JAERI-M
90-145

A MICROBIOLOGICAL STUDY ON SEWAGE SLUDGE TREATMENT

September 1990

Ngamnit SERMKIATTIPONG*, Hitoshi ITO
and Shoji HASHIMOTO

日本原子力研究所
Japan Atomic Energy Research Institute

JAERI-Mレポートは，日本原子力研究所が不定期に公刊している研究報告書です。
入手の問合わせは，日本原子力研究所技術情報部情報資料課（〒319－11茨城県那珂郡東海村）あて，お申しこしください。なお，このほかに財団法人原子力弘済会資料センター（〒319-11茨城県那珂郡東海村日本原子力研究所内）で複写による実費頒布をおこなっております。

JAERI-M reports are issued irregularly.
Inquiries about availability of the reports should be addressed to Information Division, Department of Technical Information, Japan Atomic Energy Research Institute, Tokai-mura, Naka-gun, Ibaraki-ken 319-11, Japan.

編集兼発行　日本原子力研究所
印　　刷　日立高速印刷株式会社

A Microbiological Study on Sewage Sludge Treatment

Ngamnit SERMKIATTIPONG*, Hitoshi ITO
and Shoji HASHIMOTO

Department of Research
Takasaki Radiation Chemistry Research Establishment
Japan Atomic Energy Research Institute
Watanuki-cho, Takasaki-shi, Gunma-ken

(Received August 3, 1990)

Isolation and identification of salmonellae in sewage sludge cake and radiation sensitivities of the isolated strains were studied. Disinfection of the sludge by heat or radiation and effect of such treatment on composting were also carried out. Five groups of O-antigen and seven serotypes of salmonellae were identified from the sludge cakes. D_{10} values of the salmonellae in phosphate buffer were ranged from 0.16 to 0.22 kGy and those in sludge were about three times larger. Total bacterial counts and coliforms in the sludges were determined to be 4.6×10^7 - 5.1×10^9 and 1.3×10^5 - 1.1×10^9 colony forming unit (cfu/g). After irradiation at 20 kGy by gamma ray or electron beam, decrease of total bacterial count was 5 - 7 log cycles and a dose of 5 kGy was enough to eliminate all of the coliforms. Coliforms decreased rapidly by heating at 65°C, but only one log cycle decrease was observed in total bacterial count. By heating at 100°C, total bacterial count decreased rapidly. Two peaks were observed in CO_2 evolution curves of radiation disinfected sludge composting, but only one peak in heat disinfected sludge composting.

Keywords : Sewage Sludge, Salmonellae, Radiation, Disinfection, Composting, Gamma Ray, Electron Beam, Coliforms

---

* Office of Atomic Energy for Peace, Thailand

下水汚泥処理の微生物学的研究

日本原子力研究所高崎研究所研究部
Ngamnit SERMKIATTIPONG*・伊藤 均・橋本 昭司

（1990年8月3日受理）

下水汚泥ケーキ中に分布する各種のサルモネラの分離と同定，並びに分離された株の放射線感受性に関する研究を行った。さらに，汚泥の熱あるいは放射線殺菌と，それらの処理のコンポスト化に与える影響についても研究を行った。5グループのO－抗原と7種の血清タイプのサルモネラが汚泥ケーキから同定された。これらのサルモネラの燐酸緩衝液中におけるD_{10}値は0.16から0.22kGyの範囲であり，汚泥中ではこの3倍程度高い値となった。汚泥中の総菌数と大腸菌群数は4.6×10^{7}から5.1×10^{9}並びに1.3×10^{5}から1.1×10^{9} cfu／gであった。ガンマ線あるいは電子線による20kGyの照射で総菌数は5～7桁減少し，大腸菌群を検出限界以下にするには5kGyの照射で十分であった。大腸菌群は65℃の加熱で急速に減少したが，総菌数は1／10に減少したのみであった。100℃の加熱では総菌数は急速に減少した。放射線殺菌した汚泥のコンポスト化において，炭酸ガス発生曲線は2つのピークを示したが，熱殺菌の場合では1つのピークが観察されたのみであった。

高崎研究所：〒370-12 群馬県高崎市綿貫町1233
* タイ原子力庁

Contents

目　　次

INTRODUCTION

The amount of sludge generated from waste water and sewage treatment facilities is more than 26,000 tons/year in Bangkok. Three means have been generally used for sludge disposal described as follows (1).

1) discharge into lake, sea or ocean
2) utilization for land fills
3) utilization as a fertilizer and soil conditioner

The utilization of sludges as a fertilizer and soil conditioner is a common method of resource conservation. However, most of the sludges contain pathogenic viruses, bacteria, protozoa and parasites. These organisms are harmful to crops, grazing animals and public (1,2).

In Thailand, salmonellae are very hazardous pathogenic bacteria and 80 % of sludge from hospitals and 50 % from food industries are contaminated by salmonellae (3). Hess and Breer also reported that the sludge contained salmonellae in more than 90 % of samples and the maximum number of organisms was 10^7 cfu per liter. Moreover salmonellae in sewage sludge spread on grass might survive up to 72 weeks (4). It was also reported that about 10^5 salmonellae existed in 100 grams (dry weight) of sludge (2).

Various methods are known for elimination of pathogenic microorganisms. Ultraviolet light is useful to kill pathogenic bacteria suspended in liquid, but ineffective for bacteria contained in solid. Chlorination have been reported to be inefficient, as viruses associated with solids have been found to be protected against inactivation.

Heat treatment of sludge in slurry state is effective. However this treatment is energy intensive and generates bad odor(5). Ionizing radiation requires much less energy and change in component of sludge is small compared with heat pasteurization. Moreover, the operation procedures for irradiation are fairly simple and the sludge can be continuously and completely disinfected (2).

The direct land application of sludge, may cause odor or sanitary problems to the neighbors even after disinfection. The other problem in direct land application is harmful to plants due to rapid fermentation of the sludge in soil. Stabilization of easily decomposable materials in sludge is necessary to solve

these problems (6). Composting is widely known to be useful to stabilize sludge and also useful to reduce pathogenic microorganisms by heat generated during aerobic fermentation. However, long time process is required to finish composting because it is necessary to keep high temperature to reduce pathogenic microorganisms during composting period, and such high temperature also decrease activity of composting bacteria. Moreover, uniform heating is very difficult especially in large scale fermentors and there is high possibility of regrowth of pathogenic bacteria. The optimum temperature to get high rate of composting is ranged from 40 to 50°C as reported previously (6). It is possible to reduce composting time by separating disinfection process from composting process because the optimum temperature can be selected after disinfection.

In this paper, isolation and identification of salmonellae in sewage sludge and radiation sensitivities of the isolated strains are reported in PART I. Disinfection of sewage sludge by heat or radiation and effect of such treatments on composting are also reported in PART II.

