Remediation of explosive-polluted soil in slurry phase by aerobic biostimulation

Baoping Xin, Mengyue Shen, Hina Aslam, Feng Wu
Department of Environment and Energy, School of Chemical Engineering and Environment, Beijing Institute of Technology, Beijing 100081, P. R. China

E-mail: xinbaoping@bit.edu.cn

Abstract. There is a great volume of polluted soil by 2,4,6-trinitrotoluene (TNT) manufacturing wastewater containing dozen of nitrocompounds in China. In this study, biostimulation was used for remediating the explosive-polluted soil in aerobic bioslurry by monitoring the removal of total organic carbon (TOC). The results showed that the pulp density had almost no effect on TOC removal; whereas the acetone addition evidently improved remediation efficiency of the polluted soil by intrinsic microorganism, and the TOC removal increased from 25% to 38.4% when dose of acetone increased from 0% to 4% (v/v). The maximum TOC removal of 49.1% was achieved through further adjusting pH at 9.0 and temperature at 30 ^{o}C. The second order reaction fits well removal dynamics of TOC under the optimum conditions. With the average conditions, liquid phase TOC decreased from 3404 to 3144 mg/L and solid phase TOC dropped from 1022 to 104 mg/L, leading to toxicity decline by 35%; the optimum condition witnessed 48.9% of TOC removal from 4500 to 2300 mg/L in liquid phase, causing toxicity drop by 62%.

1. Introduction

Nitroaromatic explosives have been extensively used for both military and civilian purposes for a much long time [1, 2]. The explosives can accumulate at the sites and surrounding soils during production, packing, storage, application, ordnance demilitarization, demolition procedures, and destruction of outdated and faulty ammunition, resulting in serious soil pollution by the target explosive compounds [1, 2]. The high toxicity, mutagenicity, and recalcitrance of these energetic compounds have enabled the remediation of explosive-polluted soil to be more urgent [1, 2]. Among such compounds, 2,4,6-trinitrotoluene (TNT) is a predominant contaminant [3, 4]. TNT contamination of soil occurs because of the manufacture, loading, assembly, packing and military related activities [5, 6]. Due to the potential adverse effect of the compound on human health and ecosystem, remediation of the TNT-polluted soil has been draw growing concerns in recent years [2,3,6], especially the bioremediation processes based on aerobic or anaerobic TNT transformation were developed by monitoring the residue, metabolism and fate of TNT in soils or soil slurries[7, 8, 9, 10, 11, 12].

In addition of the polluted soils by direct settlement and mixture of TNT as a target pollutant, quite a few explosive-polluted soils originated from TNT manufacturing wastewater in China. TNT

production generated a massive volume of wastewater, known as red water, pink water and yellow water [13, 14, 15]. TNT manufacturing wastewater is characteristic of high COD, high chromaticity and high toxicity, containing dozens of organic pollutants including raw materials, intermediates, by products, final products and their isomers, sometimes even more than hundred of ones; however, the content of TNT in the TNT manufacturing wastewater is always rather low [16, 17, 18]. In the early 10-20 years of TNT production in the last century, many of TNT manufacturing wastewater was discharged into man-made soil ponds and pits without any treatment by TNT manufacturing factories of China for self purification. After evaporation and infiltration of water, these massive of organic pollutants accumulated in the bottom of pits and ponds and permeated into the surrounding soils, leaving vast volume of explosive-polluted soils in China. The explosive-polluted soils by TNT manufacturing wastewater greatly differ from the TNT-polluted soils by direct settlement and mixture of TNT. The former covers dozens of organic pollutants rather than TNT alone. In fact, the concentration of TNT in the explosive-polluted soil is very low. Under the circumstances, obviously it is not incompetent to assess remediation efficiency of the polluted soil through monitoring residual concentration of TNT; in contrast, the total organic carbon (TOC) is more suitable to reflect the removal efficiency of nitro-compounds pollutants as an integrated index. Moreover, the resulting treated soil should receive the biological toxicity test to evaluate the ultimate remediation effect. However, there are few reports about remediation of the polluted soils by TNT manufacturing wastewater based on TOC analysis and corresponding toxicity test.

Bioremediation is a low-cost, efficient, environmentally friendly way for remediation of polluted soil [2]. The most widely used bioremediation procedure is biostimulation through addition of carbon source, electron donors, inorganic nutrients such as N and P to stimulate degrading activity of intrinsic microorganisms which has adapted to the pollutants [6]. Solid phase bioremediation methods such as composting and land farming have been used to treat TNT-contaminated soils. However, the prolonged incubation time due to the limited mass transfer in solid phase has forced researchers to resort to slurry phase bioremediation where a mixture of contaminated soil, water and co-substrates is treated, indicating higher removal efficiencies as compared to solid phase [19, 20, 21]. In the present works, remediation of the explosive-polluted soils by TNT manufacturing wastewater in slurry phase by aerobic biostimulation was performed for the first time. For evaluating the potential of slurry biostimulation in remediation of explosive-polluted soils by TNT manufacturing wastewater, four aspects studies were conducted, 1) removal efficiencies of the organic pollutants from soil by slurry biostimulation under different conditions based on TOC analysis; 2) removal dynamics investigation of the organic pollutants from soil; 3) removal mechanisms exploration of the organic pollutants from soil; 4) toxicity decrease evaluation of the polluted soils using luminescent bacteria toxicity test.

