World Academy of Science, Engineering and Technology
International Journal of Bioengineering and Life Sciences
Vol:10, No:11, 2016

Isolation of a Bacterial Community with High Removal Efficiencies of the Insecticide Bendiocarb

Eusebio A. Jiménez-Arévalo, Deifilia Ahuatzi-Chacón, Juvencio Galíndez-Mayer, Cleotilde Juárez-Ramírez, Nora Ruiz-Ordaz

Abstract**—**Bendiocarb is a known toxic xenobiotic that presents acute and chronic risks for freshwater invertebrates and estuarine and marine biota; thus, the treatment of water contaminated with the insecticide is of concern. In this paper, a bacterial community with the capacity to grow in bendiocarb as its sole carbon and nitrogen source was isolated by enrichment techniques in batch culture, from samples of a composting plant located in the northeast of Mexico City. Eight cultivable bacteria were isolated from the microbial community, by PCR amplification of 16 rDNA; *Pseudoxanthomonas spadix* (NC_016147.2, 98%), *Ochrobacterium anthropi* (NC_009668.1, 97%), *Staphylococcus capitis* (NZ_CP007601.1, 99%), *Bosea thiooxidans*. (NZ_LMAR01000067.1, 99%), *Pseudomonas denitrificans*. (NC_020829.1, 99%), *Agromyces* sp. (NZ_LMKQ01000001.1, 98%), *Bacillus thuringiensis*. (NC_022873.1, 97%), *Pseudomonas alkylphenolia* (NZ_CP009048.1, 98%). NCBI accession numbers and percentage of similarity are indicated in parentheses. These bacteria were regarded as the isolated species for having the best similarity matches. The ability to degrade bendiocarb by the immobilized bacterial community in a packed bed biofilm reactor, using as support volcanic stone fragments (tezontle), was evaluated. The reactor system was operated in batch using mineral salts medium and 30 mg/L of bendiocarb as carbon and nitrogen source. With this system, an overall removal efficiency (η_{bend}) rounding 90%, was reached.

Keywords**—**Bendiocarb, biodegradation, biofilm reactor, carbamate insecticide.

I. Introduction

PESTICIDES are recalcitrant pollutants that resist different levels of degradation either chemical, biochemical or photochemical, because of their chemical characteristics. Therefore, their half-life in the environment can be high, creating problems in the ecosystems in which they are applied. An example is the bendiocarb (2, 2-dimethyl-1,3-benzodioxol-4-yl methylcarbamate), whose empirical formula is $C_{11}H_{13}NO_4$ [1]; like other carbamates, this insecticide inhibits the enzyme acetylcholinesterase, necessary for normal transmission of nerve impulses [2].

Bendiocarb is available in different commercial forms: granules, wettable powders, powders, pellets, pressurized liquids and pet collars. The most frequent mode of application is by spraying [3]. In agriculture, it is used for seed treatment and granular formulations for controlling soil pests and foliage, mainly maize and sugar beet [4]. Bendiocarb is also used to eliminate domestic and industrial pests, such as mosquitoes, flies, red ants, spiders and cockroaches among others. The domestic and industrial use of insecticides, including bendiocarb has been increased in tropical countries because of the dissemination of diseases whose transmission vectors are insects (dengue, chikungunya, and zika) [5]-[7].

Studies about resistance at insecticides showed that carbamates (bendiocarb and propoxur) are the most effective group of insecticides against *Aedes aegypti* and *Aedes albopictus*, which are known vectors of the dengue, chikungunya, and zika diseases, with shorter times of mortality and lower resistance [8].

As for toxicity, toxicological studies of bendiocarb have increased in recent years since the World Health Organization recommended its use within a group of 12 insecticides for malaria control and against a variety of insects. Cases of intoxication in humans have occurred mainly during application of the insecticide, causing weakness, blurred vision, headache, nausea, abdominal cramps, chest discomfort, miosis, sweating, muscle tremors and incoordination, decreased pulse, low blood pressure, heart irregularities, giddiness, confusion, slurred speech, and loss of reflexes. One of the toxicological studies suggests that the bendiocarb treatment causes an increase in cell death, liver perisinuosoidal fibrosis, and steatosis in populations of nontargeted organisms, especially when it has been applied for prolonged periods [9].

