Improvement of biodegradability of explosives using anaerobic- intrinsic bioaugmentation approach

M.M. Amin[1], H. Khanahmad[2], F. Teimouri[3]*, M. Sadani[4], M.A. Karami[5], I. Rahimmanesh[2]

[1]*Environment Research Center, Research Institute for Primordial Prevention of Non- communicable disease, Isfahan University of Medical Sciences, Isfahan, Iran, and Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran*

[2]*Department of Genetics, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

[3] *Isfahan University of Medical Sciences, Isfahan, Iran. Health Faculty, Yazd University of Medical Sciences, Yazd, Iran.*

[4]*Shahid Beheshti University of Medical Sciences, Tehran, Iran.*

[5]*Lorestan University of Medical Sciences, Khoramabad, Iran.*

A successful anaerobic intrinsic bioremediation (bioaugmentation) was carried out on 2,4,6-trinitrotoluene (TNT) and Pentaerhytritol tetranitrate (PETN) contaminated soil, using indigenous bacteria. Two soil pans were enriched by sewage sludge and one pan was mixed by monorhamnolipid biosurfactant both of which are economically suitable substrates for anaerobic in situ explosive bioremediation. Preliminary concentrations of TNT and PETN were 1000 and 200 mg/kg. The results of this study showed that in order to increase the explosives degradation with more resistant to biodegradation such as PETN, the usage of biosurfactant could be effective. Inoculation of indigenous bacteria had a significant effect on TNT and PETN remediation efficiency and increased them to 99.1% and 91% in the presence of biosurfactant. Seven indigenous strains were identified as *Planomicrobacterium flavidum, Pseudomonas auroginosa, Entrobactor asburiae, Azospirillium, Rhizobium, Methylobacterium and Pseudomonas denitrificant* strains. It is logical that these isolates may have potential for TNT and PETN degradation. Monorhamnolipid might be effective in the improvement of explosives degradation due to impact on the cell membrane of bacteria. The results of this study have shown that intrinsic bioremediation has the potential to reduce the time and costs for in situ explosive bioremediation.

Keywords: Bioaugmentation - intrinsic bioremediation - 2,4,6-trinitrotoluene - Pentaerhytritol tetranitrate

INTRODUCTION

The large scale manufacturing and use of a variety of synthetic chemicals continuously pollutes soil, water, and air which have direct or indirect adverse impacts on our and animals' health[1]. They have significant concerns such as carcinogenicity and potential for bioaccumulation in living systems [2]. According to the estimated annual production of 108 tones, nitro-aromatics are considered as important industrial chemicals [3]. Environmental pollution by explosive residues from TNT and NG is widespread and causes long term health problems. Average contaminated sites may contain more than 10 g of TNT per kg in soil and 100 mg/l in water. TNT and metabolites have high potential for toxicity and mutation on prokaryotes and eukaryotes. It has been estimated that 3200 sites in Germany require environmental management [1]. Toxicity of Explosives is exhibited by symptoms such as irritation, methemoglobinaemia, disturbed heart function, kidney trouble and malfunction of vascular system [2]. Exposure to TNT is known to cause rashes, mucus and blood disorders. Toxic effects such as liver damage and anemia have been reported by workers who worked in manufacturing and handling of TNT [4]. The harmless concentration of TNT in the soil is <30mg/kg [5]. PETN is widely used as a powerful explosive and is classified as a great concern by DoD in the U.S.A. Short term exposure to PETN may affect the cardiovascular system, resulting in a decrease in blood pressure. PETN is known to be "toxic to aquatic organisms" by U.S DoD because of its wide spread use and the potential environmental impact [6]. Several physico-chemical methods [7-11] are available for explosive remediations from aqueous solutions. Some methods such as incineration are currently used, but are expensive and may lead to the formation of by-products that are more toxic than the primary compounds. Biological methods are more cost-effective and reduce toxicity of the soil due to the enzymes produced by specific bacteria [12]. Bioremediation has been considered as a valuable option for remediation of explosive-contaminated soil. Natural bacteria are present in the environment [13] and have an exceptional ability to exploit various compounds for their growth. Most organisms contain redox enzymes, which are able to transform nitro-aromatics to amines. Several enzymes have roles in biodegradation of explosives, but nitro-reductase enzymes are very important since they help with the detoxification of nitro-aromatic

