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DECONTAMINATION OF POLLUTANTS IN AQUATIC SYSTEM: 2. ADAPTING CERTAIN BACTERIA STRAINS FOR ORGANOPHOSPHORUS PESTICIDES DEGRADATION

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ABSTRACT: Much effort has been given at the present to the use of microorganisms in the decontamination of organophosphorus pesticides (OP's). Here we studied the role of repetitive exposure to low doses of OP and the influence of glucose as energy source on the activities of *E. coli* strains isolated from local environment for biodetoxification of OP. Results indicated that adapted *E. coli* strains were superior in their OP detoxification activity than wild. An isolated strain of *E. coli* from water samples (Wx2) showed the maximum activity among other strains for paraoxon biodetoxification. Adding glucose to the wild and adapted strains did not enhance their OP's biodegradation activities. Bioreactor column for OP's detoxification was build using promising adapted strain. The bioreactor achieved 57.49% biodegradation efficiency of the applied 0.1 mM paraoxon concentration. The efficiency of the bioreactor was maintained up to 30 days from its construction. The results suggest that adaptation of this strain of bacteria by exposure to the concentrations of the organophosphate insecticides can enhance its efficiency in degradation of these compounds that may introduced an efficient, cheap and environmental friendly technique for pollutants decontamination.

Key words: Organophosphorus pesticides, bioremediation, isolated bacteria, adaptation, and bioreactor.

Organophosphate pesticides constitute a major part of the chemicals used to control different pest species in agricultural. This group of insecticides is well recognized as one of highly toxic pesticide groups. The past and present extensive use of this group has been the reason for a serious environmental and health problems. Yet, the need to develop safe, convenient, and economically feasible methods for removal and detoxification of these compounds from the environment has been urgent.[1, 2]

The traditional practices to detoxify OP rely on chemical treatment, incineration and landfills. Those methods are not environmentally desirable because of their negative impacts. Besides, such methods do not provide a convenient solution for contamination problems. It has been found that microbial hydrolysis of several OP insecticides, are 40-2450 times faster than chemical hydrolysis by 0.1 N NaOH at 40 °C,[3] such activity persisted even at temperatures as high as of 50 °C. Isolates from specific contaminated areas of naturally occurring bacteria, showed variable capabilities for metabolizing OP's.[4] This provides the possibility for a safe and in-situ detoxification of these compounds. [4, 5, 6]

The capabilities of different bacterial species for OP's biodegradation and the influence of several factors on such capabilities were assessed by several investigators; *Flavobacterium sp.*[7, 8], *Pseudomonas sp.*[9, 10], *Arthrobacter sp.* and *Bacillus sp.*[11]; and *E. coli*, [12, 13].

In this report, we describe the ability of certain *E. coli* bacterial strains that we have isolated from contaminated spots of Marriute Lake for OP's biodegradation. The role of adaptation of these bacteria for OP's decontamination, as well as the effect of glucose as energy source on their activity for biodetoxification of OP were investigated. Using such adapted strains in building bioreactor columns for OP's detoxification has been described.

Material and Methods

Chemicals: Parathion 99.5% (dimethyl- *p*-nitrophenyl phosphorothioate) and Paraoxon 98% (diethyl- *p*-nitrophenyl phosphate) were purchased from Chem. Service, Inc, West Chester, PA, USA. Nutrient broth (NB), buffer A (50 mM citrate-phosphate 100 mM NaCl and 0.05 mM $CoCl_2$ pH 8.0) and Phosphate-Citrate buffer pH 8.0 were also used.

Bacterial strains: Strains of *E. coli* bacteria were collected from water and sediment samples that are located along Marriute Lake of Alexandria and identified according to Bergey's manual of systematic bacteriology[14, 15] as previously described. [4]

Adaptation of certain strains: Adaptation of isolated *E. coli* 3W, Sd1, Sx_3, Wx_2 strains for OP degradation capabilities were done by exposure of those bacteria to series of concentrations of methyl parathion ranged from 50 µg to 20mg $ml^{-1}$of N.B media, 20% methanol. Series concentrations of methyl-Parathion were prepared in 5 ml N.B media, 20 %methanol (50, 250, 500, 10^3, $5x10^3$, 10^4 and $2x10^4$ µg ml^{-1}). Suspensions (100µl) of bacteria of different strains in 5 ml sterilized water were inoculated with 50 µg ml^{-1} parathion, at 37°C for 48h. Then 100 µl of the above suspensions was transferred to a 5 ml o nutrient broth medium containing 250 µg

