Fate of Endocrine Disruptors in Sewage Sludge

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Project period: 2002-2005

OBJECTIVES

Recycling of sewage sludge has been increasing in Japan. In FY2002 the total amount of sewage sludge generated was 2,105 kt of dry solids (DS) and around 14% of this amount (293 kt-DS) was recycled for agricultural use. On the other hand, there is a growing concern that hazardous organic compounds may be present in sewage sludge. Nonylphenolic compounds (NPCs) and estrogens are important groups of such compounds because of their endocrine-disrupting activities. Nonylphenols (NPs) and nonylphenol n ethoxylates (NPnEOs, n = 1 - 15) of shorter ethoxylate chain are hydrophobic and tend to be adsorbed by the sludge, hence land application of this sludge compost may contaminate soil and water environments.

This study proposes an extraction method for analyzing endocrine disruptors in sewage sludge, and reveals the fate of endocrine disruptors in the land application of sewage sludge compost. The study also reveals the relationship between the fate of endocrine disruptors in the sewage sludge treatment process and the operating conditions.

RESULTS

In FY2004, we developed a method of measuring NPs, NPnEOs and NPnECs by LC/MS/MS (Liquid Chromatograph / Mass Spectrometry / Mass Spectrometry). We also conducted a laboratory experiment to study the fate of NPs in the sewage sludge composting process and a survey on the occurrence of NPCs in sewage sludge compost in an actual sewage treatment plant. The research results were as follows.

1. Development of method of measuring NPs, NPnEOs and NPnECs by LC/MS/MS

Quantitative analysis of NPnECs in sewage sludge by HPLC was difficult and thus LC/MS/MS analysis was required. NPs and NPnEOs themselves could be measured by HPLC easily, but various other substances contained in sludge often made it difficult to clearly separate their peaks. Therefore we developed a method of measuring NPs, NPnEOs and NPnECs by LC/MS/MS. The flowchart in Fig. 1 shows the basic procedure of measurement.





2. Fate of NPs in sewage sludge composting process

A laboratory-scale experiment was carried out to study the fate of NPs in the sewage sludge composting process. The experimental conditions, apparatus and results are shown in Table 1, Fig. 2 and Fig. 3, respectively. Under the condition of 35°C, NPs were degraded rapidly. Degradation was delayed at 50°C and almost ceased at 70°C. It is reported that 50 and 100 mg/l of 4-NP was reduced by up to 66% by aerobic thermophilic treatment (60°C, 10 days) in a laboratory-scale experiment (Banat et al., 2000), while another study suggested that NP degradation decreased over 65°C in a composting experiment (Moeller et al., 2003). In most composting facilities, the composting

temperature is expected to exceed 65°C mainly in order to inactivate pathogens. However, this operation may suppress the NP degrading microbial activities in the composting plant. Further research is needed to clarify the obstacle to biodegradation of NPs in the composting process and, if possible, to enhance the degradation potential. Table 1 Experimental conditions

		Run 1	Run 2	Run 3
Temperature	°C	35	50	70
NPs ⁽¹⁾	µg/g-dry	150	60	60
Materials	Sludge ⁽²⁾		0.4	
	Sawdust		0.1	
	Return compost		0.5	
Air flow	L/min		1	

(1) Calculated initial NPs concentration (background + spike)

(2) Dewatered anaerobically digested sewage sludge





Fig. 2 Compost experiment apparatus

Fig. 3 NPs change in composting at different temperatures Occurrence of NPCs in sewage sludge compost in actual sewage treatment plant

Samples of 17 composts were collected from various prefectures in Japan and analyzed for NPs and NPnEOs (n = 1 – 15). The concentration ($\mu g/g$ -dry) of NPs, total NPnEOs (n = 1 – 15) and total NPCs ranged from 6.5 to 110, from 8.3 to 82.3 and from 23.6 to 174.9, respectively (Fig. 4). NPnEOs of n > 5 (ethoxylate chains longer than n = 5) were not detected in more than 50% of the composts and NPnEOs of n > 9 were not detected in more than 75% of the composts (Fig. 4). These results suggested that sewage sludge composts contained NPnEOs of which ethoxylate chains were shortened. All five composts made from anaerobically digested sewage sludge contained higher concentrations of NPs than all 7 composts from raw excess sewage sludge (no anaerobic digestion) and both groups were significantly different statistically (p < 0.05, Mann-Whitney's U test) (Table 2). It is obvious that anaerobically digested sludge Table 2 NPs concentration and type of sludge caused the high concentration of NPs in the compost.



