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Effect of C/N Ratio on Composting Treatment of TNT-Contaminated Soil

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Abstract: This research was conducted to estimate the effect of C/N ratio control on composting of TNT (2,4,6-trinitrotoluene)-contaminated soil. Glucose or acetone was selected to control C/N ratio of the contaminated soil. The C/N ratios of the controlled experiment and no controlled one were 26.0 and 6.6, respectively. During 45 days, the degradation efficiency (96.0 or 91.8%) of acetone or glucose C/N ratio controlled soil was higher than that (78.4%) of no C/N ratio controlled case. The first order degradation rate constant of glucose or acetone C/N ratio control was 0.0641 or 0.0820/day. This constant was over twice 0.0356/day of no C/N ratio control. The C/N ratio control with glucose or acetone also showed a rather high CO_2 evolution than that without C/N ratio control. It was proven that C/N ratio control for composting of TNT-contaminated soil improved the treatment efficiency.

Keywords: TNT, composting, C/N ratio control, glucose, acetone

Introduction

The nitroaromatic compound TNT (2,4,6-trinitrotoluene) consists of a benzene ring with a methyl group ($-CH_3$) at the 1 position and nitro group ($-NO_2$) at the 2,4 and 6 positions. The molecular weight of TNT is 227.15 g/mole and it has a specific gravity of 1.654. It has a melting point of 80.65°C and a boiling point of 240°C at which point it explodes. It exists as colorless orthorhombic crystals of as yellow monoclinic needles. The water solubility of TNT is 120 mg/*l*. TNT is extremely soluble in variety organic solvents including dimethylformamide, acetone, dimethyl sulfoxide, and benzene.[1]

TNT is introduced into soil and water ecosystems mainly by military and manufacturing activities. TNT and its metabolites are known to be toxic to bacteria, aquatic and terrestrial plants, invertebrates, terrestrial mammals and mammalian cells, and some genotoxic effects have also been demonstrated.[2,3] TNT is also toxic to a number of organisms including humans and may be carcinogenic. Because of its toxic and recalcitrant properties, the contamination of soil and groundwater by TNT represents a significant international environmental problem.[4] Concerns about the environmental fate of TNT residues have intensified because the recent vegetation of contaminated sites could allow TNT, its metabolites and plant-produced TNT intermediates to be introduced into the food chain.[5] The DoD (United States Department of Defense) has identified more than 1,000 sites contaminated with explosives, of which over 95% were contaminated with TNT and 87% exceeded permissible groundwater contaminant levels.[6] In spite of unavailable information for TNT-contaminated soils in Korea, widespread use of TNT on military sites and explosives producing plants may result in numerous occurrences of TNT-contaminated sites.

Composting has been identified as an emerging ex-situ biological technology for organic compounds contaminated soils. In composting process, the contaminated soil must be mixed with a bulking agent, a source of inoculum, and an additional carbon supply, nitrogen and oxygen or air must also be supplied to increase treatment efficiency.

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Microorganisms for composting require adequate levels of carbon sources and other nutrients, including nitrogen, phosphorous, sulfur, and other trace minerals and growth factors. Among these, carbon and nitrogen are usually the limiting substrates, while other elements and nutrients are abundant in composting processes. The optimal ratios for different composting materials generally ranged from 20 to 35.[7)] The chemical formula of TNT is $C_7H_5N_3O_6$ and its C/N ratio is 2. Therefore, the C/N ratio control can be considered in order to promote treatment of TNT-contaminated soils, which usually contain a significant amount of nitrogen. Thus, this research was performed to estimate the effect of C/N ratio control on composting of TNT-contaminated soil.

Materials and Methods

Materials & Experimental Apparatus

The materials used for this research were soil, sewage sludge, and TNT. The soil was sieved through a 2 mm sieve to remove large soil fraction such as stone and gravel. Dewatered sewage sludge was obtained from Nanji Composting Facility at Nanji publicly owned treatment works in Korea. Table 1 shows physical and chemical characteristics of the soil and sewage sludge used for this research. Sewage sludge was used to supplement energy and nutrient sources in contaminated soils. The soil texture was classified as a loamy sand.

