

# Improved removal of Trinitrotoluene (TNT) from contaminated soil by inducing aerobic process: kinetic and chemical byproducts

Mohammad Amin Karami<sup>1</sup>, Bahram kamarehie<sup>1</sup>, Mansour Ghaderpoori<sup>1</sup>, Ali Jafari<sup>1</sup>, Ahmad Akrami<sup>2</sup>✉, Fatemeh Baghban Shahri<sup>2</sup>

1. Nutrition Health Research Center, Department of Environment Health, School of Health, Lorestan Medical Sciences University, Khoramabad, Iran
2. Department of chemistry, Tehran medical sciences branch, Islamic Azad university, Tehran, Iran

**Date of submission:** 31 Dec 2016, **Date of acceptance:** 13 Nov 2017

## ABSTRACT

This study describes the biological degradation of TNT by using induced aeration. Three plastic reactors were used. In each reactor 3 kg of soil were used. In order to increase the porosity of the soil, sawdust was added to soil. Textile wastewater treatment plant sludge was also added to soil. TNT at the concentrations of 1000 mg/kg of soil was added thereafter. Rhamnolipid biosurfactant at the concentration of 60 mg/L was added to related reactors. Aeration interval was at every 3 to 5 days. Every two weeks, sampling of soil were done to analyze the explosives. Samples were analyzed by HPLC. The results showed that at the end of 120 days, TNT removal efficiency in induced aeration in reactors containing sludge and biosurfactant was 98 percent. COD removal efficiency in induced aeration in reactors amended by rhamnolipid was 58 percent and in reactors to which rhamnolipid was not added was 41 percent. Follow-up kinetic studies revealed that explosives removal follow the pseudo first order reaction. The pseudo first-order rate constants of rhamnolipid amended experiments were at least 3.89 orders of magnitude higher for TNT than those found for experiments without rhamnolipid. Application of Rhamnolipid biosurfactant could have a protective effect against the toxicity of explosives for bacteria. Textile sludge from wastewater treatment plant can decrease the time needed for explosive removal. Growth of bacteria and degradation of explosives showed that explosives have been used as a nitrogen source.

**Keywords:** TNT, sludge, rhamnolipid, induced aeration

## Introduction

A large number of chemical pollutants entering into the environment through industrial and agricultural activities, have potential risk to living systems in terms of toxicity, carcinogenicity and ability for accumulation in organs.<sup>1</sup> At present, thousands of organic compounds are used and only a few of them have been characterized in terms of their effects on human health and the environment. Among these compounds, explosives are of great concern. Explosives, chemical or a combination of chemicals, release high amounts of energy during explosion.<sup>2</sup> Among explosive

compounds, 2,4,6-trinitrotoluene (TNT) is the predominant contaminant at ammunition plants, testing facilities and military zones. Exposure to TNT causes detrimental health effects including allergies, liver damage, skin irritation, anemia, toxicity and carcinogenicity.<sup>3,4</sup> The EPA has classified TNT as class C (possible human carcinogen) in drinking water equivalent levels of 20 mg/L.<sup>4</sup> Due to relatively low water solubility (140 mg/l at 25 °C),<sup>2</sup> TNT is a relatively persistent chemical.<sup>5</sup> With regard to high prevalence of TNT to soil particles, its removal from the soil by eco-friendly and cost-effective methods is necessary.<sup>6</sup> Several methods such as burning, biological removal, advanced oxidation, and oxidation with zero valent have been practiced for explosives removal.<sup>6</sup> Burning and biological removal are the most common methods for explosives removal. Since the cost of soil excavation and

