150 r/min. As revealed in Fig. 3, there was obvious effect of temperature on Chlorpyrifos degradation. When the temperature increased from 15°C to 30°C, the degradation rate increased. The highest degradation rate obtained at temperature of 30°C. Actually, it was biochemical reactions by enzymes during the organic biodegradation process. Higher temperature could induce the inactivation of enzymatic; whereas lower temperature could restrain the effectivity of enzymatic. Also, the growth of bacteria was affected by temperature directly. The bacterial grew well at optimal temperature of 30°C, which accordingly increased the activity of Chlorpyrifos degrading enzymes.

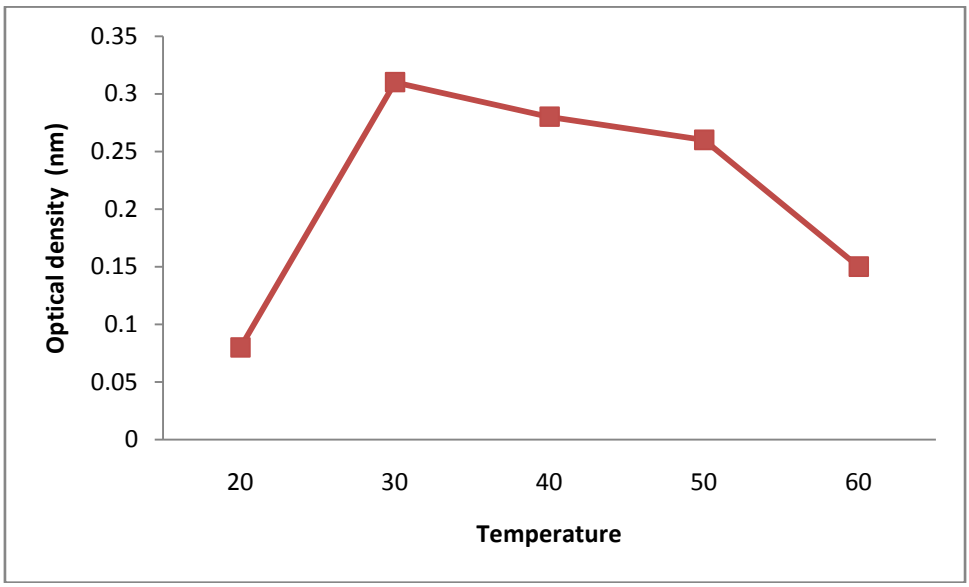

Fig 3: Effect of Temperature on Chlorpyrifos degradation by *Bacillus* Sp.

Effect of Incubation period on Pesticide degradation

The effect of incubation period showed that 48 hours time period was optimum incubation period for pesticide degradation. Along with increase in incubation period there was decrease in pesticide degradation.

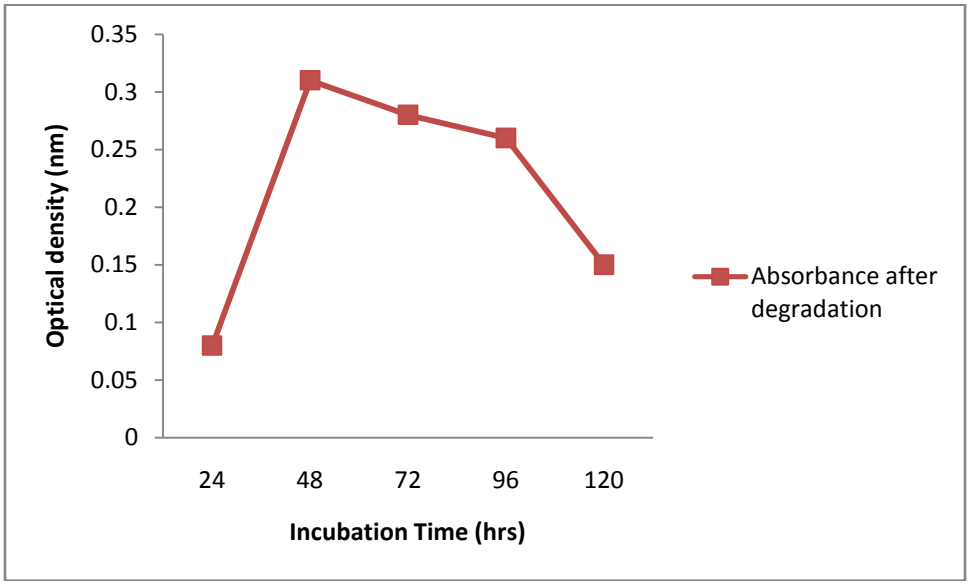

Fig 4: Effect of incubation period on Chlorpyrifos degradation by *Bacillus* Sp.