Fig. 4 Distribution of NPCs in sewage sludge composts in Japan (sample n = 17)

REFERENCE

Banat, F. A., S. Prechtl, and F. Bischof. (2000). Aerobic thermophilic treatment of sewage sludge contaminated with 4nonylphenol. Chemosphere 41:297-302.

Moeller, J., and U. Reeh. (2003). Degradation of nonylphenol ethoxylates (NPE) in sewage sludge and source separated municipal solid waste under bench-scale composting conditions. Bull. Environ. Contam. Toxicol. 70:248-54.

Recycling of Organic Wastes by Using the Bio-Solids Treatment System

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Project period: FY2002–2005

OBJECTIVES

Large quantities of waste woods and grasses are produced during civil engineering works and maintenance of green sites such as road slopes, levees, airports and parks, yet such wastes is scarcely used because of the lack of effective means. On the other hand, wastewater treatment plants consume large amounts of energy and organic substances, therefore organic wastes have great potential as a substitute for such energy and organic substances. Moreover, bio-solids are a valuable microbiological resource containing many kinds of minerals and microorganisms.

This study aims to develop a method of co-fermentation of organic wastes and bio-solids in the existing anaerobic digester of a wastewater treatment plant, to contribute to the recycling of organic wastes.

METHODS AND RESULTS

In FY2004, we examined anaerobic fermentation with sewage sludge to develop a process for recycling organic waste, especially wood waste, combined with sewage treatment. A lab-scale experiment indicated that wood waste could provide a source of acetate production by fermentation. Moreover, the liquid composting process was applied for sewage sludge. It was found that efficient composting was achieved when the temperature was appropriate. From the microbiological point of view, the population dynamics as well as the treatment ability was important to estimate and optimize the processes.





Development of technologies to utilize sewage sludge ash targeting its inorganic component

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Project period: FY2004-2007

OBJECTIVES

Although large amounts of sewage sludge are discharged and incinerated, the properties of incinerator ash remain unclear. This research aims to clarify the characteristics of sewage sludge and incinerator ash to recover the phosphorus effectively and to develop technologies for using the ash and sludge as construction materials. Sludge melting is one means of using sewage sludge and the quality of molten slag is now being standardized. We analyzed the components of slag which hinder the application of these standards.

METHODS AND RESULTS

In FY2004, we carried out the following analyses to evaluate the forms in which phosphorus may exist in sludge and ash. First, we quantified elements by using ICP and examined the surface and bulk mineral phase using SEM-EDS and XRD to clarify the properties of sewage sludge ash. Sequential extraction tests were also carried out to separate the elements contained in sludge into five fractions.

To evaluate the components of slag, we carried out leaching tests and chemical content tests following the method indicated in JIS.

The results were as follows:

- Incinerator ash contains a large proportion of crystalline components. The major crystalline components were almost the same type, regardless of the type of incinerator or sewage treatment method.

- The results of sequential extractions implied that the predominant form of phosphorus in incinerator ash is residual form (decomposed with HNO₃ and HF microwave). Activated sludge and digested sludge contain more soluble or



Fig.1 Extractability of P in sludge and ash (% of total P)

organically bound phosphorus than incinerator ash does (Fig. 1).

- The results of the leaching and content tests of molten slags showed that these slags satisfy environment quality standards and the regulation of α -Fe content prescribed by JIS.