2. Materials and methods

2.1. Soil samples

The explosive-polluted soil was collected from the discarded settling ponds for storing the TNT manufacturing wastewater from a TNT manufacturing factory, Gansu province, northwest China. The non-polluted soil was also collected from the same areas as background control. The both of soils were air-dried and sieved through a 0.25-mm sieve and stored in closed containers at room temperature for the property exploration and bioremediation experiments.

2.2. Remediation of polluted soil in slurry phase by aerobic biostimulation

2.2.1 Addition of acetone to improve bioavailability of explosives

The slurry was prepared by mixing 50 g of the polluted soil with 100 mL of deionized water in 250 mL flasks, i.e. solid-to-liquid ratio being 1:2 (w/v). The pH value of the slurry was adjusted to 7.0 with 0.5 mol/L NaOH or H_2SO_4; subsequently, different volume of acetone was added into the above slurry to final doses of 0%, 2%, 4% and 8% (v/v), respectively. The slurries with varied amount of acetone were incubated at a shaker (25 ^oC, 120 rpm) as aerobic biostimulation. During remediation of polluted soil by biostimulation, 2 mL of slurry was sampled periodically, and then extracted with enough fresh acetone to release all the organic matters into the aqueous solution, followed by volatilizing the acetone as organic extraction agent from the aqueous solution at a high temperature of 56.2 ^oC for 20 hours. The resulting solution was analyzed to determine the total organic carbon (TOC) to valuate removal efficiency of total contents of organic explosive pollutants in the soil under different acetone doses.

2.2.2 TOC removal efficiency of slurry under different initial pH

The slurry containing 50 g of soil, 100 mL of deionized water and 4% of acetone was prepared. The slurries were then adjusted with 0.5 mol/L NaOH or H_2SO_4 to pH 5.0, 7.0, 9.0 and 10.5, respectively. The slurries with varied initial pH were incubated at a shaker (25 ^oC, 120 rpm) to start the aerobic biostimulation remediation process and the TOC of slurries were monitored after extraction by enough acetone and following volatilization of acetone as described above.

2.2.3 TOC removal efficiency of slurry under different temperatures

The slurry containing 4% of acetone was prepared and adjusted to pH 9.0, followed by incubation at 120 rpm at different temperatures (20 ^oC, 25 ^oC, 30 ^oC and 35 ^oC). During incubation with varied temperatures, the TOC of slurries recorded to reflect the effect of temperatures on remediation efficiency of the explosive-polluted soil by aerobic biostimulation.

2.2.4 TOC removal efficiency of slurry under different pulp densities

The slurries with different pulp densities were prepared by mixing 100 mL of deionized water with 25, 50 and 100 g of polluted soil, respectively. The slurries were then supplemented with acetone to final concentration of 4% (v/v) and adjusted to pH 9.0. The slurries with different pulp densities were incubated at shaker (30 ^oC, 120 rpm) for aerobic degradation of explosives. The TOC of slurry were measured based on the above procedures to explore remediation efficiency of the polluted soil by aerobic biostimulation under different pulp densities.

All the experiments were carried out in triplicate. The sterile soil by autoclave (121.3 ^oC, 30 min) received the same procedures as the non-treated soil to serve as controls for assessing the possible abiotic remediation. The non-explosive soil also received the same as the explosive-polluted soil to serve as another controls for assessing the removal of non-explosive TOC in soil by biostimulation.

2.3. Mechanisms responsible for remediation improvement under optimum conditions

The remediation performance of slurry phase containing 50 g of polluted soil and 100 mL of deionized water under optimum conditions (4% of acetone, pH 9.0, 30 ^oC) by aerobic biostimulation was compared with that under average conditions (no addition of acetone, pH 7.0). During remediation, the

pH value and ORP value of both slurry bioreactors were detected, the concentrations of NO_2^- and NO_3^- of the liquid phases of both slurry bioreactors were monitored, the TOC of both solid phase and liquid phase of both slurry bioreactors were measured, the total number of bacteria of both slurry bioreactors was counted.

2.4. Toxicity testing of remediated slurry under optimum and average conditions

2 ml of slurry was sampled periodically from both bioreactors, and then mixed with 4 ml of acetone for at least 4 hours at 120 rpm a shaker for extraction, followed by centrifugation at 10000 rpm for 10 min to remove the solid soil. Subsequently, the supernatant was heated at 56.2 ^oC for at least 20 hours for completely volatilize acetone, the resulting solution was analyzed for toxicity test using the Freshwater Luminescent Bacterium Vibrio-qinghaiensis sp. based on the method described by [22]. The dilution factor for 50% of luminescent inhibition as EC50 value was obtained for reflecting the toxicity of liquid sample.