Table 1. Characteristics of soil and sewage sludge

Item		Soil	Sewage sludge
pH		7.6	6.8
Volatile solids (%)		3.9	59
Cation exchange capacity (meq/100 g dry matter)		18.6	32.1
Chemical composition (%, dry weight basis)	C	0.42	31.31
	H	0.50	2.63
	O[a)]	2.26	19.12
	N	0.04	4.37
	S	0.16	1.42
	Ash	96.62	41.15
Soil textures (%)	Sand	73.4	
	Silt	17.3	
	Clay	9.3	
		Loamy sand	

[a)] O = 100 − (C + H + N + S) − Ash.

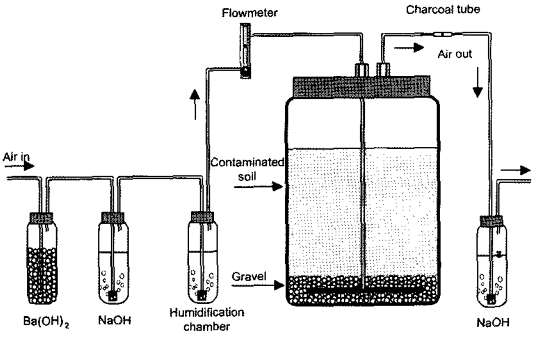

Fig. 1. Schematic diagram of experimental apparatus.

Composting system consisted of a reactor, two CO_2 removal trap, a humidifier, and a trap for collecting CO_2 evolved from biodegradation (Fig. 1). Carbon dioxide was removed from the incoming air so that CO_2 in the exiting air was attributed entirely to decomposition. A humidifier containing distilled water was used to prevent any aspirated alkali solution from entering the reactor and to raise the moisture content of the incoming air to nearly 100% relative humidity. CO_2-removed and humidified air was entered into the reactor through a perforated stainless bottom plate. Volatile compounds from the reactor were collected using a charcoal tube (SKC Cat. No. 226-01) in order to determine volatilization rate.

Experimental Condition

Composting reactor was placed in an incubator in which temperature was maintained at 30°C in order to minimize effect of exterior temperature variation. Air was introduced into the reactor in 200 $l/\mathrm{min \cdot m^3}$. High TNT concentration may also reduce degradation rate if the TNT is toxic to the microbial population. Therefore, TNT as target contaminant was spiked at about 1,300 mg/kg of sample on dry basis. The TNT contaminated-samples in all experiments were consisted of contaminated soil and sewage sludge in the ratio of 1 : 0.5 on wet weight basis.

Glucose or acetone was selected to control C/N ratio of the TNT-contaminated soil. The C/N ratio

Table 2. Experiment condition for this research

Experiments	C/N ratio	Remarks
Glucose	26.0	C/N ratio controlled with acetone
Acetone	26.0	C/N ratio controlled with glucose
No control	6.6	without C/N ratio control

of experiment without the control was 6.6. TNT is extremely soluble in a variety organic solvents, especially acetone of which solubility is 130 g/100 g-acetone. Acetone was found to delay the self-heating of the composts but did not inhibit degradation of the TNT. The optimal C/N ratio for composting ranged from 20 to 35. The C/N ratio controlled with acetone or glucose was adapted to 26.0. This C/N ratio was corresponded to four times of that without C/N ratio control (Table 2).

Sampling was carried out as the method described by Hwang (1999).[8] In order to minimize sampling errors, the samples were taken at three depths. These subsamples were integrated to one sample by mixing.