compounds [14]. However, enzymes for complete breakdown of nitro-compounds are rare [3]. It seems that anaerobic metabolism of nitro-aromatics may provide a treatment for contamination with nitro-compounds, while most of the aerobic studies showed only the modification of these compounds [15]. The biodegradation of TNT under anaerobic conditions occurs by reduction of the nitro group to form the corresponding mononitroso, monohydroxylamino and monoamino derivatives. These monoamino derivatives were further transformed into diamino and triamino derivatives through a reductive mechanism [16-19]. Also, other studies have shown that denitrifying bacteria are able to reduce PETN to precursor derivates [20]. Overall, the general goals for bioremediation are to enhance indigenous bacteria activities by the addition of nutrients or aeration (biostimulation) or the addition of microorganisms (bioaugmentation). Bioaugmentation has proven for remediation of PAHs in sediments with poor intrinsic degradation potential [21]. Hence, for efficient and complete biodegradation, solubilization of hydrocarbons with biosurfactants prior to bioaugmentation is advantageous [22]. Many studies related to bioaugmentation have been based on the use of certain species which were isolated from contaminated soils [23]. Although isolated bacteria can degrade explosives, most authors accept the importance of the growth of natural consortia rather than select specific strains. The latter may survive under laboratory conditions, but usually could not survive or grow in full scale conditions [8]. Nowadays, more than 10000 km^2 of land area in the border provinces of southern-southwest and west of the country of Iran are affected by pollution caused by Iran-Iraq war. Despite the fact that we are over three decades past the war, large areas are not suitable for human applications. Khuzestan is one of the provinces which were repeatedly invaded by Iraqi army and in which soil contamination is higher than other cities. Geographic and climatic characteristics show that most of these areas are located in the wet zone, low rainfall and high humidity [24].

In this study, the natural explosive degrading bacteria in the soil were isolated and inoculated into the soil. The advantages of this method may be that the natural bacterial species in the soil do not change. Considering that a large area of the country is contaminated with explosives, another main purpose of this study was to perform the least expensive and most efficient way so that it can be offered on a large-scale as in situ bioremediation. Characterization of explosives degradation bacterial

community, isolation and the influence of dominant indigenous bacteria inoculation on degradation rates (bioaugmentation effect), were the other objectives that were evaluated in this study.

EXPERIMENTAL

Biosurfactant

Monorhamnolipid biosurfactant was purchased from the National Institute for Genetic Engineering and Biotechnology, Institute of Chemistry and Chemical Engineering, which was produced through fermentation by P. aeruginusa strains. The concentration of used rhamnolipid in this study was 120 mg/l [25].

Chemical analysis

TNT and PETN were analyzed using an HPLC system, a Model 486 UV detector and a Nova pak C_{18} guard column. The analytical column was an ODS_2 optimal column (25cm × 4.6 mm id, 5μm) from capital HPLC. The sample was injected into the HPLC system with the following condition: Acetonitrile- water mixture (75:25 v/v) as the mobile phase at a flow rate of 1.0 ml/min. Injection volume for all samples was 20 μl and the wavelength for the UV detector was 210 nm and 230 nm for PETN and TNT detections, respectively. All trace analysis quality or gradient grade solvents were purchased from Merck Company.

Soil sampling, preparation and extraction

After screening, the soil was manually contaminated with explosives. TNT concentrations in the soil were 1000 mg/kg and PETN concentration was 200 mg/kg. The soil had a pH of 6.4. Sampling of the soil was taken periodically during the experiment. Grab sampling was taken from top 3 cm of soil and dried before analyses. Sample preparation was performed according to EPA method 8330. Total extractable explosives were determined by drying 10 g of the homogenized soil pan in ambient air and transferring it to an Erlenmeyer. Acetonitrile (20 ml) was added and vial was placed in a shaker for 18 hr. Then 5 ml of supernatant was filtered through PTFE filter and ultimately explosives were quantified by the HPLC system [26].

Preparation of anaerobic soil pans

Three soil pans were used in this experiment. A pan set consisted of a plastic pan (30cm × 20 cm × 15cm in height) that was placed in a slightly larger pan. The bottom of smaller pans was perforated with 2-mm- diameter holes spaced 8 cm apart to allow for the drainage of fluids. Fluid drainage was recycled

to each pan again. Each small pan contained 4 kg of contaminated soil. In order for soil amendment to occur, screened saw dust was used (soil/saw dust ratio was 1:1). Water was added not only for supplementing the moisture but also for creating and keeping the anaerobic condition, once a week (to maintain a free water surface 2 cm above the surface of the soil).