2.5. Analysis methods

The TOC of the soil was measured using the methods described by [23], the TN of the soil was determined according to [24], the pH of the soil was monitored based on description of [25]. For reflect the pollution characteristic of soil by TNT manufacturing wastewater, the soil was extracted with acetone solution (acetone/water, 1/1 in volume) at 10 g soil/50 mL, and the acetone-extracted fraction was analyzed by TOC meter (1020A, OI, USA) after the acetone was volatilized from the liquid solution to obtain the TOC originating from the explosive wastewater; the acetone-extracted fraction was analyzed by TON meter (IL500, HACH, USA) to assess the soluble TON from the explosive nitro-compounds; the dose of TNT of the acetone-extracted fraction was also measured using HPLC methods described by [26] to examine the concentration of the polluted soil.

The pH value of slurry phase was measured directly using a precise pH meter, the ORP value was determined by portable ORP meter. The concentrations of NO_2^- and NO_3^- of liquid phases of slurry bioreactor were analyzed using an ion chromatograph (ICS-1500, Dionex, USA) after removal of the solid matters from slurry samples by centrifugation. The TOC value of slurry phase known as the total TOC was analyzed with TOC meter (1020A, OI, USA) after all the organic matters of the slurry samples were extracted into liquid solution by enough acetone and subsequently the acetone was volatilized from the liquid solution. The TOC of liquid phase of slurry bioreactor was achieved with the same procedure as the total TOC except for the extraction step by acetone. The TOC of solid phase of slurry bioreactor was obtained by deducting TOC of liquid phase from the total TOC. The number of bacteria was obtained by colony count method grown in LB media.

3. Results and discussion

3.1. Properties of the explosive-polluted soil by TNT manufacturing wastewater

The TOC and TON of the non-polluted soil were relatively low, suggesting that the soil from northwest China was infertile (Table 1). In contrast, the TOC and TON of the polluted soil were much higher (Table 1), demonstrating that the soil was seriously polluted by nitro-organic compounds. Because the nitro-explosives including TNT were easily extracted from the soil using acetone, the acetone-extracted fraction could reflect the pollution by nitro-compounds from TNT manufacturing wastewater more accurately. The very high TOC and TON of the acetone-extracted fraction of the polluted soil further demonstrated that the soil suffered from the strong explosive pollution (Table 1).

Although the polluted soil might contain dozens of nitro-compounds from the TNT manufacturing wastewater, TNT was not detected in the acetone-extracted fraction using HPLC method (Table 1), it was because that TNT in the TNT manufacturing wastewater is generally low and TNT transforms into other compounds in the soil. It was concluded that the polluted soil by TNT manufacturing wastewater was different from the TNT-polluted soil. As a result, TOC rather than residual TNT was used to assess the remediation efficiency of the polluted soil in the present studies. There were quite a few of studies about remediation of TNT-polluted soil based on monitoring residual TNT [2, 3, 6]; however, there was no report about remediation of polluted soil by TNT manufacturing wastewater based on TOC analysis.

3.2. Improved effect of addition of acetone on TOC removal from slurry

Bioremediation of explosive-polluted soil has been draw increasing attentions in recent dozens of years [2]. Bioremediation includes biostimulation and bioaugmentation, the former was accomplished by stimulating the growth and activity of the intrinsic microorganisms to enhance biodegradation of organic contaminants through addition of exogenous oxygen and inorganic nutrients; the latter was carried out by injection of the competent biodegrading microorganisms to further accelerate the biodegradation of pollutants, besides the exogenous oxygen and inorganic nutrients [6]. Because the high cost of growing certain biodegrading microorganisms and the weak competitiveness of exogenetic microorganisms in fighting the intrinsic ones, biostimulation possesses a greater application future in treating polluted soil compared with bioaugmentation [27, 28]. Therefore, in this present works the biostimulation was utilized to remediation the explosive-polluted soil in aerobic bioslurry due to the high efficiency of aerobic biodegradation and the greater mass transfer of bioslurry reactor. Several crucial parameters affecting biostimulation such as acetone addition, pH adjustion, temperature variation, pulp density change were optimized for remediation of the polluted soil based on TOC analysis.

Table 1. The properties of the polluted soil by TNT manufacturing wastewater and of the non-polluted soil as background.

Items	**TOC of soil (mg/g)**	**TON of soil (mg/g)**	**pH of soil**	**TOC of extracted fraction (mg/g)**	**TON of extracted fraction (mg/g)**	**TNT of extracted fraction (mg/g)**
Non-polluted soil	14.42	2.29	8.4	1.37	0.21	0
Explosive-polluted soil	31.74	8.33	8.5	14.92(13.55)*	5.87 (5.66) *	0

* The data in the brackets are the actual TOC and TON of extracted fraction from the explosive pollutants.

