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Biodegradation of malathion by a bacterium isolated from the Arvandkenar region in Iran

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ABSTRACT

The global use of pesticides has resulted in the contamination of various ecosystems worldwide. The impact of these pesticides can be reduced through bioremediation. The factors that influence the biodegradation rate include the isolation of efficient bacteria for use in remediation and the determination of optimal biodegradation conditions. In this study, malathion degrading bacteria were isolated from agricultural soil samples taken from the Arvandkenar region in Iran. To optimize the biodegradation of malathion by an isolated strain, the effect of four parameters (temperature, salinity, NH_4Cl and K_2HPO_4) was evaluated while considering protein concentrations at different times. The malathion remaining in the media was measured using the gas chromatography method. A gram-negative bacterium strain BNA1 with malathion biodegrading ability was isolated from the soil sample which showed a 99% similarity to *Serratia marcescens*. The optimum biodegradation condition occurred at a temperature = 30 ˚C, salinity = 0 %, NH_4Cl = 0.25 g/L and K_2HPO_4 = 0.25 g/L. A biodegradation efficiency of 65% was obtained under the above-mentioned condition. The results of this study revealed the significant capability of BNA1 in the biodegradation of malathion. Therefore, the use of an isolated strain may be considered as an important tool in the bioremediation of pesticide-contaminated soil.

1. Introduction

Organophosphate pesticides (OPPs) are one of the most important groups of pesticides that are widely used in agriculture and landscape pest control [1]. OPPs are esters of phosphoric acid and its derivatives. The general chemical structure of its molecule is similar among OPPs, consisting of a central phosphorus atom and the characteristic phosphoric or thiophosphoric bond [2]. As a result of the global use of pesticides, various ecosystems have been contaminated [3]. For example, diazinon was found in 46.6% of the samples, while malathion was detected in 13.3% of the samples collected from Qazvin Province in Iran [4]. Moreover, organophosphorus pesticides have also been detected in Iran's surface water (e.g. Babolrood River of Mazandaran Province) [5]. Nowadays, almost all people are exposed to pesticides through environmental pollution and/or food contamination [6]. Acute exposure to OPPs can cause neurotoxic effects by inhibiting the acetylcholinesterase enzyme [7]. Yet, other toxicities (e.g. immunotoxicity, genotoxicity, and carcinogenicity) have also been observed in chronic exposure to lower doses of OPPs. Severe environmental pollution is a result of the widespread use of OPPs and since OPPs do not remain at the original sites, they can enter different ecosystems. For this reason, concerns regarding the presence of these substances in food and the environment are growing [8]. OPPs degradation is achieved by applying physio-chemical and biological methods. Microbial degradation of pesticides is

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the principle way to prevent the accumulation of these compounds in the environment [9]. Biodegradation is an attractive remediation technique and it still remains the most cost-effective and natural solution for the complete treatment of contaminated soils. The successful application of bioremediation procedures depends on the presence of suitable degrading strains and physio-chemical conditions [10]. Although numerous malathion degrading bacteria have been isolated from various environments [11, 12-13], successful bioremediation depends on environmental parameters and operational factors, which can be optimized to achieve more biodegradation efficiency. Moreover, the development of new bioremediation strategies for domestic use depends on isolating and optimizing native bacterial strains. The aim of our study was to isolate a malathion degrading bacteria from the agricultural soil of the Arvandkenar region and investigate the effect of environmental variables on the biodegradation of malathion by an isolated strain.

