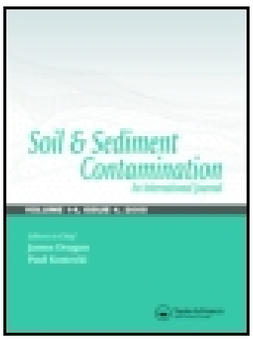

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# Remediation of 2,4,6-trinitrotoluene Persistent in the Environment – Areview

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**ABSTRACT**

Nitroaromatic explosives such as trinitrotoluene (TNT), dinitrotluene (DNT) and other metabolites present the greatest concern to the environment and public health due to their mutagenic properties and persistence. Remediation of soil contaminated by explosive residues has received increased interest in recent years in many countries due to their prolific use since the World War I and II. There is an urgent need to identify appropriate and effective technologies to attenuate/remediate TNT-contaminated sites and ensure safe environment. This article presents an overview of the technologies commonly adopted for remediation of TNT contaminated sites with particular emphasis on ways to enhance biodegradation especially with the addition of surfactants or biosurfactants.

**KEYWORDS**

2, 4, 6-trinitrotoluene; aerobic bioremediation; anaerobic bioremediation; biosurfactants

## Introduction

Presence of nitroaromatic explosive residues in the environment poses a great threat to surface water, subsurface soil and groundwater.2,4,6-Trinitrotoluene (TNT)is popular among other explosives bothin production and usage (Rahal and Moussa 2011; Snellinx et al. 2002; Spain, Hughes, and Knackmuss 2000).TNT and its derivatives such as 2,4-dinitrotoluene (2,4-DNT)and 2,6-dinitrotoluene (2,6-DNT) are used in rockets, missiles and as intermediates in the manufacture of polyurethanes, smokeless gun powder, dyestuffs and photographic chemicals. These compounds are released through firing of munitions, industrial effluents, disposal of ordnance, open incineration and through leaching from unlined impoundments. Other nitro compounds such as nitroglycerin (NG), nitrobenzene (NB) nitrocellulose(NC) are also used widely in the manufacture of industrial products like lubricating oils, dyes, and synthetic rubber (EPA 2017; Kalderis, Juhasz, and Boopathy 2011; Rodgers and Bunce 2001).The explosive residues in the soil range from trace quantities to as high as 14000 mg/kg (ATSDR, 1995; MMR, 2001).

TNT has been known as the worst of the contaminants because of its mutagenic properties, low mobility and its persistent nature. In the United States, TNT has been identified in 1200 sites with at least 20 sites projected for inclusion in the National Priorities list. The historical practices have resulted in extensive contamination of soil and groundwater at many current and former U.S. Department of Defense (DOD) and U.S. Department of Energy (DOE) sites. (Adrian et al., 2003; Schmelling and Gray 1995).

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Although usage of TNT is prevalent in many countries, limited information is available on contaminated sites and extent of contamination. It has been stated by Eisentraeger et al. (2007)that sites contaminated during World War II still contain high levels of TNT. Red water, the effluent from TNT manufacturing, is also a major source of TNT contamination in soil, groundwater and surface water/sediment at army ammunition plants (EPA 2005). A single TNT manufacturing plant can generate as much as 1.5–2 million litres of wastewater per day that contains metabolites of TNT. Explosive wastewater is generally discarded outside the manufacturing facilities on the ground or in unlined lagoons that leach the explosive in the soil as well as in groundwater and surface water.

As TNT and its metabolites are toxic to the environment, risk associated to living organisms is higher. TNT particularly affects the central nervous system in the form of seizures and cause immune system dysfunction. Humans are exposed to TNT and its metabolites through occupational hazards at explosives manufacturing units, in unidentified disposal sites and during military operations. Even at minuscule concentration, TNT is responsible for organ failures such as bone marrow and kidney failures. It causes hepatitis, dermatitis, anaemia, and cyanosis and is classified as a possible human carcinogen (Class C) (Lachance et al. 2004; Mercimek et al. 2015; Nyanhongo et al. 2005). TNT is a single-ring nitroaromatic compound that is a crystalline solid at room temperature. The persistence of TNT in the subsurface is a result of three electron withdrawing nitro groups that are resistant to electrophilic attack by oxygenases and hydrolysis (Rodgers and Bunce 2001). Though TNT reduction occurs naturally under both aerobic and anaerobic conditions, the rate of reduction depends upon the redox potential, pH and electron accepting conditions of the environment (Habineza, Zhai, and Mai 2016). With octanol-water partition coefficient ($K_{ow}$) of 1.6 and soil organic carbon-water coefficient ($K_{oc}$) of 300, TNT can rapidly be adsorbed to soil thus making remediation of contaminant sites challenging. Hence, numerous efforts have been undertaken in finding an appropriate remediation technology or sequence of technologies for cleaning up TNT contaminated areas.