✉ Ahmad Akrami  
ahmadakrami@gmail.com

**Citation:** Improved removal of Trinitrotoluene (TNT) from contaminated soil by inducing aerobic process: kinetic and chemical byproducts. J Adv Environ Health Res 2017; 5(3): 139-145

energy consumption in burning method is high, the usage of this method on explosives removal is not cost-effective.<sup>6,7</sup> Compared with other methods, biological methods for remediation of explosive contaminated sites can be more cost-effective and significantly reduce toxicity of the soil. Moreover, microorganisms can utilize explosives as a source of carbon and nitrogen.<sup>8</sup> However, relatively low solubility of explosives<sup>9</sup> could be a limiting factor in their biodegradation. Thus addition of external agents such as surfactants to contaminated soil, at concentrations above their critical micelle concentration (CMC) values, can be a feasible approach to enhance the solubility and therefore, increase their biodegradation.<sup>10</sup> During the past decade, application of biosurfactants has increasingly improved as possible candidates because of their biodegradability, lower toxicity and greater diversity than the available synthetic surfactants.<sup>11</sup> In recent years, rhamnolipids (a common biosurfactant) have been used commonly in many industries such as petrochemical, pharmaceutical, biomedical and food processing industries. Rhamnolipid not only increased the solubility of hydrophobic compounds, but also reduced the toxicity to bacteria leading to increase the biodegradability of hydrophobic compounds.<sup>12, 13</sup>

Accessibility to nutrients are another important factor in explosives bioremediation other than bioavailability. Application of sewage sludge as a nutrient source has been reported in various studies.<sup>14</sup> Therefore in the present work, sewage sludge was used as nutrient and seeding agent. To the best of our knowledge there have been no studies done on the effects of rhamnolipid in bioremediation of TNT in Iran. However, the effects of different variables such as addition of surfactant rhamnolipid, nutrients and aeration were studied. Also kinetics studies on reductive degradation of TNT in contaminated soil were investigated.

## Materials and Methods

### *Preparations of reactors*

Three soil-pan (1 to 3) experiments were conducted. Each pan experiment was prepared

by placing 3000 g of contaminated soil in a square plastic pan (30 cm × 20 cm × 20cm in height). The bottoms of these pans were perforated to allow drainage of fluids during and after flooding phases, via 2-mm-diameter holes spaced 2 cm apart in a square grid as described by Boopaty.<sup>6</sup> Each of the plastic pans were placed inside a slightly larger plastic pan (33 cm × 22 cm × 22 cm) to provide secondary containment of deionized water.

Pans 1 served as controls but were operated on a periodic cycle consisting of flooding for 2 d with deionized water, followed by draining and aeration for several days. Pans 2 and 3, were designed to be biologically active treatments.

### *Soil preparation*

Clean natural soil was used. The physical and textural characteristics of this soil is provided in Table 1. Contaminated soil was then prepared by dissolving an appropriate quantity of TNT in water/acetonitrile solution and a known weight of soil was added with continuous mixing. The contaminated soil was stored at room temperature for 7 days. Since addition of amendment can improve soil management properties,<sup>6</sup> sawdust was added to the contaminated soil. In this study, contaminated soil represented nearly 95% of the total soil mixture, while the sawdust amendment comprised the remaining 5% (dry-weight basis). Activated sludge from a textile wastewater treatment plant was added to pans 2, 3, except that rhamnolipid at CMC of 60 mg/l was added to the pan 3. Each of the mentioned Pans had a TNT concentration of 1000 mg/kg. Kinetic studies were conducted for each set of experiments. The length of soil aeration depended on the general drying conditions in all of the aerated pans. All of specified biological Pans were operated on a periodic cycle consisting of flooding with deionized water for 2 d, followed by draining and aeration for several days.

### *Aeration method*

Aeration in pans were carried out by forced aeration; 12 cubic air stones were used at the

bottom of each pan to distribute the air. The air stones were connected with a blower, which provided air into the reactor from the bottom.

### **Biosurfactant**

The biosurfactant used in this study was rhamnolipid (C<sub>32</sub>H<sub>58</sub>O<sub>13</sub>) obtained from the Genetic Engineering and Biotechnology Institute (Iran). It is an extracellular natural substance produced during precisely controlled fermentation processes using certain bacterial strains.<sup>15</sup> Its molecular weight is 650 g/mol.