I. ISOLATION AND IDENTIFICATION OF SALMONELLAE FROM SLUDGE AND RADIATION SENSITIVITIES OF ISOLATED STRAINS

1. Materials and Methods

1.1 Sewage sludge cake

Activated sludge cakes (sludge A and B) treated by polymer flocculant and dewatered by centrifugation or filter press were collected from two sewage treatment facilities in Takasaki-city.

1.2 Isolation of salmonellae

Salmonellae were isolated by modified method described by Cowan and Steel and FAO/WHO methods (7,8) as shown in Fig.1.

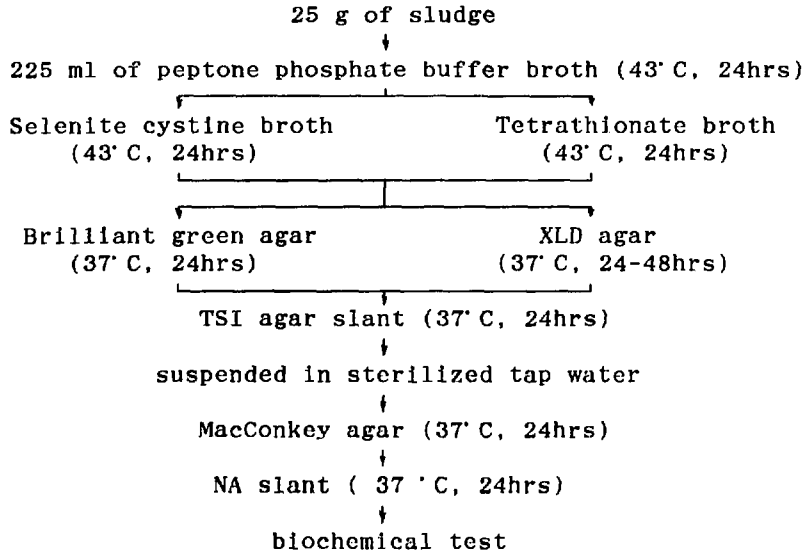

Fig. 1 Isolation method of salmonellae in sludge cakes

Sludge cake was weighed in a bottle and diluted ten times by peptone phosphate buffer broth. Ten bottles of these mixtures were incubated at 43° C for 24 hours. One ml from each bottle was transferred to each tube of 9 ml of selective enrichment media of tetrathionate broth and selenite cystine broth. These tubes were incubated at 43° C for 24 hours. One loopful suspension in each tube was streaked on two kinds of plate agar; Brilliant green agar and Xylose Lysine Deoxycholate agar (XLD). These plates were incubated at 37° C for 24 to 48 hours. The suspicious colonies were transferred to Triple Sugar Iron (TSI) slant agar tubes and incubated at 37° C for 24 hours. For pure isolation, one loopful bacteria in each tube was mixed with 6 ml of sterilized tap water. The suspension was mixed well, then streaked on a MacConkey agar plate and incubated at 37° C for 24 hours. To get pure culture for identification, single colony was transferred to each tube of nutrient agar slant and incubated at 37° C for 24 hours.

1.3 Identification of salmonellae

Various biochemical characteristics of isolated strains were examined according to " Bergey's Manual of Determinative Bacteriology " (9). After examination of biochemical characteristics of isolated strains, serological test by using Salmonella polyvalent O-antiserum and O-group antisera were also performed. As for the agglutination test, a small amount of Salmonella polyvalent O-antiserum was dropped on a clean slide and smeared well with the bacterial cells. It took about 1 minute to appear the agglutination reaction. After salmonellae were classified into different O-groups. Typing was performed by WHO National Salmonella and Shigella Center using O, H and Vi antisera by the method of Kauffmann (10).

1.4 Radiation sensitivities of salmonellae

1) Radiation sensitivities of salmonellae in phosphate buffer

Three groups of salmonellae (S.oranienburg, S.hadar and S. panama) were isolated from sludge A. Each group of salmonellae and S.typhimurium (type strain) were studied for radiation sensitivities by the method reported by Ito et. al (11) as described below.

Pure cultures of each strain were grown for 16 hours in 100 ml of nutrient broth under aeration at 30° C. Cells at the stationary

phase were harvested by centrifugation, washed twice with 0.067 M phosphate buffer of pH 7, and then resuspended in the same buffer. These suspensions with a concentration of about 10^8 to 10^9 cells/ml were irradiated with atmospheric air at dose rate of 1.2 kGy/hr using Co-60 source. Determination of total counts of salmonellae was conducted using 0.01 % tween 20 for serial hundred-fold dilutions and subsequently plated on the surface of Difco nutrient agar. The plates were incubated at 30°C and 37 °C, 1 day for S.oranienburg and S.typhimurium, and 30°C, 1 day for S.hadar and S.panama.

2) Radiation sensitivities of salmonellae in sewage sludge

S.panama isolated from sludge A and S.typhimurium were studied for radiation sensitivities in sewage sludge. Pure cultures of each strain were grown for 16 hours in 100 ml of nutrient broth under aeration at 30°C. Cells at the stationary phase were harvested by centrifugation and mixed well in 50 ml of 1 % peptone aqueous solution. The suspension was poured into the flask which contained 60 g of sterilized sludge and mixed well by glass rod. The mixed sludges were dried until the moisture content became the same as before inoculation. Five grams of each sludge cake was put into a sterilized polyethylene bag. Each bag was sealed and irradiated by gamma ray. The dose rate determined with a Fricke dosimeter was 3 kGy/hr at the distance of 56 cm away from Co-60 source. After irradiation, each bag was appropriately diluted in 0.01 % Tween 20 and 0.2 ml of the suspension was spread on the surface of Difco MacConkey agar plates. The plates were incubated at 37°C for 1 day and relative survivals were determined by colony counting.

1.5 Recovery of irradiated cells

Recovery of irradiated cells was conducted mainly by the method of Stapleton et al. (12). S.typhimurium was grown for 16 hours in 100 ml of nutrient broth under aeration at 30°C. Cells at the stationary phase were harvested, washed twice with 0.067 M phosphate buffer of pH 7, and then resuspended in the same buffer. After adjusting the concentration to about 10^8 to 10^9 cells/ml, these suspensions were irradiated by gamma ray. All suspensions were immediately put in ice bath after irradiation and diluted quickly in 0.01 % tween 20 aqueous solution. The appropriate dilution was spread on the surface of nutrient agar plates and ammonium-salt glucose agar plates. Two series of different culture media plates were incubated at various temperatures for 1 day and reincubated continuously at 30°C, 1 day for nutrient agar and 5 days for ammonium-salt glucose agar.

Relative survival at the various temperatures was determined by colony count.