The TOC variation of soil in the slurry as a function of time under different doses of acetone was illustrated in Figure 1. Addition of acetone evidently improved the remediation of polluted soil by intrinsic microorganism. The maximum removal efficiencies of TOC increased from 25% to 38.4% when dose of acetone increased from 0% to 4% (v/v); however, higher 8% of acetone did not achieve further increase in removal efficiency. In the bioremediation of organic compounds-polluted soil,

surfactants are usually utilized to release pollutants from soil for improving the bioavailability. In explosive-polluted soil, the explosives are subject to be strongly adsorbed by the soil particles, resulting in low bioavailability and poor bioremediation efficiency. In the current study, the cheap acetone as extraction agent was used to dissolve the explosives from soil particle for degradation by the intrinsic microorganism. The results showed that 4% of acetone is sufficient for improving bioavailability of the polluted soil, achieving an increase of 13.4% in removal efficiency of TOC from 25% to 38.4%. Moreover, it was speculated that 4% of acetone did not harm the activity of the intrinsic microorganism, which was proved in the following studies about bioremediation mechanisms.

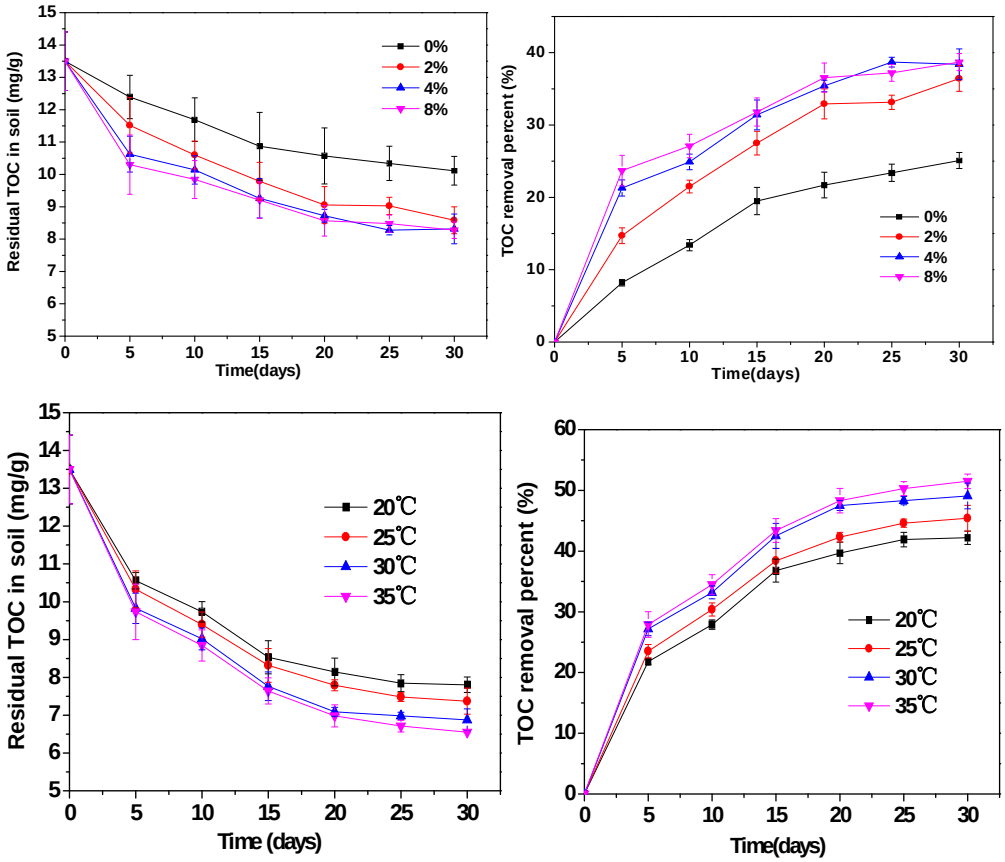

Figure. 1 Time-course for TOC removal percent and residual TOC concentration in the soil of bioslurry in the presence of different volume of acetone (v/v)

3.3. TOC removal efficiency of slurry under different initial pH

The initial pH value has a moderately influence on TOC removal, and the initial pH 9.0 achieved the maximum TOC removal of 45.4% after 30 days of incubation, witnessing an increase of 7.0% in removal efficiency of TOC from 38.4% at pH 7.0 (Figure 2). The results suggested that the weak alkaline of pH 9.0 was fit for the growth of intrinsic bacteria which played an important role in TOC removal; whereas lower pH of acidic condition such as 5.0 or higher pH of alkaline condition such as

10.5 adversely affected activity and growth of intrinsic bacteria, resulting in lower removal efficiency of TOC.

3.4. TOC removal efficiency of slurry under different temperatures

The temperature exhibits a relatively small effect on TOC removal. Although increase in temperature promoted TOC removal, the removal efficiency of TOC rose from 45.4% to 49.1 when temperature went up from 25 ^{o}C to 30 ^{o}C, only 3.7% increase. The highest temperature of 35 ^{o}C achieved the maximum TOC removal of 51.5% , but the 30 ^{o}C was recommended as the optimum temperature for bioremediation of the polluted soil due to the lower energy requirement for maintaining lower temperature.