Due to its toxicity, bendiocarb is not authorized for use in the European Union, according to the Directive 91/414/EEC and Regulation (EC) No 1107/2009. In the United States, the company that produced bendiocarb voluntarily canceled the registration of all bendiocarb-based products after that the preliminary risk assessment reported by the US Environmental Protection Agency (EPA) showed the risks when mixing or applying the pesticide. In other countries, bendiocarb is registered as a biocide for indoor use, which means that it can be applied by any person, and its use is allowed, mainly of Africa, Asia, and Latin America [10]. Therefore, it is very important to find efficient technologies to eliminate pollution by insecticides, and bioprocesses using microbial consortia could be suitable alternatives.

In this work, it was possible to isolate a bacterial community capable of efficiently removing, one of the most worldwide used pesticides, the bendiocarb insecticide.

Eusebio A. Jiménez-Arévalo, Deifilia Ahuatzi-Chacón*, Juvencio Galíndez-Mayer, Cleotilde Juárez-Ramírez and Nora Ruiz-Ordaz are with the Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Wilfrido Massieu esq. cda Miguel Stampa s/n, CP 07738 México (*corresponding author (phone: 52-55-7296000 ext. 57844; e-mail: dahuatzi@hotmail.com).

II. Materials and Methods

A. Chemicals

All components used in culture media were obtained from Merck (Darmstadt, Germany). Bendiocarb standard was acquired from Chem Service Inc, Pennsylvania. The solvents used for HPLC were purchased from J.T. Baker. For biodegradation experiments, a commercial formulation of the insecticide Ficam W, Bayer, México, was used.

B. Culture Media

Mineral salts medium (MSM) was used for all biodegradation experiments. The medium contains, in g L^{-1}, KH_2PO_4, 0.20; K_2HPO_4, 0.40; $MgSO_4$, 0.20 and $CaCl_2$, 0.02. It was complemented with trace elements, obtaining a final concentration (in mg L^{-1}) of $FeSO_4.7H_2O$, 0.55; $MnSO_4.7H_2O$, 0.34; $ZnSO_4.7H_2O$, 0.23; $CoCl_2.6H_2O$, 0.065; $Na_2MoO_4.2H_2O$, 0.034. The insecticide Ficam W was added to MS medium to reach bendiocarb concentration of 30 mg L^{-1} (MS-Ficam W medium).

For isolation and examination of the colonial morphology of the isolated bacteria constituting the microbial community, Standard Plate Count Agar medium was used. For DNA extraction of the isolated bacteria, they were propagated in Luria-Bertani medium (1.0% Tryptone, 0.5% Yeast Extract, 1.0% NaCl; pH 7.0).

To conserve the microbial community and the bacterial isolates, they were cultivated on MSM medium complemented with bendiocarb (30 mg L^{-1}) and harvested by centrifugation at 13,000 rpm for 5 min. The culture medium was decanted and the pellet obtained was suspended on 200 µL of glycerol and cryopreserved at -70°C in a Revco ultra-low freezer.

C. Identification of the Bacterial Isolates

DNA was extracted from pure bacterial strains which showed morphological differences when growing on Standard Plate Count Agar. By PCR amplification (GeneAmp PCR System 2400, Applied Biosystems, USA), using fd1 [11] and 1492r [12] primers, 16S rDNA fragments of about 1500 bp were obtained. They were sequenced by Macrogen Inc, Korea.

For bacterial strain identification, amplicons sequences were compared with 16S rDNA sequences stored in the NCBI GenBank.

Reported species showing the highest similarity were regarded as the isolated strains.