Three treatments that were investigated in this study include:

1- A pan consisted of contaminated soil + activated sludge as an enrichment for the soil (soil/sludge ratio was 1:0.25) which was only added at the startup of the pan operation.

2- A pan consisted of contaminated soil + activated sludge as an enrichment for the soil (soil/sludge ratio was 1:0.25) + monorhamnolipid biosurfactant in which the two latter (sludge and biosurfactant) were only added at the beginning of pan operations.

3- A pan served as the control in which no substrate was added and explosives were used as the main substrates.

Total DNA extraction

In this study, the boiling method for extraction of DNA was used, because a number of studies show that by performance of this method, good quality of DNA could be obtained. Isolated colonies were grown on nutrient agar plated, mixed with sterile 100 μl Milli – Q water and boiled for 15 min. Then, the solution was centrifuged at 13000 rpm for 10 min and its supernatant was used as the DNA template for PCR analyses [12].

Amplification of DNA

After extraction of total DNA from the soil, bacterial 16srDNA was amplified with universal eubacterial primers that consist of F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′- TACGGYTACCTTGTTACGACT-3′), which are targeted to universally conserved regions and permit the amplification of an approximately 1,500-bp fragment [27]. PCR reactions were carried out in 25 μl of microtubes consisting of 1μl of each primer, 2.5 μl 10 × buffer, 1μl $MgCl_2$, 0.5 μl dNTPs, 17.5 μl distilled sterile H_2O, 0.5 μl Taq DNA polymerase and 1 μl of extracted DNA. Thermocycling conditions were as follows: samples were heated at 95ºC for 5 min (1 cycle), 94ºC for 30 sec, 58ºC for 30 sec, 72ºC for 1 min (30 cycles) and 72ºC for 5 min. PCR products were visualized on a 1% agarose gel and quantified with an appropriate molecular weight marker.

Bioaugmentation experiments

In this stage of experiment, natural floras (dominant indigenous degrading bacterial population), which have already been isolated and identified by the PCR method were used. These microorganisms were multiplied under sterile conditions by nutrient broth and incubated (37ºC for 24 hr). Then, optical density was measured by spectrophotometer and inoculated to the anaerobic pans. In order to maintain anaerobic conditions, water was added to each pan once a week.

RESULTS AND DISCUSSION

Effect of Intrinsic Bioaugmentation on TNT and PETN transformation rates

First, microorganism adaptation was performed about three months (TNT and PETN concentrations were 200 and 50 mg/kg respectively). After the degradation rate became constant, the operation of the pans was performed by increasing the TNT and PETN concentrations to 1000 and 200 mg/kg of soil. Figure 1 and 2 shows the effect of bioaugmentation of indigenous bacteria on TNT and PETN degradation rates.
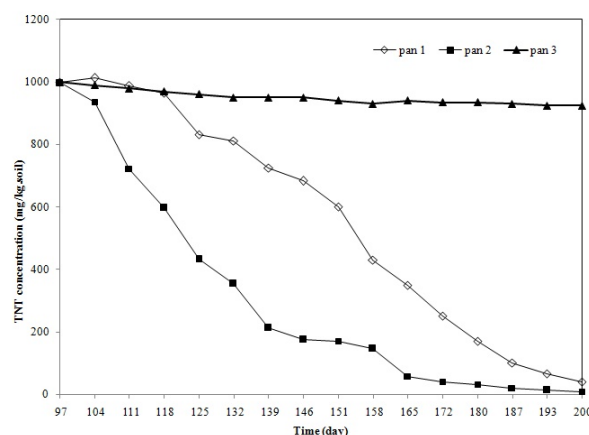