The objective of this paper is to review the existing TNT remediation technologies and understand the extent and limitations of each technology with main focus on biological degradation of TNT. TNT-transforming cultures that produce surfactants termed as biosurfactants show promising results for enhanced remediation of TNT. The paper suggests future research directions for an appropriate and effective technology using biosurfactants while treating TNT contaminated sites.

## Fate and transport of TNT

The predominant physico chemical properties of TNT such as solubility, vapour pressure and Henry's law constant (Table 1) combined with the soil properties such as pH and redox potential dictate the fate and transport of TNT in the environment (EPA 2017).TNT buried in soil or present on the surface persist for many years depending upon factors such as soil organic matter, concentration of TNT, oxygen concentration, incubation period, and microbial activity (Shemer et al. 2017). The presence of nitro groups ($NO_2^-$) form hydrogen bonds with water thereby increasing the aqueous solubility of TNT and its affinity for charged surfaces. In the presence of fine or colloidal particles, TNT's affinity for surfaces, especially which have high cation exchange capacity increases. Small quantities of TNT can

**Table 1.** Physico-chemical properties of 2,4,6-Trinitrotoluene.

| S.NO | PROPERTY | VALUE |
|---|---|---|
| 1 | Chemical Formula | $C_7H_5N_3O_6$ |
| 2 | Molar Mass | 227.13g/mol |
| 3 | Color | Yellow |
| 4 | Boiling Point | 240°C (explodes) |
| 5 | Melting Point | 80.1°C |
| 6 | Water Solubility | 130 mg/L (at 20°C) |
| 7 | Density | 1.654 g/cm$^3$at 20°C |
| 8 | Vapor Pressure | 7.2 x $10^{-9}$ bar at 20°C |
| 9 | Henry's Law Constant | 4.57 x$10^{-7}$ atm-m$^3$/mol at 20°C |
| 10 | Octanol-water partition coefficient | 1.86 |

also reach groundwater, surface water and marine environments. Compared to explosives such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), the solubility of TNT is higher (130 mg/L) and hence TNT particles have the ability to dissolve slowly over time causing groundwater contamination. Processes such as dissolution and adsorption govern the transport of TNT while volatilization path is trivial because of TNT's low vapor pressure.

TNT may either remain as a free product or dissolve in aqueous media or sorb onto organic matter or can be degraded by direct photolysis. Direct photolysis is a result of absorption of light energy by TNT and its derivatives, and the process is strongly influenced by the light intensity and wavelength. Depending upon reaction pH and TNT concentration, hydrolysis of TNT can be considered as one of the remedial routes for contaminated soils. However, it is unlikely to prompt and maintain alkaline pH condition for hydrolysis to take place. 1,3,5-Trinitrobenzene (1,3,5-TNB) is one of the primary photodegradation products of TNT during photolysis. TNT has been found to show higher sorption affinity of 170 L/kg compared to 8 L/kg for RDX and 30 L/kg for HMX. Covalent binding of TNT to soil and humic substances results in irreversible sorption and hence removal of TNT would be difficult. The only viable option is biodegradation apart from hydrolysis and photolysis. TNT is broken down by biodegradation in water but at rates much slower than photolysis. TNT degradation products include 2-amino4,6-dinitrotoluene (2ADNT), 4-amino 2,6-dinitrotoluene (4ADNT), 2,4 diamino 6-nitrotoluene (2,4-DANT) and 2,6 diamino 4-nitrotoluene(2,6-DANT) (Clausen and Nic Korte, 2011). They in turn combine with soil organic matter and undergo further oxidation and have the potential to contaminate the groundwater by continuous leaching (Eriksson et al. 2004; Pichtel 2012; Wang, Thiele, and Bollag 2002).