D. Packed Bed Support

Fragments of volcanic stone (named tezontle in México) were used as the porous support in the packed bed reactor. This vesicular stone has the appropriate and desirable characteristics for a material to be used as a support. Due to its porous structure is a low-density material. However, this rock shows great mechanical strength. Porous fragments were considered ellipsoidal bodies with three characteristic radii: a, b, c. The calculated particle volume was used to determine the equivalent diameter of the volcanic stone fragments [13]. The average value was 6.18 ± 1.57 mm.

E. Packed Bed Reactor (PBR)

A column with a porous glass diffuser (pore diameter of 40-100 µm) with glass cover sealed and a neoprene gasket for secure operation was used as PBR. The total operating capacity of the reactor was 500 cm^3.

The column has ports for sampling, input of liquid medium, and output of exhausted air and liquid medium. Air and liquid medium were concurrently supplied at the base of the packed column. Fig. 1 shows a diagram of the PBR.

The use of packed-bed reactors in wastewater treatment is very common and convenient because the attached cell mass (biofilm) resists elevated concentrations of toxic compounds [14].

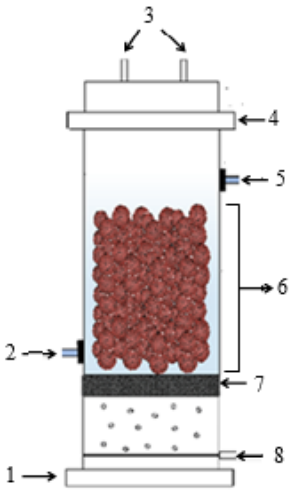

Fig. 1 PBR: Base (1), inflowing liquid (2), air vent (3), glass cover (4), outflowing liquid (5), packed-bed support material (6), sintered glass plate for air dispersion (7), air input (8)

An abiotic saturation of the packed support with bendiocarb was carried out before inoculating the selected microbial community into the PBR. In this process, the reactor was continuously fed with MSM medium containing bendiocarb, until the concentrations of the insecticide (spectrophotometrically measured) at the inlet and the outlet of the reactor remained equal.

F. Determination of Bendiocarb by Spectrophotometry

For a rapid evaluation of bendiocarb removal, the insecticide concentration was spectrophotometrically estimated by measuring the absorbance of properly diluted samples, at ʎ=230 nm (Beckman DU650 spectrophotometer).

G. Determination of Bendiocarb by HPLC

For bendiocarb determination by HPLC, the following procedure was used. From centrifuged samples, bendiocarb concentration was determined using a Shimadzu SPD-10A HPLC System equipped with a Zorbax SB Phenyl C18 Agilent Technologies column and a UV detector (ʎ=226 nm). The flow rate of the mobile phase was one mL min^{-1}. The mobile phase consisted of acetonitrile/water (60-40%) [15].

H.Determination of Chemical Oxygen Demand (COD)

Samples were adequately diluted and analyzed using Hach Method 8000 [16].

I. Culture Enrichment by Successive Transferences

For enrichment culture, Erlenmeyer flasks of 500 mL were used, with 100 ml of MSM supplemented with Ficam W equivalent to 30 mg bendiocarb L^{-1}, 10 g agricultural soil and fragments of volcanic rocks. The flasks were incubated under stirring at room temperature and two weeks later was drained 90% of the medium and it was substituted with new medium.

3 mL of medium, that was discarded, was appropriately diluted to determine the concentration of the insecticide by UV spectrophotometry and HPLC. Rock fragments were maintained in the flask, repeating the procedure every two weeks until the selected microbial community was obtained.