Fig. 1. Effect of intrinstic bioaugmentation on TNT degradation rate

As shown in Figure 1, with the passage of time, TNT degradation rate was decreased and became almost constant. The maximum degradation efficiency was about 40% and 83% in pans 1 and 2, respectively. On the other hand, TNT concentration decreased to 543 and 162 mg/kg in pans 1 and 2. Also, PETN concentration decreased to 142 and 63 mg/kg in pans 1 and 2, respectively (Fig.2).
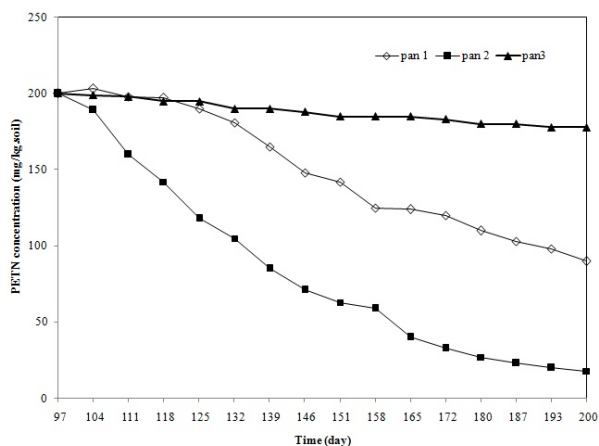
Fig. 2. Effect of intrinstic bioaugmentation on PETN degradation rate

However, as Figure 2 shows, according to the chemical structure, PETN has shown greater biological resistance and its decomposition rate was lower than TNT decomposition rate. After two months of pan operations, degradation rates were relatively constant. Then, bioaugmentation effect was investigated by the inoculation of dominant degrading bacteria that have already been identified by the PCR method. After bioaugmentation, TNT concentration decreased to 40 and 9 mg/kg with the removal percentage equivalent to 96% and 99.1% in pans 1 and 2, respectively (Fig.1). Also, bioaugmentation had a positive effect on PETN degradation rate, as PETN concentration decreased to 90 and 18 mg per kg, the equivalent of 55 and 91 percent in pans 1 and 2, respectively (Fig.2) . In general, the results show that PETN degradation rate is less than TNT degradation. However, inoculation had a positive effect on two explosive degradation, especially in the second pan. It can be interpreted that more degradation rate in pan 2 is related to the presence of monorhamnolipid biosurfactant, since it would be able to increase bioavailability of microorganisms to pollutants and also could be able to change cell membrane permeability of bacteria. This results is confirmed by Muter et al. [23] who reported that bioaugmentation has a significant effect on soil samples with high initial concentrations of TNT (500 mg/kg), in particular for soil samples amended with 50% and 100% nutrient solutions. Also, Elis et al. [28] demonstrated the value of bioaugmentation when evidence indicates the absence of organisms which are capable of complete conversion of cis-DCE to ethene. Also, Zhang and Miller [29] reported that 300 mg/l rhamnolipid increased the mineralization of octadecane about 4 times more than initial value. The results obtained by Manickam et al. [30] confirmed that the halogenated compounds

biodegradation efficiencies were increased by 30-50% in 2 days compared to degradation in the absence of biosurfactant. In the control pan (pan 3), the TNT and PETN concentrations remained around 1000 and 200 mg/kg throughout the study indicating the removal of TNT in the soil was biological and was not a chemical or physical process.

Nitrite release from TNT and PETN transformations

Nitrite measurement was performed to detect the release of nitro-group from TNT and PETN during demineralization. The results of nitrite assays are presented in Figure 3. As can be seen, before the microbial inoculation, native soil bacteria were able to use TNT and PETN as the only sources of nitrogen that have been responsible for the disappearance of explosives in the soil.
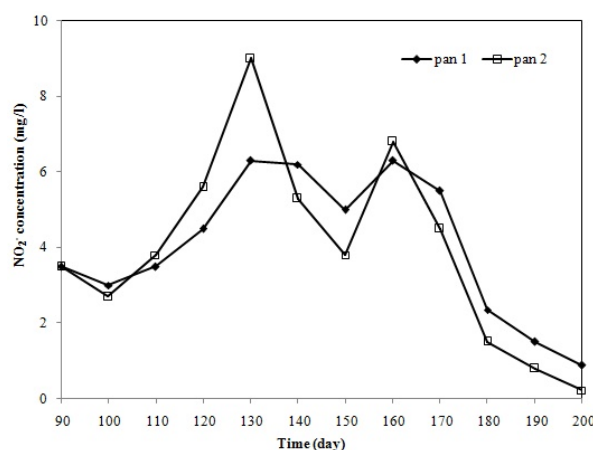

