Journal of Agroecology and Natural Resource Management
Print ISSN: 2394-0786, Online ISSN: 2394-0794, Volume 2, Number 2; April-June 2015 pp. 125-129
© Krishi Sanskriti Publications
http://www.krishisanskriti.org/janrm.html

Biodegradation of Pesticide Carbendazim by Microbial Consortium

Rachitha R[1], Dharanendra Chivate[2] and T.H. Udayashankara[3]

[1,2]Under Graduate Student, Department of Environmental Engineering, Sri Jayachamarajendra College of Engineering (SJCE), Mysore-570 006, Karnataka, India
[2]Department of Environmental Engineering, Sri Jayachamarajendra College of Engineering (SJCE), Mysore-570 006, Karnataka, India
E-mail: [1]rachitha09@hotmail.com, [2]dharansjce@gmail.com, [3]hugstumkur@gmail.com

Abstract—*A bacterial consortium capable of degrading the pesticide carbendazim was obtained by the enrichment of soil samples collected from paddy fields at Palahalli, Mysore. The introduction of a microbial consortium has a higher possibility of success in biodegradation than that of a single strain because such a consortium has a higher ability to adapt to these stresses. The microbial consortium obtained from the enrichment process was acclimated in a rotary shaker with 10ppm of Carbendazim. The cells were harvested after acclimation and were used for degradation. By the means of a spectrophotometer, the changes in the bacterial population following the degradation of pesticide were observed. Using Thin Layer Chromatography method, the amount of Carbendazim being degraded by the microbial consortium cells was determined. Microbial consortium capable of degrading simultaneously different types of pesticides is useful for cleaning up pesticide contaminants. This microbial consortium could be useful for bioremediation at sites contaminated with these pesticides.*

Keywords: *Biodegradation, carbendazim, microbial consortium, pesticide.*

1. INTRODUCTION

Rice is the staple food for many people in the world. To increase the yield of rice in paddy fields, farmers continuously and excessively use many types of pesticide to control pests (insects, weeds and diseases) coupled with crop rotation, leading to the simultaneous accumulation of several types of pesticide in paddy fields [1]. Since rice is an irrigated crop, the use of pesticides directly affects the surrounding aquatic environment. Also, during pesticide treatment only 10 % reaches the target crop and the rest reaches the environment [3]. These chemicals can be removed from the environment by physicochemical (e.g.,evaporation, volatilization, hydrolysis, oxidation, photolysis and incineration) and biological processes [7]. And carbendazim (methyl benzimidazol-2-ylcarbamate) is the most widely used benzamidazole fungicide, used for most of the rice crops worldwide [11]. MBC is a systemic fungicide with both curative and protective activities against a wide range of fungal diseases [3]. It is a stable compound a long half-life in the environment (WHO 1993). Hence, it can persist at application sites and easily induce cumulative effects; its residues in fruits, plants and soils could be harmful to human health through food chains [12]. Some studies indicated that carbendazim could do harm to the liver to some extent (WHO 1993) and it has been documented as mutagenic and has teratogenic effects on mammals at single, low level doses [9]. Studies have also reported that carbendazim could be found in paddy field soil and run-off water in the central region of Thailand. Therefore, the degradation of carbendazim has received extensive attention [4]. Carbendazim may be degraded by ways of both photolysis and biodegradation [11].Bioaugmentation is an important process for the removal of these pesticides. Holtmann(1997) reported that carbendazim in soil was decomposed mainly by microorganisms [7]. The isolation and screening of efficient carbendazim degrading microorganisms is a useful approach in bioremediation of carbendazim contamination [12]. The bacteriaintroduced to the contaminated sites fail to degrade the pollutants due to their poor survival or low activity in the environment as they are present as single strains [8]. On the other hand, the introduction of a microbial consortium has a higher possibility of success in biodegradation that that of a single strain because such a consortium has a higher ability to adapt to the stresses like pH, temperature, predation and competition [9]. A microbial consortium contains both the degraders of target compounds and strains that can utilize the metabolic intermediates of the target compounds. Also the individual members of the consortium can work synergistically during degradation thus increasing the efficiency of the degradation process [10].