2. Materials and methods

2.1. Isolation and identification of malathion- degrading bacteria

Soil samples from the Arvandkenar region in Iran were collected from different areas. The samples were transferred to sterilized bottles and kept at 4˚C. About 5 g of the collected samples were transferred to a flask containing 50 ml of mineral salts medium (MSM), supplemented with 200µl economically formulated malathion (EFM). After detecting bacterial growth, 1 ml of medium was transferred to a new flask; this process was repeated four times. The MSM medium composition was set as follows: NH_4Cl, 1 g/L; K_2HPO_4, 0.5 g/L; $MgSO_4.7H_2O$, 0.2 g/L; $FeSO_4.7H_2O$, 0.01 g/L; $CaCl_2$, 0.01 g/L; and 1 ml trace mineral solution [14]. All media were incubated at 130 rpm at 35˚C on a rotary shaker. At the end of enrichment, the bacterial strains were isolated by serial dilutions on nutrient agar plates and purified strains were identified using biochemical and molecular tests. The DNA of the selected strain was extracted by a kit (Roche®-Germany). The strain identification was done through the amplification of the 16S rDNA gene by PCR using 27F (5-AGA GTT TGA TCC TGG CTC AG-3) and 1510R (5-GGT TAC CTT GTT ACG ACT T-3) primers. The reactions were cycled in a Primus 25 advanced® thermocycler with an initial denaturation step at 95˚C for 5 min followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 52˚C for 1:30 min, and extension at 72˚C for 1 min; a final extension step was at 72˚C for 15 min. The DNA sequences were compared using BLAST at:
http://blast.ncbi.nlm.nih.gov/Blast.cgi.

2.2. Malathion biodegradation by isolated strains

The biodegradation ability of purified strains on malathion was assessed from the medium in flasks containing 50 ml MSM supplemented with EFM as the sole carbon and energy source. The same medium without the inoculation of the bacteria was carried out as a control group.

2.3. Optimization of malathion biodegradation

To achieve maximum malathion biodegradation rates, optimization was performed using one factor at a time in the experimental design. The effect of the four factors, which included temperature, salinity, NH_4Cl and K_2HPO_4 concentrations, on malathion biodegradation was evaluated. The different levels of the studied factors and the order of the optimized factors are shown in Table 1.

Table 1. The different levels and order of the studied factors for biodegradation optimization of malathion by BNA1.

Factor	
Temperature	25, 30, 35 and 40˚C
Salinity	0, 0.5, 1, 1.5 and 2 %
NH_4Cl	0.250, 0.500, 0.625, 0.875 and 1.125 g/L
K_2HPO_4	0.125, 0. 250, 0.312, 0.437 and 0.562 g/L

EFM (4mL/L) was used as the sole carbon and energy source at mediums. The isolated strain with a final OD_{600nm} ≈ 0.20 was incubated to the mediums (130 rpm). The same medium without inoculation with the bacteria was used as the control group. During the optimization process, 1ml of medium of each flask was used for the analysis of protein concentrations at different times [15]. In order to evaluate the accuracy of the optimization process, the optimized culture conditions were studied.

2.4. Extraction and analysis of malathion

Firstly, the culture medium was centrifuged at 8000 rpm (9500 g) for 15 min; the supernatant was then transferred to a conical flask and 25 ml of ethyl acetate was added and shaking was performed for 15 min. The organic phase was separated by a separator funnel, and the next extraction was performed with 25 ml of the solvent. The two extracts were mixed and then dehydrated using Na_2SO_4; the volume was adjusted to 50 ml. The extracted samples were then analyzed by GC-FID (HP-5MS fused silica capillary column (30 m x 0.25 mm ID x 0.25 µ). Nitrogen was used as the carrier gas. Injector and detector temperatures were set to 250˚C. The oven temperature program was from 80˚C to 120˚C at a rate of 10˚C/min [16].

2.5. Statistical analysis

ANOVA was used to compare the effects of temperature, salinity, NH_4Cl, and K_2HPO_4 concentrations on bacterial growth rates. All analyses were performed using SPSS 16 software.

3. Results and discussion

Among isolated strains, BNA1 (i.e. gram negative, rod shaped with round creamy colony) had a positive effect on the biodegradation of malathion. Some characteristics of BNA1 are presented in Table 2.