3.5. TOC removal efficiency of slurry under different pulp densities

The pulp density has no effect on TOC removal. The four pulp densities ranging from 25% to 100% (solid-to-water, w/v) harvested almost the same removal efficiency of about 49% after 6 weeks incubation. So, high pulp density was recommended for bioremediation of the polluted soil because the higher pulp density meant lower water consumption, smaller bioreactor volume and cheaper remediation cost.

3.6. TOC removal dynamics under the optimum remediation conditions

The dynamics analysis of TOC removal is important for regulation and control of the bioremediation process. For this purpose, under the optimum conditions (4% of acetone addition, 50% of pulp density, pH 9.0 and 30 ^{o}C) linear relationship between TOC at certain time and incubation time (T) was established as zero order reaction, the linear relationship between In (TOC) and incubation time (T) was set up as first order reaction, the linear relationship between TOC^{-1} and incubation time (T) was plotted as second order reaction [29]. It was found that the second order reaction fits well the experimental data owing to the greatest R^2， although the other models can also describe the dynamics.

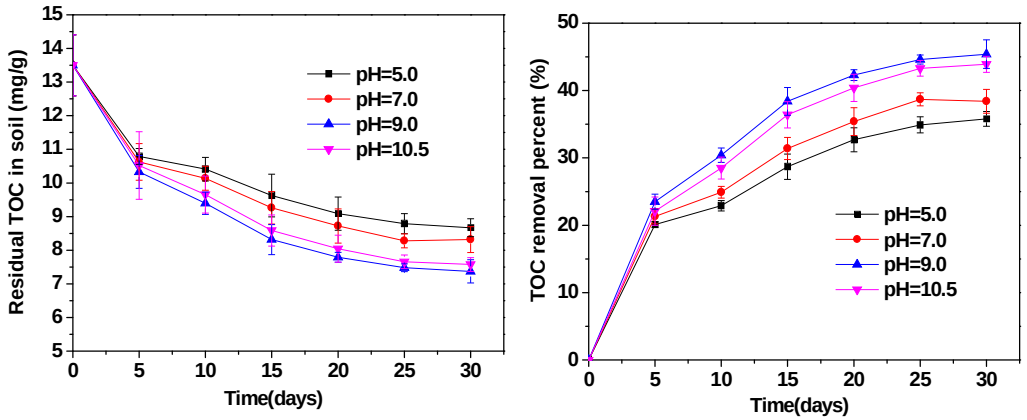

Figure 2. Time-course for TOC removal percent and residual TOC concentration in the soil of bioslurry under different initial pH.

3.7. Mechanisms exploration of remediation improvement under optimum conditions

For exploring the remediation mechanisms, the variation of both pH and ORP under optimum conditions was compared with that under average conditions. It was found that final pH value of both bioreactors approached the same due to the great buffer capacity of the soil (Figure 3). Different from pH, the ORP of both bioreactors kept fluctuation during the bioremediation process (Figure 3), indicating that the oxidation-reduction reaction occurred although the intermediates and details were unknown. However, greater variation range of the ORP with the optimum conditions suggested stronger oxidation-reduction reaction, and the lower ORP values might imply more reducing ambient originated from addition of acetone.

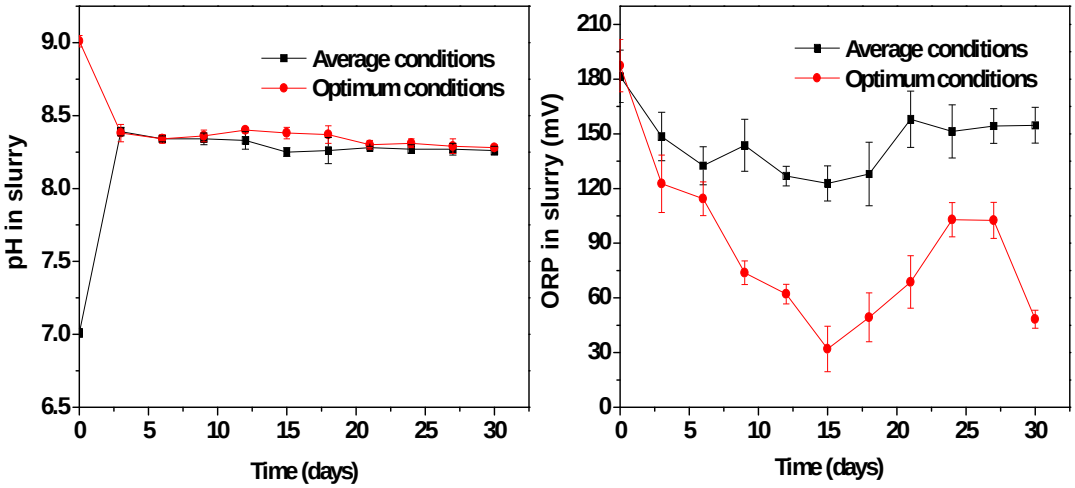

Figure 3. Comparison of both pH and ORP variation as functions of remediated time in slurry between the optimum and average conditions.