In this study, a microbial consortium capable of degrading the pesticide carbendazim was obtained by the enrichment of soil sample. The change in the microbial population during the biodegradation process was analyzed by spectrophotometer and the degradation that took place was monitored by thin layer chromatography method.

2. MATERIALS AND METHODS

2.1 Enrichment of microbial cultures capable of degrading Methyl-[benzimidazol-2-yl] carbamate (MBC)

Soil samples were collected from three different fields at a distance of 1-1.5km apart, in Palahalli, Mysore which were treated with carbendazim pesticide for two months. The screening process for the isolation of the bacteria capable of degrading carbendazim pesticide was obtained by incubating conical flasks containing 1g of the soil sample (along with 10 ml nutrient broth and 40 ml distilled water) at room temperature in a rotary shaker for 24hours at 140 rpm. Addition of 10ppm of pesticide carbendazim was added to the conical flasks after 24 hours and the continuous addition of this at an interval of 24 hours was carried out for a period of 10 days. This was centrifuged and the supernatant was obtained and the precipitate was used for further work. The isolation of the bacterial culture was done with the diluted the samples upto 10^8. This was spread on the petriplate containing the nutrient agar and these plates were inoculated at 37^0C for 48 hours and observed for the bacterial growth.

2.2 Preparation of Defined Microbial Consortium and Inoculation

The colonies obtained from the serial dilution plates were purified by subculturing. Purified bacterial isolates were inoculated to sterile nutrient broth under sterile conditions and incubated in a shaker for 48 hours. The cells were then separated by centrifugation at 8000rpm for 15 minutes at 4^0C. The cell mass was resuspended in 5ml minimal media (KH_2PO_4- 0.675 g, K_2HPO_4- 5.455g, NH_4NO_3- 0.25 g per liter).

2.3 Acclimation of Bacterial Isolates to the Pesticide Carbendazim

Optical densities for all the isolates obtained from the previous process were determined using a spectrophotometer at 600nm. All the individual isolates were mixed at equal OD_{600}. The cell suspension was acclimated to carbendazim by adding 10 ppm of the pesticide and the addition was made daily upto 72 hours. Incubation was done in a shaker at ambient temperature at 150 rpm. The cells were harvested by centrifugation at 8000rpm for 15minutes at 4^oC. Cells were resuspended in minimal medium and were used for degradation studies.

2.4. Biodegradation Tests

0.5 ml of the above obtained cells were added to 10 different conical flasks containing 20 ml of the sterile minimal medium each. Different concentrations of pesticide, 2ppm, 5ppm, 10ppm, 15ppm and 20 ppm were added to the conical flasks with minimal medium and consortium. These flasks were subjected to shaking for 24 hours at 150rpm daily for 10 days. 1ml of the suspension from each conical flask was drawn daily at 24 hours interval and the optical density was measured at 600nm.

2.5. Extraction of Carbendazim

The remaining quantity of the sample, after determining the optical density, were dissolved to approximately double the quantity by using chloroform and was shaken well. The chloroform with the residual carbendazim pesticide was left open to atmosphere until complete evaporation took place. The evaporated samples containing the residual pesticide traces were used for residue analysis.

2.6 Thin Layer Chromatography

Chromatography is used to separate mixtures of substances into their components. It has a stationary phase (a solid or a liquid supported on a solid) and a mobile phase (a liquid or a gas). The mobile phase flows through the stationary phase and carries the components of the mixture with it. Different components travel at different rates. In TLC the silica gel (or the alumina) is the stationary phase. The stationary phase for thin layer chromatography also often contains a substance which fluoresces in UV light. The mobile phase is a suitable liquid solvent or mixture of solvents. Saturating the atmosphere in the TLC tank with the vapors of the mobile phase allows for the proper movement of the solvents.

To measure the different concentrations of MBC that has been degraded by the microbial consortium, thin layer chromatography method was carried out by immersing the TLC plates loaded with extracts from each day's sample. Also, 5µl of the standard pesticide solution of concentration 1ppm was loaded onto the TLC plates for comparison purpose. This was run in the TLC tank containing mobile phase (hexane: tetrahydrofuran: ethyl acetate: chloroform in the ratio 28: 6: 1/4: 1/4). Later, the TLC plates were observed under the UV light at 305 nm. The pesticide degradation ability was then analyzed by measuring the area under the spot and analyzing calibration curve prepared for standard carbendazim under similar conditions.