Table 2. Differential biochemical and phenotypic characteristics of strain BNA1

Characteristic	Strain BNA1
Morphology	rod-shaped
Motility	+
Anaerobic growth	+
pH	4-10
Temperature (˚C) growth at	
4˚C	-
40˚C	+
45˚C	-
50˚C	-
Oxidase	-
Catalase	+
Gelatinase	+
Ureas	-
Citrate	+
Amylase	-
Reduction of Nitrate to Nitrite	+
Reduction of Nitrite to N_2	-
Nitrification	+
Indole	-
Growth on MacConkey Agar	+

The sequences of the BNA1 were deposited in the GenBank (Accession number: KT351729) and showed a 99% similarity to *Serratia marcescens*. It has been reported that several bacterial genera such as Bacillus [17], Stenotrophomonas sp. [18], *Enterobacter aerogenes* [13] and *Alcaligenes faecalis* [19] may participate in the efficient degradation of some OPPs molecules. Serratia sp. strains have been attracting much attention because of their wide range of biodegrading activities against a large number of chemicals such as methyl parathion [20], palmarosa oil [21], polycyclic aromatic hydrocarbons (PAHs) [22], diazinon [23], deltamethrin [24] and biosurfactant production [25]. There are also reports on the biodegradation of malathion by different strains of Serratia sp. [26]. To our knowledge, studies related to malathion degradation by *Serratia marcescens* have not been reported in Iran. Based on previous studies, environmental conditions are important for the degradation of xenobiotic materials such as pesticides [27-28]. The effects of the four medium components of temperature, salinity, nitrogen, and phosphorus sources on the biodegrading ability of BNA1 were studied. The results of a one-way ANOVA on the protein concentrations are shown in Table 3

Table 3. Results of one-way ANOVA based on the protein concentration

Factors	F	*p*-value
Temperature (˚C)	75.51	0.00
Salinity (%)	17.52	0.00
NH_4Cl (mg/L)	28.08	0.00
K_2HPO_4 (mg/L)	15.56	0.00

In general, all selected factors showed a significant affect ($p< 0.05$) on bacterial growth (Figures 1, 2, 3 and 4). The optimized levels for the considered factors were determined as follows: temperature = 30˚C, salinity = 0%, NH_4Cl = 0.25 g/L and K_2HPO_4 = 0.25 g/L. A biodegradation efficiency of 65% was achieved under the suggested conditions. Many factors affect the biodegradation rate of OPPs. Favorable growing conditions are not the same for each strain; thus, it is necessary to determine the optimal nutritional and environmental conditions for each isolated strain. Temperature is one of the major environmental variables that influence biodegradation. Moreover, bacterial metabolism may increase with an increase in temperature. In the present study, temperature was found to be a significant factor. The BNA1 biodegradation ability was active throughout the temperature range of 25-40˚C, but the best biodegradation efficiency was achieved at 30˚C. The same results have been obtained for *Acinetobacter baumannii* [29], although *Stenotrophomonas* sp. has shown higher biodegrading efficiency at higher temperatures (40˚C) [18]. The influence of salinity on biodegradation has been studied since the 1990s and is considered an important factor affecting bioremediation [30]. In the current study, salinity was another variable that had a significant effect on the biodegrading rate. We proposed 0% salinity as the optimum level for malathion biodegradation. Some researchers have reported decreased metabolism rates by increasing salinity, which could represent a negative role of ions on the metabolism rate. The amount of nutrients in the soil is usually less than the optimal level needed for the growth of microorganisms; therefore, enriching the environment with nutrients increases the efficiency of biodegradation. Adding nutrients stimulates the microorganisms to produce essential enzymes and breakdown the contaminants. The treatment of contaminated areas with nitrogen sources increases the growth rate, reducing the lag phase and helps bacteria remain at high activity levels which eventually increase the rate of biodegradation [31]. However, as the present study shows, it has been argued that nitrogen concentration has ambiguous effects and excessive levels of nitrogen sources can be harmful or ineffective [32]. K_2HPO_4 was another

variable that showed a significant effect on malathion biodegradation. Recently, Qiu *et al*. [33] reported that phosphorus concentration was a limiting factor during the bacterial degradation of atrazine and dichlobenil. In the present study, high and low concentrations of phosphorus decreased cell growth which reflects the importance of determining optimal biodegradation conditions.