Analysis

Analytical mix standards was purchased from AccuStandard (Mix A (M8330A-R-10X) and Mix B (M8330B-R2-10X), New Haven, USA). The mixture contained 2-ADNT, 1,3-DNB, 2,4-DNT, HMX, NB, RDX, 1,3,5-TNB and 2,4,6-TNT for Mix A and 4-ADNT, Tetryl, 2,6-DNT, 2-NT, 3-NT and 4-NT for Mix B. The standards were diluted in acetonitrile to achieve a 10 ppm.

A 2.0 g sample was placed in a 10 m*l* glass vial equipped with a Teflon-lined cap. A 10 m*l* of acetonitrile was added in the vial. The target compounds in sample were dispersed by vortex mixing for 1 min and extracted in an ultrasonic bath (Branson Ultrasonic Corp., 8210R-DTM) for 18 hours. The vials were removed from the bath and allowed to settle for 30 min. A 5 m*l* aliquot was removed, placed in a glass vial and combined with 5 m*l* aqueous $CaCl_2$ (5 g/*l*). The vials were shaken and centrifuged at 2,000 g for 20 min. A 5 m*l* aliquot was filtered through a 0.45 μm PTFE filter (Millipore, JHWP02500) into a clean vial. The sample extracts were analyzed using RP-HPLC (reversed-phase high performance liquid chromatography) method (SW-846 Method 8330A) described by USEPA (1998).[9]

CO_2 evolved by biological reaction was collected in 4N NaOH as proposed by Stotzky (1979).[10] An excess of barium chloride (3N $BaCl_2$) was added to precipitate the carbonate as $BaCO_3$. After adding a few drops of phenolphtalein indicator, the samples were titrated with 1N HCl.

Results and Discussion

Effect of C/N Ratio

The effect of C/N ratio control on TNT degradation is presented in Fig. 2. The mix ratio used in all experiments was the same as 1:0.5 of contaminated soil to sewage sludge. The C/N ratio controlled experiments with glucose or acetone were compared to that (C/N = 6.6) without control. As shown in Fig. 2, C/N ratio control with glucose or acetone degraded TNT in contaminated soil more greatly. In glucose C/N ratio controlled experiment, TNT concentration decreased sharply until 20 days from initial 1,294.0 mg/kg to 147.5 mg/kg. After that time, TNT concentration was slowly decreased to 106.1 mg/kg at the end of composting, which indicated a degradation efficiency of about 92% (see Fig. 3). On the other hand, in acetone C/N ratio controlled experiment, TNT concentration decreased until 20 days from initial 1,302.4 mg/kg to 156.7 mg/kg. TNT concentration of the final compost was 51.5 mg/kg, which was slightly lower

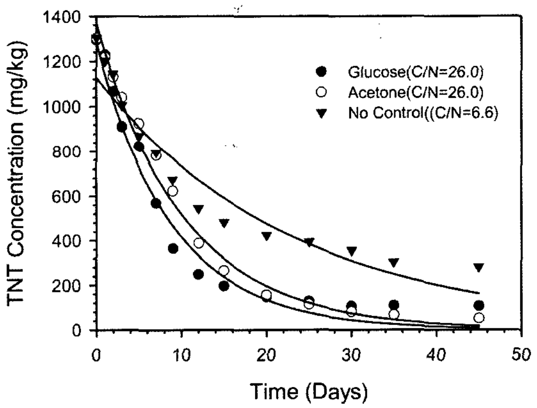

Fig. 2. Variation of TNT concentration (The legend of 'no control' means the case without C/N ratio control, and the 'glucose' or 'acetone' means C/N ratio control experiments).