2. Results an Discussion

2.1 Isolation and identification of salmonellae from sewage sludges

All of the isolates of salmonellae from sludge A and B were gram negative, short rod and motile. Cell sizes of all isolates were in the range of 0.2 to 0.4 micron wide and 0.5 to 0.8 micron long. The colonies of the isolated strains on Brilliant green agar were smooth, white color with reddish pink zone at the center and 1.5 to 2.5 mm diameter. After incubation for 1 day, the appearance of the colonies on XLD agar was transparent, smooth and round shape or slightly spindle shape of 1.5 to 2.0 mm diameter. After 2 days incubation, the colonies enlarged to 2.0 to 3.0 mm. Black color was appeared at the center of the colony because the isolated strain could produce hydrogen sulfide from sodium thiosulphate and change ferric ion in the culture media into ferrous sulfide.

Table 1 shows results of biochemical characteristics and serological test using Salmonella polyvalent O-antiserum and O-group antisera. Fourteen isolates of the salmonellae were detected from 250 g of sludge A. Four groups of salmonellae namely B, C_1, C_2 and D were found and 5 serotypes of salmonellae were isolated as shown in Table 2. As for sludge B, 18 isolates of the salmonellae from 50 g of sample were detected. Four groups of salmonellae namely B, C_1, D and E were found and 4 serotypes of salmonellae were identified as shown in Table 2.

2.2 Radiation sensitivities of salmonellae

1) Radiation sensitivities of salmonellae in phosphate buffer

The survival curve of S. typhimurium irradiated in phosphate buffer is shown in Fig. 2. There is no difference between two different incubation temperatures after irradiation. D_{10} value calculated from the slope of line is 0.16 kGy and induction dose is 0.13 kGy. Fig. 3 shows survival curve of S. oranienburg in phosphate buffer. The survival curve is almost linear and there is no effect of incubation temperature after irradiation. D_{10} value calculated is 0.20 kGy. Fig. 4 shows survival curves

Table 1 Biochemical characteristics of salmonellae isolated from sludge A

Salmonella	1	2	3	4
Phenylalanine	-	-	-	-
MR test	+	+	+	+
VP test	-	-	-	-
Indole test	-	-	-	-
Lysine decarboxylase	+	+	+	+
Malonate	+	+	+	+
Urea	-	-	-	-
Citrate as C source	+	+	+	+
H_2S from TSI	+	+	+	+
Acid from glucose	+	+	+	+
mannitol	+	+	+	+
lactose	-	-	-	-
sucrose	-	-	-	-
xylose	+	+	+	+
arabinose	-	-	-	-
Gas from glucose	+	+	+	-
O-multiantigen	+	+	+	+
Serum type	C_1	C_2	D	B
No. of isolates	6	2	5	1

Table 2 Various serotypes of salmonellae isolated from sludge A and B

Sample	O group	Serotypes
sludge A	B	S.I.4,12:d:-
A	C_1	S.oranienburg
A	C_1	S.isangi
A	C_2	S.hadar
A	D	S.panama
sludge B	B	S.I.4,12:d:-
B	C_1	S.tennessee
B	D	S.panama
B	E	S.krefeld

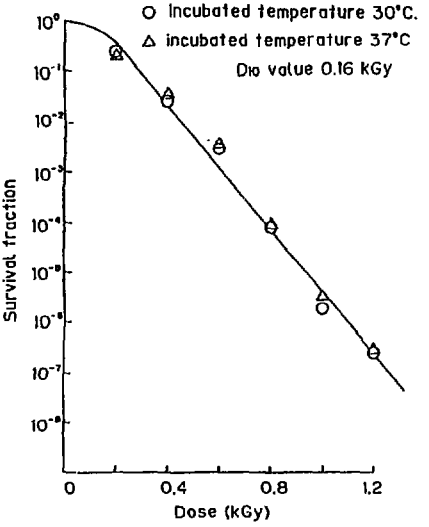

Fig.2 Survival curve of S.typhimurium irradiated in phosphate buffer

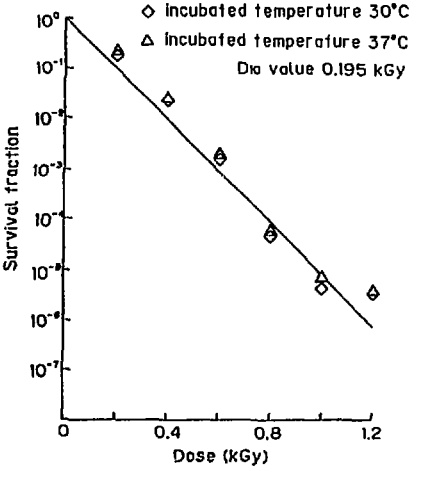

Fig.3 Survival curve of S.oranienburg irradiated in phosphate buffer

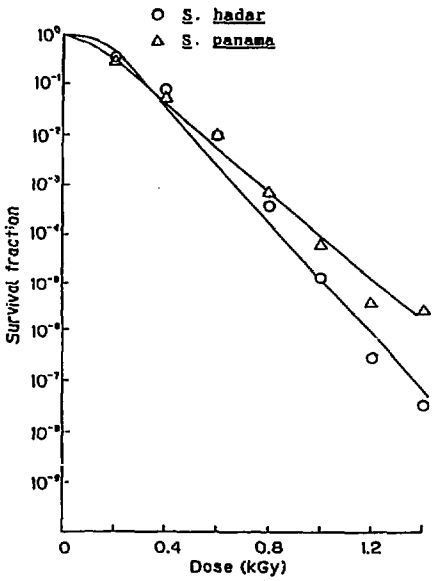

Fig.4 Survival curves of S.hadar and S.panama irradiated in phosphate buffer

of S. hadar and S. panama irradiated in phosphate buffer. The curves show sigmoid shape with shoulder. D_{10} values of these salmonellae are 0.18 and 0.22 kGy and induction doses are 0.14 and 0.09 kGy, respectively. D_{10} values of the isolated salmonellae are summarized in Table 3. S. panama is the most resistant in comparison with other serotypes. D_{10} values of 3 serotypes of salmonellae are larger than that of S. typhimurium.

Table 3 D_{10} values of salmonellae in phosphate buffer

Serotypes	D_{10} value (kGy)
S. oranienburg	0.20
S. hadar	0.18
S. panama	0.22
S. typhimurium	0.16

2) Radiation sensitivities of salmonellae in sewage sludge

S. panama was used to study radiation sensitivities in sewage sludge because D_{10} value is the largest in the salmonellae isolated from the sludge sample. Fig. 5 shows survival curves of S. panama and S. typhimurium. These curves show sigmoid shape. D_{10} values calculated from the slope of the curves are shown in Table 4 and induction doses of S. panama and S. typhimurium are 0.96 and 0.85 kGy, respectively. Radiation sensitivities of these salmonellae are lower in sewage sludge cakes. Irradiation dose of 3 and 4 kGy are enough to decrease the level of S. typhimurium and S. panama below 6 log cycles. Radiation resistance of the salmonellae are higher in sewage sludges than in phosphate buffer which is similar to that reported by Kapila (2).