Study on Techniques for Identifying Pathogenic Microorganisms

and Analyzing Their Behavior

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Project period: FY1999-2005

OBJECTIVES

In order to prevent outbreaks of infection caused by pathogenic microorganisms contained in treated wastewater, reclaimed water and sludge, it is necessary to ensure that the treated materials are safe with respect to pathogenic microorganisms. The ultimate aim of this study is to adapt techniques of molecular biology (particularly the Polymerase Chain Reaction method) to pathogen detection methods due to the importance of developing a rapid and highly sensitive method for detecting trace levels of pathogenic microorganisms (such as viruses and protozoans) in natural water, treated wastewater and sludge. The study also aims to clarify the behavior of pathogenic microorganisms during the wastewater treatment process and in the water environment.

The study focused on the *Cryptosporidium* pathogen and virus. The main areas of this work performed during FY2004 are described below.

RESULTS

In FY2004, we investigated the adaptability of cell culture of *Cryptosporidium* followed by microscope observation or the ELISA method for evaluating its infectivity.

Sampling date	Samp1e	Concentration of Norovirus (copies/ L)			
		G1 type	G2 type		
	Influent	3. 2×10^{1}	3. 3×10^{2}		
2004/6	Treated eff.	ND	ND		
	Advanced eff.	ND	ND		
	Influent	6. 0×10^4	5. 0×10^{3}		
2005/1,2	Treated eff.	1.9×10^{4}	1.1×10^{3}		
	Advanced eff.	1.6×10^{4}	5. 1×10^{2}		

Table 1 Removal ratio of Norovirus by wastewater treatment process

ND: Not detected (5 copies/L)

As a result, the cell culture method with microscope observation or ELISA is faster than the mice infectivity test. However, the values obtained from the cell culture method fluctuated, and so the measurement accuracy needs to be improved.

As for virus detection, cellulose adsorbed coagulation and polyethylene glycol methods were investigated for concentrating viruses, and suitable methods for influent, effluent and tertiary effluent were proposed. With the concentration method and real-time PCR, we measured *Norovirus* concentration in the activated sludge process, and the removal efficiency of the process was obtained. Table 1 shows the results.

Advanced Removal of Residual Organic Matter in Secondary Effluent for Wastewater Reuse

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Project period: FY2002-2005

OBJECTIVES

Treated wastewater is regarded as an alternative water resource in urban areas; however, residual organic matter may cause problems such as regrowth of microorganisms in distribution facilities and change of biota in the water environment. This research aims to develop advanced methods of removing residual organic matter inexpensively and efficiently.

METHODS AND RESULTS

In 2004, we carried out experiments using eight columns as a trickling filter which were each packed with different media (Table 1 and Fig. 1) to analyze the effects of removing trace organic matter from the advanced treatment water of wastewater. Fractionation by gel permeation chromatograph was used for characterizing the organic matter in the secondary effluents from wastewater treatment plants with activated sludge processes.

The results of the research were as follows:

- 1) Maximum removal rates were CODcr: 36% (Fig. 2) and TOC: 21% (Fig. 3) for a continuous flow period of 230 days.
- 2) Organic matter in the secondary effluent could not be characterized.



Fig. 1 Apparatus of the column experiments

Table	1	Medias	used
raute	1	wiculas	usee

columns	packed media
Α	Activated carbon
В	Zeolite
С	Charcoal
D	Kohga stone
E	Chemo-treated charcoal
F	Mixture zeolite and charcoal
G	Mixture charcoal and kohga stone
Н	Mixture kohga stone and zeolite



Fig. 2 Change of CODcr (mg/L) in the outflow



Fig. 3 Change of TOC (mg/L) in the outflow

Status of Pollution and Fate of Polycyclic Aromatic Hydrocarbons in Lake Sediment

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Project period: 2002-2005

OBJECTIVES

Environmental pollution caused by hazardous organic substances has become a problem: the hazardous organic compounds that exist in watersheds concentrate in closed water bodies via rivers. In particular, several papers have reported on the pollution of bottom sediment of closed water bodies with polycyclic aromatic hydrocarbons (PAHs). This study reveals the status of pollution and fate of sediment with PAHs.

RESULTS

In FY2004, we conducted a series of field surveys for PAHs pollution in a lake watershed. The impact of road runoff from a bridge crossing over the center of the lake was examined. We also calculated the mass balance of benzo[a]pyrene (BAP) in the lake to clarify the main source and fate of BAP. The research results were as follows.