## Treatment technologies

A plethora of lab scale studies and field level investigations have been carried out for attenuation and complete removal of TNT, which include chemical and thermal methods of removal, biological processes such as soil slurry reactors, composting and land farming. Composting is a traditional technique where the mere mix of contaminated soil with bulking agents and organic substrates for a particular period of time aid in removal of TNT (Funk, Crawford., and Crawford 1996; Parr, Marsh, and Kla 1983). However, the main disadvantage of the compost technique is the long incubation time, cost of setting up, periodic maintenance of the system and lack of knowledge about the bacteria and fungi which is involved in the

process of composting (Larson et al. 2008). Similar to composting, land farming is a solid phase treatment process where the soil is mixed with required nutrients and moisture and allowed for TNT degradation in a natural way. However, mechanized or manual mode of aeration is essential for accomplishing effective TNT removal. Indigenous soil bacteria are capable of mineralizing TNT in land farming (Kalderis, Juhasz, and Boopathy 2011) albeit at a slower rate.

Adsorption of TNT onto adsorbents such as activated carbon or iron particles is another common technique. Nano zero valent iron (nZVI) particles have the capability to degrade TNT successfully (Hawari et al. 2000; Laine and Cheng 2007; Pennington and Patrick 1990). It has been reported that nano scale zero valent iron emulsion (EZVI) synthesized out of nZVI, immiscible substrate such as corn oil and surfactant can combinedly degrade TNT (Echols 2009). TNT reduction and its conversion into amino compounds, specifically to triaminotoluene (TAT) by metallic iron particles has been reported by Chung et al. (2010).Abiotic treatment with Fenton oxidation and metallic iron has an excellent potential to remediate explosives contaminated soil and water. Li, Comfort, and Shea (1997) and Hundal et al. (1997b)successfully reduced the concentration of TNT and RDX in soil from 3000 mg/Kg to 17.2 mg/Kg of TNT and 5.8 mg/Kg of RDX which were much below the remediation goals established for the Nebraska Ordnance Plant using abiotic methods. Shukla et al. (2018) have demonstrated high adsorption potential of 13.56 mg/Kg of TNT with synthesized zero valent iron-silica nanocomposite ($Fe/SiO_2$) which could later be separated using a magnet. In previous years, incineration was considered as the most effective method for remediation, but it can be very expensive for polluted soils and high levels of explosives could be released from the process of incineration (Symons and Bruce 2006).

TNT phytoremediation is a low cost, environment friendly approach but suffers from factors such as slow clean up times, low inherent metabolic activity of plants towards explosive compounds and phytotoxicity of explosive compounds (Hannink et al. 2002). A variety of plant systems such as yellow nutsedge, bush bean, switch grass, aquatic and wetland species and hybrid poplar are commonly used. Plant-bacterium combinations to phytoremediate contaminated soil are also being developed with *Pseudomonas* strain capable of transforming TNT to amino compounds. Inoculation of meadow brome grass (*Bromus erectus*) with this strain increased the portion of the rhizosphere community involved in nitroaromatic metabolism and led to a reduction in soil TNT levels. More recently, transgenic plants that express microbial degradative enzymes for TNT bioremediation have been developed. Transgenic plants that contain the explosives-degrading enzyme are able to germinate and grow in the presence of even high explosives concentrations (Chang et al. 2004; van Dillewijn et al. 2007). The fast-growing perennial grass Vetiver (Vetiveria *Zizanioides*) shows promising results of upto 95% removal of TNT from contaminated soil with TNT concentration of 200 mg/Kg in a period of 6 months. Three metabolites 2ADNT, 4ADNT and 1,3,5 TNB were detected in shoots showing translocation of contaminant post phytodegradation (Das 2014; Truong, Van, and Pinners 2008) Further research is required to determine whether transgenic plants are capable of contributing to the degradation of TNT.

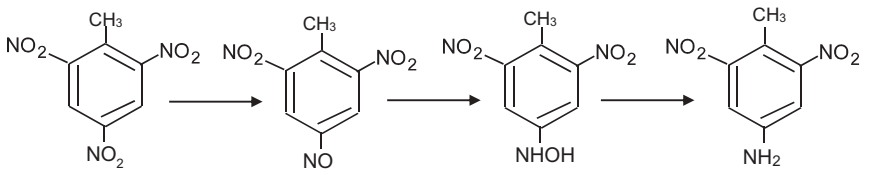
## Biological treatment

TNT can be degraded by biological agents which include microorganisms, fungi and yeasts that have the capability of transforming TNT into non-toxic end products (Serrano-Gonzalez.