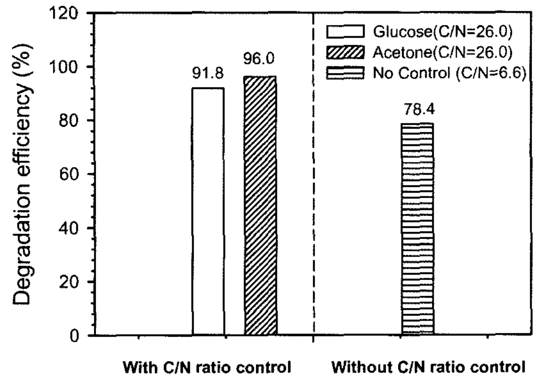

Fig. 3. Degradation efficiency of TNT according to C/N ratio control.

than that of glucose C/N ratio controlled experiment. The degradation efficiency (96.0%) of acetone-C/N ratio controlled experiment was about 4% higher than 91.8% of glucose C/N ratio controlled one. 78.4% of the initial TNT concentration was removed in no C/N ratio controlled case. It was estimated that C/N ratio control for composting of TNT-contaminated soil improved the treatment efficiency.

TNT has been founded in particulate form (chunk) and heterogeneously dispersed in explosive contaminated sites.[11,12] Unfortunately, chunk TNT isn't broken down as easily by the microbes and remains in the soil at the end of the composting period. Radtke *et al.* (2000)[13] evaluated effect of acetone addition as pretreatment on the composting of TNT-contaminated soil. They reported that acetone-pretreated soil responded to composting significantly better than untreated soil because TNT is extremely soluble in acetone, of which solubility is 130 g/100 g of acetone. Bradley and Chapelle (1995)[14] reported that microbial TNT mineralization was significantly inhibited by the addition of complex carbon source such as cellobiose and syringate. As the result of this study, acetone addition did not inhibit degradation of the TNT. Therefore, it can be considered that the acetone addition enables soil microorganisms to access TNT easily as well as to be used as carbon source.

Table 3. Degradation kinetic parameters based on the first-order and zero-order kinetic models

	Zero-order		First-order		
Experiments	K[a] (mg/kg/day)	r[b]	k[c] (1/day)	r	Half-life (day)
Glucose	40.11	0.69	0.0641	0.91	10.8
Acetone	39.93	0.79	0.0820	0.98	8.50
No control	32.22	0.69	0.0356	0.95	19.5

[a] K: the zero order kinetic constant.
[b] r: correlation coefficient.
[c] k: the first order kinetic constant.

Kinetics

Degradation kinetic parameters based on the first order and zero order kinetic models are presented in Table 3. The correlation coefficients for the kinetic models indicated that the commonly used first order model described the TNT degradation with high correlation (r = 0.91~0.98) compared to that (r = 0.69~0.79) of the zero order model. The first order kinetic model was linearly regressed with relationship between operational period and natural log value of TNT concentration. The first order degradation rate constant of glucose or acetone C/N ratio control was 0.0641 or 0.0820/day. This constant was over twice 0.0356/day of no C/N ratio control. It was estimated that C/N ratio control in the contaminated soil composting process enhanced TNT degradation from the first order degradation rate data. The most active degradation of TNT occurred in the C/N ratio control using acetone (k = 0.0820/day).

Biological Parameters

Biological parameters including evolved-CO_2, total bacterial number, and dehydrogenase activity are predicted in Fig. 4. Fig. 4(a) shows cumulative evolved-CO_2. The C/N ratio control with glucose or acetone showed a rather high CO_2 evolution than that without C/N ratio control. However, significant difference in cumulative CO_2 was not observed between glucose and acetone addition. In case of acetone addition, cumulative evolved-CO_2 was slightly higher than that of glucose addition at early composting until 20 days. On day 45, total amount of evolved-CO_2, however, was slightly higher as 13,262 mg/kg for glucose addition than 12,668 mg/kg for acetone addition.

Total bacterial number in Fig. 4(b) reflected TNT degradation and CO_2 generation rate well (see Fig.