1. Status of pollution of PAHs in lake sediment

Lake sediments were collected by core sampling or grub sampling and river water SS were collected by on-site filtration of river water at the points shown in Fig. 1. PAHs concentrations were determined by the GC-MS method and were as shown in Tables 1 and 2.





Table 1 PAHs in the lake sediments

Table 2 PAHs in river water SS

Aromatic				Concentration of PAHs [ng/g-dry]			Annatio					Concentration of PAHs [ng/g-dry]				
ringe	PAHs and abbreviations		River AA	River AD	AD Near the Bridge		Aromatic	PAHs and abbrevi	PAHs and abbreviations		River AA River AB River AD River AE Channel AF					
nings			AA1	AD1	AC1	AC3	rings				AB1	AD1	AE1	AF1		
2	Naphthalene	NAP	20	74.2	40.6	55	2	Naphthalene	NAP	273	520	22	30	188		
	Acenaphthylene	ACL	8	26	17.1	23		Acenaphthylene	ACL	0	0	0	0	16		
	Acenaphthene	ACN	9	11.4	4	9		Acenaphthene	ACN	0	0	0	0	7		
3	Fluorene	FLU	21	37	11	25	3	Fluorene	FLU	163	452	31	30	39		
	Phenanthrene	PHE	104	103	29	93		Phenanthrene	PHE	470	1236	51	115	215		
	Anthracene	ANT	18	25	15	24		Anthracene	ANT	62	164	19	14	21		
	Fluoranthene	FLR	172	154	39	165	4	Fluoranthene	FLR	688	1725	148	185	220		
4	Pyrene	PYR	184	130	32	167		Pyrene	PYR	839	2072	137	219	217		
4	Benz[a]anthracene	BAA	66	47	9	63		Benz[a]anthracene	BAA	225	480	37	61	68		
	Chrysene	CHR	104	75	20	97		Chrysene	CHR	519	1299	76	143	137		
	Benzo[b+k+j]fluoranthene	B(bjk)F	49	45	9	58	5	Benzo[b+k+j]fluoranthene	B(bkj)F	993	2137	167	291	99		
	Benzo[e]pyrene	BEP	60	55	10	77		Benzo[e]pyrene	BEP	426	897	57	104	113		
5	Benzo[a]pyrene	BAP	63	46	7	72		Benzo[a]pyrene	BAP	612	1362	101	174	100		
	Perylene	PRL	161	871	603	769		Perylene	PRL	816	6405	896	537	544		
	Dibenz[a,h]anthracene	DBA	12	11	1.2	16		Dibenz[a,h]anthracene	DBA	473	170	165	157	154		
6	Indeno[1,2,3-cd]pyrene	INP	93	87	11	122	6	Indeno[1,2,3-cd]pyrene	INP	6400	1223	3363	2729	17		
0	Benzo[ghi]perylene	BPR	80	68	10	100	0 6	Benzo[ghi]perylene	BPR	613	1051	78	127	130		
	Total PAHs without Perulana	Σ16PAHs	1062.7	9934	264.8	1165.9		Total PAHs without Pendene	Σ16PAHs	12755	14789	4453	4378	1740		

PAHs concentrations per VSS (Fig. 2) showed that sediment (AA1) of the influent river AA received the highest PAHs load and sediment (AC1) under the bridge was the second highest. The PAHs profile of AC1 (Fig. 3) showed high contents of PHE, FLR, INP and BPR, which is similar to the profile of road and roof dust (Murakami et al., 2003). Therefore, it was considered that the concentrations of PAHs in the sediment under the bridge with heavy traffic were high because of road runoff.



Fig. 2 PAHs in the lake sediments

Fig. 3 PAHs profiles in the lake sediments

2. Fate of benzo[a]pyrene (BAP) in lake sediment

A one-box model of BAP mass balance in a lake watershed was adopted (Fig. 4). According to the calculation results shown in Table 3, 606.2 g BAP enters the lake and 488.7 g (80.6% of influent) is discharged to the effluent river. The residual 117.5 g BAP (19.4% of influent) precipitates to the lake sediment, of which 12.4 g (2.1% of influent) decomposes and 105.1 g (17.3% of influent) accumulates in the sediment. When dredging is conducted, BAP of 286.6 g (44.3% of influent) is removed. This means an extra reduction of 163.5 g (27% of influent) BAP, resulting in a negative net BAP balance and improvement of sediment. It was also revealed that the main sources of BAP were rivers and that the amount of BAP removed by sediment dredging was larger than that accumulated in the sediment.