Table 1. Properties of soil samples used in this study

Parameter	Value (%)
Clay	16
Sand	34
Silt	46
Total carbon	4

### **Chemicals**

All chemicals used were of analytical grade; TNT was from Zarrinshahr Chemical Industries (Esfahan, Iran) and was purified by recrystallizing. All other chemicals were obtained from Sigma–Aldrich and Merck.

### **Sampling and chemical analyses**

Samples of soil and liquid filtrates were taken periodically during the experiment for analyses of TNT. Sampling was done once every two weeks. The three grab samples were collected from the top 3 cm of soil in the pans during each sampling event. And liquid filtrates were collected after drainage events at the bottom of larger pans for all of biological pans. The TNT in soil samples were extracted in accordance with the US EPA Method 8330.<sup>16</sup>

Soil samples were air-dried; then 5 g (mixture of three grab samples) of soil was transferred to a clean glass vial and extracted with 20 ml of acetonitrile. The mixture was then centrifuged for 5 min at 3,000 rpm. The prepared sample was analyzed for TNT removal with high-performance liquid chromatography (HPLC). The HPLC system used from Waters (Milford, MA, USA), consisted of a Model 600E pump, fitted with a Rheodyne 7725i

injector valve, a Model 486 UV programmable multi wavelength detector, a data module, a Model 600E system controller, Detector and a Nova-pak C18 guard column. The analytical column was an ODS2-Optimal column (25c × 4.6mm id, 5µm) from Capital HPLC (West Lothian, UK).

A water-acetonitrile mixture (20:80, v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 20 µL and the absorbance was measured at a wavelength of 210 nm. The measurement of chemical oxygen demand (COD) was carried out according to standard methods.<sup>17</sup>

### **Analysis of biodegradation products by LC–MS**

TNT biodegradation products were analyzed by LC–MS using a Shimadzu LCMS-2010 EV (Japan) equipped with two pumps (LC-10 ADvp), controller (SCL- 10Avp), autoinjector (SIL- 10ADvp) and a UV2000 UV/VIS detector. The analytes were separated on 250 mm × 4.6 mm (diameter) × 5 µm C18 Hypersil GOLD column (Thermo, Waltham, MA) by acetonitrile–water gradient elution (90:10, v/v), at a flow rate of 0.2 mL/min.

## **Results and Discussion**

### **Effect of sludge in removal of TNT from soil**

Performance of induced aeration in removal of TNT with sludge as nutrient source is shown in Fig. 1.

As shown, approximately 50% of TNT has been removed. Aerobic bioremediation due to higher removal rate and lower toxicity of the produced intermediate has more advantages than anaerobic degradation.<sup>18</sup> In a study conducted by Widring et al, it was found that TNT at an initial concentration of 4000 mg/kg in soil, decreased to 1 mg/kg within 12 months.<sup>6</sup> In their study, wood chips were used as bulking agents and the aeration method was similar to our study. In the present work, textile wastewater treatment plant sludge was used as nutrient and microbial inoculum. It seems that wastewater sludge contain nutrients that can accelerate the degradation process. Compared with the results of Widring et al,<sup>6</sup> TNT degradation in our study has progressed at a

slower rate. Since the bacteria in textile wastewater treatment sludge, were encountered previously with aromatic compounds, it seems that the use of these bacteria can shorten the time required for explosives degradation. In a study conducted by Innemanova et al<sup>14</sup> it was found that addition of sludge caused 32.6% elimination of TNT. Compared to results of the present work, lower removal of TNT in their experiment may be related to the study circumstances. Their study was conducted in anaerobic conditions while present work was done in aerobic conditions. Since the bacteria have higher growth rates in aerobic conditions, a substantial increase in the bacterial density may have contributed to the higher rate of contaminant removal predicted in the present work.

