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Enhance biodegradation of pentaerythritol tetranitrate (PETN) anaerobic/aerobic biological treatment by biosurfactant

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Pentaerythritoltetranitrate (PETN) is explosive that show more toxic to some microorganisms than other explosives. Some chemical and Structural character of PETN such as very low solubility in water can cause some problem to biological treatments in success. This study was undertaken to evaluate the potential for using anaerobic/aerobic sequences to degrade PETN and the influence of rhamnolipids biosurfactant for PETN biodegradation improvement. The results showed that the anaerobic treatment with rhamnolipid biosurfactant caused an almost 74% transformation of PETN and 30 days aeration of the reactor led to an elimination of most of the remaining PETN (almost 98%). in the control experiments without biosurfactant a 24% PETN removal was observed. Rhamnolipids can enhance solubility of PETN and disperse it in aqueous solution and cause homogeneous distribution in soil can cause microbial PETN degradation enhancement and more denitrated metabolites disappearance.

Keywords: Explosive, PETN, Rhamnolipid, Anaerobic/Aerobic degradation

INTRODUCTION

Manufacture, handling, and disposal of Army munitions facilities cause Contamination of soil and water. Moreover, the current emphasis on demilitarization, disposal of unwanted weapons systems adds to the pollution problem [1]. PETN is widely used as a powerful military explosive and more sensitive to shock and friction than other secondary explosives like TNT, HMX and RDX. It is commonly used in a mixture with them to produce Semtex plastic explosive and improvised explosive devices (IEDs) [2].

PETN is also used as coronary vasodilator disease in the treatment of heart disease. Some research show that PETN to be five times more toxic to some microorganisms than RDX thus can be classified as munitions constituent of great concern [3].

Past methods for disposing of explosive wastes have included incineration, chemical oxidation, Adsorption, dumping at sea and dumping at specified landfill areas. Limitations containing the retention of untreated and undefined compounds harm to fragile ecosystems, air pollution by incinerations, and disposal on land may lead to soil and groundwater contamination that will affect humans, and animals [4].

Recent researches have focus on biotreatment processes because they are ecologically harmless due to low energy consumption, low emissions, and preservation of the biological activity of the soil [5]. Although biological treatments are attractive alternatives to contaminated soil but some chemical and Structural character of PETN can cause problem in success of process. Structurally, the nitrate ester linkage such PETN is extremely rare in nature and therefore that is considered recalcitrant to biodegradation and represents a major bioremediation challenge [6]. On the other hands, PETN show very low solubility in water and high solubility in acetone.

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Because of the low solubility of explosive compounds, an external co-substrate is commonly added to stimulate the growth of the explosive degrading bacteria.

The use of surface active agents like surfactants is a possible solutions to limited pollutant bioavailability [7].

Surfactants are organic molecules that usually include a hydrophobic part and a hydrophilic part. The hydrophilic part makes surfactants soluble in water, while the hydrophobic part makes them tend to concentrate at interfaces. Enhancement of xenobiotic compound solubility is one of the main mechanisms that surfactants can improve the bioavailability of hydrophobic organic pollutants. This so called 'solubilisation' is caused by the presence of micelles. Formation of micelles is specific characteristic of surfactants that consist of small aggregates of surfactant molecules. In the center of the micelles, hydrophobic compounds will dissolve, whereas more hydrophilic molecules may be present in the core and the shell of the micelles. The results of older study shows that in many case transport of micellar hydrocarbon to the aqueous phase can be very rapid [8].

Applications of rhamnolipid biosurfactant for improvement of xenobiotic bioremediation examined in other study like, Zhang and Miller (1992) for octadecane [9], Beal and Betts (2000) for hexadecane [10], Noordman et al. (2002) for hexadecane [11], Al-Awadhi et al. (1994) for oil-contaminated desert sands [12], Rahman et al. (2003) for n-alkanes in petroleum sludge [13] and Park et al., 1990 for olycyclic or polynuclear aromatic hydrocarbons (PAHs) [14].

Some studies of PETN biotransformation were primarily focused aerobic bacterial species under nitrogen-limiting conditions that showed it to be capable of aerobic growth using PETN as the sole nitrogen source [15]. Other study was evaluating the potential using indigenous bacteria for PETN degradation under anaerobic conditions [6]. The results of that study showed in bioremediation process, PETN underwent sequential reductive denitrification Processes, resulted releasing of nitrite and nitrate in each denitrification step but any report don't resulted of complete denitrification of PETN in bioremediation processes. In the other hand, low solubility and other chemical characters of PETN can cause that the rate of biological process was relatively slow [16].