Fig. 4 Model of BAP mass balance in a lake

<u>REFERENCE</u>

M. Murakami, F. Nakajima and H. Furumai (2003). Distinction of size-fractionated road and roof dust based on PAH contents and profiles. Journal of Japan Society on Water Environment 26:837-842

Study on Technology for Using Waste Woods and Grasses for Revegetation

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Project period: FY2002–2005

OBJECTIVES

Large amounts of waste woods and grasses are produced from public works. This study aims to develop technologies for effectively using such wastes to produce materials for planting at green sites, and to propose a system for recycling such wastes in a closed area.

METHODS AND RESULTS

In FY2004, we carried out an investigation to clarify the amount of waste woods and grasses produced in an area. Also, experiments to develop technologies for using waste woods for spraying materials to produce greenery on slopes were started, and the results showed that the method using steam explosion for modifying wood structure is at a practical level. The progress of the experiments on greening slopes is shown in Figs. 1 and 2.



Fig. 1 Experiment field C after 5 months



Fig. 2 Change in height of Japanese Mallotus

Fate of Pathogens in Sewage Treatment Plant in Monsoon Asia

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Recycling Research Team

Project period: 2003-2006

OBJECTIVES

Rapid population growth, urbanization, delayed sewerage system construction and intensive rainfall in Monsoon Asia have polluted urban water environments with pathogens originating from human feces. To evaluate the associated risks to people living in Monsoon Asia, the sources, routes and fate of pathogens in the water environment must be investigated. Most sewage treatment in Monsoon Asia is performed by lagoons, and the characteristics of the removal or inactivation of pathogens by these lagoons must be evaluated in terms of the effects of temperature, sunlight intensity and amount of rainfall. Lagoons are sometimes backed up by wetlands, and this supplemental process must also be performed efficiently.

The first aim of this research is to develop indicator microorganisms which are representative of the three pathogen groups: bacteria, protozoa and viruses. Then, these indicators will be used to evaluate the effects of the type of lagoon, the wetland and the climate conditions on the fate of pathogens.

The main works performed during FY2004 are described below.

RESULTS

In FY2004, lagoons in Thailand in the Mekong watershed were surveyed. Two lagoons in Khon Kean and the Asian Institute of Technology (AIT) were selected for this research to compare the effects of sewerage system. Each house has a septic tank in Khon Kean, whereas AIT has its own sewerage system with flush toilets. As the results in Table 1 show, the concentrations of *Giardia* and *Norovirus* at AIT are much higher than at Khon Kean. The concentration of *Norovirus* at both lagoons is much higher than that in Japan, but *Norovirus* and Giardia are treated by lagoons effectively.

		Total coli form (cfu or MPN/ml)	NorovirusG1 (copies/L)	NorovirusG2 (copies/L)	Giardia (cysts/L)	Cryptosporidium (oocysts/L)		
	Date	04/06~05/2,3	04/06~	12,05/01	04/06~05/01			
	Influent	4.4E+2∼2.3E+5	1.1E+4~9.7E+5	4.1E+2∼1.6E+5	ND~1.5E+2 [7/8]	ND [0/8]		
kean	Aerated	4.0E+1~3.0E+4	ND~1.0E+3	ND~1.7E+4	ND~2.2E+1 [2/8]	ND [0/8]		
Khon	Effluent	3.8E+1∼2.2E+4	ND~2.9E+2	ND~1.0E+4	ND~1.0E+0 [1/8]	ND [0/8]		
	Mean removal ratio(%)	83.2	94.2	85.0	99.9	_		
	Influent	2.1E+4~9.0E+6	4.5E+4~3.5E+6	1.7E+4~2.0E+6	2.6E+2~1.8E+4 [9/9]	ND~1.6E+1 [5/9]		
⊢	Effluent1	4.6E+3~1.6E+6	1.0E+5~2.2E+6	1.3E+4~1.3E+6	1.1E+1~4.1E+2 [9/9]	ND~2.0E+0 [2/9]		
A	Effluent2	7.0E+1~5.0E+3	3.6E+1~4.2E+5	ND~6.0E+4	3.0E+0~2.8E+1 [9/9]	ND [0/9]		
	Mean removal ratio(%)	99.6	65.7	92.3	99.2	100		