3. RESULTS AND DISCUSSIONS

Carbendazim, a representative benzimidazolic compound, is a systemic and broad-spectrum fungicide and widely used for the control of plant fungal diseases on paddy rice, cotton, vegetables and fruits which results in considerable pollution to the water and soil and threatens to ecosystem and human health. Microbial bioremediation of contaminated environment has gained much attention. Pesticide accumulation is a serious problem affecting the environment. The biodegradation of pesticides, such as 4-nitrophenol [9], 1, 3-dichloropropene [13], dichlorodiphenyltrichloroethane[15] and endosulfan[3] by a microbial consortium has been reported. Furthermore, the biodegradation of herbicide mixtures such as those of 2, 4-dichlorophenoxyacetic acid (2,4-D)and 2-methyl-4-chlorophenoxyacetic acid [10], and (RS)-2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop) and (RS)-2-(2,4-dichlorophenoxy)propionic acid (dichlorprop) [8] has also been studied. A microbial consortium capable of

simultaneously degrading different types of pesticide is useful for cleaning up pesticide contaminants.

In this study, the fungicide, carbendazim (methyl-2-benzimidazole carbamate, MBC), was selected as target pesticide. It is used extensively throughout the world to control pests in paddy fields, and their detectable residues in paddy fields have been reported [11].Due to the intensive application of carbendazim and its adverse effects, there is a need to find a way to enhance their degradation on soil.

Choice of the bacteria / consortium is an important factor in any pesticide degradation. In our study we isolated a bacterial consortium from contaminated soil by enrichment technique. The consortium thus obtained was able to degrade MBC very efficiently .The presence of these bacteria in the initial microbial consortium suggests that they might contribute to the complete degradation of the pesticide. After this consortium was acclimated and MBC began to be degraded, the cell numbers of this consortium increased to a detectable value during incubation. On the other hand, there is another possible explanation for this phenomenon. Malghani et al. (2009) suggested that a lag time is observed when cells are transferred from a rich culture medium to a poor one [10]. When the substrate in the medium is changed, some enzymes are induced to metabolize the new substrate, which was not present in the previous medium. It was also reported that the addition of readily utilizable carbon sources, such as glucose or amino acids, inhibits, decreases or has no significant influence on the mineralization of xenobiotic substrates [2] [3] [14]. In our studies also, acclimation was found to be necessary.

In an attempt to degrade this systemic and broad spectrum pesticide, microorganisms were isolated from soil with a history of carbendazim spray. A mixed microbial population was obtained by acclimation of contaminated soil. During acclimation, the pesticide, carbendazim was added daily for 10 days. The microorganisms capable of breaking carbendazim or utilizing carbendazim as a sole source of carbon survived i.e. the microorganisms having the machinery to mineralize carbendazim survived. These isolates were isolated on nutrient agar and resolved in to individual isolates. These isolates were maintained on nutrient agar at 4°C. The bacterial strains isolated were screened for the degradation of carbendazim. The fungicide, carbendazim was added at 10 ppm level. It was observed that the consortium could utilize carbendazim. All the individual isolates resolved were grown in nutrient broth and harvested. These individual isolates were mixed at equal $O.D_{600}$ to obtain a defined microbial consortium. The defined microbial consortium obtained above was induced with carbendazim for 72h with daily addition of carbendazim. This step was necessary to induce the enzyme systems involved in the degradation of carbendazim. Only induced consortium was used in all experiments and induction was done before experimental set up. viz.,. the induced cells were not stored for long time at refrigerated conditions.

4. DEGRADATION OF DIFFERENT CONCENTRATIONS OF CARBENDAZIM BY THE DEFINED MICROBIAL CONSORTIUM

4.1. Degradation of Lower Concentrations of Carbendazim

Lower concentrations of carbendazim 2ppm and 5 ppm were degraded very fast. Residue was not observed at 24h of incubation.

4.2. Degradation of 10 ppm of Carbendazim

At 10 ppm level of carbendazim, degradation started early. There was no initial lag. There was 20 % degradation by 24h. Degradation progressed with time. Another 20% of the substrate disappeared by the end of another 24h (Fig. 1). By 240h, only 10 % of the residue was remaining. Good growth was observed which reached maximum by 72h of incubation (Fig. 2). Growth decreased with decrease in substrate level.