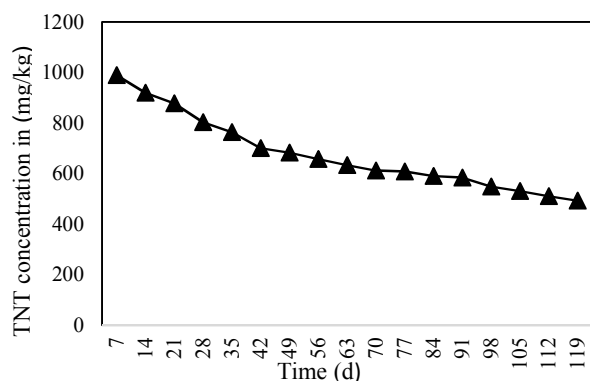


Fig. 1. Disappearance of TNT in presence of sludge in induced aeration (TNT= 1000 mg/kg)

Effect of rhamnolipid on the removal of TNT The effectiveness of rhamnolipid in removal of TNT by induced aeration is shown in Fig 2. It is clear that in the presence of rhamnolipid, TNT removal rate increased to 99 percent. By comparison it can be seen that TNT removal was at least 1.98 orders of magnitude higher than those found for experiments not amended with rhamnolipid. Additionally, rhamnolipid not only provided micelles for higher solubility of TNT, but also increased its emulsification, which ultimately increased its availability for the microorganisms. Low solubility of explosives is the main factor that decreased their degradation in previous studies. Furthermore, because of the intensive binding of explosive with soil particles,<sup>19</sup> application of an

agent is required in order of increasing TNT availability, for consequent increase in TNT degradation. Thus increasing the removal of TNT in the reactor containing rhamnolipid can be attributed to increased TNT desorption from soil. Aggregation of TNT in rhamnolipid micelles can reduce its toxicity. Chrzanowski et al conducted a similar study, which showed that the toxicity of 4-chlorophenol intensely microbial growth and further removal of decreased by aggregation in biosurfactant micelles, which in turn led to an increase in 4-chlorophenol.<sup>10</sup>

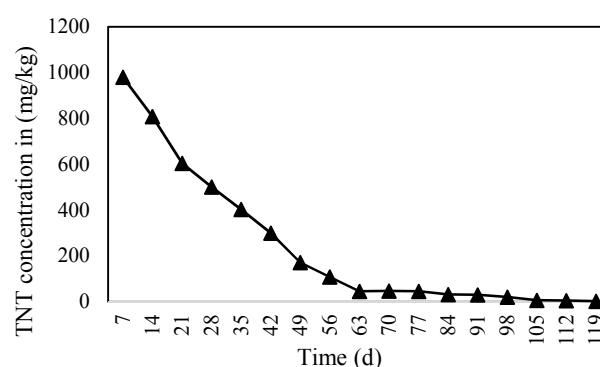


Fig. 2. Disappearance of TNT in presence of rhamnolipid in induced aeration (TNT= 1000 mg/kg)

#### ***Effect of sludge on COD reduction***

Performance of activated sludge in the removal of COD is shown in Fig. 3. It was found that maximum COD removal rate was 41 percent.

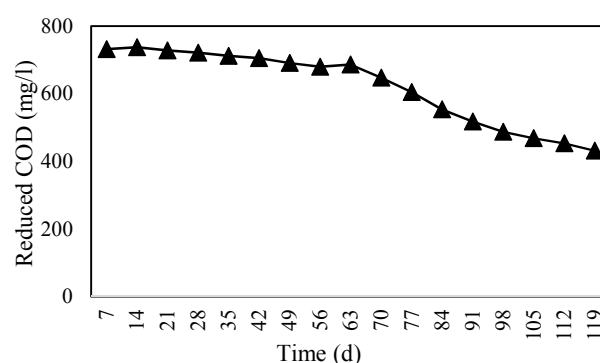


Fig. 3. Effect of sludge on COD removal in induced aeration. No rhamnolipid was added