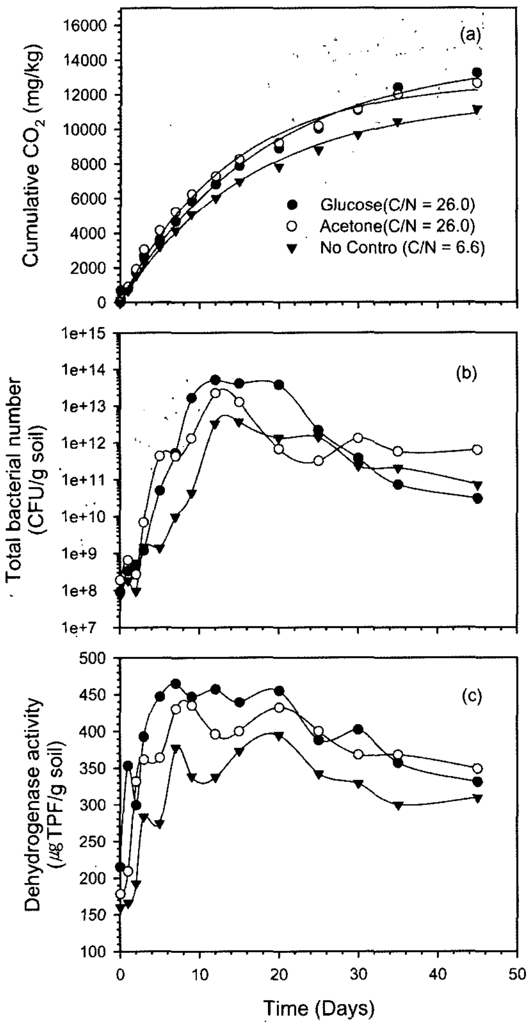

Fig. 4. Variation of (a) cumulative evolved-CO_2, (b) total bacterial number, and (c) dehydrogenase activity.

2 and 4(a)). Maximum growth was observed in glucose C/N ratio control. The total bacterial number of initial samples in all experiments existed from about 10^7 CFU (colony forming unit)/g to 10^8 CFU/g on dry weight basis. It was dramatically increased to about 10^{12} CFU/g to 10^{13} CFU/g until 15 days, and thereafter decreased gradually to about 10^{10} CFU/g to 10^{11} CFU/g. In case of C/N ratio control with glucose or acetone, total bacterial number was more promptly increased than that of no C/N ratio control in the early stage.

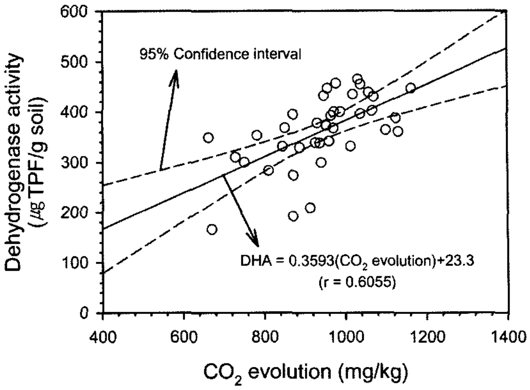

Fig. 5. Relationship between dehydrogenase activity and CO_2 evolution (n=39).

Microbial population size is not always a good indicator of microbial function in soil system, because many of the microbes found in the system cannot be isolated and those grown on a nutrient-rich medium plate may have been inactive in the soil.[15)] Therefore, dehydrogenase activity (DHA) was used as an indicator of microbial activity in this research. Dramatic increase of DHA was observed in all experiments at the early stage. DHA in the glucose-C/N ratio control significantly increased from the initial 215 to 465 μg TPF (triphenylformazan)/g on day 7 and showed the highest value, and thereafter decreased gradually to 331 μgTPF/g (Fig. 4(c)). This means that microbial activity expressed as DHA was the greatest in the early stage. A similar increasing trend was observed in the acetone-C/N ratio control. As described previously, this trend of DHA was largely corresponded to those of total bacterial number. However, the DHA was get to the top more rapidly compared to total bacterial number.