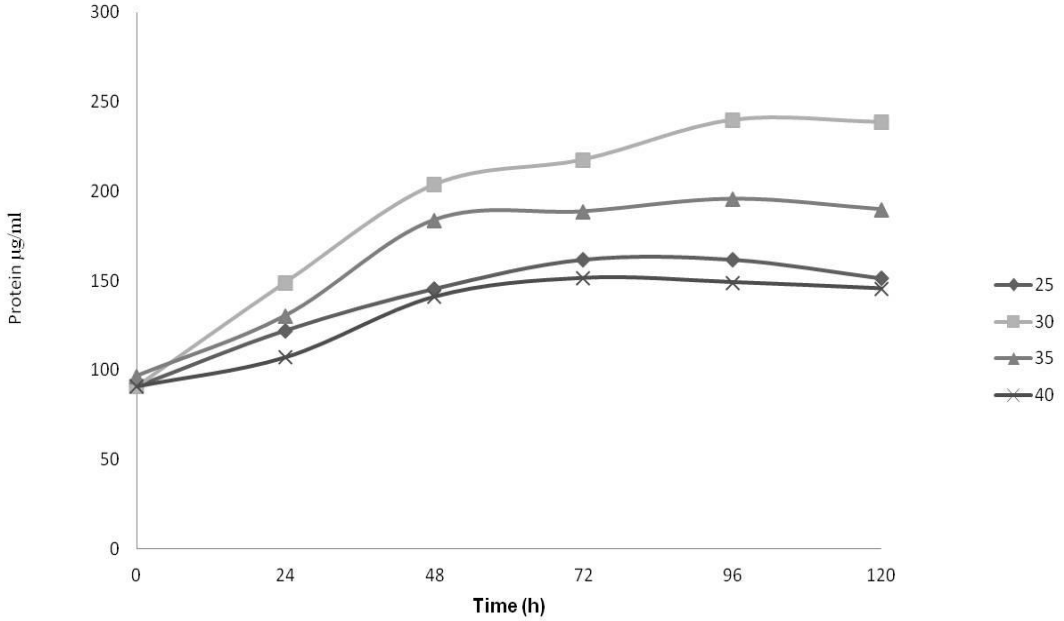

Fig. 1. Protein concentration of BNA1 during the experiment under different temperatures.

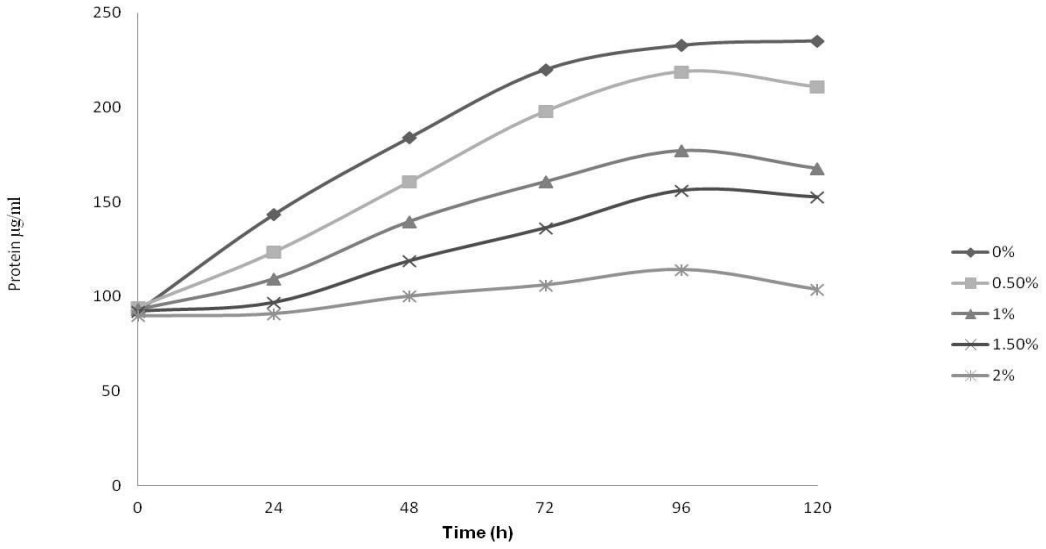

Fig. 2. Protein concentration of BNA1 during the experiment under different salinity.