In aerobic bioremediation, the nitro group maybe release from the nitro-explosives in the form of NO_2^- which further transforms into NO_3^-. So, the variation of NO_2^- and NO_3^- of the slurry reactor under optimum conditions was compared with that under average conditions for investigating the bioremediation mechanisms (Figure 4). During bioremediation, removal of nitro group from the explosives caused increase of dose of NO_2^- in liquid phase; whereas oxidation of NO_2^- into NO_3^- under aerobic conditions resulted in decrease of concentration of NO_2^-, so there was a dynamic balance with NO_2^-. Like NO_2^-, a dynamic balance also occurred with NO_3^- through generation of NO_3^- from oxidation of NO_2^- and consumption of NO_3^- for growth of microorganisms. Under the average conditions, both NO_2^- and NO_3^- set at dynamic balance; therefore, the concentrations of both NO_2^- and NO_3^- kept almost unchanged over the period of bioremediation (Figure 4). However, the case was completely different with the optimum conditions. On one hand, the stronger growth of the intrinsic bacteria consumed much more NO_3^-, leading to continuous decline of dose of NO_3^- (Figure 4). On the other hand, the higher activity of the intrinsic bacteria achieved faster removal of nitro group from the explosives, resulting to accumulation of NO_2^- in liquid solution (Figure 4).

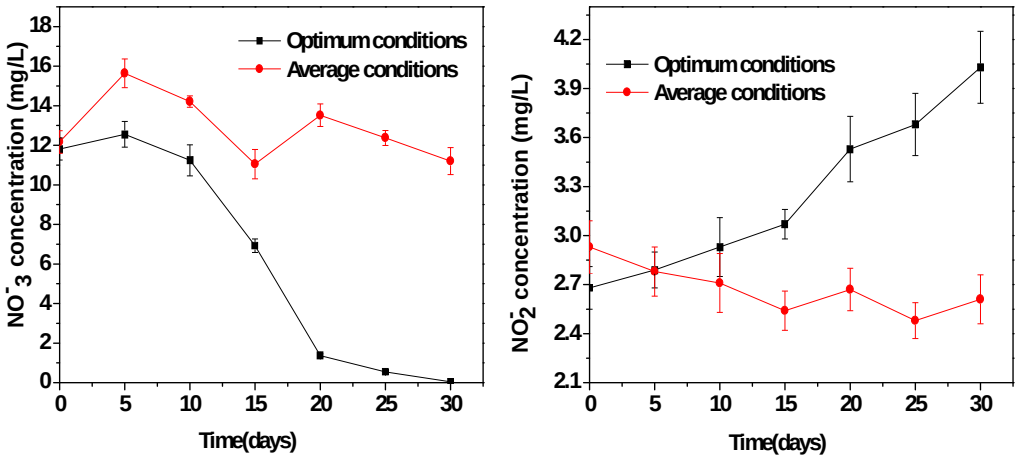

Figure 4. Comparison of both NO_2^- and NO_3^- concentration variation as functions of remediated time in slurry between the optimum and average conditions.

Under the average conditions, the liquid phase TOC decreased from 3404 to 3144 mg/L (i.e. 260 mg/L in removal amount) and the solid phase TOC dropped from 1022 to 104 mg/L (i.e. 918 mg/L in removal amount) after 30 days treatment, respectively, working together to achieve a 26.6% of total TOC removal of slurry from 4426 to 3248 mg/L (Figure 5). The results demonstrated that the solid bioremediation played a more important role than the liquid remediation under the average conditions, although the liquid phase covered 77% of the total TOC. In contrast with the average conditions, the optimum condition witnessed 48.9% of TOC removal from 4500 to 2300 mg/L in the liquid phase because of release of organic matters from the solid phase by acetone (Figure 5), leaving the solid phase free of bioremediation.

3.8. Toxicity testing of remediated slurry under optimum and average conditions

Both of the remediated slurries under the optimum conditions or average conditions witnessed a decrease of toxicity accompanied by bioremediation (Figure 6) indicating that the low-cost and simple aerobic biostimulation was substantially efficient for remediation of the explosive-polluted soil. With the average conditions, the dilution factor for 50% of luminescence inhibition decreased from 3.62 to 2.12, toxicity fell by 35% (Figure 6). In contrast, with the optimum conditions the dilution factor dropped from 3.43 to 1.32, toxicity decline by 62% (Figure 6). Although the optimum conditions achieved a lower toxicity than the average conditions due to the higher TOC removal, the further studies was needed for complete removal of toxicity of the polluted soil by supplement of organic carbon source for co-metabolism.