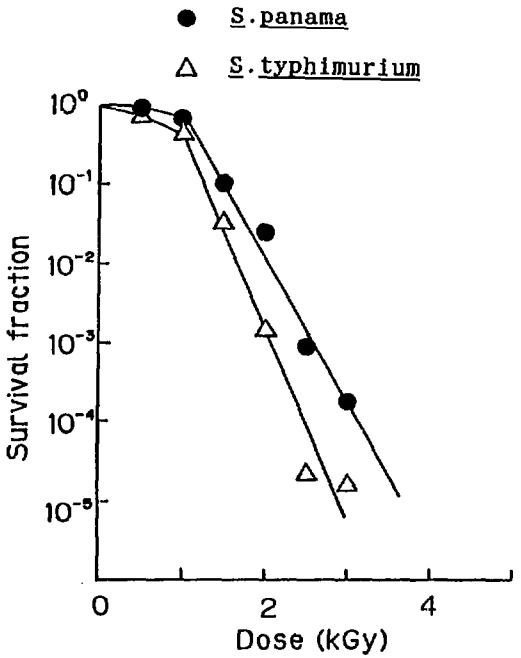

Fig.5 Survival curves of S.panama and S.typhimurium irradiated in sewage sludge

Table 4 D_{10} values of salmonellae in sewage sludge

Serotypes	D_{10} value (kGy)
S. panama	0.55
S. typhimurium	0.41

2.3 Effects of culture media and incubation temperature on recovery of irradiated cells

Fig. 6 shows survival curves of S. typhimurium irradiated in phosphate buffer and incubated at 30° C on nutrient agar plate and ammonium-salt agar plate. It can also be seen that the surviving fraction shows higher value when incubated on nutrient agar. These results show that the irradiated cells are able to recover at higher rate on a nutritious medium than in a basal medium. It was also reported that cells held at the same temperature in inorganic salt-glucose medium did not recover even incubated for a sufficient time (12). This finding suggests that there might be a special nutritious requirement for the recovery process after irradiation.

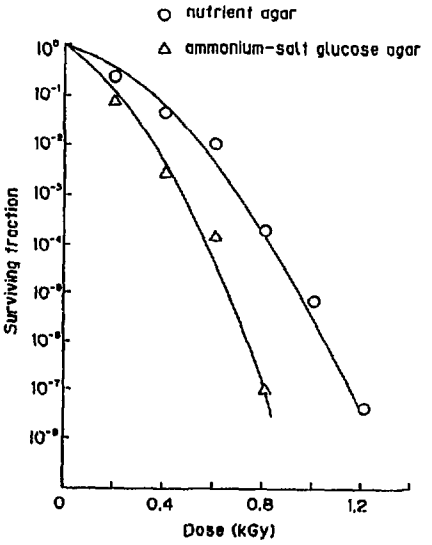

Fig.6 Survival curves of S.typhimurium incubated at 30 °C on different culture media

Fig. 7 shows a survival curve of S.typhimurium irradiated at 0.6 and 1.0 kGy in phosphate buffer. The irradiated cells are plated on nutrient agar or ammonium-salt glucose agar and incubated at various temperature. High recovery of irradiated cells on both culture media is observed around 37° C The level of survival fraction on nutrient agar is about 2 log cycles higher than an ammonium-salt glucose agar.

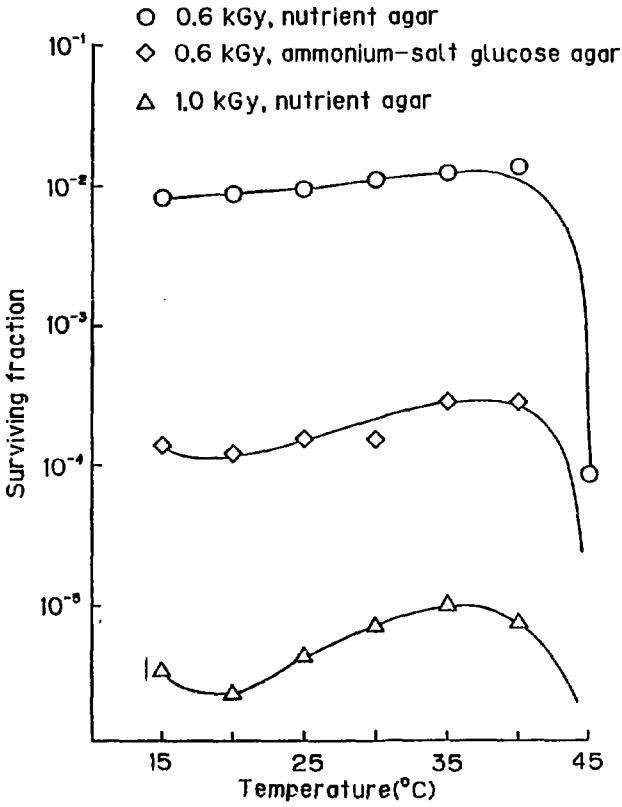

Fig.7 Surviving fraction of S.typhimurium incubated on different culture media at various temperature

II. DISINFECTION OF SEWAGE SLUDGE BY HEAT OR RADIATION TREATMENT AND COMPOSTING OF DISINFECTED SLUDGES

1. Materials and Methods

1.1 Sewage sludge cakes

Sludge cakes from the same sewage treatment facilities in Takasaki-city were used. Moisture content of the sludge A mainly used in this study was ranged from 76.1 to 78.5 % and volatile solid content, measured after heating at 600° C for 4 hours, was ranged from 76.6 to 80.0 %

1.2 Heat treatment

Five grams of each sludge cake was put into a sterilized polyethylene bag. Each bag was sealed and heated in water bath at temperature ranged from 50 to 100° C for various time from 15 minutes to 5 hours.

1.3 Radiation treatment

1) Gamma ray irradiation

Five or ten grams of sludge cake was put into a sterilized polyethylene bag. Each bag was sealed and irradiated by Co-60 gamma ray. The dose rate was determined by a Fricke dosimeter.

2) Electron beam irradiation

Preparation of sludge for electron-beam irradiation and evaluation of radiation dose were carried out mainly by the method of Hashimoto et. al. (14) as follows.