#### ***Effect of rhamnolipid on COD reduction***

The COD is a gross parameter of concentration of organic materials in a solution, therefore any reduction in COD level reflected

mineralization.<sup>20</sup> The effect of rhamnolipid at CMC of 60 mg/l on mineralization of TNT was evaluated. Fig. 4 shows the effect of rhamnolipid on removal of COD. As fig. 4 indicated, COD removal in presence of rhamnolipid was 58 percent. Compared to Fig. 3 it can be seen that the reactor containing rhamnolipid had a better performance in removal of COD. Also Fig 3 shows that until day 63, the reduction of COD was insignificant

and only 6 percent was removed. Although, the TNT concentration decreased, and eventually transformed to relevant metabolites, there was no significant reduction in COD until 63 days. In reference to fig. 4, it was found that in the presence of rhamnolipid, COD reduction was observed from the beginning of the experiment, and at the end of 120 days COD removal reached 58 percent.

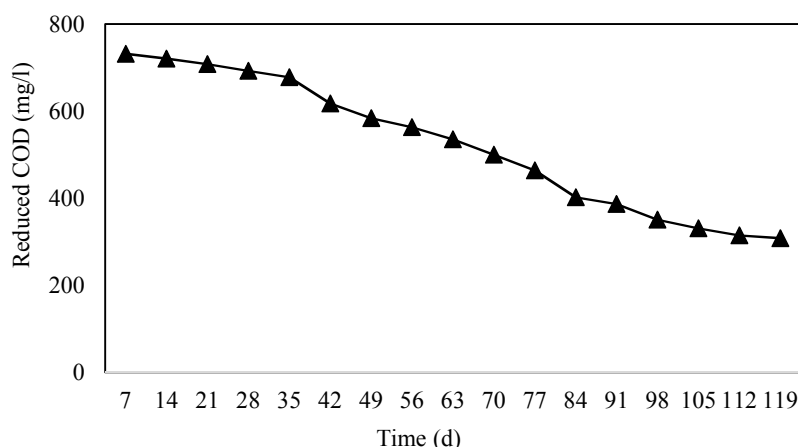


Fig. 4. Effect of rhamnolipid on COD removal in induced aeration. (rhamnolipid concentration, 60 mg/l)

By comparison it was found that, COD removal in fig. 4 was 1.5 fold higher than that of the Fig. 3. It seems that applications of rhamnolipid increased the solubility of TNT, and thus, facilitated its biodegradation which ultimately lead to reduction of COD. Sponza and Gök<sup>21</sup> previously found that the addition of rhamnolipid surfactant (15 mg l<sup>-1</sup>) to petrochemical wastewater increased the removal efficiencies of PAHs and soluble COD.

### Kinetic studies

In order to determine the kinetics of TNT degradation, different concentration of TNT were used. Results of study showed that, TNT biodegradation followed pseudo first order kinetics (Table 2). Rate constant  $k_{obs}$  [d<sup>-1</sup>] is calculated from the slope of the line for  $\ln [C_0/C]$  vs. reaction time.

$$dC/dt = -k_{obs}t \quad (1)$$

$$C = C_0 \exp(-k_{obs}t) \quad (2)$$

Where  $C_0$  = initial explosives concentration (mg/kg);  $k_{obs}$  = rate constant (d<sup>-1</sup>);  $t$  = degradation time (d).

In general, removal rate of TNT was higher in experiments where rhamnolipid was added. As seen in table 2, the pseudo first-order rate constants of the rhamnolipid amended experiment was at least 3.89 orders of magnitude higher than those found for experiments not amended with rhamnolipid.

Table 2. Reductive degradation of TNT. Experimental data was fit to the pseudo first-order kinetic equation

TNT Concentration (mg/kg)		With rhamnolipid	Without rhamnolipid
Equation	R <sup>2</sup>	0.97	0.95
$C = C_0 \exp(-k_{obs}t)$	$k_{obs}$	0.023	0.0059

This can be related to the addition of rhamnolipid, which resulted in increasing the solubility of TNT and then enhancing its

biodegradation. Surfactants can increase the surface area of hydrophobic materials, increasing their water solubility and subsequently increasing the biodegradation of complex hydrocarbons.<sup>15</sup>

In the present work, LC-MS analysis of TNT revealed that two reduction metabolites, 2-amino-4, 6-dinitrotoluene and 4-amino-2, 6-dinitrotoluene were observed during TNT

metabolism. The same results have been reported by others.<sup>22,23</sup> The possible metabolic pathway proposed for TNT biodegradation in accordance to our results, was also reported by others.<sup>22,24</sup> The possible mechanism involved reduction of one nitro group to form a hydroxylamino group, and subsequent reduction of the other nitro group to an amino group.