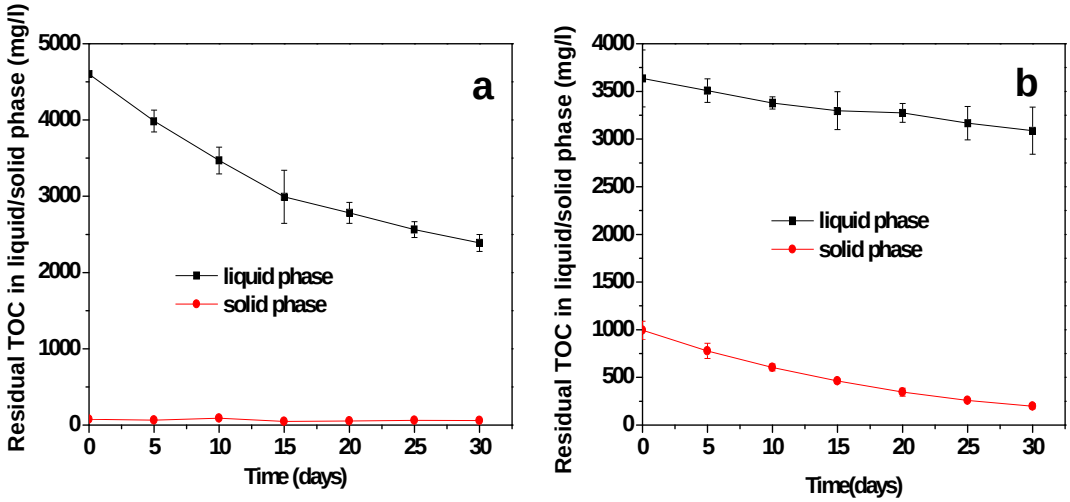

Figure 5. Variation of TOC in both liquid and solid phases as function of remediated time in optimum conditions (a) and average conditions (b)

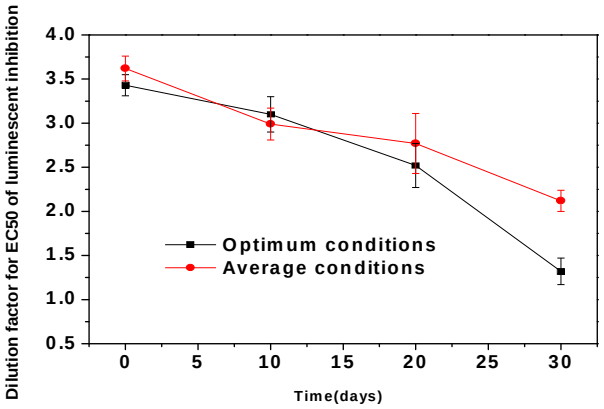

Figure 6. Comparison of toxicity change of the explosive-polluted soil with remediation progress under the optimum and average conditions

4. Conclusion

The maximum removal efficiencies of TOC increased from 25% to 38.4% when dose of acetone increased from 0% to 4%. The initial pH 9.0 achieved the maximum TOC removal of 45.4% from 38.4% at pH 7.0. The removal efficiency of TOC further rose from 45.4% to 49.1 when temperature went up from 25°C to 30 °C. The second order reaction fits well TOC removal dynamics under the optimum conditions. Solid bioremediation played a more important role than the liquid remediation under the average conditions; whereas the optimum condition witnessed complete TOC removal in the liquid phase, causing toxicity decline by 62%.

Acknowledgements

We really appreciate the Shandong Fund of Sciences and Technology for Environment Protection and Basic Research Fund of BIT for providing us the financial support. We also appreciate professor Ma

Mei (Research Center for Environmental Sciences and Ecology, CAS) for the kind help in toxicity test.

References

[1] Jerger D E, Woodhull P 2009 *Applications and costs for biological treatment of explosives-contaminated soils in the U.S. I*n: Spain J C, Hughes J B, Knackmuss H J (eds) Biodegradation of nitroaromatic compounds and explosives(Lewis, New York) pp 395–423

[2] Lewis T A, Newcombe D A, Crawford R L, 2004 Bioremediation of soils contaminated with explosives *J. Environ. Manage*. **70** 291-307

[3] Ayoub K, van Hullebusch E D, Cassir M, Bermond A 2010. Application of advanced oxidation processes for TNT removal: A review. *J. Hazard. Mater*. **178** 10–28.