Five grams of sludge cake was put in a polyethylene bag and flatted by a steel roller to get thickness of 1.0 mm. Each bag was sealed, fixed on the conveyer and irradiated by passing under the scan horn as shown in Fig. 8. The electron accelerator used for irradiation was the Cockcroft-Walton type (Nisshin Highvoltage Co.). A slit of 3 cm wide was used to get a uniform dose rate distribution. The electron beam current and the speed of the conveyer were controlled to obtain the desired dosages. To evaluate the irradiation dose of the sludge sample, a commercial CTA film dosimeter (Fuji FTR-125, 8 mm wide, 0.125 mm

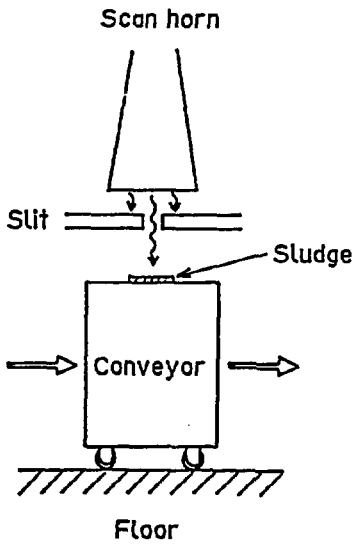

Fig.8 Irradiation apparatus for electron beam

thick) was used. Two 5 cm pieces of CTA film were put on the surface and opposite side of the bagged sludge, which was fixed on the conveyer and irradiated by passing under the scan horn several times. After irradiation, the film was allowed to stand for 2 hours and measured optical density with a spectrophotometer (Shimazu Model UV-210A) at a wavelength of 280 nm. Absorbed dose was calculated from the change of the absorption.

The dose rate distribution at the conveyer surface was measured with the same film dosimeter. A 30 cm length of film was fixed on the stationary conveyer under the slit. After irradiation, distribution of optical density was measured by a spectrophotometer (FDR-01, Nisshin Electric Co. Ltd.).

Depth dose curve was obtained by 12 polyethylene bags of sludge, each bag contained 10 g of sludge and was pressed into a thin sheet of 1 mm thick. The first bag was trimmed out along the rims, measured the area and weighed to calculate mass thickness. CTA films with 5 cm long were placed between piled bags and irradiated by electron beam. Optical density of the irradiated film was also measured to calculate the dose of each film.

1.4 Composting

Experimental apparatus for composting was the same as reported by Kawakami and Hashimoto (14) as shown in Fig. 9. Ten grams of sludge was mixed well with five grams of perlite and 1 gram of seed compost. pH of the mixture was adjusted by adding 80 mg of sodium carbonate. The mixture was put in the glass fermentor and kept fermenting at a constant temperature of 40 or 50° C for 6 days. Aeration was performed from the bottom of the fermentor during fermentation period using humid air to keep a constant moisture content of the mixture. The state of fermentation was observed by measuring CO_2 concentration in the exhaust gas using an infrared-type analyzer (Beckman, Model B-64).

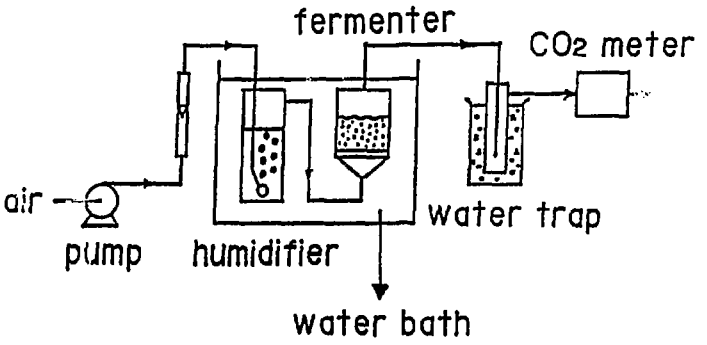

Fig.9 Flow chart of composting

1.5 Enumeration of bacteria

Total bacterial count in irradiated sludge by gamma ray was determined according to the method of Ito et. al. (11). After irradiation or heating, disinfected water with 0.01 % Tween 20 was added to each bag and homogenized with a Stomacker Lab-Blender-400 (Seward Laboratory UAC House) for one minute. The mixture was diluted to get optimum number of colonies on the agar plate. Each 0.2 ml of suspension was spread on the surface of

Difco-nutrient agar plate for total bacterial count and Difco-MacConkey agar for coliforms. The plates were incubated at 30° C for 3 days and 37° C for 18 to 28 hours, respectively.

2. Results and Discussion

2.1 Disinfection of sludge by heat

As shown in Fig. 10, the initial load of total bacterial count was about 1.5×10^8 cfu/g. Total bacterial count decreased about 1.5 log cycle by heating at 50, 65 and 80° C for 1 hour. Total bacterial count did not change after heating more than 1 hour at 50 and 65° C, but gradually decreased at 80° C. Total bacterial count decreased more than 5 log cycles within 1 hour when heated at 100° C and then gradually decreased. Coliforms gradually decreased by heating at 50° C for 1-5 hours. By heating at 65° C for 5 minutes, number of coliforms were below detectable level.

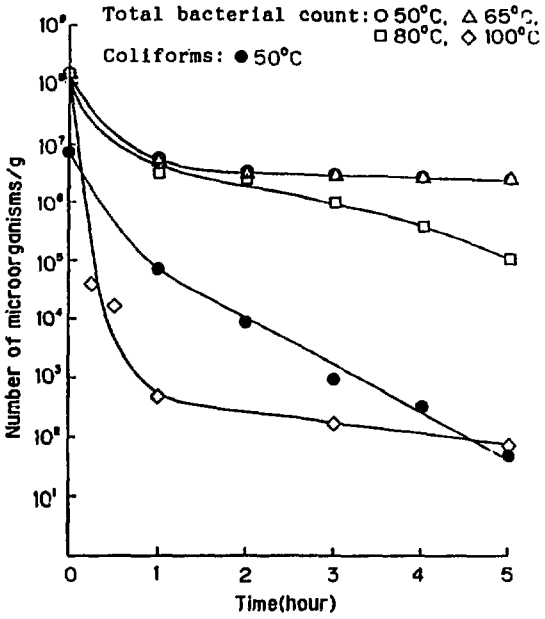

Fig.10 Inactivation of total bacteria and coliforms by heat

2.2 Disinfection of sludge by radiation

1) Disinfection by gamma ray

Two samples of sewage sludge A collected at different times were used. As shown in Fig. 11, the initial load of total bacterial count of the first sample was 4.7×10^7 cfu/g and coliforms was 2.4×10^5 cfu/g. After irradiation, total bacterial count decreased to 4.5×10^2 cfu/g at dosage of 20 kGy. Coliforms decreased to 3.3×10^2 cfu/g at 1 kGy and undetectable level at 2 kGy. The initial load of total bacterial count in second sample was 5.3×10^8 cfu/g and coliforms were 1.3×10^5 cfu/g. Total bacterial count decreased approximately 6 log cycles at 20 kGy. As for coliforms, the number decreased to be 30 cfu/g at 1.5 kGy and were below detectable level at 2 kGy. Even though the initial value of total bacterial count is smaller, the first sample required higher dose to get the same level of total bacterial count compared with the second one. This may be attributable that the first sample is contaminated by radiation resistant bacteria, e.g. Pseudomonas sp., Deinococcus sp. and Bacillus sp., as reported by Ito et al. (11).