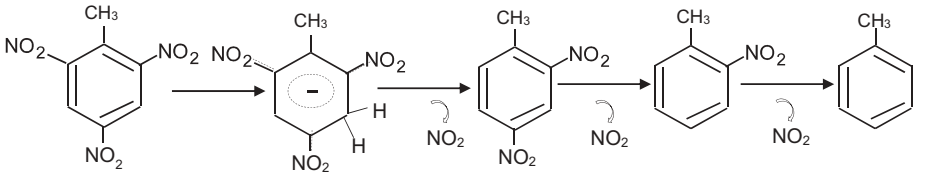
et al. (2018). A wide variety of bacterial isolates such as *Pseudomonas sp.,Desulfovibrio sp., Bacillus sp. and Staphylococcus sp*. have the ability to transform TNT under aerobic and anaerobic conditions through reductive pathways (McFarlan, Sara, and Yao 2016). However, anaerobic consortia have been reported to be more effective than pure cultures of the aerobes. Either aerobic or anaerobic, biotransformation is typically co-metabolic in nature. The reduction pathway results in the formation of nitroso, hydroxylamino and amino derivatives of TNT via a series of electron transfers. Oxygen insensitive enzymes (Type I) such as nitroreductase, aldehyde oxidase, dihydrophilic amide dehydrogenase aid in the reduction process (Nyanhongo et al. 2005). The derivatives formed through this process are equally harmful to humans and environment unless complete reduction to the amino group occurs. Another class of enzymes, oxygen sensitive (Type II) enzymes prevalent in *Clostridium sp*. and *E. coli sp*. produce a nitroanion radical that could react with oxygen to form a superoxide radical and affect the original nitroaromatic compound (Gonzalez-Perez et al. 2007; Nyanhongo et al. 2005).

Complete microbial transformation does not occur through oxidative or aerobic degradation. Aerobic metabolism is limited to dinitro aromatic compounds such as 2,4-DNT or 2,6-DNT or mono nitroaromatic compounds due to the presence of three electron withdrawing nitro groups in TNT causing high electron deficiency to the aromatic ring (Claus 2014). This causes decline in ring fission and hence microbial activities are involved primarily in elimination of nitro groups from aromatic ring. Aerobic bacteria help in eliminating the nitro groups by ring oxygenation and by partial reduction to hydroxylamino derivatives. When partially reduced form of TNT reacts with oxygen, more toxic azoxytetranitrotoluenes are formed. Figure 1 shows the pathway for aerobic metabolism of TNT through reduction to amino compounds and through Meisenheimer complex resulting in toluene formation (Pak et al. 2000). Formation of hydride-Meisenheimer complex also plays a significant role in the direct metabolism of 2,4,6- TNT where the aromatic ring is reduced to dinitroderivative by the addition of hydride ions. *Pseudomonas sp., Rhodococcus erythropolis, Enterobacter cloacae* are few strains reported to reduce TNT to its hydride Meisenheimer complex with concomitant release of nitrite and NADPH oxidation. Various species of fungi like white-rot fungi, *Phanerochaete chrysosporium* and *Stropharia sp*. also degrade TNT under aerobic conditions (Esteve-Nunez, Caballero, and Ramos 2001).

In the anaerobic system (Figure 2), the nitro moieties of TNT can be successfully reduced to nitroso, hydroxylamino and finally amino groups with the help of anaerobic bacteria like *Clostridium sp. Desulfovibrio sp*. and *Methanococcus sp*. One of the nitro groups in TNT is reduced to hydroxyl amine group (-NHOH), which is further reduced to 2-amino 4,6-dinitrotoluene (2ADNT) or 4-amino 2,6-dinitrotoluene (4ADNT). 2ADNT and 4ADNT are the most common intermediates during continuous TNT reduction process. The intermediates are further reduced to 2,4-diamino-6-nitrotoluene (2,4-DANT) followed by reduction to 2,4,6 triaminotoluene (TAT) with the aid of heterotrophic bacteria. TAT appears as a dead-end metabolite unless amended with co-substrates or additional electron acceptors (Ederer, Lewis, and Crawford 1997; Moshe et al. 2009). The role of *Clostridium acetobutylicum* in anaerobic TNT bioremediation systems received considerable attention in the past due to their ability to rapidly reduce the nitro groups in TNT to hydroxylaminonitrotoluenes subsequently followed by dihydroxylaminotoluene (Hughes, Wang., and Zhang 1999). Hydroxylamines can be either




