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ACTIVITY ASSAY OF BACTERIAL MIXED CULTURE IN BIOLOGICAL TREATMENT OF ORGANOCHLOROPESTICIDES

Iman H. Gatea*, Eman H. Khudhair, Amina G. A. Bid, Amal A. Halob and S. Kh. Maied

Directorate of Environment and Water Research, Ministry of science and Technology, Baghdad- Iraq.

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***Corresponding Author**
Dr. Iman H. Gatea
Directorate of Environment and Water Research, Ministry of science and Technology, Baghdad- Iraq.

ABSTRACT

Some pesticides are readily degraded by microorganisms; others have proven to be recalcitrant. A diverse group of bacteria, including members of the genera Alcaligenes, Flavobacterium, Pseudomonas and Rhodococcus, metabolize pesticides. Microbial degradation depends not only on the presence of microbes with the appropriate degradative enzymes, but also on a wide range of environmental parameters. In this research two bacterial isolates defined consortium with Propachlor biodegradation capacity, were isolated from soil samples from pesticides contaminated site. These consortia: Streptomyces sp.Y7and *Rhodococcus sp.S3* produced a significant increment of the specific degradation activity compared to the individual culture with 20 mg L-1 of Propachlor as the only carbon source, consortium of *Streptomyces spY7* and *Rhodococcus* spS3 were able to grow with different concentration of Propachlor. The microorganisms present in the special growth medium provided with Propachlor as a sole carbon source were found to possess the ability to degrade 84% of it after 48 h. of incubation at 30°C with shaking at 120 rpm and optimum pH was7 and 35 mg L-1of pesticide was the favorable concentration. Therefore the selected bacterial isolates could be considered one of the most promising bacterial groups for COP biodegradation in contaminated environment.

KEYWORDS: Chlorinated organopesticides; Bacteria; Degradation, Propachlor, Contaminated soils.

INTRODUCTION

Pesticides are unique in that they are purposefully released into the environment to kill selected species. The chlorinated pesticides are now largely banned chemicals which may still pose a threat to human health as well as the wider environment. Along with other persistent organochlorines such as chlorinated biphenyls, chlorofluorocarbons, dibenzodioxins and dibenzofurans, the chlorinated pesticides have the potential to cause significant damage to the natural ecosystem by interfering with reproductive processes, thus influencing the biodiversity of non-target organisms, as well as allowing resistant gene pools within pest communities to dominate. The fate of pesticides in the environment is determined by both biotic and abiotic factors caused by microorganisms or their enzymes.[2] Pesticides are degraded in the environment principally by the action of microorganisms, a process termed biodegradation, where biodegradation is defined as the breakdown of a substance to smaller products. Unfortunately, COP and its residues still remain in soils more than 20 years after use ceased. Consequently COP residues have been transported to water and sediments by surface runoff[13] and have been accumulated into food chains.[11] It was perhaps fortunate that analytical techniques suitable for the detection of very low levels of these organochlorines were developed earlier than for many other compounds[5] A number of factors influence availability, though effects are primarily due to either low solubility and/or strong adsorption to the soil matrix.[12] The chemical structure of a pesticide, its size and functional groups all determine the water solubility of the compound. Chloro-N iso Propylacetanilide (Propachlor) is an acetamide, herbicide widely used to protect corn, onion, cabbage rose bushes and ornamental plant.

MATERIAL AND METHODS

Soil samples were obtained from AL- Jadirya gardens and at depth of (10-30) cm.The soil had been exposed to pesticides for more than 10 years. 5 g soil samples were added into 100 ml nutrient broth medium containing 20 mg L -1 Propachlor, The suspension was incubated at 30°C with shaking at 120 rpm for72 h. then it was spread on the plate Minimal Salt medium (MSM) which was used for growth of the microorganisms and pollutants removal assays. It consisted of ($g\,L^{-1}$): L-asparagine, 0.5; K_2HPO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 0.01 supplemented with 20 mg L, Propachlor as isolation medium). After inversion incubation at 30°C for 3 days, pick vigorous growing colony on the plate for streaking and purified by repeated streaking. Lastly, isolates were inoculated onto enrichment plate for preservation. Isolates were identified by Using methods in (10) to its morphology,

physiological and biochemical characteristic in rich medium-Broth. All media were sterilized by autoclaving at 121°C for 15 min.[6]