Table 1 Concentration and removal ratio of pathogens

ND: Not detected

Study of Occurrence of Antibiotic Resistance Bacteria in Water Environment

Masaaki Ozaki and Mamoru Suwa

Recycling Research Team Project period: FY2004–2006

OBJECTIVES

In recent years, outbreaks of antibiotic-resistant bacteria have become a public health problem. The widespread use of antibiotics by humans and for livestock breeding has increased the risk of contamination of water environments by bacteria that are resistant to antibiotics. The aim of this study is to clarify the antibiotic resistance of bacteria in the water environment by investigating the concentration of antibiotic-resistant bacteria of treated wastewater, etc. As the sewered population has increased, the impact of treated wastewater on the water environment has grown.

The main works performed during FY2004 are described below.

RESULTS

In FY2004, we investigated the concentration of antibiotic-resistant $E. \ coli$ in the wastewater of a municipal treatment plant (MTP) and a hospital. The results are shown in Table 1. The ratio of antibiotic-resistant $E. \ coli$ was not higher in the wastewater treatment process. There were many $E. \ coli$ strains that showed sensitivity of antibiotic resistance to Ampicillin, Tetracycline and Cefdinir in the wastewater of the MTP and hospital. Also, it was observed that the wastewater treatment process tended to contain $E. \ coli$ strains having resistance to many antibiotics.

	_	Influen	Ł	Treated offwort			
		Innuen	l	Treated enfuent			
		Concentration of E. c	oli (cfu/mL)	Concentration of E. coli (cfu/mL)			
			Ave.		Ave.		
	Conc. of E. coli	$2.7\! imes\!10^4 - 6.0\! imes\!10^4$	$3.9\! imes\!10^4$	$1.3\! imes\!10^1\!-2.0\! imes\!10^2$	$7.8 imes 10^1$		
ater	Conc. of LVFX-resistant E. coli	$4.2 \times 10^2 - 6.2 \times 10^2$	$4.9\! imes\!10^2$	$8.0 imes 10^{-2} - 2.6 imes 10^{0}$	$0.9\! imes\!10^{0}$		
Wastew	LVFX resistance ratio (%)	1.0 - 1.6	1.3	0.3 - 1.3	0.9		
	Conc. of ABPC-resistant E. coli	$2.0 imes 10^3 - 4.4 imes 10^3$	$3.2\! imes\!10^3$	$1.7\! imes\!10^{0}-7.8\! imes\!10^{0}$	4.8×10^{0}		
	ABPC resistance ratio (%)	6.3 - 16.3	11.3	3.9 - 13.1	8.5		
	Conc. of E. coli	$1.0 imes 10^3 - 7.3 imes 10^3$	$4.2 imes10^3$	$3.2 \times 10^{0} - 4.0 \times 10^{1}$	$2.2\! imes\!10^1$		
al: ater	Conc. of LVFX-resistant E. coli	$4.0 imes 10^1 - 1.2 imes 10^3$	$6.2\! imes\!10^2$	1.0×10^{-1}	1.0×10^{-1}		
Hospit Wastew	LVFX-resistance ratio (%)	4 - 16.4	10.2	0.3 - 3.1	1.7		
	Conc. of ABPC-resistant E. coli	$4.4 imes 10^2 - 2.7 imes 10^3$	$1.6 imes10^3$	$6.8 imes 10^{-1} - 2.3 imes 10^{0}$	$1.5\! imes\!10^{0}$		
	ABPC-resistance ratio(%)	37.0 - 44.0	40.5	5.7 - 21.1	13.4		

Table 1 Ratio of antibiotic-resistant E. coli