III. Results and Discussion

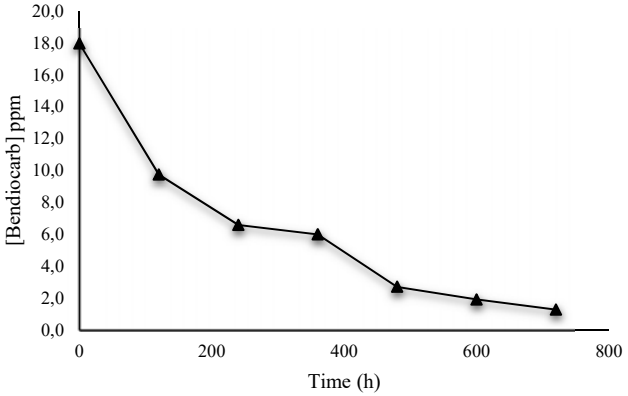

Fig. 2 Bendiocarb biodegradation by the microbial community isolated from soil samples of a composting plant, after a culture enrichment period

Soil samples from a composting plant Mexico City located in the northeast of Mexico City were used. After a period of 720 h of successive transfers in batch cultures, the community obtained, could efficiently remove the insecticide. The results are shown in Fig. 2.

Once the abiotic process was finished in the PBR, the selected community was inoculated in the reactor and cultivated in batch during 168 h, then the repeated batch culture stage was started to allowing colonization and selection of the best microbial community for remove the insecticide. The duration of each batch run depended on the remainder concentration of the insecticide. The samples were taken every 24 h. When bendiocarb concentration, measured by spectrophotometry, was lower and remained unchanged, a replacement of the medium exhausted was made with fresh medium.

The removal efficiencies and the volumetric removal rates of the bendiocarb were gradually improved after each batch run. Five repeated batches were made; in the last run, a bendiocarb removal efficiency of 90%, measured by HPLC, was obtained. The volumetric removal rate was 12.6 mg L^{-1} d^{-1}.

The COD removal efficiency reached values higher than 70%. About 30% of the remaining COD could be attributed to residual bendiocarb residues and also to degradation products.

The system's ability to remove high insecticide concentrations was evaluated by testing 60 and 120 mg L^{-1} of bendiocarb. The results, after five repeated batches, are shown in Table I. It can be observed that although the removal efficiency of bendiocarb was maintained, the COD removal efficiency increased from 71.4% to 85%. Likewise, higher volumetric removal rates of the insecticide and COD were obtained, pointing out that the microbial community was enriched with the most suitable bacteria for the degradation of bendiocarb.

TABLE I
VOLUMETRIC REMOVAL RATES AND REMOVAL EFFICIENCIES IN BENDIOCARB BIODEGRADATION

Bendiocarb initial concentration (mg L^{-1})	η_B (%)	η_{COD} (%)	$R_{V,B}$ (mg L^{-1} d^{-1})	$R_{V,COD}$ (mg L^{-1} d^{-1})
30	90	71	12.6	3.12
60	90	84	17.3	11.6
120	90	85	29.5	21.6

Bendiocarb (η_B), COD (η_{COD}) removal efficiencies and Bendiocarb ($R_{V,B}$), COD ($R_{V,COD}$) volumetric removal rates in the bioreactor operating in repeated batch culture.

Eight predominant cultivable bacterial strains were identified, the results are shown in the Table II.

Biodegradation studies on the insecticide bendiocarb are scarce. It has been reported that *Arthrobacter* sp. can degrade carbamates, including carbofuran, carbaryl and bendiocarb [17], [18]. The degradation of bendiocarb by *Arthrobacter* sp. was enhanced in the presence of *Pseudomonas* sp., which alone did not have the capacity to degrade the carbamates [18]. However, as far we know, there are no reports about the use of bendiocarb by the bacterial strains identified in this work or their participation in bendiocarb biodegradation processes.

All individual bacterial strains were able to grow in liquid MSM medium and on agar plates, using the components of the insecticide Ficam W, as carbon and nitrogen source.