a) Pathway for aerobic metabolism of TNT through reduct ion




b) Pathway for aerobic metabolism of TNT through Meisenheimer complex

**Figure 1.** (a)Pathway for aerobic metabolism of 2,4,6-TNT through reduction. (b) Pathway for aerobic metabolism of 2,4,6-TNT through Meisenheimer complex.
(Adapted in part from Esteve-Nunez, Caballero, and Ramos 2001)

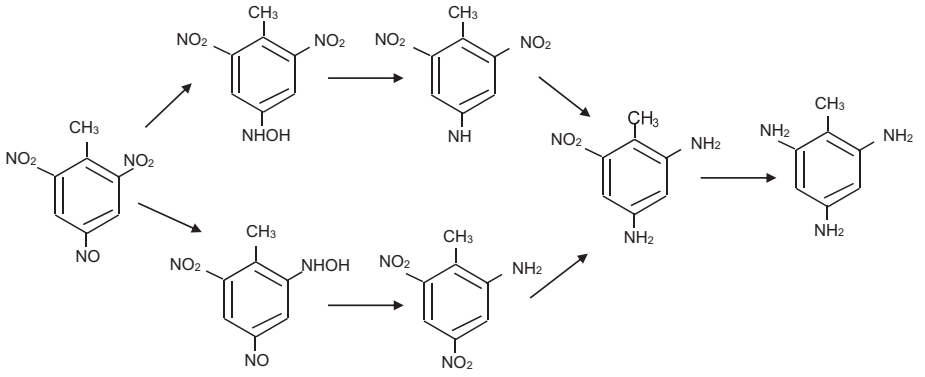


**Figure 2.** Pathway for anaerobic metabolism of 2,4,6-TNT.
(Adapted in part from Esteve-Nunez, Caballero, and Ramos 2001)

reduced to amines or bound with the organic fraction of soils and form azoxytoluenes. They are often more mutagenic than their parent nitro compounds, and their accumulation in remediation systems may represent a concern, depending on their eventual fate.

Isolate *Pseudomonas putida* was used by Stenuit and Agathos (2010) for degrading TNTsince it maintains low intracellular TNT concentration to overcome the toxicity. In the case of *Pseudomonas sp.*, the released nitrate from the aromatic ring and the reduced ammonium converts 85% of nitrogen associated to cells as organic nitrogen (Esteve-Nunez, Caballero, and

Ramos 2001). *Clitocybula dusenii* TMb12 and *Stropharia rugosa-annulata* DSM11372 are the most active strains used to mineralize 42 and 36%, TNT, respectively. Recently, *Acinetobacter* species has been demonstrated as a resourceful species using TNT as a growth substrate (Solyanikova et al. 2012). The list of isolated organisms from TNT contaminated soil and their ability to transform TNT is given in Table 2. *Bacillus* cells possess the ability to grow out of the surrounding and can initiate TNT transformation even after 5-years of storage at room temperature (Nyanhongo et al. 2009). *Pseudomonas putida* can remove 89% TNT from clayey soil in the slurry phase within 15 days of incubation showing *P. putida* as a high potential organism for TNT removal from clayey soil. The screening experiments showed 68.1–92.3% TNT removal can be achieved possibly by changing the factor levels such as glucose, slurry concentration, inoculum size, temperature and yeast extract (Sheibani, Naeimpoor, and Hejazi 2011).