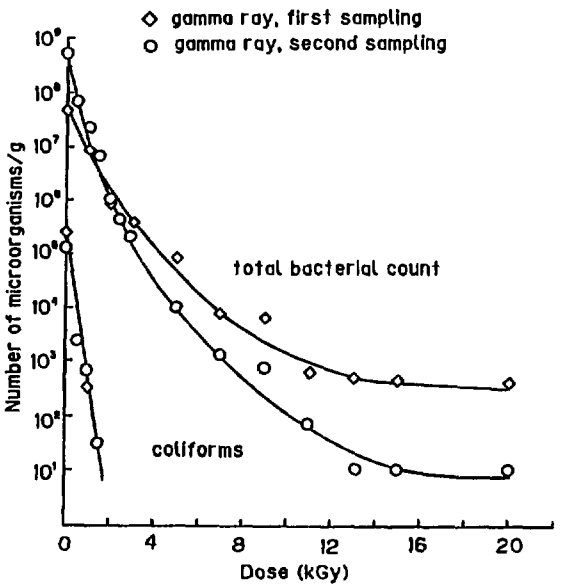

Fig.11 Inactivation of total bacteria and coliforms by gamma ray irradiation (sludge A)

Fig. 12 shows the surviving curves of total bacterial count and coliforms in sludge B. These samples were dewatered by centrifugation and filter press. Total bacterial count of centrifuged sludge was determined to be 5.1×10^9 cfu/g and coliforms were 1.1×10^9 cfu/g before irradiation. Total bacterial count decreased to 28 cfu/g at 20 kGy. Coliforms decreased to 1.3×10^2 cfu/g at 3 kGy and could not be detected at 5 kGy. Total bacterial count of the sample dewatered by filter press was 1.1×10^9 cfu/g and coliforms 6.9×10^7 cfu/g. At 20 kGy, total bacterial count decreased to 2.5×10^2 cfu/g. Coliforms decreased to 73 cfu/g at 2.5 kGy and could not be detected at 3 kGy.

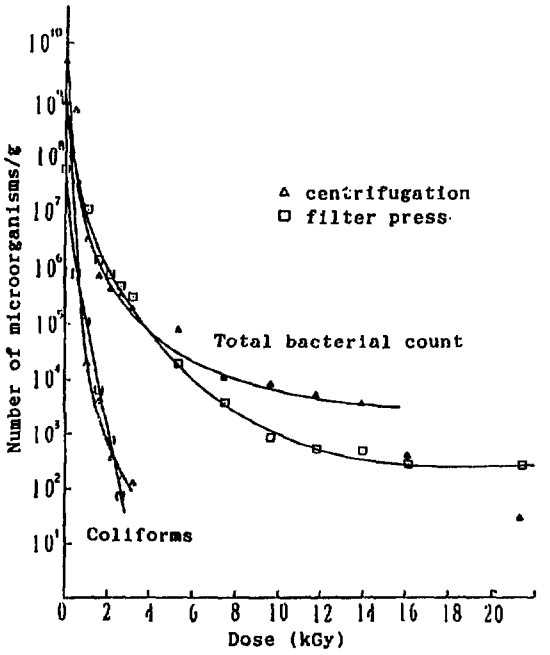

Fig.12 Inactivation of total bacteria and coliforms by gamma ray irradiation (sludge B)

There was large difference between initial bacterial loads depend on the sample. But, it was reported that total bacterial counts in sewage sludge varied with the range between 10^6 to 10^9 microorganisms/g in sewage treatment plants of many countries (2).

Total bacterial counts of all samples diminished rather rapidly with an increase of the radiation dose, but slowly after 5 kGy. At 10 kGy, 5 to 6 log cycles of total bacterial counts can be reduced. This is almost the same as the result on the radiation treatment of sludge from Atladara plant (2).

In sludge A and B, coliforms were almost inactivated by the dose ranged from 2 to 5 kGy. In the case of sewage sludge from Atladara, 6 kGy of gamma radiation was necessary in maximum to eliminate coliforms completely (2). Our finding suggests that inactivation of coliforms requires lower dosage than Atladara plant, however the initial load of coliforms is effective factor concerned.

2) Disinfection by electron beam

Fig. 13 shows the dose rate distribution at the conveyer surface under the slit. The dose rate distribution is almost rectangular and the value at the plateau is 0.73 kGy/sec.

Depth dose curve is shown in Fig. 14. The density of the sludge was 1.23 g/cm^3. The distribution of absorbed dose along the path shows that the maximum dose is given at about 0.4 g/cm^2 of mass thickness. Sample thickness should be selected to be less than 0.6 g/cm^2.