TABLE II
PREDOMINANT BACTERIAL STRAINS

Bacteria	NCBI accession numbers	percentage of similarity
Pseudoxanthomonas spadix.	NC_016147.2	98
Ochrobacterium anthropi	NC_009668.1	97
Staphylococcus capitis	NZ_CP007601.1	99
Bosea thiooxidans	NZ_LMAR01000067.1	99
Pseudomonas denitrificans	NC_020829.1	99
Agromyces sp.	NZ_LMKQ01000001.1	98
Bacillus thurengiensis	NC_022873.1	97
Pseudomonas alkylphenolia.	NZ_CP009048.1	98

Reported species showing the highest similarity were regarded as the isolated strains.

There are reports on the ability of some of the strains isolated in this work to degrade several pollutants; for example, *Pseudoxanthomonas spadix* can metabolize benzene, toluene, ethylbenzene, and o-, m-, and p-xylene (BTEX) [19]. *Ochrobacterium anthropi* can degrade polychlorinated biphenyls [20] and glyphosate [21]. *Pseudomonas denitrificans* can remove polycyclic aromatic hydrocarbons (PAHs) [22], *Agromyces* sp. [23] can degrade nylon oligomers, *Bosea thiooxidans* oxidizes thiosulfate [24] and can remove lindane [25]. *Staphylococcus capitis* reduce the toxic hexavalent chromium into its nontoxic trivalent form [26]. *Bacillus thuringiensis* has the ability to degrade 2,4-dichlorophenoxyacetic acid (2,4 D) [27], cyhalothrin [28] and chlropyrifos [29]. *Pseudomonas alkylopholia* could be involved in the biofilm formation since there are studies showing its ability to produce an extracellular polymer matrix that provides mechanical stability to the biofilm and prevent the dehydration of biofilms [30]. Glycocalyx formation protects the attached cells against toxic compounds and rough environmental conditions [31].

IV. Conclusions

A microbial community capable of effectively remove the insecticide bendiocarb was isolated, opening the possibility of evaluating the degradation using different strains, bioprocesses and bioreactors to improve the bendiocarb removal rates and efficiencies. The remaining COD values indicate that it was not possible to eliminate all the COD in the PBR operating in repeated batch culture.