This study was undertaken to evaluate the potential for using anaerobic/aerobic sequences to degrade PETN with the aim to aerobic incubation after anaerobic resulted in a further decrease of derivatives, presumably due to oxidative transformations. On the other hand, investigate the influence of rhamnolipids biosurfactant on PETN biodegradation rate and process.

MATERIALS AND METHODS

PETN-contaminated soil

The soil used in the laboratory experiments was obtained from garden soil containing 5.5% organic matter. The PETN concentration was 200 mg/kg soil.

Before use, the soil was air dried and ground to pass a 2 mm sieve before use. According to the dissolved experiments [17], PETN dissolved in organic solvent (acetone) was transferred into dried and sieved soil and was evenly distributed to obtain a final concentration of 200 mg/kg in soil matrix.

Laboratory batch experiment

For aerobic and anaerobic soil bioremediation, six soil-pan experiments were conducted. Each pan was prepared by placing 3000 g of contaminated soil in a square polyethylene with aluminum foil as Surface coatings for aerobic and 4000 g for anaerobic. This mass of soil filled the pans approximately two-thirds full. For Improvement of soil porosity 40 g sown dost was mixed thoroughly into 1000 g of the soil.

A total of 3 sets of treatment treatments were conducted; set-1, were control samples with no amendment added; set-2 contained activated sludge; set-3 contained biosurfactant only; set-4 contained biosurfactant and activates sludge as a source of microorganisms.

The bottoms of the pans were perforated to allow drainage of fluids during and after flooding phases, via 1.5 mm diameter holes spaced 2 cm apart in a square grid. Each pan was placed inside a slightly larger glass pan to provide nutrient solutions and sampling of drainages water for COD and PETN concentrations.

Anaerobic pan continuously flooded with deionized water to maintain anaerobic conditions in the pan. During the aerobic phase air was introduced into the soil slurry system. Air was supplied twice a day for 20 minutes, through a diffuser.

The PETN and other contaminant concentrations, bacterial growth, pH, oxygen uptake, nitrate, and ammonia were monitored periodically in all reactors.

During the set 3and 4, rhamnolipid biosurfactant were added to stimulate biological activity and improve the solubility of PETN. The duration of the anaerobic phase was 80 days. This was followed by an aerobic phase of 20 days. At the start of the aerobic phase, 500 mL of the mixed bacterial culture Obtained from activated sludge was added. Each

treatment involved the same laboratory conditions with identical set-up and sampling and analysis procedures.

Chemical analyses

Nitrate and ammonia concentrations were analyzed by colorimetric methods using Hach water analyses reagent kits [18].

PETN was supplied by a local explosives producer in Isfahan city. Other chemicals were of analytical grade and were obtained from Sigma-Aldrich and Merck.

Analysis of PETN transformation was carried out using a using a Waters HPLC system (Milford, USA) equipped with a UV detector.

Water–methanol–acetonitrile mixture (40:50:10, v/v/v) was used as the mobile phase at a flow rate of 0.8 mL min -1. The injection volume was 20 µL and the absorbance was measured at a wavelength of 210 nm. The detection limit was 0.1 mg L-1. Final confirmation of target products made whereby LC/MS.

RESULTS AND DISCUSSION

PETN biodegradation under anaerobic – aerobic condition

PETN-contaminated soil spiked was treated anaerobically by percolated for 80 days with tap water. After 80 days the water was drained off and reactor was flushed with air, and the soil was treated aerobically for 30 days. other study use combination of anaerobic and aerobic treatment for explosive bioremediation, for example D. Bruns-Nagel and et al in 1998 use Anaerobic/Aerobic Composting for 2,4,6-Trinitrotoluene biotransformation [19].

The changes in PETN concentrations over the 80 days anaerobic and 30 days aerobic incubation period in are shown in Fig1. Analysis of the soil mixture showed that the anaerobic treatment with rhamnolipid biosurfactant (80 days) caused an almost 74% transformation of PETN and 30 days aeration of the reactor led to an elimination of most of the remaining PETN (almost 98%).

It should be mentioned that a 24% PETN removal was observed in the control experiments (without biosurfactant) which might be a result of treatment with natural organism or physical and chemical elimination. It is notable that bacterial consortium in all pans was same.

The results show that, although soil microbes could degrade PETN without biosurfactant, the addition of biosurfactant enhanced microbial PETN degradation.

Many studies on biodegradation of nitroaromatic Specially PETN in soil have shown that their slow release from the soil matrix to the aqueous phase is often the rate limiting step in the process, on the other hand, PETN has a low solubility (<40 mg/L), therefore the initial concentration of crystallized PETN in the sediment is expected to have minimal effect on microbial activity and biodegradation rate [6].