Parameters such as evolved-CO_2, total bacterial number, and dehydrogenase activity are biological indicators. Relationship between CO_2 evolution and DHA was estimated in Fig. 5. The relationship at all experiments (n = 39) was developed as the first order equation, which was [(DHA as μgTPF/g of soil) = 0.3593 × (evolved-CO_2 mg/kg of soil) + 23.3] with r of 0.6055 and $p<0.05$. Frankenberger and Dick (1983)[16)] reported the DHA showed a significant positive correlation with microbial activity as CO_2 evolution in glucose amended soils (r = 0.83, $p<0.05$). Hwang (1999)[8)] also reported that

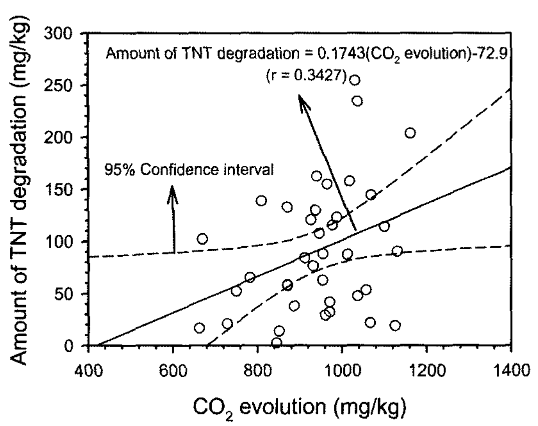

Fig. 6. Relationship between amount of TNT degradation and CO_2 evolution (n=39).

DHA showed positive correlation with CO_2 evolved in diesel contaminated soils (r = 0.59, $p<0.01$). Van Der Waarde *et al.* (1995)[17] reported that bacterial numbers are indicative of a stimulated biodegradation process, but do not represent an accurate measurement of the actual biodegradation. DHA appeared to have a good relationship with CO_2 production in several soils.

Fig. 6 shows correlation coefficient between the amounts of TNT degradation and evolved-CO_2. As shown in Fig. 6, there was weak correlation between the two parameters. The relationship between the amounts of TNT degradation and evolved-CO_2 in all experiments (n = 39) was developed as the first-order equation, which was [(degraded-TNT mg/kg soil) = 0.1743 × (evolved-CO_2 mg/kg of soil) − 72.9] in r of 0.3427 and $p<0.05$. CO_2 analysis used in this study do not reveal whether carbon fraction of CO_2 evolved from composting reactors was the carbon by TNT degradation or that by biodegradation of sewage sludge as organic amendment. Hawari *et al.* (1997)[18] reported that although TNT undergoes extensive biotransformation, very little (<10%) or no mineralization takes place. In order to evaluate mass balance, Pennington *et al.* (1995)[19] performed composting of TNT-contaminated soil, which was amended with radiolabelled TNT before composting. The mass balance results indicated that TNT was not mineralized to volatile organic compounds and almost no radiolabelled CO_2 was produced.

Conclusions

The C/N ratio was controlled into 26.0 to estimate the effect of C/N ratio control on composting of TNT-contaminated soil. Glucose or acetone was used to control C/N ratio of the contaminated soil. The C/N ratio of no controlled soil was low as 6.6, of which value was out of general C/N ratio for composting.

During 45 days, the degradation efficiency (96.0 or 91.8%) of TNT in acetone- or glucose-C/N ratio controlled soil was higher than 78.4% of no C/N ratio controlled case. C/N ratio control with glucose or acetone degraded TNT in contaminated soil more greatly. The first order degradation rate constant of glucose or acetone C/N ratio control was 0.0641 or 0.0820/day. This constant was over twice 0.0356/day of no C/N ratio control. It was proven that C/N ratio control in the contaminated soil composting process enhanced TNT degradation from the first order degradation rate data. From the results of biological parameters including evolved-CO_2, total bacterial number, and dehydrogenase activity, it was estimated that these parameters were related with degradation of TNT and could be used as biological indicators.

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