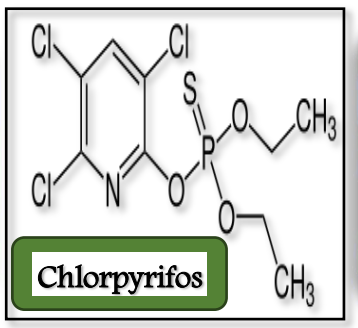
Optimization of Chlorpyrifos Biodegradation and Elimination from Contaminated Water/ Wastewater Using Specialized Marine Bacteria

[1] Nidal Mohammed Zabermawi*, [2] Ebtesam Abd El-Hamid El-Bestawy*

[1] * *Corresponding Author,* Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia.

Email: nzabermawi@Kau.edu.sa, Phone: +966505658458, ORCID: https://orcid.org/ 0000-0003-1216-7003

[2] Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University, 163 Horria Ave. El-Shatby, P.O. Box 832, Alexandria, Egypt. Phone : +2/1224979468, E-mail: ebtesamelbestawy@alexu.edu.eg, ORCID: https://orcid.org/0000-0002-5351-9696



INTRODUCTION

- Chlorpyrifos (CPS), is an organophosphate pesticide that is widely applied to control foliage and soil-born insects in residential settings, golf course turf, as a structural termite control agent, and on agricultural crops.
- Pollution of water courses with Chlorpyrifos can lead to severe negative environmental impacts and high human health risk.
- The World Health Organization classifies Chlorpyrifos as Class II, moderately hazardous pesticide to humans. United States (EPA) listed Chlorpyrifos as a hazardous substance under the Clean Water Act.
- Chlorpyrifos exposure may lead to acute toxicity at higher doses, results mainly from interference with the acetylcholine neurotransmission pathway, leading to a range of persistent health effects neuromuscular symptoms such as seizures, unconsciousness, paralysis, and suffocation from lung failure.
- Mild poisoning can result in eye-watering, increased saliva and sweating, nausea, headache, muscle spasms or weakness, vomiting, or diarrhea, and impaired vision.
- Moreover, developmental effects appear in fetuses and children even at very small doses, where they are more likely to experience muscle weakness rather than twitching; excessive saliva rather than sweat or tears; seizures; and sleepiness or coma.
- Therefore, effective procedures to control its levels in water courses is a must.
- There are several technologies to treat Chlorpyrifos-contaminated sites, including landfills, reprocessing, combustion, chemical treatment, and pyrolysis. These methods are inefficient and expensive as well as polluting the environment by releasing toxic products into microbial habitats.
- Biodegradation using microorganisms is a more efficient and sustainable method for degrading and removing toxic compounds from soils and waterbodies.
- A number of microbes are known of degrading Chlorpyrifos to date. Among those microorganisms, *Arthrobacter*, *Enterobacter*, *Xanthomonas*, *Streptomyces*, *Stenotrophomonas*, *sphingomonas*, *Bacillus*, *Synechocystis*, *Pseudomonas*, *Actinobacteria*, and *Klebsiella* have been confirmed as potential Chlorpyrifos degraders.

AIM OF THE WORK

- The present study aimed to investigate the ability and efficiency of for biodegrading and eliminating Chlorpyrifos from contaminated water/ wastewater using selective indigenous marine bacteria in a free-living batch-mode process.

MATERIALS & METHODS

- Synthetic wastewater samples supplemented with three elevated levels of Chlorpyrifos (100, 250 and 500 mg/L) were treated in a batch mode using individual free-living cultures of six pure marine indigenous bacterial isolates (E 7, 8, 9, 11, 12 and 13) that were previously isolated and identified in a previous work of the authors.
- The remediation bioassays were performed for 7 consecutive days, where samples were aseptically drawn at 24-h interval for analysis.
- Chlorpyrifos residual concentrations (RCs) were determined using GS-Mass and its removal efficiencies (REs%) were calculated to determine the most efficient Chlorpyrifos degrading candidate(s).

RESULTS

- Extremely high Chlorpyrifos removal was achieved by almost all the tested cultures (6), reaching almost complete elimination (100%) at all the 3 tested concentrations.
- Unexpectedly, increasing Chlorpyrifos concentration, enhanced the removal efficiency (RE). Results recorded 97.0-99.3, 99.0-100 and 100% REs of Chlorpyrifos at 100, 250 and 500 mg/L, respectively, within only 48 h.
- The current study's findings confirmed that the proposed bioremediation process by almost all the selected cultures, is a highly active, promising, renewable, and inexpensive biotechnology for the treatment of a wide range of contaminated aquatic media.