Amin et al. (2017) investigated anaerobic intrinsic bioremediation on 1000 mg/Kg of TNT using seven native strains of *Planomicrobacterium flavidum, Pseudomonas aeruginosa, Entrobactor asburiae, Azospirillium, Rhizobium, Methylobacterium*, and other *Pseudomonas* strains. Achtnich et al. (2000) reported that reduced TNT metabolites binds to the soil with most reduced metabolite like TAT binding to humic material appears to exhibit irreversible covalent bonding. *Clostridium sp*. and *Desulfovibrio sp*. has been extensively studied for anoxic metabolism of TNT and they have the ability to reduce TNT anaerobically.Numerous experiments on TNT metabolism by a single isolate *Pseudomonas savastanoi* provided effective reduction of TNT to 2,4-DNT (Martin et al. 1997). Previous reports demonstrated TNT transformation by *Clostridium* strains isolated from a long-term bioreactor fed with explosive (TNT, RDX, HMX) contaminated soils without oxygen (Lewis et al. 1996). Under anaerobic condition, TNT gets converted into two compounds 2-NHOH-4,6 DNT and 4-NHOH-2, 6 DNT. Then these compounds lose one nitro group to form 2,4-ADNT and finally gets converted to TAT.TAT is produced with aromatic compounds lacking nitrogen. Wang et al. (2010) reported that ethanol was employed to reduce TNT in fermentation process under anaerobic conditions. The J.R. Simplot anaerobic bioremediation system is a proven technology to destroy nitroaromatic and energetic materials without evolution of any toxic intermediates at the end of the experiment. In this technology, carbon source, contaminated soil, buffers are mixed and left for some time to deplete the dissolved oxygen and create anaerobic conditions. Either aerobic or anaerobic, the toxicity of nitroaromatic compounds might limit the practice of biological methods especially when TNT concentrations are higher and not easily bioavailable.

**Table 2.** Biologicalstudies of 2,4,6-Trinitrotoluene degradation.

| S. NO | ORGANISMS | REACTION | REFERENCE |
|---|---|---|---|
| 1 | *E. coli AB1157* | Reduction of TNT to TAT | Chang et al. 2004 |
| 2 | *Clostridia and Desulfovibrio Clostridium acetobutylicum Clostridium aaterianums Clostridium bifermentans LIP 1 Clostridium bifermentans CYS 1* | Reduction of TNT to TAT | Gonzalez-Perez et al. 2007 |
| 3 | *Methanococcus sp.* | Reduction of TNT to DANT | Esteve-Nunez, Caballero, and Ramos 2001 |
| 4 | *Lactobacillus sp.* | Reduction of TNT to TAT | Esteve-Nunez, Caballero, and Ramos 2001 |
| 5 | *Pseudomonas putida* | Reduction of TNT to 2ADNT and 4ADNT | Stenuit and Agathos 2010 |
| 6 | *Bacillus cereus* | Reduction of TNT | Habineza, Zhai, and Mai 2016 |
| 7 | *Enterobacter* | Formation of polar products | Bae, Autenrieth, and Bonner 1995 |
| 8 | *Pseudomonas sp.* | Meisenheimer complex | Duque et al. 1993 |

## Enhanced treatment with surfactants

Limited bioavailability is one factor contributing to long-term persistence of TNT in the environment. The main reason for the persistence of TNT in soil is due to its low redox potential and sorption to soil surfaces. The metabolites of TNT such as 2ADNT, 4ADNT can bind covalently in soil organic matter. Under such conditions, surfactants facilitate desorption of TNT from the soil matrix, thereby increasing the bioavailability and enhancing biodegradation of TNT contaminated soil. Surfactants are surface-active molecules with an ability to concentrate at the interfaces of the soil matrix and lower the binding of contaminants. Addition of surfactants to contaminated soil, at concentrations above their critical micelle concentration (CMC) values will be a successful feasible approach to enhance the solubility and therefore, increase their biodegradation. Biosurfactants of microbial origin have been recognized as partial or total substitutes for synthetic surfactants because of their low toxicity and high biodegradability. The effect of a surfactant on biodegradation is a combination of the solubilizing power of the surfactant and the bioavailability of the substrate within the surfactant micelles. Incorporation of surfactant addition into treatment technologies would improve bioavailability and shorten cleanup times. In the present context, a biosurfactant is a surface-active biomolecule produced by microorganisms isolated from contaminated soils.

Biosurfactants play an important role in biodegradation and bioremediation due to their biocompatibility, digestibility and biodegradability properties when compared to artificial and chemical surfactants. They are active at extreme temperatures, pH and salinity and can be produced from industrial wastes and from by-products. This last feature makes cheap production of biosurfactants possible and allows utilizing waste substrates and reducing their polluting effect at the same time. Some anaerobic microorganisms possess the ability to produce their own surfactants. They are grouped as glycolipids, lipopeptides, phospholipids, fatty acids and neutral lipids, polymeric and particulate compounds. Biosurfactants such as rhamnolipids are used in agrochemical, fertilizer, food, petroleum, and mining industries including beverages and cosmetics sectors (Marchant and Banat 2012). Rhamnolipids increase the solubility of hydrophobic compounds and also reduce the toxicity to bacteria leading to increase in the biodegradability of TNT.