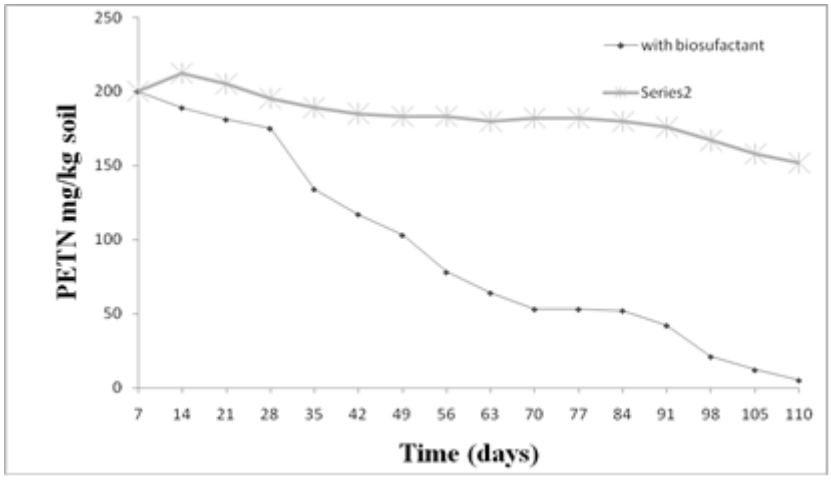

Fig1: The changes in PETN concentrations over the anaerobic and aerobic soil treatment

Because of PETN crystal character, distribution of it at soil is very heterogeneous, resulting in different initial concentrations in soils that has problematic effect in bioremediation [16].

Biosurfactant such as rhamnolipids can enhance solubility of PETN and disperse it in aqueous solution and cause homogeneous distribution in soil. On the other hand, biosurfactant is also biodegradable and can act as a carbon source and Co-metabolism have led to higher rate of biodegradation of PETN. Previous research has shown that Tween 80 surfactant canEnhance TNT biotransformation rate. Also the results of study shows that addition of Tween80 in high concentrations can cause toxic effect and inhibits microbial function but rhamnolipid biosurfactant

because of biologic character don't have any toxic effect in microorganism [20].

Table1 exhibits the PETN degradation intermediates in the aerobic and anaerobic process with and without biosurfactant. (Inoculated bacterial liquid culture derived from PETN-contaminated soil without enrichment and selection).

Table 1. Changes in PETN, PETriN and PEDN over time (inoculated: bacterial liquid culture derived from PETN-contaminated soil without enrichment and selection)

Bioremediation	Anaerobic		Aerobic	
Denitrated metabolites After 7 Weeks	Without biosurfactant	With biosurfactant	Without biosurfactant	With biosurfactant
	PETriNPETriN, PEDN, PEMN		PETriN	PEMN
Denitrated metabolites After 14 Weeks	PETriN, PEDN	PEDN, PEMN	PETriN	-

As PETN degraded, the Denitrated metabolites consist of PETriN, PEDN and PEMN are generated. This metabolite is the same of other mentioned in pervious study [21].

The presence of PETriN, PEDN, PEMN and the potential presence of pentaerythritol suggest that three or four nitro groups are sequentially removed from PETN via biological reactions [6].

Same PETN degradation, the removals of the three intermediates in the presence or absence of rhamnolipid in anaerobic and aerobic were different. In anaerobic treatment in the tab with biosurfactant PETriN, PEDN, PEMN are detected but in tab without biosurfactant PETriN was detected only. These results indicate that in tab with biosurfactantdenitrification process is more completed than tab without biosurfactant.

Although after the end of anaerobic phase with biosurfactant shows higher removal of PETN but the denitrification metabolites (PEDN, PEMN) still was found. Only during the aerobic phase did the denitrification metabolites disappear completely. These results can due to humification and bounding the denitrification metabolites to the soil. These observations agree with results of previous study that shows the effects of aerobic process after anaerobic for complete disappearance of TNT denitrification metabolites after the final aerobic treatment [22].

CONCLUSSION

The results of the study demonstrate that the anaerobic–aerobic soil treatment with rhamnolipid biosurfactant additions represents an efficient strategy for PETN biotransformation in soils.

Rhamnolipids can enhance solubility of PETN and disperse it in aqueous solution and homogeneous distribution in soil that cause microbial PETN degradation enhancement.

Anaerobic treatment followed by an aerobic phase results in irreversible binding of denitrification metabolite of PETN with soil and complete disappearance of them.

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