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ml^{-1} of Parathion and incubated at 37°C for another 48h. The previous step was repeated with higher concentrations of methyl parathion (500, 10^3, 5×10^3, 10^4 and 2×10^4 µg ml^{-1}) to obtain highly adapted strains. All chosen strains showed growth up to 2×10^4 µg ml^{-1} of parathion except Sx_1 that showed no growth above 10^4 µg ml^{-1} concentration.

Growing and preparing bacterial cells for biodegradation: The adapted strains of bacteria were grown as described by Kearney *et al.*,[5] and Mulbry *et al.*,[6] using N.B liquid media supplemented with (0.1µg ml^{-1}) ampicilin. Cells were harvested after 48h of growing by centrifugation at 4°C, 5000 rpm for 10 min, washed twice with 40ml of 150 mM buffer phosphate pH 8.0 and re-centrifuged at the same conditions. Pellets obtained were weighted and suspended in 10 ml of buffer phosphate.
One colony of wild *E.coli* strain was grown following the previously mentioned method for adaptation of isolated bacteria to compare their capability for OP biodegradation.

Assaying the biodegradation capabilities: The capability of adapted and wild isolated bacteria for OP biodegradation was tested by incubating ten µl (≈ 2.0 mg) of the harvested bacterial suspension in 50mM citrate-phosphate buffer, pH 8.0 containing a range (10 to 250 µg ml^{-1}) of Paraoxon concentrations at 37°C for 48h. The degradation product, *p*-nitrophenol was measured spectrophotometrically at 410 nm.

Effect of glucose on the OP degradation capability of isolated bacteria strains: This was assessed by growing colonies of wild and adapted strains of isolated *E.coli* as previously described in NB liquid media containing 4% D- Glucose.[5, 6] The influence of glucose addition on the capability of these bacteria on Paraoxon degradation was assayed as described above

Bioreactor degradation of Paraoxon: A bioreactor was built as we described before.[16] One and a half gram (wet weight) of *E.coli* strain (Wx_2) cells were suspended in 70 ml of buffer A. The suspension was circulated overnight through a 2.5 x 20 cm column (Kimble, Vineland, NJ), packed with 20 g of SIRAN glass beads (bed height of approximately 9 cm) at room temperature and flow rate of 23 ml/h using a peristaltic pump (Metering Pump, HiloFlow, LTD, London). The OD_{600} of cell suspension was measured before and after cell loading. After draining the cell suspension from the column, 2.5% of glutaraldehyde solution in buffer A was circulated through the column for 2 h at room temperature and 23ml/h flow rate to crosslink the cells. The column was then washed with buffer A overnight at the same conditions. The bioreactor column then is ready for assaying of OP's degradation.

Determination of Paraoxon detoxification in bioreactor: Paraoxon (0.1 mM) in buffer A containing 5 % methanol was introduced from the top of the column at a desired rate by a precision flow peristaltic pump. The effluent solution was reentered to the column three times, and then collected and subsequently analyzed for *p*-nitrophenol. The column efficiency for Paraoxon biodegradation was assayed after 10, 14, and 30 days of column bioreactor construction.

Results and Discussion

Efficiency of Wild and Adapted Isolated *E. coli* Bacteria for OP Biodegradation: As shown in Figures 1, the biodegradation efficiency of wild *E.coli* strains for Paraoxon ranged between 82.5 and 5.3 %, whereas those of the adapted strains (Figure 2) were between 100 and 22.5%. This increase in the degradation activity due to the adaptation was obvious in Wx_2 and Sd1 strains. Also, Wx2 strain showed enhancement of biodegradation, which ranged between 82.5 to 32.3 %; and 100 to 43.3 % for wild and adapted strains respectively.