Effect of pesticide concentration on Pesticide degradation

The effects of pesticide concentration were investigated at a pH of 6.0, rotation speed of 150 r/min at pH-6. As revealed in Fig. 5, there was obvious effect of temperature on Chlorpyrifos degradation. The effect

of pesticide concentration showed that 0.5% concentration was optimum concentration for pesticide degradation. Along with increase in concentration there was decrease in pesticide degradation.

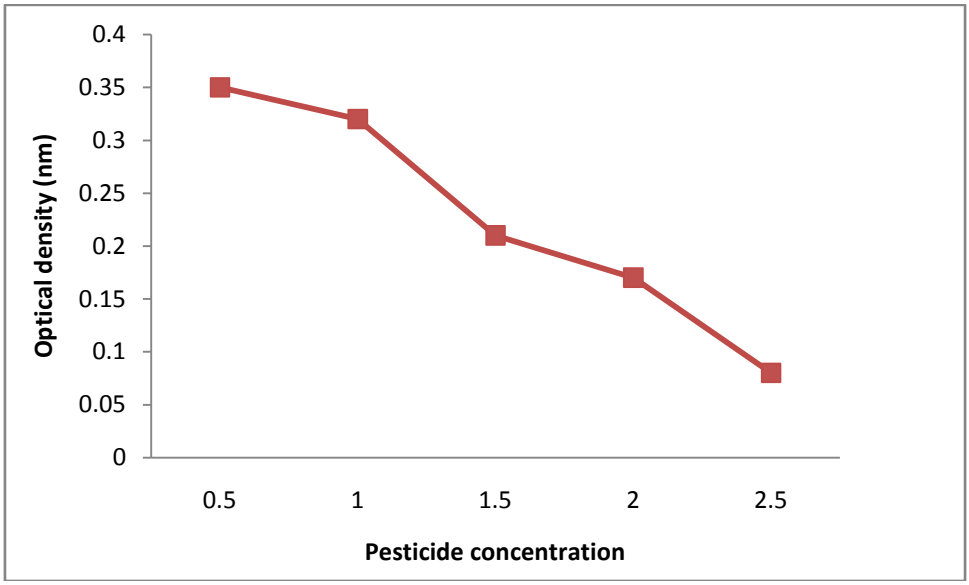

Fig 5: Effect of pesticide concentration on Chlorpyrifos degradation by *Bacillus* Sp.

IV. CONCLUSION

In this present work, Chlorpyrifos degrading bacteria strain were isolated from farm soil that showed degradation of Chlorpyrifos in a laboratory based study. Successful enrichment and isolation of bacterial strain capable to grow on Chlorpyrifos is carried out. As Chlorpyrifos is the only source of carbon in the medium, it is concluded that, the isolated bacterial strain is able to degrade Chlorpyrifos. The results have valuable application for Chlorpyrifos bioremediation in polluted sites.

REFERENCES:

[1]. Aleagha, M.M., Pedram, M., Ebadi, A.G., Afsari, H. (2011). Measurement of chlorpyrifos residues (organophosphate insecticide) in apple fruit in Damavand region. *Ameri. J. of Sci. Res.* 13:79-81

[2]. Anuja, G. (2005). Isolation, Screening and Selection of efficient chlorpyriphos degrading bacteria. M.Sc.(Agri.) *Thesis*, University of Agricultural Sciences, Dharwad.

[3]. Anwar, S., Liaquat, F., Khan, Q. M., Khalid, Z.M., Iqba, S. (2009). Biodegradation of chlorpyriphos and it's hydrolysis product 3,5,6-trichloro-2-pyridinol by Bacillus pumilus strain C2A1. *J. Hazard Mater.* 168:400-405.

[4]. Awad, N.S., Sabit, H. H., Salah, E., Abo-Aba, M. and Bayoumi, R.A. (2011). Isolation, characterization and fingerprinting of some chlorpyriphos-degrading bacterial strains isolated from Egyptian pesticides-polluted soils.