Fig. 3. Nitrite concentrations released during TNT and PETN demineralization

The first step in anaerobic metabolism of nitro-aromatics is reduction. Then, deamination occurs, which removes all nitro groups linked to the ring. Finally, toluene and ammonia form as end products. Of course, toluene could be degraded by denitrifiers or other microorganisms [15]. After inoculation of dominant bacterial populatioin, nitrite concentrations increased and then decreased to 0.89 and 0.22 mg/l in pans 1 and 2, respectively. Nitro-explosives are typically biodegraded by one or more known mechanisms. One or more nitro groups can be reduced to hydroxylamino groups and then N-N bond may be cleaved, releasing a nitro group to nitrite ion as a final product [1]. Nitrite ion is an unstable form of nitrogen and may quickly be converted into ammonium ion and ammonia. This result also confirmed the role of biosurfactant in the improvement of the degradation process due to the higher nitrite concentration in pan 2 than in pan 1. Also, the inoculated bacterial population had a major responsibility for mineralization of these

compounds. This result is in accordance with those obtained by Boopathy and Kulpa [19] that demonstrated certain Pseudomonas sp. can use TNT as a nitrogen source through the removal of nitrogen nitrite from TNT and the further reduction of the released nitrite to ammonium which is incorporated into carbon skeleton. Wittch et al. [see 4] 333noted that unstable reduced derivatives of TNT produced by microorganisms have been found to release nitrite by rearomatization and/or condensation.

Identification of Isolates

PCR analysis has become one of the most popular technologies for the biodiversity assessments. In this study, PCR technology was used to identify the bacterial community structure in the system after 150 days of adaptation and operation. Visualized gel picture of a 16 S rDNA picture for TNT and PETN degrading isolates have been shown in Figure 4.
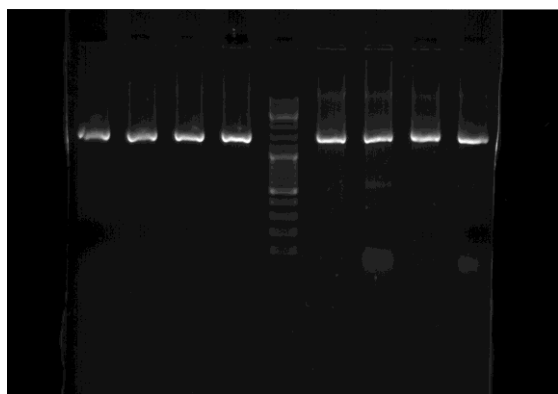

Fig. 4. Visualized gel picture of a 16 S rDNA picture for TNT and PETN degrading isolates

As seen in Figure 4, all targeted DNA had amplification of an approximately 1,500-bp fragment. Seven bacterial strains were isolated from anaerobic pans. In pan 1, two bacterial isolates were capable of growing and were identified as Planomicrobacterium flavidum strain and Pseudomonas auroginosa sp.JB2. Differential experiments show that anaerobic pan 2 had the highest number of bacterial strains. In pan 2, five bacterial isolates were identified as Entrobactor asburiae, Azospirillium, Rhizobium, Methylobacterium and Pseudomonas denitrificant strains**.** Bacteria isolated in this study show that indigenous bacteria are capable of using TNT and PETN as sole sources of nitrogen and energy. Previous studies show that degrading strains are often species of Pseudomonas and rhodococcus that are able to degrade herbicides, phenols, polyaromatic hydrocarbons etc [31]. These results are in agreement with those of Hoffsommer et al (1978) in Naval Surface Weapons Center who reported that TNT in the laboratory under control

condition could be biologically transformed into amin isommers with the pure bacterial strains such as Ps. denitrificans [32] and those of Zhang et al [33] who isolated Azospirillium zeae and Rhizobium to treat TNT in red water. He found that Azospirillium might play a key role in reducing the concentration of ammonium. The latter had the ability of nitrogen fixation in the bioreactor [33]. The biotransformation processes ultimately depend on enzyme action. Identification of the enzymes capable of transforming explosives is necessary for bioremediation. Glenn et al. [34] reported that Ps. auroginosa JB2 could degrade 2,4- Dinitrotoluene and aromatics via deoxygenase enzyme and Aken et al. [35] found that Methylobacterium strain have the capacity to metabolize TNT, RDX and HMX explosives. Previously, Bink et al. [36] have reported that Entrobactor Cloacae PB2 isolated from explosive contaminated soil containing only PETN as a nitrogen source. In this study, this is the first report of Entrobactor asburiae and Planomicrobacterium flavidum strains with the ability to degrade TNT and PETN as sole nitrogen and energy sources and therefore it is logical that these isolates may have the potential for TNT and PETN degradation by enzymes potential production (such as nitroreductase, deoxygenase,..). The other isolates have been described to be able to degrade explosives. The results suggest that the indigenous bacteria can effectively degrade explosives in anaerobic conditions.