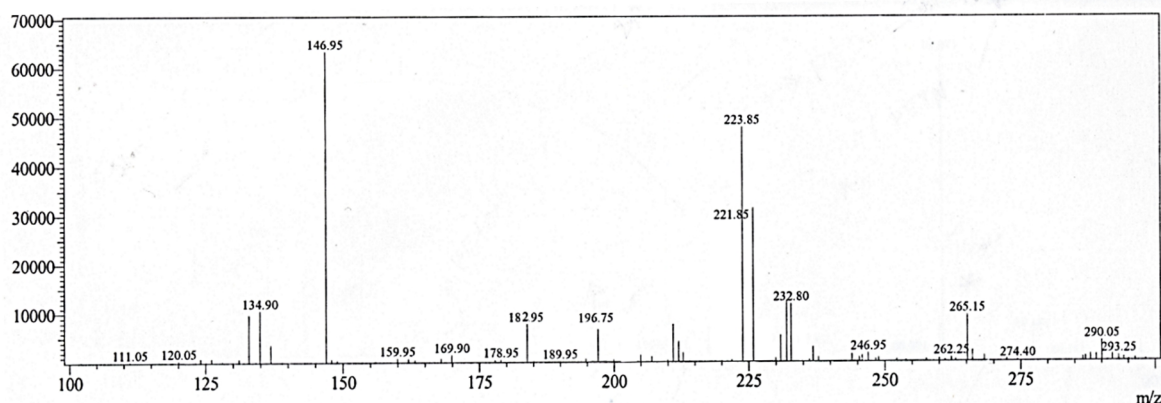


Fig. 5. Chromatogram analysis of the TNT degradation

## Conclusion

Application of rhamnolipid was effective in TNT biodegradation. By the formation of micelles, rhamnolipid reduced the TNT toxicity and increased the solubility of TNT. Due to adapted bacteria, application of wastewater sludge can be useful in bioremediation of polluted areas, and reduced the required time for remediation of pollutants. Based on results obtained, usage of this method is recommended in remediation of explosives contaminated areas.

## Conflict of Interests

Authors have no conflict of interests.

## Acknowledgements

The authors are thankful to Dr. Nezam Mirzaiee for his friendly support.

## References

1. Singh A, Kuhad R C, Ward P. *Advances in Applied Bioremediation*: Springer Dordrecht Heidelberg London New York 2009; 14-18.
2. Meyers S, Shanley ES. Industrial explosives - a brief history of their development and use. *J Hazard Mater* 1990; 23(2): 183-201.
3. Rosenblatt DH, Burrows EP, Mitchell W.R. , Parmer, D.L. *Organic explosives and related compounds*, In O. Hutzinger, *The handbook of environmental chemistry*. Berlin: Springer-Verlag, 1991.
4. Sheibani G, Naeimpoor F, Hejazi P. Statistical factor-screening and optimization in slurry phase bioremediation of 2,4,6 -trinitrotoluene contaminated soil. *J Hazard Mater* 2011; 188 (1-3): 1-9.
5. Pennington JC, Barnnon JM. Environmental fate of explosives. *Thermochemica Acta* 2002; 384(1-2): 163-172.
6. Widrig DL, Boopathy RJ, Manning JR. Bioremediation of TNT- contaminated soil: a laboratory study. *Environ Toxicol Chem* 1997; 16(6): 1141-1148.
7. Clark B, Boopathy R. Evaluation of bioremediation methods for the treatment of soil contaminated with explosives in Louisiana Army Ammunition Plant, Minden, Louisiana. *J Hazard Mater* 2007; 143(3): 643-648.
8. Boopathy R. Effect of food-grade surfactant on