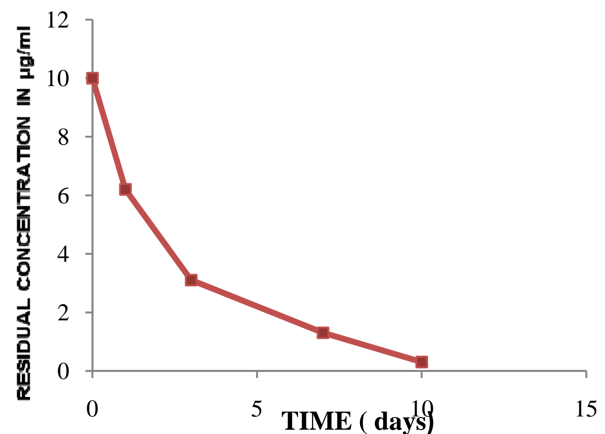

Fig. 1: Degradation of 10ppm Carbendazim

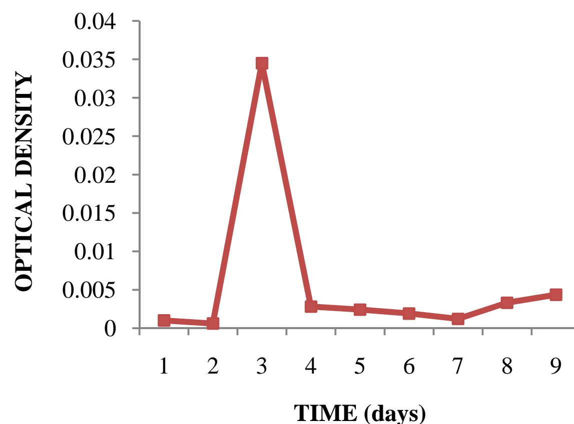

Fig. 2: Growth of microbial consortium during degradation of 10 ppm of carbendazim.

4.3. Degradation of 15 ppm of Carbendazim.

Degradation of 15 ppm of carbendazim also followed the same pattern. Degradation started without any lag. By the end of 24h, Only 8 ppm of carbendazim was remaining indicating that 7 ppm of carbendazim was degraded by the end of this period. The degradation improved with incubation time (Fig. 3). By the end of 7 days of incubation, only 0.5 ppm was remaining indicating almost complete degradation of carbendazim. Growth of the consortium was good (Fig. 4) indicating the capability of the consortium in using carbendazim as sole source of carbon and energy.

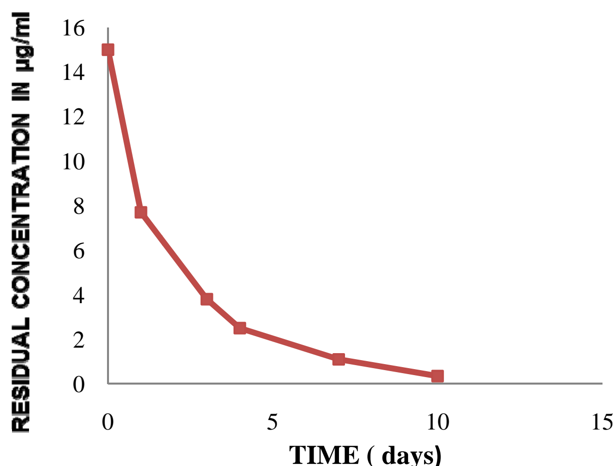

Fig. 3: Degradation of 15ppm Carbendazim.

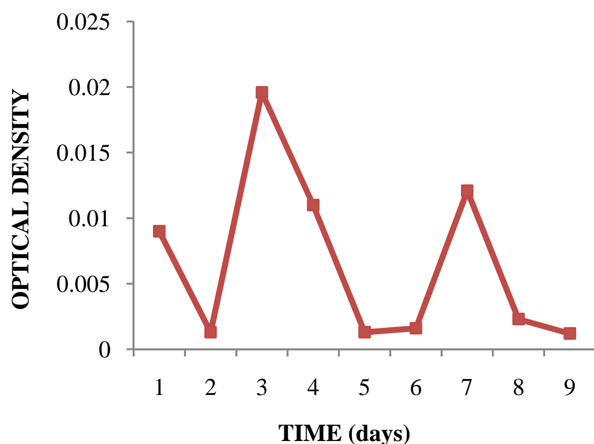

Fig. 4: Growth of the microbial consortium during degradation of 15 ppm of carbendazim.