CONCLUSION

- All the tested six isolates, {*Bacillus pacificus* (E7 and E8), *Bacillus cereus* (E9) and *Bacillus paramycoides* (E11 and E13) and 12 (Unidentified)} were found to be most effective and active in the degradation and removal of the target insecticide Chlorpyrifos.
- The highest Degradation Efficiency ranged between 99.3-100% by the tested strains, equivalent to 0.69-0.27 mg/L (690- 270 µg/L) RCs. However, Chlorpyrifos residues are still much higher (1,380-540-Fold) than its maximum permissible limit (MPL) of 0.5 µg/L stated by Environmental Quality Standards (EQS)- European Union (Water Framework Directive, SCHEER Opinion 2022) for Chlorpyrifos in surface waters (freshwater and saline) to protect aquatic ecosystems. This is mainly because in the present study, extremely high concentrations (100-500 mg/L) were tested to simulate the worst Scenario that might happen.
- Biological remediation using Chlorpyrifos degrading- bacterial system is highly effective, low-cost, and readily available technology for Chlorpyrifos removal.
- It also offers an attractive chance to decontaminate the environment from toxic pollutants, produce high quality effluents, complying with the environmental regulations for the safe drainage into open environment.
- Results evidenced that marine environment is a natural, rich and renewable source of bacteria with marvelous metabolic capabilities for efficient bioremediation of Chlorpyrifos -contaminated aquatic environments or wastewater.

Table 1: Residual Concentrations (mg/L) and Removal Efficiencies (RE%) of Chloropyrifos at its Elevated Concentrations (I, II, III) from the Liquid Medium Augmented by Different Marine Bacteria

Conc. I	Residual Concentration (mg/L)													
Exposure Time (Days)	Control		7		8		9		11		12		13	
	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%
	Initial Conc. I: 100 mg/L													
1	1.18	98.8*	6.59	93	7.99	92	2.76	97	3.68	96	4.55	95	10.50	89
2	1.72	98	2.69	97	0.69	99.3**	0.70	99**	1.58	98**	1.43	99**	2.61	97**
3	14.62	85	21.98	78	5.06	95	24.88	75	14.25	86	49.59	50	33.58	66
4	11.84	88	1.56	98**	2.87	97	2.83	97	26.69	73	20.76	79	52.67	47
5	2.68	97	3.74	96	3.27	97	2.88	97	7.78	92	7.22	93	13.91	86
6	19.0	81	48.82	51	70.81	29	87.82	12	6.77	93	35.38	65	31.58	68
Conc. II	Initial Conc. II: 250 mg/L													
Exposure Time (Days)	Control		7		8		9		11		12		13	
	RC	RE	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%
	Initial Conc. 250 mg/L													
1	3.80	98**	1.37	99	1.21	100**	2.76	99	1.82	99	45.78	82	3.40	99
2	5.00	98	1.00	100**	11.90	95	1.15	100**	1.41	99**	1.01	100**	1.86	99**
3	4.84	98	7.15	97	46.36	81	23.88	90	4.81	98	7.94	97	9.57	96
4	4.00	98	12.28	95	22.56	91	5.03	98	8.43	97	5.54	98	9.50	96
5	20.41	92	13.95	94	31.17	88	17.50	93	15.42	94	17.71	93	23.61	91
6	27.84	89	26.77	89	2.63	99	3.80	98	27.80	89	140.6	44	56.76	77
Conc. III	Initial Conc. III: 500 mg/L													
Exposure Time	Control		7		8		9		11		12		13	
	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%	RC	RE%
1	0.43	100**	2.00	100**	0.46	100**	0.12	100**	9.22	98	10.88	98	5.36	99
2	0.60	100**	3.28	99	0.70	100**	1.37	100**	0.27	100**	0.65	100**	0.66	100**
3	9.79	98	9.55	98	15.16	97	4.71	99	8.87	98	21.38	96	27.25	95
4	6.11	99	14.10	97	5.98	99	44.6	91	9.23	98	5.37	99	2.71	99
5	6.96	99	26.12	95	7.78	98	3.41	99	7.12	99	14.11	97	11.72	98

Table 1. Similarity Percentages to the Nearest Neighbors of the Selected Isolates

Isolate No.	Nearest Neighbor(s)	Gen Bank accession of the Nearest Neighbor	Similarity %
7	*Bacillus pacificus* strain MCCC 1A06182	NR157733.1	100
8	*Bacillus pacificus* strain MCCC1A06182	NR157733.1	99.85
9	*Bacillus cereus* strain ATCC 14579	NR074540.1	99.9
11	*Bacillus paramycoides strain* MCCC 1A04098	NR157734.1	98.44
13	*Bacillus paramycoides strain* MCCC 1A04098	NR157734.1	99.16