CONCLUSION

The results of the study demonstrate that TNT and PETN are biodegradable under anaerobic conditions by indigenous bacteria in the presence of sewage sludge as enrichment source, but monorhamnolipid biosurfactant can rapidly increase TNT and PETN degradation rates so that TNT and PETN degradation rates increased to 83% and 68.5% in comparison with 40% and 29% in the absence of biosurfactant. Inoculation of indigenous bacteria have a significantly positive effect on the efficiency of the remediation process, to the extent that TNT removal efficiencies increased to 96% and 99.1% (TNT concentration was less than 30 mg/kg) and PETN removal efficiencies increased to 55% and 91% in pans 1 and 2, respectively. TNT removed significantly higher than PETN in anaerobic conditions. Seven indigenous strains were more potent for explosives degradation which was identified as *Planomicrobacterium flavidum, Pseudomonas auroginosa, Entrobactor asburiae, Azospirillium, Rhizobium*, *Methylobacterium* and *Pseudomonas denitrificant* strains.

Monorhamnolipid biosurfactant might be effective in the improvement of degradation due to the impact on the cell membrane of bacteria. However, knowledge of the metabolic pathways and enzymes is needed so that the microorganisms can degrade explosive compounds more efficiently and effectively.

REFERENCES

1. S.N. Singh, Biological remediation of explosive residues. Springer, Switzerland, pp.179-180, 2014.
2. A. Singh, R.C. Kuhad, O.P. Ward, Advances in applied bioremediation. Springer, Switzerland, pp. 38-43, 2009.
3. M. Kulkarni, A. Chaudhari, *J. Environ. Manage.*, **85,** 496 (2007).
4. A.G. Rahal, L.A. Moussa, *Aust. J. Basic Appl. Sci.*, **5,** 8 (2011).
5. N. Čėnas, A. Nemeikaitė-Čėnienė, A. Marozienė, J. Šarlauskas, V. Vilutienė, J. Baublys, EUExcert (Certifying Expertise in European Explosives Sector), 2001, p. 1.
6. L. Zhuang, Ph.D Thesis, University of Waterloo, 2007.
7. R.J. Spanggord, C.D. Yao, T. Mill, *Environ. Sci. Technol.*, **34,** 497 (2000).
8. J.D. Rodgers, N.J. Bunce, *Water Res.*, **35,** 2101 (2001).
9. P. Kanekar,P. Dautpure, S. Sarnaik, *Indian J Exp Biol.*, **41,** 991 (2003).
10. S.M. King, Dessertation, University of Orlean, 2012.
11. G.El Diwani, S. El Rafie, S. Hawash, *Intl J Environ. Sci. Technol* , **6,** 619(2009).
12. N.E. Georgie, Ph.D Thesis, RMIT University, 2011.
13. P. Innemanová, R. Velebová, A. Filipová, M. Čvančarová, P. Pokorný, J. Němeček, New biotechnology, 32, 6, 702 (2015).
14. M. Erkelens, E. M.Adetutu, M. Taha, L. Tudararo-Aherobo, J. Antiabong , A. Provatas, *J. Environ. Manage,* **110,** 69 (2012).
15. R. Boopathy, C.F. Kulpa, J. Manning, *Bioresour. Technol.,* **63,** 81 (1998).
16. T. Borch, W. P. Inskeep, J. A. Harwood, R. Gerlach, *Environ. Sci. Tech.*, **39,** 7126 (2005).
17. S.S.-B. Moshe, Z. Ronen, O. Dahan, N. Weisbrod, L. Groisman, E. Adar, *Environ. Pollut.*, **157,** 2231 (2009).
18. H.-Y. Kim, G.N. Bennett, H.-G. Song, *Biotechnology Letters.*, **24,** 2023 (2002).
19. R. Boopathy, C.F. Kulpa, *Can.J. Microbiol.*, **40,** 273 (1994).
20. L. Zhuang, L. Gui, R.W. Gillham, *Chemosphere.*, **89,** 810 (2012).
21. A.L. Juhasz, R. Naidu, *Int. Biodeterior. Biodegrad.,* **45,** 57 (2000).
22. S.N. Singh, and R.D. Tripathi, Environmental bioremediation technologies: Springer Science & Business Media, 2007.
23. O. Muter, K. Potapova, B. Limane, K. Sproge, I. Jakobsone, G. Cepurnieks, *J. Environ. Manage.*, **98,** 51(2012).
24. M.M. Amin,, M. Giahi, M. Mansourian, *Int. J. Environ. Health Eng.*, **4,** 16 (2015).
25. E. Kaczorek, S. Moszyńska, A. Olszanowski, *Biodegradation*, **22,** 359 (2011).
26. A.A.C. Numbera. Method 8330B Nitroaromatics and Nitramines Esters by High Performance Liquid Chromatoghraphy (HPLC), 2006.
27. L. Muckian, R. Grant, E. Doyle, N. Clipson, *Chemosphere.*, **68,** 1535 (2007).
28. D.E. Ellis, E. J. Lutz, J. M. Odom, R. J. Buchanan, C. L. Bartlett, M. D. Lee, *Environ. Sci. Technol.*, **34,** 2254 (2000).
29. Y. Zhang, R.M. Miller, *Applied and Environmental Microbiology.*, **58,** 3276 (1992).
30. N. Manickam, A. Bajaj, H.S. Saini, R. Shanker, *Biodegradation*, **23,** 673 (2012).
31. R. Behki, E.Topp, W. Dick, P. Germon, *Appli. Environ. Microbiol.*, **59,** 1955 (1993).
32. J. Hoffsommer, J. Kaplan, D. Glover, D. Kubose, C. Dickinson , Biodegradability of TNT: a three-year pilot plant study, DTIC Document, NAVAL SURFACE WEAPONS CENTER. Dahlgren,Virginia, 22448, 1978,
33. M. Zhang, G.-h. Liu , K. Song, Z. Wang, Q. Zhao, S. Li, *Chem. Eng. J.*, **259,** 876 (2015).
34. G.R. Johnson, R.K. Jain, J.C. Spain, *J. Bacteriol.*, **184,** 4219 (2002).
35. B. Van Aken, J.M. Yoon, and J.L. Schnoor, *Appli. Environ. Microbiol.*, **70,** 508 (2004).
36. P.R. Binks, C.E. French, S. Nicklin, N.C. Bruce, *Appli. Environ. Microbiol.*, **62,** 1214 (1996).