4.4. Degradation of 20 ppm of Carbendazim

Degradation of 20 ppm of carbendazim showed a slightly different pattern. Although there was no lag in the degradation of 20 ppm of carbendazim but complete degradation took slightly longer time. Degradation of 20 ppm took 8 days. Even by the end of 8 days, 1 ppm was still remaining (Fig. 5). Growth was good (Fig. 6) and more than 15 ppm.

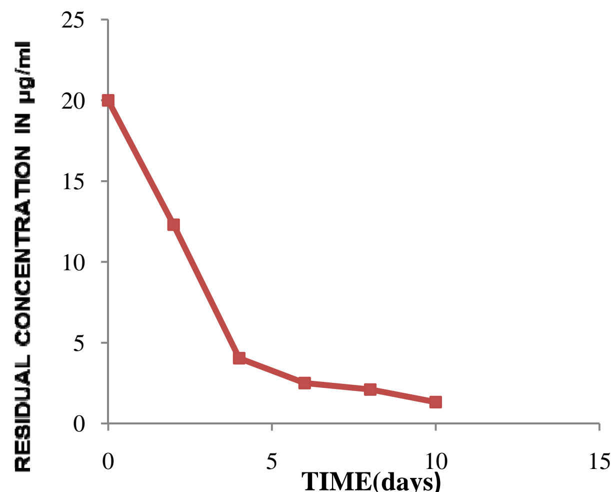

Fig. 5: Degradation of 20 ppm carbendazim

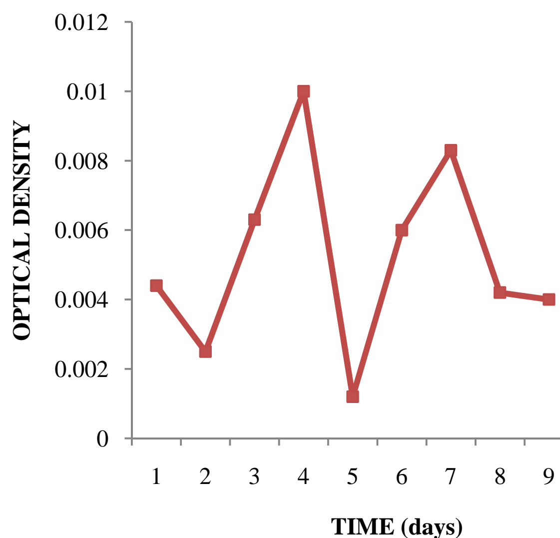

Fig. 6: Growth of the microbial consortium during degradation of 20 ppm of carbendazim.

The consortium was able to degrade up to 20 ppm of MBC completely. Lower concentrations were degraded with in 24h incubation indicating the efficiency of the isolated consortium. Thus, a bacterial consortium isolated from paddy soil samples that had the ability to degrade MBC A rapid degradation of MBC was observed at lower concentrations. Several reports have also demonstrated the MBC degradation ability of bacteria and fungi at high levels [16].In this study, our microbial consortium had the ability to degrade these pesticides at concentrations reaching the solubility limit.

We observed that the microbial consortium could degrade approximately 99% of 10ppm concentration of carbendazim and 95% degradation of 20ppm pesticide concentration

The high potential of this consortium to degrade carbendazim was clarified from this study being conducted. The effect of supporting materials on MBC degradation can also be further investigated to increase the efficiency of degradation. To develop the pesticide degradation system using microbial consortium, the effect of environmental parameters, such as temperature, pH, and nutrients concentration, on degradation ability, stability of the degradation ability during long term preservation needs to be investigated. The use of microbial consortium can therefore serve as a possible bioremediation technique for soils contaminated by carbendazim.

5. ACKNOWLEDGEMENT

We are grateful to Dr. T.H Udayashankara, Professor, SJCE, Mysore, Karnataka, for his immense support and guidance in the course of fulfillment of our project.