For mixed culture, three flasks containing MSM (100 ml each) were prepared, pesticides was added sterilized and heavily inoculated. After inoculation, the two cultures of the tow species were incubated in a shaker incubator at 120 rpm and 30°C for 120 h after which, biomass and extracted COP were determined. Similar set of experiment was done with, different initial pH and medium containing (5-40) mg L -1 Propachlor. Control medium without inocula were maintained in parallel. After incubation for 48 h., the sample was extracted by adding 60mL of dichloromethane and shaking for two minutes. It is critical to shake all samples in the same manner. The dichloromethane settles to the bottom of the separatory funnel and then is decanted through a sodium sulfate tube into a collection vessel. Acetone and dichloromethane usually are used in a 1:1 combination as the extraction solvent. The acetone is needed to adequately penetrate into particles so that compounds contained in the particle can be extracted.[16]

RESULTS AND DISCUSSION

Different bacterial isolates were isolated when initial pesticide concentrations (20mg L-1) was added in MSM, an increment of the microbial growth was detected as biomass in all the cultures tested. Also a diminution of the residual propachlor concentration was determined in the growth medium in relation to the abiotic controls. The cultures exhibited considerable ability to degrade Propachlor. Biochemical characteristics of highest Propachlor -degrading strains isolated from soil were studied. The bacterial isolated strains were identified as *Streptomyces* sp. *Y7* and *Rhodococcus sp S3*.The actinomycetes are Gram positive bacteria with great potential to bioremediate xenobiotic.[3] Bacteria of the genus Rhodococcus can degrade a wide range of organic pollutants and catalyse many useful biotransformations.[4] Chemical pollutants which can be degraded by Rhodococci range from simple hydrocarbons, which are used as sole carbon sources in the isolation of Rhodococci[8], through chlorinated hydrocarbons, aromatic hydrocarbons and nitroaromatics to chlorinated polycyclic aromatics such as polychlorinated biphenyls (PCBs) The two isolates, *Streptomyces* sp. Y7 and Rhodococcus isolated from organochlorine pesticides (OPs) contaminated soil was selected for their capacity to grow in presence of Propachlor as only carbon source; after growing at 30°C, the isolates recorded 54% and 60% respectively consumption of pesticide after 72h.

Table(1). Production of surfactants may be a mechanism for enhancing the solubility of the pesticide and hence its ease of biodegradation.[1]

Table [1] Primary screening of degrading bacterial isolates

Bacterial isolate	Gram stain	Biomass g\l	Conception of pesticide%
S5	-ev bacilli	0.63	32
S3	+ev bacilli	0.92	54
D4	-ev rods	0.54	27
D8	-ev bacilli	0.41	28
Y7	+ev short rods	0.82	60
Mixed of S3 with Y7		1.8	66

Degradation rates are strongly influenced by a wide variety of environmental factors, substrate availability, moisture content, temperature and pH.[12] In this research survival of the selected microbes for the experiment duration (48 h) is attributed to the use of pesticides as sole carbon and energy sources, suggesting that the pesticide could not been toxic for this microorganism and that would not either be accumulated toxic intermediary metabolites that had an inhibiting effect on the growth. Beside, these bacteria were adapted to extreme pollution and starvation conditions where they can stand and survive. This can explain the viability of our selection (treated and control) for such short period of time (5 days) is highly expected.

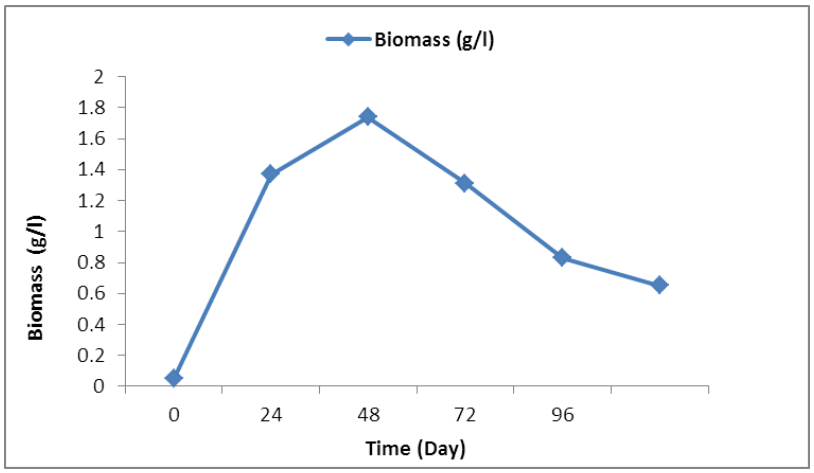

Figure 1. Effect of incubation time on the bacterial growth of *mixed culture in* medium amended with 20 mg/l of propachlor.

Growth curve experiments were performed with different pH values in order to determine the optimum pH that stimulates the growth of the mixed culture in laboratory scale. Result shown that pH7 was the best one to get highest biomass 1.8 g (figure 2).