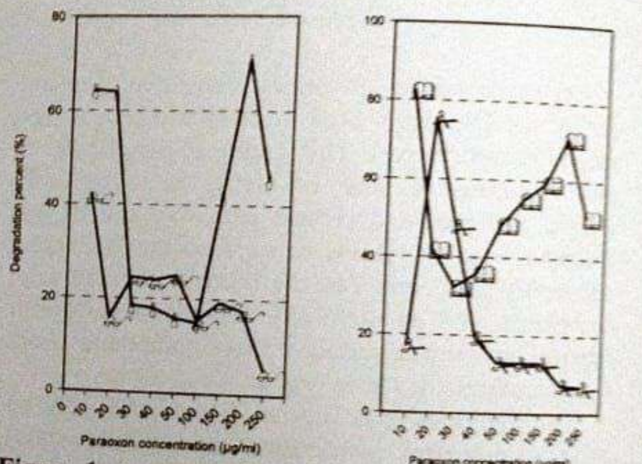

Figure 1: Paraoxon degradative ability of wild *E. coli* isolates from sediments of Marriute Lake in (Left Panel) Sd1 ▲ and Sx3 □, and from water of lake in (Right Panel) 3W# and Wx2 ♦. experimental conditions were as described under material and methods.

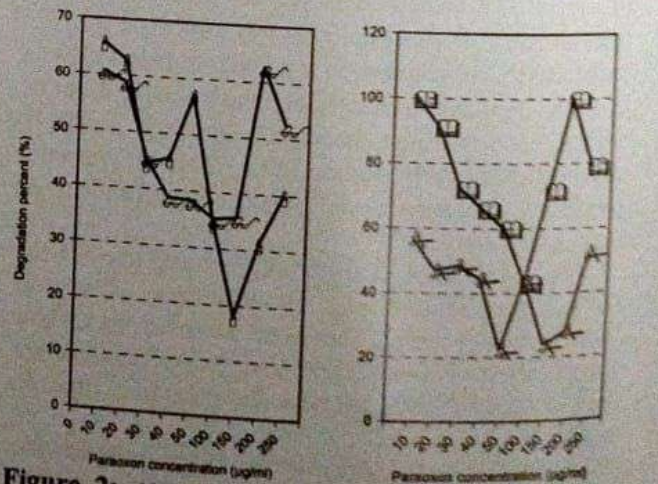

Figure 2: Ability of adopted E. coli strains isolated from Marriute Lake for Paraoxon biodegradation, experimental conditions were as described under material and methods. (Left Panel): sediment isolated strains, Sd1 ▲ and Sx3 □, (Right Panel): water isolated strains, 3W# and Wx2 ♦

It is known that the efficiency of bacteria to degrade OP pesticides is generally due to their content of the enzyme organophosphorus hydrolase (OPH) that is encoded by a plasmid (*opd*) gene.[17] McLoughlin *et al.*,[12] studied this and other genes that enabled *E.coli* to grow using Paraoxon as the sole phosphorus source. Kim *et al.*,[13] significantly enhanced efficiency of degrading Coumaphos (CP) in cattle dip waste (CDW) using a dense, nongrowing cell population that functions without the addition of nutrients required for growing cell cultures. A recombinant strain of *E.coli* containing the *opd* gene for organophosphate hydrolase (OPH), was capable of active hydrolysis of OP neurotoxins. The activity of the present strains of *E. coli* in the degradation of Paraoxon most likely is due to the same gene. In this case adaptation may act through the effect on this gene expression. Details of this effect remain for further studies.

Effects of Glucose on the Capabilities of Isolated Bacteria For Paraoxon degradation:

The influence of glucose on the efficacy of isolated *E. coli* strains for Paraoxon biodegradation is shown in Figures (3 and 4). As it can be seen adding glucose did not show enhancement of the capabilities of the present strains for Paraoxon biodegradation compared to the glucose-free preparations. Ethoprophos (10 mg L^{-1}) was shown not to be affected by the addition of glucose or succinate to the growth media.[10] That suggest that such substrates do not affect either the gene expression or the activity of the degrading enzyme.