[4] Esteve Núñez A, Caballero A, Ramos J L 2001 Biological degradation of 2,4,6-trinitrotoluene. *Microbiol. Mol. Biol.* Rev. **65** 335-352

[5] N Samotokin B, Marmiroli M (eds) Advanced science and technology for biological trinitrotoluene. Microbiol *Mol. Biol. Rev* 65 335–352

[6] Gerth A, Hebner A 2007 *Risk assessment and remediation of military and ammunition sites. In: Marmiroli decontamination of sites affected by chemical and radiological nuclear agents NATO science series* vol 75 Springer, Heidelberg pp 45–57

[7] Stenuit B A, Agathos S N, 2010 Microbial 2,4,6-trinitrotoluene degradation: could we learn from (bio)chemistry for bioremediation and vice versa? *Appl Microbiol Biotechnol* **88** 1043–1064

[8] Dillewijn P V, Caballero A, Paz J A, Gonzaalez M M., Oliva J M, Ramos J M, 2007 Bioremediation of 2,4,6-trinitrotoluene under field conditions *Environ. Sci. Technol*. **41** 1378-1383

[8] In B H, Park J S, Namkoong W, Hwang E Y, Kim J D 2008 Effect of co-substrate on anaerobic slurry phase bioremediation of TNT-contaminated soil Korean J. Chem. Eng. **25** 102-107.

[9] Dalgren K E, Waara S, Düker A, von Kronhelm T, van Hees P A W 2009 Anaerobic bioremediation of a soil with mixed contaminants: explosives degradation and influence on heavy metal distribution, monitored as changes in concentration and toxicity. *Water Air Soil Pollut*. **202** 301–313

[10] Newcombe D A, Crawford R L 2007 Transformation and fate of 2,4,6-trinitrotoluene (TNT) in anaerobic bioslurry reactors under various aeration schemes: implications for the decontamination of soils. *Biodegradation* **18** 741–754

[11] Sheibani G, Naeimpoor F, Hejazi P 2011 Statistical factor-screening and optimization in slurry phase bioremediation of 2,4,6-trinitrotoluene contaminated soil *J. Hazard. Mater*. **188** 1–9

[12] Muter O, Potapova K, Limane B, Sproge K., Jakobsone I, Cepurnieks G, Bartkevics V 2012 The role of nutrients in the biodegradation of 2,4,6-trinitrotoluene in liquid and soil. *J. Environ. Manage*. **98** 51 -55

[13] Barreto-Rodrigues M, Silva F T, Paiva T C B 2009 Combined zero-valent iron and fenton processes for the treatment of Brazilian TNT industry wastewater *J. Hazard. Mater*. **165** 1224–1228

[14] Maloneya S W, Adriana N R, Hickeyb R F, Heineb R L 2002 Anaerobic treatment of pink water in a fluidized bed reactor containing GAC *J. Hazard. Mater*. **92** 77–88

[15] Zhao Q L, Ye Z F, Zhang M H 2010 Treatment of 2,4,6-trinitrotoluene (TNT) red water by vacuum distillation *Chemosphere* **80** 947–950
[16] Ciecone V J, 1982 ADA115218
[17] Patterson J 1976 PB265042
[18] Spanggord R J 1978 sADA059434
[19] Park C, Kim T H, Kim S, Lee J, Kim S W 2003 Bioremediation of 2,4,6-trinitrotoluene contaminated soil in slurry and column reactor *J. Biosci. Bioeng.* **96** 429–433
[20] Popesku J T, Singh A, El-Alawi Y, Ward O P 2006 Trinitrotoluene removal in a soil slurry and soil box systems by an oil-degrading mixed bacterial culture. World *J. Microbiol. Biotechnol.* **22** 1075–1081
[21] Robles-González I, Fava F, Poggi-Varaldo H M 2008 A review on slurry bioreactors for bioremediation of soils and sediments *Microb. Cell Fact.* **7** 5–21
[22] Ma M, Tong Z, Wang Z, Zhu W 1999 Acute Toxicity Bioassay Using the Freshwater Luminescent Bacterium Vibrio-qinghaiensis sp Nov. Q67. Bull. *Environ. Contam. Toxicol,* **62** 247-253
[23] Karvelas M, Katsoyiannis A, Samara C 2003 Occurrence and fate of heavy metals in the wastewater treatment process *Chemosphere* **53** 1201–1210
[24] Bremner J M., Mulvaney C S 1982 Nitrogen—total. In A. L. Page, R. H. Miller R H, & D. R. Keeney (Eds.), *Methods of soil analysis.* Part 2 (2nd ed.). Agron Monogr Vol. 9 Madison (WI: ASA and SSSA) ppS 595–624
[25] Wang et al 2010 A rapid selection strategy for an anodophilic consortium for microbial fuel cells *Journal of Bioresource technology*
[26] Guo X J, Xin B P, Ma X T, Xia Y T, Chen S, Yang Y P 2009 Biodegradation of Trinitrotoluene by a Bacterial Consortium Containing Klebsiella sp. and Burkholderia sp.. Chinese Journal of Catalysis, 30, 1261-1268
[27] El Fantroussi S, Agathos S N 2005 Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Curr. Opin. *Microbiol* **8** 268–275
[28] Van Dillewijn P, Wittich R M, Caballero A, Ramos J L 2008 Type II hydride transferases from different microorganisms yield nitrite and diarylamines from polynitroaromatic compounds. *Appl. Environ. Microbiol* **74** 6820–6823
[29] Alberty R A, Silbey R J 2001 Physical chemistry (third edition). John Wiley & Sons, Inc. pp 656–703