Although a wide variety of research has now been published on a variety of biosurfactants, rhamnolipids produced by the gram-negative *P. aeruginosa* are commonly studied due to their ability to be produced from a variety of substrates. Immiscible carbon sources such as vegetable oil, crude oil, olive oil and waste frying oil have shown good results for the production of biosurfactants (Abdel-Mawgoud, Lépine, and Deziel 2010). Hydrophilic sources, such as glycerol, sucrose and glucose have also been used for their production, but the yield of biosurfactants would be lesser than with hydrophobic or immiscible carbon sources such as n-alkanes and oils (Nitschke, Costa, and Contiero 2011).Yeast extract, peptone, ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate, and malt extracts are good nitrogen sources. Using either glucose, glycerol, waste frying oils or vegetable oils as the carbon source, different strains of *P. aeruginosa* produce biosurfactants with good yield ranging from 0.7 (Amani et al. 2010) to 150 g/L (He et al. 2017). This bacterium has the ability to produce several different molecules with alkyl chains ranging from 8 to 12 carbon atoms such as mono rhamnolipid and di rhamnolipid (Gunther et al. 2005). Biosurfactants like glycolipid, sophorolipids are

produced by yeast of the genus *Candida*; rhamnolipids are produced by *P. aeruginosa*. Among the sophorolipids, length of alkyl chain, the degree of unsaturation and the number of acyl groups differ.

Synthetic surfactants such as Triton X-100 and Tween 80 can also be used for the same remediation purposes. Boopathy (2002) investigated TNT degradation with the surfactant Tween 80 which served as an additional carbon substrate for the microorganisms. The study highlights the potential of Tween 80 in desorbing TNT from soil and makes it bioavailable to the microbes. It was observed that TNT and its metabolite, 4-amino-2, 6-dinitrotoluene were degraded faster in a period of 35 days. However, the addition of Tween 80 alone did not result in complete TNT removal. Molasses, the carbon source, was added to improve the remediation. Sadani et al. (2016) demonstrated explosive biotransformation in soils using rhamnolipid. Biosurfactant rhamnolipids improved the solubility of the explosive Pentaerythritol Tetranitrate (PETN), enhanced dispersion in aqueous solution and promoted homogeneous distribution in soil. Out of the many explosive-transforming cultures containing Proteobacteria of the genera *Achromobacter, Stenotrophomonas, Pseudomonas, Sphingobium, Raoultella, Rhizobium*, and *Methylopila, Achromobacter spanius* S17 and *Pseudomonas veronii* S94 possess high TNT transformation rates and act as biosurfactant producers especially when an additional carbon source is generally added. Recent study by Amin et al. (2017)demonstrated concomitant removal of TNT and PETN from the soil in aerobic conditions with the aid of rhamnolipid biosurfactant. Sadani et al. (2016) showed increased solubility of TNT and enhanced biodegradation rates with rhamnolipids.

## Conclusion

Contamination of soil and groundwater with explosive residues is prevalent in many countries due to intensive military activities and improper industrial disposals. Among the many explosive compounds, 2, 4, 6 TNT is predominant in use and is classified as a Class C (human carcinogen) compound by United States Environmental Protection Agency (EPA 2017). Fate and distribution of TNT is mainly controlled by sorption and biodegradation. Technologies including incineration, composting, chemical oxidation and adsorption have their limitations. TNT, though being persistent in the soil, is still susceptible to microbial attack. To enhance the bioavailability of TNT to microbes, addition of external agents such as surfactants or bio surfactants seems to be essential. Nitroaromatic explosives especially TNT requires a co substrate for complete transformation of TNT to TAT and further to polymerization products. Surfactants, though effective, can lead to operating problems such as sorption onto soil surfaces, foaming and precipitation. However, it is understood that biosurfactant sorption at the soil-water interface would be responsible for enhancing desorption kinetics and biodegradation kinetics. Biotechnological advancements on the remediation of explosive contaminated soil or groundwater using biosurfactants is therefore essential and should be the scope for further studies.

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