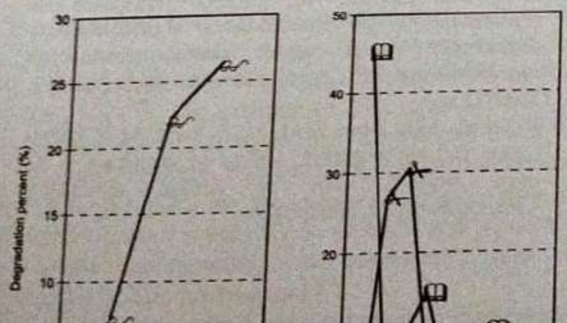

Figure 3: Effects of Glucose addition on the degradative ability of wild isolated strains, (Left Panel): sediment isolated strain Sx3 □, (Right Panel): water isolated strains, 3W# and Wx2 ♦

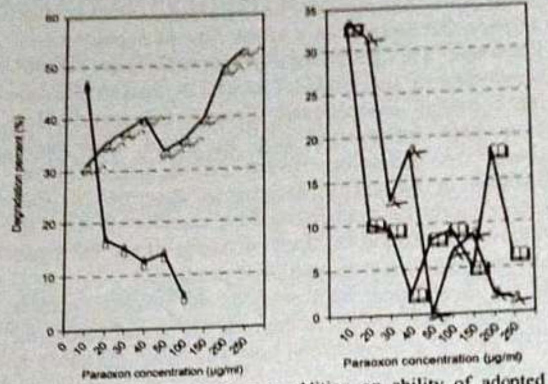

Figure 4: Effects of Glucose addition on ability of adopted strains for biodegradation of Paraoxon, (Left Panel): sediment isolated strains, Sd1 ▲and Sx3 □, (Right Panel): water isolated strains, 3W # and Wx2 ♦.

Biodegradation of Paraoxon Using Bioreactor Immobilized by Adapted *E. coli* Strain (Wx_2):

Since, the adapted *E.coli* strain (Wx_2) was proved to be the highest strain in its ability to degrade Paraoxon, it was chosen as promising strain to build a bioreactor column. The biodegradation activity of the column for 0.1 mM Paraoxon was shown in Figures 5. As it can be seen, there is an enhancement in biodegradation efficiency of this strain immobilized to glass beads in the bioreactor. Such efficiency persisted up to 30 days after the bioreactor construction. These results are in agreement with our published data on the biodetoxification of Coumaphos,[16] in Figure 5, 57 % of 0.1 mM paraoxon degradation was achieved using the bioreactor described her. In our previous study using Coumaphos, approximately 80% degradation was achieved when 0.2mM Coumaphos used similar bioreactor device.[16]

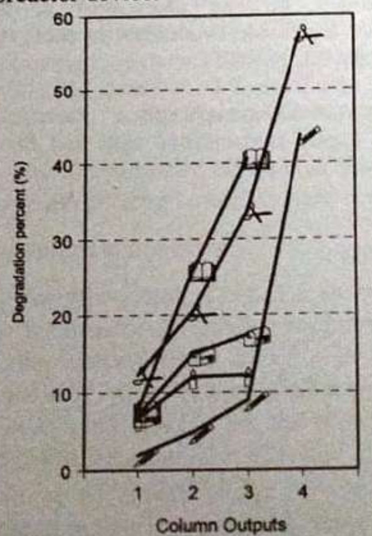

Figure 5: Degradative ability of bioreactor immobilized by promising adapted strain (Wx2), the biodegradation of Paraoxon (0.1mM in citric/phosphate with 5% methanol, pH 8) was assayed after zero time♦, 10 days ■, 14 days▲, 30 days ●. Conditions were as described under materials and methods.

Research described in this report demonstrates remarkable ability of local isolates of *E.coli* to degrade OP insecticide s of the type of parathion and paraoxon. This presents a potential in view of the possibility of bioremediation of OP insecticides, in the Egyptian environment. Several OP insecticides have been intensively used in Egypt over many years.[18] A technology based on the use of local bacteria in bioreactor designs as described in this report could be manifested in a number of ways to remove insecticide residues of this group of OP from environmental sectors and food products.

In the mean time, improving of the present bacteria regarding their ability to degrade OP insecticides and their use in a commercial way for those purposes is open for future research.

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