ПОДОБРЕНА БИОДЕГРАДАЦИЯ НА ЕКСПЛОЗИВИ ЧРЕЗ АНАЕРОБНО БИОУСКОРЯВАНЕ

М.М. Амин[1], Х. Канахмад[2], Ф. Теймури[3]*, М. Садами[4], М.А. Карами[5], И. Рахимманеш[2]

[1]*Изследователски център по околна среда, Изследователски институт за предпазване от незаразни болести; Департамент по екологично здраве, Медицински университет в Исфахан, Иран*
[2]*Департамент по генетика, Училище по медицина, Медицински университет в Исфахан, Иран*
[3] *Исфахански университет по медицински науки, Исфахан, Иран. Факултет по здравеопазване, Университет по медицински науки "Язд", Язд, Иран.*
[4] *Университет по медицински науки Шахид Бехещи, Техеран, Иран·*
[5] *Университет по медицински науки Лорестан, Хорамабод, Иран·*

(Резюме)

Извършена е успешна анаеробна биоремедиация на 2,4,6-тринитротолуен (TNT) и на пентаеритритол тетранитрат (PETN), заразени с нативни бактерии. Две почвени проби са обогатени с активна утайка, като едната от тях се смесва с биосърфактанта моно-рамнолипид. И двата субстрата са подходящи за *in situ* биоремедиация на експлозиви. Началните концентрации на TNT и PETN бяха1000 и 200 mg/kg. Резултатите от това изследване показват използването на биосърфактанта води до повишаване биодеградацията на резистентен експлозив, като PETN. Инокулирането с нативни бактерии има значителен ефект за биодеградацията на TNT и PETN, която достига 99,1 и 91 % в присъствие на биосърфактант. Седем микробни щама са идентифицирани като *Planomicrobacterium flavidum, Pseudomonas auroginosa, Entrobactor asburiae, Azospirillium, Rhizobium, Methylobacterium и Pseudomonas denitrificans*. Монорамнолипидът е ефективен за подобряване деградацията на експлозивите поради въздействието му въз клетъчните мембрани. Резултатите от това изследване показаха, че присъщата биоремедиация има потенциал за намаляване на времето и разходите при *in situ* обезвреждането на експлозиви.