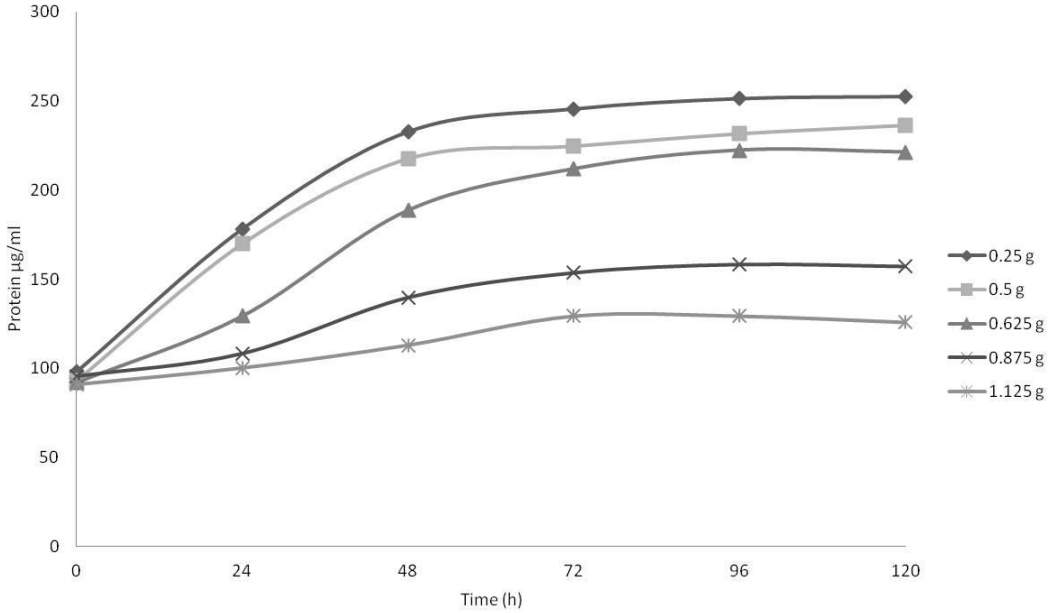

Fig. 3. Protein concentration of BNA1 during the experiment under different NH_4Cl concentrations.

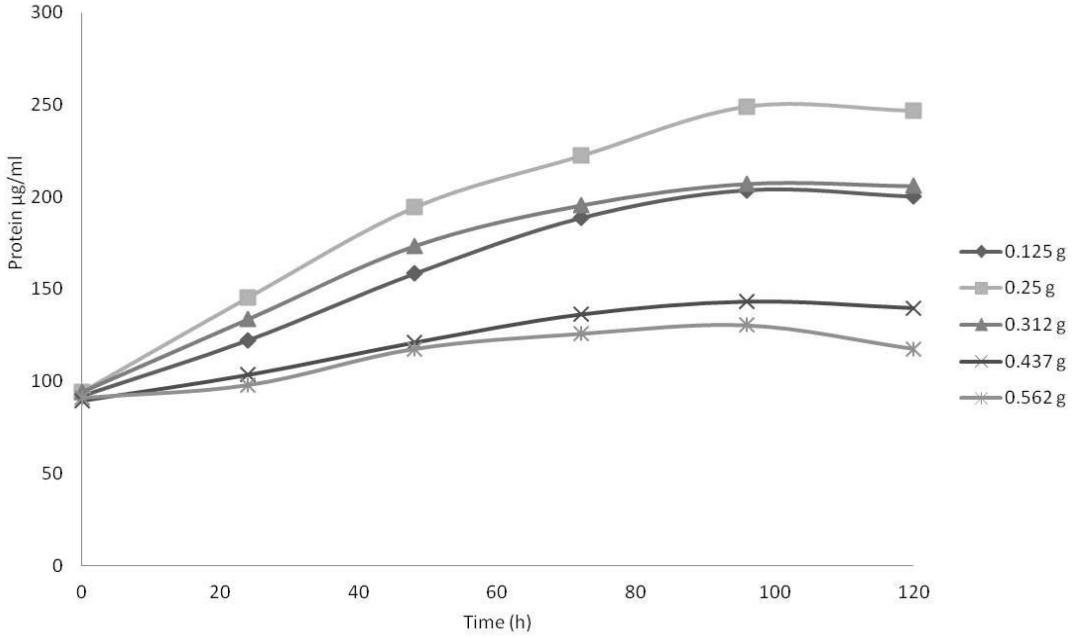

Fig. 4. Protein concentration of BNA1 during the experiment under different K_2HPO_4 concentrations.