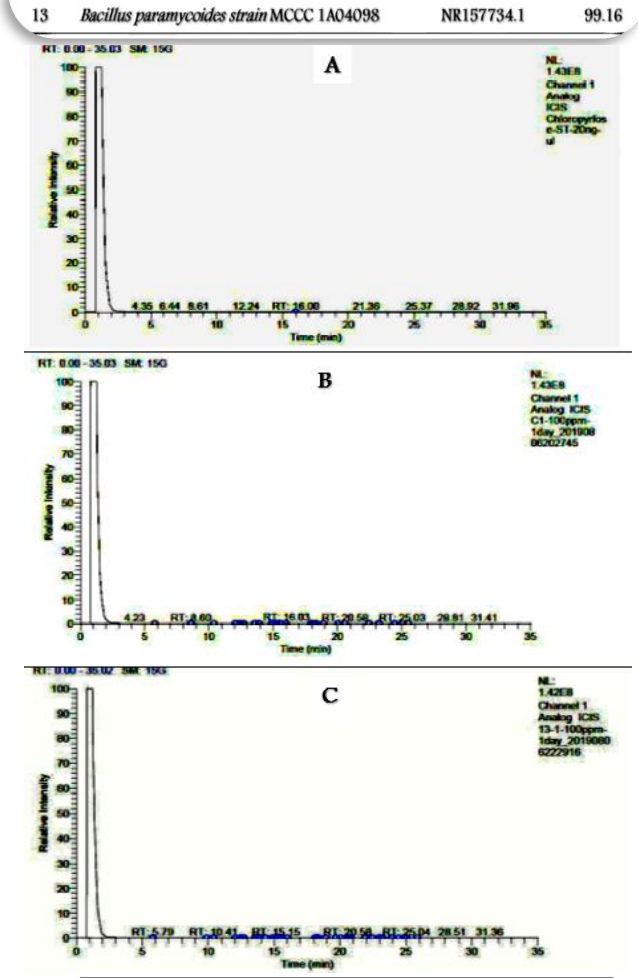
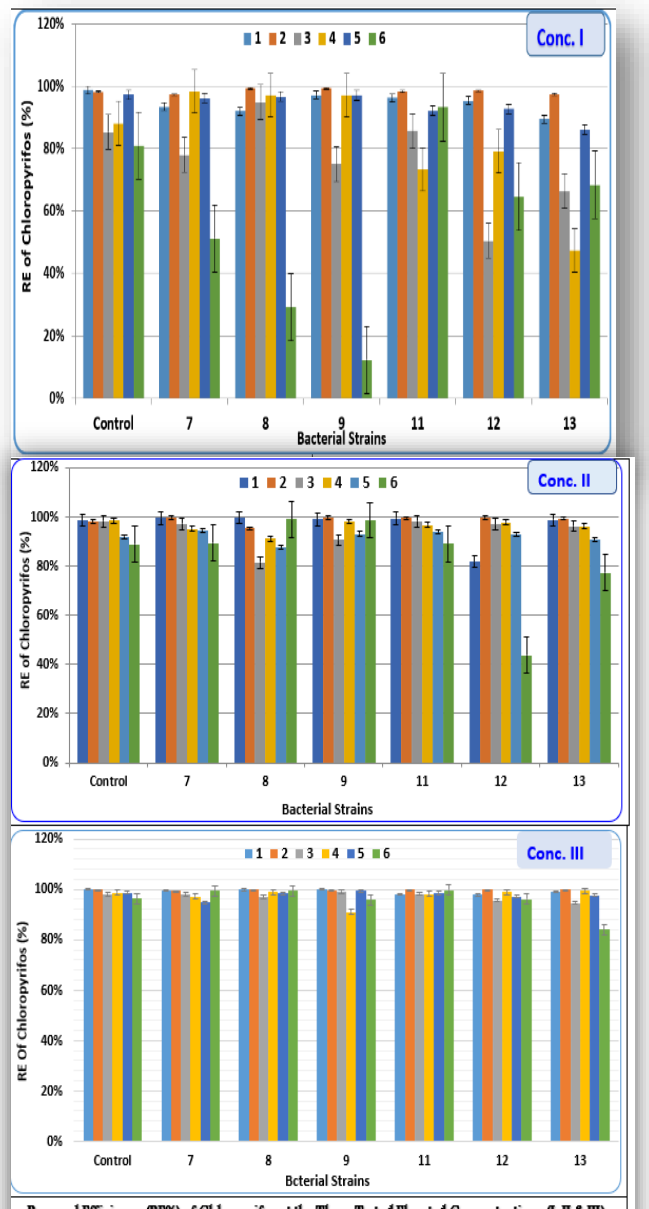


Fig. 1. GC-Mass Chromatograms of Bacterial Degradation of Chlorpyrifos; A) Chlorpyrifos Standard 20 ng/µL; B) Chlorpyrifos Control Solution with Concentration of 100 ng/µl without Bacteria and C) Treated Solution with Chlorpyrifos Concentration of 100 ng/µl Augmented with Bacterial Strain No 6 Showing the Disappearance of Chlorpyrifos peak



Removal Efficiency (RE%) of Chlorpyrifos at the Three Tested Elevated Concentrations (I, II & III) from the Liquid Medium Augmented by Different Selected Marine Bacteria at Different Exposure Times