- bioremediation of explosives-contaminated soil. *J Hazard Mater* 2002; 92(1): 103-114.
9. Zhuang L, Gui L, Gillham RW. Biodegradation of pentaerythritol tetranitrate (PETN) by anaerobic consortia from a contaminated site. *Chemosphere* 2012; 89(7): 810-816.
  10. Chrzanowski Ł, Owsianiak M, Szulc A, Marecik R, Piotrowska-Cyplik A, Olejnik-Schmidt AK, et al. Interactions between rhamnolipid biosurfactants and toxic chlorinated phenols enhance biodegradation of a model hydrocarbon-rich effluent. *Int. Biodeterior. Biodegradatio* 2011; 31;65(4):605-11.
  11. Avramova T, Sotirova A, Galabova D, Karpenko E. Effect of Triton X-100 and rhamnolipid PS-17 on the mineralization of phenanthrene by *Pseudomonas* sp. cells. *Int. Biodeterior. Biodegradatio* 2008;62(4):415-20.
  12. Christodoulatos C, Bhaumik S, Brodman BW. Anaerobic biodegradation of nitroglycerin. *Water Res* 1997; 31(6): 1462-1470.
  13. Meng M, Sun W, Geelhaar LA, Kumar G, Patel AR, Payne GF, et al. Denitration of glycerol trinitrate by resting cells and cell extracts of *Bacillus thuringiensis/cereus* and *Enterobacter agglomerans*. *Appl. Environ. Microbiol* 1995;61(7): 2548-2553.
  14. Innemanová P, Velebová R, Filipová A, Čvančarová M, Pokorný P, Němeček J, et al. Anaerobic in situ biodegradation of TNT using whey as an electron donor: a case study. *New biotechnology* 2015;32(6):701-9.
  15. Mazaheri Assadi M, Tabatabaee MS. Biosurfactants and their use in upgrading petroleum vacuum distillation residue: A review. *Int. J. Environ. Res* 2010; 4(4): 549-572.
  16. Numbera AA. Method 8330B nitroaromatics, nitramines, and nitrate esters by high performance liquid chromatography (HPLC). US Environmental Protection Agency, Washington, DC. 2006.
  17. Federation WE, American Public Health Association. Standard methods for the examination of water and wastewater. American Public Health Association (APHA): Washington, DC, USA. 2005.
  18. Son A, Lee J, Chiu PC, Kim BJ, Cha DK. Microbial reduction of perchlorate with zero-valent iron. *Water Res* 2006;40(10):2027-32.
  19. Lewis TA, Newcombe DA, Crawford RL. Bioremediation of soils contaminated with explosives. *J Environ Manage* 2004;70(4):291-307.
  20. Moussavi G, Aghapour AA, Yaghmaeian K. The degradation and mineralization of catechol using ozonation catalyzed with MgO/GAC composite in a fluidized bed reactor. *Chem Eng J* 2014;249:302-310.
  21. Sponza DT, Gök O. Effect of rhamnolipid on the aerobic removal of polyaromatic hydrocarbons (PAHs) and COD components from petrochemical wastewater. *Bioresource technology* 2010;101(3):914-924.
  22. Lin HY, Yu CP, Chen ZL. Aerobic and anaerobic biodegradation of TNT by newly isolated *Bacillus mycoides*. *Ecological engineering* 2013;52:270-277.
  23. Montpas S, Samson J, Langlois É, Lei J, Piché Y, Chênevert R. Degradation of 2, 4, 6-trinitrotoluene by *Serratia marcescens*. *Biotechnology letters* 1997;19(3):291-294.
  24. Nyanhongo GS, Schroeder M, Steiner W, Gübitz GM. Biodegradation of 2, 4, 6-trinitrotoluene (TNT): An enzymatic perspective. *Biocatalysis and Biotransformation* 2005;23(2):53-69.