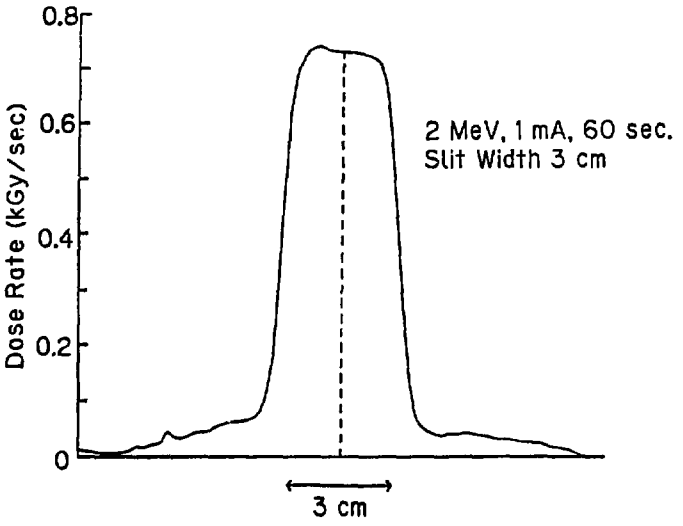

Fig.13 Dose rate distribution on the conveyer surface

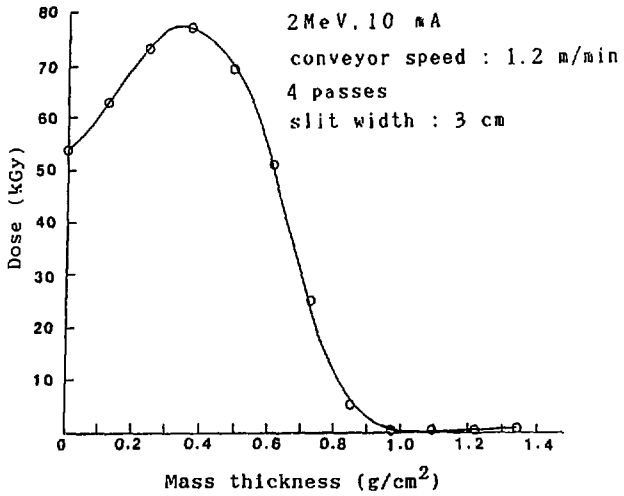

Fig.14 Depth dose curve

Inactivation curves of total bacterial count and coliforms by electron beam in sludge A are shown in Fig. 15. Total bacterial count was 3.8 x 10^8 cfu/g in unirradiated sludges. A dose of 6 kGy can reduce total bacterial count approximately 4 log cycles. At about 20 kGy, total bacterial count decreased to 25 cfu/g or by 7 log cycles. Sinskey et al. reported that a dose of 6 kGy using electron beam resulted in approximately 5 log cycles reduction in the total plate count (15). In comparison, our experiment requires higher dose to reduce the same number of total bacterial count than Sinskey et al.

Effect of electron beam on coliforms in sludge cakes is also shown in Fig. 15. The initial load of coliforms were 2.3 x 10^7 cfu/g and decreased linearly with increase of the radiation doses. Irradiation of 4 kGy was enough to inactivate all of the coliforms.

2.3 Effect of heat or radiation treatment on composting

Fig. 16 shows the rate of CO_2 evolution at composting temperature of 40° C. CO_2 evolution curves of irradiated and untreated sludge

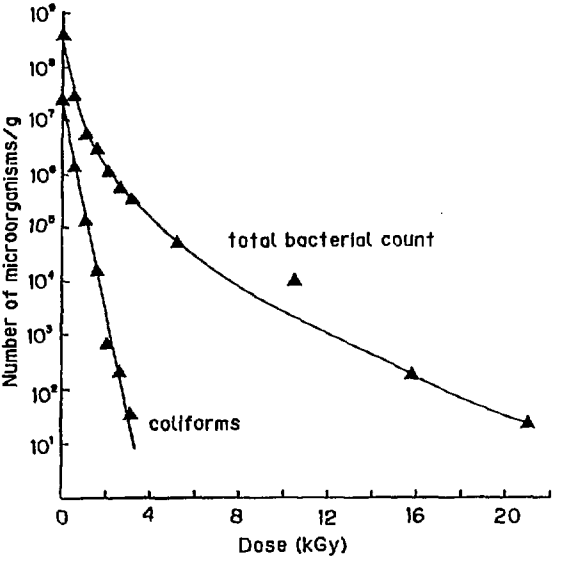

Fig.15 Inactivation of total bacteria and coliforms by electron beam irradiation

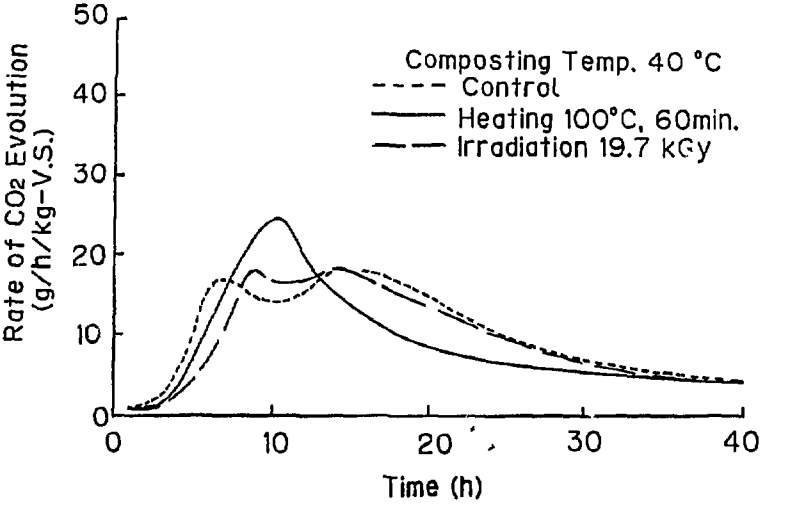

Fig.16 CO_2 evolution by fermentation at 40 °C

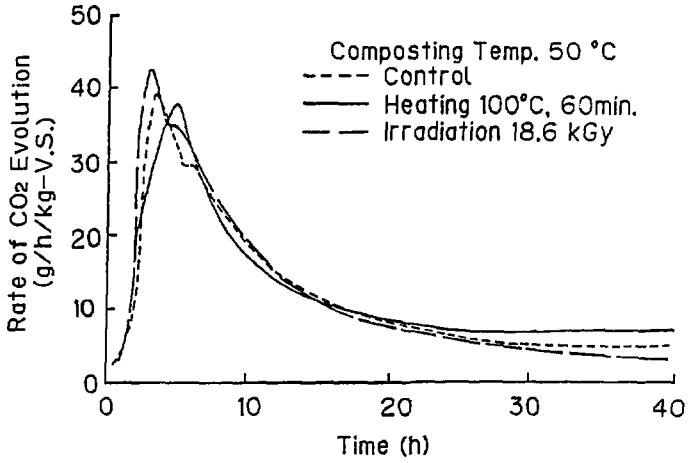

Fig.17 CO_2 evolution by fermentation at 50 °C

samples have two peaks, but the curve of heated sample has only one peak. Fig. 17 shows the rate of CO_2 evolution at composting temperature of 50°C. CO_2 evolution curves of irradiated and untreated sludge have one peak and shoulder, but only one peak without shoulder is found in heated sludge. The differences of curves show that the change of sludge component by heat treatment is large compared with those by radiation treatment.

Fig. 18 shows organic carbon conversion during composting at 40°C. The organic carbon conversion is defined as the ratio of carbon evolved as CO_2 to the amount of organic carbon contained in the sludge initially. The conversion increases rapidly with the composting time but slowly after 25 hours. The values of organic carbon conversion of all treatments are almost the same after 40 hours in spite of different shape of CO_2 evolution curve in Fig. 16. As shown in Fig. 19, composting temperature at 50 °C, organic carbon conversions are slightly higher compared with those at 40°C.