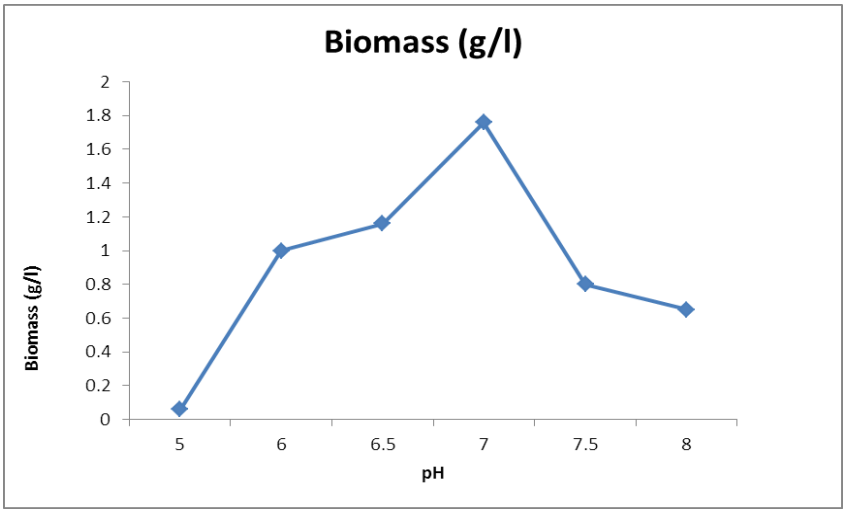

Figure (2). Effects of various pH values on growth curve of mixed culture

Streptomyces sp. and *Rhodococcus* sp. were able to grow as a mixed culture with different concentration of COP (Figure 3). In all cases, biomass production was higher than 0.07 mg l-1and reached a maximum 3.8 g l_1 with initial concentration of 35 mg l-1after 48 h of incubation at 30°C and 120 rpm. concentration of the pesticide must not be so high as to be toxic, nor so low such that biodegradation does not proceed due to a lack of induction of appropriate degradative enzymes and uptake mechanisms, failure to induce sufficient enzyme activities, or problems in providing \ sufficient energy even for cell maintenance.[7,15]

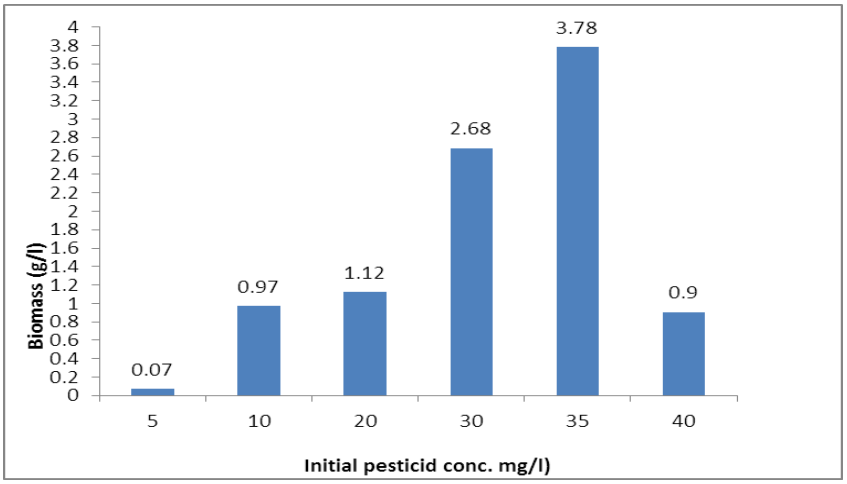

Figure (3) Growth of mixed culture of *Streptomyces sp and Rhodococcus sp.*in mineral salt medium containing different concentrations of (Propachlor).

These results would indicate that the growth of *Streptomyces* sp. Y7 in supplemented liquid medium was not affected by the pesticide concentrations assayed, suggesting that the microorganism could tolerate or maybe degrade the pesticide by producing the necessary dehalogenase enzymes as was demonstrated by.[14]

CONCLUSION

The present study reports the identification of bacterial isolates, *Streptomyces* sp. Y7 and *Rhodococcus sp S3* which were capable of utilizing propachlor as a source of carbon. Utilization of xenobiotic compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. Results from the present study suggest that the isolated *Streptomyces* sp. *Y7 and Rhodococcus sp S3* were able to grow as a mixed culture in medium in the presence of added pesticide (35 mg/L) and may therefore be used for bioremediation of pesticide-contaminated soil. Mixed cultures have shown to be more suitable for bioremediation compared with pure cultures because their biodiversity can enhance environmental survival and increase the catabolic pathways available for contaminant biodegradation.

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