4. Conclusions

In conclusion, our results revealed the significant capability of BNA1 to remediate malathion in polluted soil. The experimental design was effective in determining the optimal biodegradation condition. Also, an isolated strain may be considered as an important tool in the bioremediation of pesticide-contaminated soil.

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References

[1] Wu, H., Zhang, R., Liu, J., Guo, Y., Ma, E. (2011). Effects of malathion and chlorpyrifos on acetylcholinesterase and antioxidant defense system in Oxya chinensis (Thunberg) (Orthoptera: crididae). *Chemosphere*, *83*(4), 599-604.

[2] Eleršek, T., Filipič, M. (2011). Organophosphorus pesticides-mechanisms of their toxicity. *PM Stoytcheva*. 243-260

[3] Singh, B. K., Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS microbiology reviews*, *30*(3), 428-471.

[4] Karyab, H., Mahvi, A. H., Nazmara, S., & Bahojb, A. (2013). Determination of water sources contamination to diazinon and malathion and spatial pollution patterns in Qazvin, Iran. *Bulletin of environmental contamination and toxicology*, 90(1), 126-131.

[5] Shayeghi, M., Shahtaheri, S. J., & Selsele, M. (2001). Phosphorous Insecticides Residues in Mazandaran River Waters, Iran (2000). *Iranian Journal of Public Health*, 30(3-4), 115-118.

[6] González-Alzaga, B., Lacasaña, M., Aguilar-Garduño, C., Rodríguez-Barranco, M., Ballester, F., Rebagliato, M., Hernández, A. F. (2014). A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicology letters*, *230*(2), 104-121.

[7] Huen, K., Bradman, A., Harley, K., Yousefi, P., Barr, D. B., Eskenazi, B., Holland, N. (2012). Organophosphate pesticide levels in blood and urine of women and newborns living in an agricultural community. *Environmental research*, *117*, 8-16.

[8] Lukaszewicz-Hussain, A. (2010). Role of oxidative stress in organophosphate insecticide toxicity–Short review. *Pesticide biochemistry and physiology*, *98*(2), 145-150.

[9] Arbeli, Z., Fuentes, C. L. (2007). Accelerated biodegradation of pesticides: An overview of the phenomenon, its basis and possible solutions; and a discussion on the tropical dimension. *Crop protection*, *26*(12), 1733-1746.

[10] Shahriari Moghadam, M., Ebrahimipour, G., Abtahi, B., Ghassempour, A. (2013). Isolation, identification and optimization of phenanthrene degrading bacteria from the coastal sediments of Nayband Bay. *Jundishapur journal of microbiology*, *6*(9). 650-663

[11] Imran, H., Altaf, K. M., Kim, J. G. (2006). Degradation of malathion by Pseudomonas during activated sludge treatment system using principal component analysis (PCA). *Journal of environmental sciences*, *18*(4), 797-804.

[12] Abo-Amer, A. (2007). Involvement of chromosomally-encoded genes in malathion utilization by *Pseudomonas aeruginosa* AA112. *Acta microbiologica et immunologica Hungarica*, *54*(3), 261-277.

[13] Mohamed, Z. K., Ahmed, M. A., Fetyan, N. A., Elnagdy, S. M. (2010). Isolation and molecular characterisation of malathion-degrading bacterial strains from waste water in Egypt. *Journal of advanced research*, *1*(2), 145-149.

[14] Schlegel HG. (1992). Allgemeine Mikrobiologie: Auflage, Georg Thieme Verlag.

[15] Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254.

[16] Nadalian, B., Shahriari Mogadam, M., Ebrahimipour, G. H. (2016). Biodegradation of malathion using mixed culture of *Serratia marcescens* BNA1 and *Pseudomonas aeruginosa* BNA2. *Iranian journal of health and environment*, *8*(4), 525-534.

[17] Romeh, A. A., Hendawi, M. Y. (2014). Bioremediation of certain organophosphorus pesticides by two biofertilizers, *Paenibacillus* (*Bacillus*) *polymyxa* (*Prazmowski*) and *Azospirillum lipoferum* (*Beijerinck*). *Journal of agricultural science and technology*, *16*(2), 265-276.