References

[1] A. Wood, "Compendium of Pesticide Common Names, Index, Bendiocarb", London, Accesed Nov. 2016.
[2] Pesticide Action Network UK, Pesticides News, no. 68, pp. 20-21, Jun. 2005.
[3] R.E.D. Facts. "Bendiocarb", EPA, USA, pp. 1-5. Sept. 1999.
[4] C. R. Worthing, "The Pesticide Manual: A World Compedium," 7th ed. Lavenham Press Ltd, Lavenham, Suffelk, 1983.
[5] S. Lozano-Fuentes, M.H. Hayden, C. Welsh-Rodriguez, C. Ochoa-Martinez, B. Tapia-Santos, K.C. Kobylinski, C.K. Uejio, E. Zielinski-Gutierrez, L.D. Monache, A.J. Monaghan, D.F. Steinhoff and L. Eisen., "The dengue virus mosquito vector *Aedes aegypti* at high elevation in Mexico," *Am. J. Trop. Med. Hyg*., vol. 87, no. 5, pp. 902-909, Nov. 2012.
[6] M.D. Martin Cetron, "Revision to CDC'S Zika travel notices: minimal likelihood for mosquito-borne Zika virus transmission at elevations above 2,000 meters," *M.M.W.R*., vol. 65, no. 10, pp. 267-268, March. 2016.
[7] M. Dhimal, I. Gautam, H.D. Joshi, R.B. O'Hara, B. Harens, U. Kuch, "Risk factors for the presence of chikungunya and dengue vectors (*Aedes aegypti* and *Aedes albopictus*), their altitudinal distribution and climatic determinants of their abundance in central Nepal," *PLoS. Negl. Trop. Dis.,* vol. 9, no. 3, e0003545, March. 2015.
[8] Secretaría de Salud. Centro Nacional de Programas Preventivos y Control de Enfermedades (CENAPRECE). "Monitoreo de Resistencia a Insecticidas (Adulticidas) Utilizados en el Programa de Enferemedades Transmitidas por Vectores en México," 2016.
[9] E. Petrovova, H. Purzyc, D. Mazensky, L. Luptakova, N. Torma, I. Sopoliga, D. Sedmera, "Morphometric Alterations, Steatosis, Fibrosis and Active Caspase-3 Detection in Carbamate Bendiocarb Treated Rabbit Liver," *Environ. Toxicol*., vol. 30, no. 2, pp. 212-222, Feb. 2015.
[10] K. A. Delahaut, IPM Outreach Specialist, "Bendiocarb to lose registration," University of Wisconsin Urban Horticultural Website, Dec. 2001.
[11] M. Miyajima, M. Matsuda, S. Haga, S. Kagawa, B.C. Millar, J.e. Moore, "Cloning and sequencing of 16S rDNA and 16S-23S rDNA interna spacer region (ISR) from urease-positive thermophilic Campylobacter (UPTC)," *Letters in Applied Microbiology*," vol. 34, pp. 287-289, Jan. 2002.
[12] D. J. Lane, "16S/23S rRNA sequencing," in *Nucleic acid techniques in bacterial systematics*, E. Stackebrandt, M. Goodfellow, Ed. New York. John Wiley and Sons, 1991, pp. 115-175.
[13] S. P. Galíndez-Nájera, M.A. Llamas-Martínez, N. Ruiz-Ordaz, C. Juárez-Ramírez, M.E. Mondragón-Parada, D. Ahuatzi-Chacón, J. Galíndez-Mayer, "Cyanuric acid biodegradation by a mixed bacterial culture of Agrobacterium tumefaciens and *Acinetobacter* sp. in a packed bed biofilm reactor," *J. Ind. Microbiol. Biotechnol*, vol. 36, pp. 275-284, 2009.
[14] A. Gómez-De Jesús, F.J. Romano-Baez, L. Leyva-Amezcua, C. Juárez-Ramírez, N. Ruiz-Ordaz, J. Galíndez-Mayer, "Biodegradation of 2,4,6-trichlorophenol in a packed-bed biofilm reactor equipped with an internal net draft tube riser for aeration and liquid circulation," J. Hazard. Mat., vol. 161, pp. 1140-1149, Apr. 2008.
[15] T. Pérez-Ruiz, C. Martínez-Lozano, M.D. García, "Determination of N-methylcarbamate pesticides in environmental samples by an automated solid-phase extraction and liquid chromatographic method based on post-column photolysis and chemiluminescence detection," *J. Chromatogr. A.*, vol.1164, pp. 174-180, July 2007.
[16] Hach Water Analysis Handbook. Hach Company, Loveland. 2012.