We are grateful to Dr. H.K Manonmani of Microbiology Department at Central Food, Technology and Research Institute, Karnataka, India, for providing us with her valuable guidance and immense support throughout the course of our project

REFERENCES

[1] Ahmed S, Ismail A, Houssien A., 2010, Dissipation and persistence of Fungicides, Carbenazim and Metalaxyl in Egyptian Soil under Biotic and Abiotic Conditions., *Journal of Applied Sciences Research,* 6(8), 1240-1246

[2] Alexander M. 1981. Biodegradation of chemicals of environmental concern. *Science* 211, 132–138.

[3] Awasthi, N., N. Manickam, and A. Kumar, 1997. Biodegradation of endosulfan by a bacterial co-culture. *Bull. Environ. Contam. Toxicol*. 59:928-934.

[4] Bidlan, R. and Manonmani, H. K. 2002. Aerobic degradation of dichlorodiphenyl-trichloroethane (DDT) by Serratiamarcescens DT-1P. *Process Biochem*. 38, 49-56.

[5] Burrows L A, Edwards C., 2004, The Use of Integrated Microcosms to Assess the Impact of Carbendazim on Soil Ecosystems, *Ecotoxicology,*13, 143-161

[6] Fang H.,WangY.,Gao C., Hu Yan.,Dong B., Yu Y, 2010, Isolation and characterization of Pseudomonas sp. CBW capable of degrading carbendazim , *Biodegradation.*.

[7] Holtman. M .A, D. Y. Kobayashi.,1997, Identification of Rhodococcuserythropolis isolates capable of degrading the fungicide carbendazim, *Application of microbiology and biotechnology*.

[8] Katayama, A. and F. Matsumura, 2004. Degradation of organochlorine pesticides, particularly endosulfan by Trichodermaharzianum. *Environ. Toxicol. Chem*. 12:1059-1065.

[9] Laha, S. and Petrova, K. P. (1998). Biodegradation of 4-nitrophenol by indigenous microbial populations in Everglades soils. *Biodegradation*, 8, 349-356.

[10] Malghani S, Chatterjee N, Yu HX, Luo Z, 2009, Isolation and identification of profenofos degrading bacteria. *Brazilian Journal of Microbiology*;40: 893-900.

[11] Nagase H.,PattanasupongA,Sugimoto E.,Tani K.,Nasub M., Hirata K.,Miyamoto K.,2006, Effect of environmental factors on performance of immobilized consortium system for degradation of carbendazim and 2,4-dichlorophenoxyacetic acid in continuous culture*"., Biochemical Engineering Journal*.

[12] Pattanasupong A, Nagase H ,Inoue M, Hirata K, Katsuji,Nasu M and Miyamoto K, 2004, Ability of a microbial consortium to remove pesticide, carbendazim and 2,4-dichlorophenoxyacetic acid, *World Journal of Microbiology & Biotechnology* 20, 517-522.

[13] PattanasupongA,Nagase H, Inoue M, Hirata K,Tani K, Nasu M and Miyamoto K, 2005, Degradation of carbedazim and 2,4 Dichlorophenoxyacetic acid by immobilized consortium on a loofa sponge, *Journal of Bioscience and Bioengineering* 98, 28-33.

[14] Xiuguo W, Min S, Chunming G, Bin D, Qun Z, Hua F, Yunlong Y, 2009, Carbendazim induces a temporary change in soil bacterial community structure, *Journal of Environmental Sciences*21,1679-1683.

[15] Xiuguo W, Min S, Yiqi W, Chunming G ,Xiaoqiang C, Hua F, YunlongY,Zhang Q.,2012, Response of soil bacterial community to repeated applications of Carbendazim., *Ecotoxicology and Environmental Safety* ,75., 33–39.

[16] Yarden O, Salomon R, Katan J, Aharonson N, 1993, Involvement of fungi and bacteria in enhanced and nonenhanced biodegradation of carbendazim and other benzimidazole compounds in soil, *Can. J. Microbiol*36, 15-23.

[17] Zhang G,JiaX,Cheng T, Ma X and Zhao Yh,2005, Isolation and Characterization of a new carbendazim-degrading Ralstoniasp.strain, *World Journal of Microbiology & Biotechnology* 21,265-269.