Table 5 shows total bacterial count before and after composting at 50°C. Total bacterial count in the mixture of untreated sludge, seed compost and bulking agent before composting was 1.1×10^8 cfu/g and decreased to 1.5×10^7 cfu/g after composting, because some bacteria originated from sewage sludge were killed by heat in water bath for composting at 50°C as shown in Fig. 10. On the contrary, total bacterial counts of the mixture of other two treatments were found to increase after composting. In the

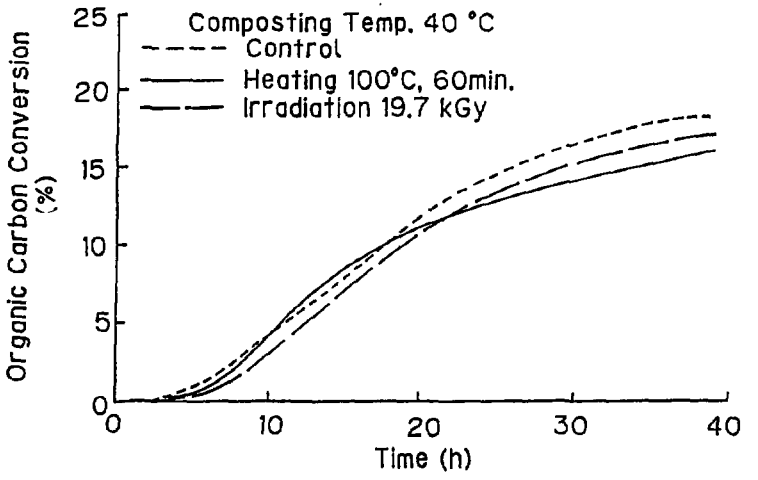

Fig.18 Organic carbon conversion at 40 °C

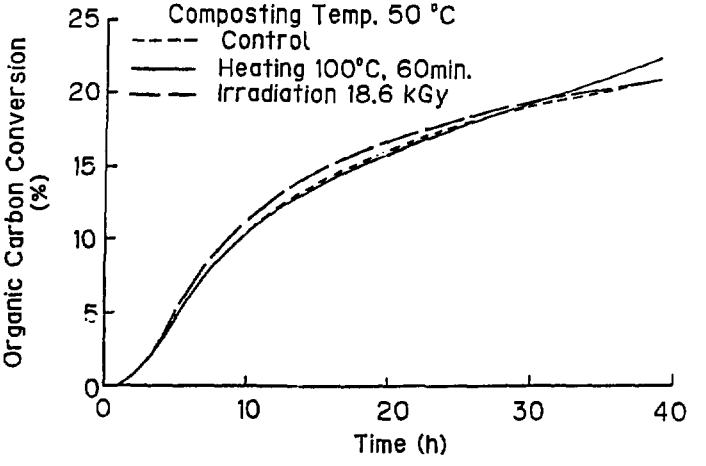

Fig.19 Organic carbon conversion at 50 °C

case of the sludges irradiated with 4.7 and 18.6 kGy, total bacterial counts were found to be 2.7 x 10^6 and 2.1 x 10^6 cfu/g before composting. These values increased to 4.7 x 10^7 and 2.4 x 10^7 cfu/g, respectively, after composting. In the case of heat treatment at 100° C for 15 min and 60 min, total bacterial counts were counted to be 8.6 x 10^6 and 2.0 x 10^6 cfu/g before composting. These values increased to 1.4 x10^7 after composting.

Coliforms were counted to be 4.4 x 10^6 cfu/g in mixture of untreated sludges before composting and became undetectable level after composting at 50° C even without irradiation. But it is reported that 10^2 to 10^4/g of coliforms are contained in conventional compost (16). Because it is very difficult to heat the raw mixture uniformly in large scale fermentors and there is high possibility of regrowth of coliforms even after composting if they remain. Irradiation of sludge before composting take more advantages than conventional composting method because of high rate of composting, less energy requirement, short time of composting and complete disinfection.

Table 5 Total bacterial counts before and after composting

Treatment	Before composting	After composting
Untreated	1.1 x 10^8 cfu/g	1.5 x 10^7 cfu/g
Irradiated		
4.7 kGy	2.7 x 10^6 cfu/g	4.7 x 10^7 cfu/g
18.6 kGy	2.1 x 10^6 cfu/g	2.4 x 10^7 cfu/g
Heated		
100° C,15min	8.6 x 10^6 cfu/g	1.4 x 10^7 cfu/g
100° C,60min	2.0 x 10^6 cfu/g	1.4 x 10^7 cfu/g

Acknowledgment

Major part of this work was carried out during the stay of one of the authors (Ngamnit Sermkiattipong) at Takasaki Radiation Chemistry Research Establishment, JAERI, from October 1987 to April 1988, under "Scientist Exchange Program" of Science and Technology Agency, Japan. Authors wish to express our thanks to Japanese Government, Drs. S. Machi, M. Takehisa,N. Tamura and S. Sato (JAERI) for giving us the chances to perform this study and to complete this report. The authors are indebted to Dr. T. Kume for Fricke dosimetry and the valuable discussion. The authors also wish to express our sincere thanks to Mr. K. Nishimura for preparing experimental apparatus using in composting sludge. Lastly, the authors are grateful to Mrs. Aroon Bangtrakulnonth (WHO National Salmonella and Shigella Center, Division of Clinical Pathology, Department of Medical Science, Nonthaburi, Thailand) for typing serotypes of salmonellae.

References

1) B.B. Wiley and S.C. Westerberg; Appl. Microbiol., 18(6), p.994(1969).
2) S. Kapila; Thesis for the Degree of Doctor of Philosophy on "Sewage sludge hygienization by gamma-radiation and radiosensitization of Salmonella typni.", M.S. University of Baroda, Baroda, India(1985).
3) S. Piadang et al.; Proceeding of the Conference of International Association on Water Pollution Control in Asia, p.399 (Bangkok,1988).
4) E. Hess and C. Breer; Proceeding of a Symposium of IAEA on "Radiation for Clean Environment", p.203 (Munich,1975).
5) G.A. Pandya et al.; Environ. Pollut., 43, p.1(1987).
6) S. Hashimoto et al; JAERI-M 83-238, (1984).
7) S.T. Cowan and K.J. Steel; "Identification of Medical Bacteria", Second Ed., Cambridge University Press, Cambridge, (1974).
8) Report of the Joint FAO/WHO Expert Consultation on "Microbiological Specifications for Foods", United Nations Environment Programme, (Geneva,1975).
9) R. E. Buchanan and N. E. Gibbons; "Bergey's Manual of Determinative Bacteriology", 8 th Ed., Williams and Wilkins, Baltimore, p.294 (1974).
10) F. Kauffmann; "Classification of Bacteriology", Copenhagen, Munksgaard (1975).
11) H. Ito et al.; Agric. Biol. Chem., 47(6), p.1239 (1983).
12) G. E. Stapleton et al.; J. Cell. Comp. Physiol., 41, p.345 (1953).
13) S. Hashimoto et al.; J. Ferment. Technol., 64(4), p.299 (1986).
14) W. Kawakami and S. Hashimoto; Radiat. Phys. Chem., 24(1), p.29 (1984).
15) A. J. Sinsky et al.; Proceeding of a Symposium of IAEA on "Radiation for a Clean Environment", p.139 (Munich,1975).
16) S. Tsuru; SHOKUHINKOGYOU, 21(18), p.21(1978, in Japanese).