[5]. Bhagobaty RK, Joshi SR, Malik A (2007). Microbial degradation of organophosphorous pesticide: Chlorpyrifos. The internet Journal of Microbiology. 4 :1.

[6]. B. K. Singh, A. Walker, J. A. W. Morgan and D. J. Wright. (2004). Biodegradation of chlorpyrifos by Enterobacter strain B-14 and its use in bioremediation of contaminated soils. *Appli Environ Microbiol, 70, 4855-4863.*

[7]. Chawla, N., Sneja. S., and Kukreja, K. (2013). Isolation and Characterization of chlorpyriphos degrading bacteria. *Indian J. Agric. Res.* 47,381-391.

[8]. D.Gulati and M. M. Nisar. Isolation and characterization of chlorpyrifos utilizing bacteria from sugarcane field soil. *J Pharm Biomed Sci. 5, 765-770.*

[9]. D. S. Bakre, A. A. Sane, G. G. Biradar, and B. B. Kaliwal. (2008). Studies on isolation, characterization and growth of chlorpyrifos degrading bacteria from farm soil. *South-Asian Journal of Multidisciplinary studies. 3, 220-227.*

[10]. Fang, H., Yu, Y.L., Wang, X., Shan, M., Wu, X.M., Yu. J.Q. (2006). Dissipation of chlorpyriphos in pakchoi-vegetated soil in a greenhouse. J. Environ.Sci., 18: 760-764.

[11]. K. Barathidasan, and D. Reetha. (2013). Biodegradation of chlorpyrifos and their intermediate metabolites identified by liquid chromatography mass spectroscopy (LC-MS). *Int J pharm Bio Sci. 4, 1277-1283.*

[12]. K.B. Rokade and G. V. Malli. (2013). Biodegradation of chlorpyrifos by pseudomonas desmolyticum ncim 2112. *Int J Pharm Bio Sci. 4, 609-616.*

[13]. Li, X.; He, J.; and Li, S. (2007). Isolation and Chlorpyrifos Degrading Bacterium, *Shengomonas sp*, strain DSP-2 and Cloning of the MPD Gene. *Research Microbiology*, 158:143-149.

[14]. Mohan, S.V., Sirisha, K. and Rao, N.C. (2004). Degradation of chlorpyriphos contaminated soil by bioslurry reactor operated in sequencing batch mode: bioprocess monitoring. J. Hazard. Mater., 116: 39-48.

[15]. Mallick, B. K., Banerji, A., Shakil, N. A. and Sethunathan, N. N. (1999) Bacterial degradation of chlorpyrifos in pure culture and in soil. Bull. Environ. Contam. Toxicol. 62, 48-55.

[16]. N. Neti and V. Zakkula. (2013). Analysis of chlorpyrifos degradation by kocuria sp. Using GC and FTIR. *Current Biotic 6, 466-472.*

[17]. P. Vijayalakshmi and M.S. Usha. (2012). Optimization of chlorpyrifos degradation by *Pseudomonas putida. J Chem Pharm Res, 4, 2532-2539.*

[18]. Racke, K. D., J. R. Coats, and K. R. Titus. 1988. Degradation of chlorpyrifos and its hydrolysis products, 3,5,6-trichloro-2-pyridinol, in soil. J. Environ. Sci. Health B 23:527-539.
[19]. Rani, S. M.; Lakshim, K. V.; Devi, S. P.; Madlian, F. and Aruna, K. (2008). Isolation and Characterization of Chlorpyrifos Degrading Bacteria from Agricultural Soil and its Growth Response. *African Journal of Microbiology* 2:26-31.
[20]. Singh, B.K.; Walker, A. Microbial Degradation of Organophosphorus Compounds. FEMS Microbiol. Rev. 2006, 30, 428–471.
[21]. Sumit, K. (2004). Bioremediation of Chlorpyrifos by Bacteria Isolated from the Cultivated Soils. *International Journal of Pharms and Biosciences*, 2: 359-366.
[22]. Yang L, Zhao YH, Zhang BX, Yang CH, Zhang X (2005). Isolation and characterization of a chlorpyrifos and 3,5,6-trichloro-2-pyridinol degrading bacterium. FEMS microbiol. Lett., 251:67-73.
[23]. Z. Y. Liu, X. Chen, Y. Shi, and Z. C. Su. (2012). Bacterial degradation of chlorpyrifos by Bacillus cereus. *J Adv Mater Res. 356, 676-680.*