[17] K. Ramanand, M. Sharmila, Neera Singh, N. Sethunathan, "Metabolism of Carbamate Insecticides by Resting Cells and Cell-Free Preparations of a Soil Bacterium, *Arthrobacter* sp.," *Bull. Environ. Contam. Toxicol*., vol. 46, pp. 380-386, 1991.
[18] Neera Singh, Anusmita Sahoo, Debjani Misra, V. R. Rao, N. Sethunathan, "Synergistic interaction between two bacterial isolates in the degradation of carbofuran," *Biodegradation*, vol. 4, pp. 115-123, Jan. 1993.
[19] Seung Hyeon Lee, Hyun Mi Jin, Hyo Jung Lee, Jeong Myeong Kim, Che Ok Jeon, "Complete Gnome Sequence of the BTEX-Degrading Bacterium *Pseudoxanthomonas spadix* BD-a59," *J. Bacteriol. asm. org*, pp. 544. Nov. 2011.
[20] Slavomíra Murínová, Katarína Dercová, "Potential Use of Newly Isolated Bacterial Strain *Ochrobactrum anthropi* in Bioremediation of Polychlorinated Biphenyls," *Water, Air, & Soil Pollution*, vol. 225, pp. 1980, Jun. 2014.
[21] I.T. Ermakova, N.I. Kiseleva, T. Shushkova, M. Zharikov, G.A. Zharikov, A.A. Leontievsky, "Bioremediation of glyphosate-contaminated soils," *Appl. Microibiol. Biotechnol*., vol. 88, no. 2, pp. 585-594, Sep. 2010.
[22] L. U. Obi, H. I. Atagana, R. A. Adeleke, "Isolation and characterization of crude oil sludge degrading bacteria," *Springer Plus*, vol.5:1946, pp. 1-13.
[23] K. Yasuhira, Y. Tanaka, H. Shibata, Y. Kawashima, A. Ohara, D. Kato, M. Takeo, S. Negoro, "6-Aminohexanoate Oligomer Hydrolases from the Alkalophilic Bacteria *Agromyces* sp. Strain KY5R and *Kocuria* sp. Strain KY2," *Appl. Environ. Microbiol*., vol. 73, no. 21, pp. 7099-7102. Nov. 2007.
[24] S. K. Das, A. K. Mishra, B. J. Tindall, F. A. Rainey, E. Stackebrandt, "Oxidation of Thiosulfate by a New Bacterirum, *Bosea thiooxidans* (strain BI-42) gen. nov., sp. nov.: Analysis of Phylogeny Based o Chemotaxonomy and 16S Ribosomal DNA Sequencing," *Int. J. Syst. Bact*., vol. 46, no. 4, pp. 981-987. Oct. 1996.
[25] K. Girish, A.A. Mohammad Kunhi, "Microbial degradation of gamma-hexachlorocyclohexane (lindane)," *African J. Microbiol Res*., vol. 7, no. 17, pp. 1635-1643. April 2013.
[26] A. Zahoor, A. Rehman, "Isolation of Cr(VI) reducing bacteria from industrial effluents and their potential use in bioremediation of chromium containing wastewater," *J. Environ. Sci*., vol. 21, no. 6, pp. 814-820. 2009.
[27] A. Barbarán-Vilela, D. Cieza-Diaz, R. Guillén-Domínguez, F. Joaquín-Neira, F. Montenegro-Saavedra, E. Geldres-Vigil, "Biorremediator effect of *Bacillus thuringiensis* on fruit crops soils contaminated with herbicides 2,4 dichlorophenoxyacetic acid," *REFI UPN*, vol.3, no. 2, pp. 7-17. Nov. 2015.
[28] S. Chen, Y. Deng, Ch. Chang, J. Lee, Y. Cheng, Z. Cui, J. Zhou, F. He, M. Hu, L-H, Zhang, "Pathway and kinetics of cyhalothrin biodegradation by *Bacillus thuringiensis* strain ZS-19," *Scientific Reports*, vol.5:8784, pp.1-10. March. 2015.

[29] A.E. Aceves-Diez, K.J.,Estrada-Castañeda, L.M. Castañeda-Sandoval, "Use of *Bacillus thuringiensis* supernatant from a fermentation process to improve bioremediation of chlorpyrifos in contaminated soils," *J. Environ. Manage.*, vol. 157, pp. 213-219. Jul. 2015.
[30] K. Lee, E. J. Lim, K. S. Kim, S. Huang, Y. Veeranagouda, B. H.A. Rehm, "An alginate-like exopolysaccharide biosynthesis gene cluster involved in biofilm aerial structure formation by *Pseudomonas alkylphenolia*," *Appl. Microbiol. Biotechnol*, vol.98, no. 9, pp.4137-4148. Feb. 2014.
[31] R. Sánchez-Sánchez, D. Ahuatzi-Chacón, J. Galíndez-Mayer, N. Ruiz-Ordaz, A. Salmerón-Alcocer, "Removal of triazine herbicides from aqueous systems by a biofilm reactor continuously or intermittently operated," *J. Environ, Management*, vol. 128, pp. 421-426. Jun. 2013.