[18] Deng, S., Chen, Y., Wang, D., Shi, T., Wu, X., Ma, X., Li, Q. X. (2015). Rapid biodegradation of organophosphorus pesticides by Stenotrophomonas sp. G1. *Journal of hazardous materials*, *297*, 17-24.

[19] Kong, L., Zhu, S., Zhu, L., Xie, H., Su, K., Yan, T., Sun, F. (2013). Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. *Journal of environmental sciences*, *25*(11), 2257-2264.

[20] Pakala, S. B., Gorla, P., Pinjari, A. B., Krovidi, R. K., Baru, R., Yanamandra, M., Siddavattam, D. (2007). Biodegradation of methyl parathion and p-nitrophenol: evidence for the presence of a p-nitrophenol 2-hydroxylase in a Gram-negative Serratia sp. strain DS001. *Applied microbiology and biotechnology*, *73*(6), 1452-1462.

[21] Mohanan, S., Maruthamuthu, S., Muthukumar, N., Rajasekar, A., Palaniswamy, N. (2007). Biodegradation of palmarosa oil (green oil) by *Serratia marcescens*. *International journal of environmental science and technology*, *4*(2), 279-283.

[22] Arbabi, M., Nasseri, S., Chimezie, A. (2009). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in petroleum contaminated soils. *Iranian journal of chemistry and chemical engineering (IJCCE)*, *28*(3), 53-59.

[23] Abo-Amer, A. E. (2011). Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. *J. microbiol biotechnol*, *21*(1), 71-80.

[24] Cycoń, M., Żmijowska, A., Piotrowska-Seget, Z. (2014). Enhancement of deltamethrin degradation by soil bioaugmentation with two different strains of *Serratia marcescens*. *International journal of environmental science and technology*, *11*(5), 1305-1316.

[25] Montero-Rodríguez, D., Andrade, R. F., Ribeiro, D. L. R., Rubio-Ribeaux, D., Lima, R. A., Araújo, H. W.,

Campos-Takaki, G. M. (2015). Bioremediation of petroleum derivative using biosurfactant produced by *Serratia marcescens* UCP/WFCC 1549 in low-cost medium. *International journal current microbiology and applied sciences, 4*(7), 550-562.

[26] Kannan, V., Vanitha, V. (2005). Influence of assay medium on degradation of malathion by *Serratia marcescens*. *Indian journal of biotechnology*, *4*(2), 277-283.

[27] Ortiz-Hernández, M. L., Sánchez-Salinas, E. (2010). Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Revista internacional de contaminación ambiental, *26*(1), 27-38.

[28] Bidlan, R., Manonmani, H. K. (2002). Aerobic degradation of dichlorodiphenyltrichloroethane (DDT) by *Serratia marcescens* DT-1P. *Process biochemistry*, *38*(1), 49-56.

[29] Azmy, A. F., Saafan, A. E., Essam, T. M., Amin, M. A., Ahmed, S. H. (2014). Biodegradation of malathion by *Acinetobacter baumannii* Strain AFA isolated from domestic sewage in Egypt. *Biodegradation*, *34*(5), 55-65.

[30] Li, H., Hu, L., Xia, Z. (2013). Impact of groundwater salinity on bioremediation enhanced by micro-nano bubbles. *Materials*, *6*(9), 3676-3687.

[31] Shahriari Moghadam, M., Ebrahimipour, G., Abtahi, B., Khazaei, N., Karbasi, N. (2014). Statistical optimization of crude oil biodegradation by Marinobacter sp. isolated from Qeshm Island, Iran. *Iranian journal of biotechnology*, *12*(1),35-41.

[32] Zhou, E., Crawford, R. L. (1995). Effects of oxygen, nitrogen, and temperature on gasoline iodegradation in soil. *Biodegradation*, *6*(2), 127-140.

[33] Qiu, Y., Pang, H., Zhou, Z., Zhang, P., Feng, Y., Sheng, G. D. (2009). Competitive biodegradation of dichlobenil and atrazine coexisting in soil amended with a char and citrate. *Environmental pollution*, *157*(11), 2964-2969.