การใช้รังสีแกมมาทำลายเชื้อโรคในตะกอนจากระบบบำบัดน้ำทิ้งในระดับกึ่งอุตสาหกรรม

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บทคัดย่อ

การศึกษาผลของรังสีแกมมาที่มีต่อจุลินทรีย์ในตะกอน จากระบบบำบัดน้ำทิ้งของโรงงานบำบัดน้ำเสียเคหะชุมชนห้วยขวาง และวชิรพยาบาล พบว่า การฉายรังสีปริมาณ 5 กิโลเกรย์ในสภาพเติมและไม่เติมอากาศ สามารถลดจำนวนแบคทีเรียทั้งหมดลงได้ 2-3 log cycles และ 1-2 log cycles ตามลำดับ สำหรับโคลิฟอร์มแบคทีเรียที่ตรวจพบในตะกอนจากระบบบำบัดน้ำทิ้งนั้น สามารถ inactivate โดยการฉายรังสีปริมาณ 3 - 4.5 กิโลเกรย์ในสภาพเติมอากาศ และ 4 - 5 กิโลเกรย์ในสภาพไม่เติมอากาศ การฉายรังสีปริมาณ 2 - 3 กิโลเกรย์เพียงพอสำหรับการ inactivate ฟีคัลโคลิฟอร์มแบคทีเรีย และเชื้ออีโคไล จากข้อมูลข้างต้นทำให้ทราบว่า ปริมาณรังสีที่ใช้สำหรับการ inactivate แบคทีเรียดังกล่าวนั้น ขึ้นอยู่กับสภาพการฉายรังสี และปริมาณของแข็งในตัวอย่าง นอกจากนี้ การฉายรังสีในสภาพเติมอากาศมีแนวโน้มช่วยในการ inactivate จุลินทรีย์ได้ดีขึ้น

สำหรับผลของรังสีที่มีต่อเชื้อโรคบางชนิดนั้นพบว่าความถี่ในการตรวจพบเชื้อซัลโมเนลลาปะปนในตะกอนจากระบบบำบัดน้ำทิ้งของโรงงานบำบัดน้ำเสียเคหะชุมชนห้วยขวางและวชิรพยาบาลนั้นสูงถึง 50% และ 75% ตามลำดับ เมื่อฉายรังสีปริมาณ 2กิโลเกรย์ในสภาพเติมอากาศและไม่เติมอากาศปรากฏว่า ตรวจไม่พบเชื้อซัลโมเนลลาในตัวอย่าง สำหรับเชื้อ *Clostridium perfringens* นั้นตรวจพบทั้งในตัวอย่างที่ฉายรังสีและไม่ฉายรังสี แสดงให้เห็นว่า รังสีปริมาณ 5 กิโลเกรย์ไม่เพียงพอที่จะกำจัดเชื้อ *C. perfringens* ได้ ส่วนเชื้อชิเกลลานั้นตรวจไม่พบทั้งในตัวอย่างที่ฉายรังสีและไม่ฉายรังสี

Gamma Radiation Inactivation of Pathogens in Sludge under Larger-Scale Condition

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ABSTRACT

The effect of gamma radiation on microorganisms in sludge from Huay Kwang Sewage Treatment Plant and Vajira Hospital showed that total bacterial counts were reduced to 2 - 3 log cycles and 1 - 2 log cycles at 5 kGy irradiation with and without aeration, respectively. Inactivation of coliform bacteria in sludge required irradiation with and without aeration at the dosages of 3 - 4.5 and 4 - 5 kGy, respectively. A dose of 2 - 3 kGy was sufficient to inactivate fecal coliform bacteria and *E. coli*. The doses used for inactivation these bacteria depend on the irradiation condition and solid content in sludge sample. Irradiation with aeration led to an increased microbial inactivation.

According to our results, the frequency of occurrence of salmonellae contaminated in sludge from Huay Kwang Sewage Treatment Plant and Vajira Hospital was 50% and 75%, respectively. A dose of 2 kGy irradiation with or without aeration , salmonellae could not be detected in any sludge. *Clostridium perfringens* organisms were also detected in non-irradiated and irradiated sludge from both sources. Moreover, a dose of 5 kGy irradiation with or without aeration was not enough to eliminate *C. perfringens*. However, no shigellae were isolated from any treatment of sludge.

1. Introduction

The amount of sewage sludge increases rapidly every year due to the expansion of population in the cities and increase of industrial activities. Special attention to the treatment of sewage sludge is therefore necessary. Utilization of sludge as a fertilizer or soil conditioner and for land filling are common method of resource conservation practised worldwide. In parallel to the increasing quantity of sludge, contamination of the sewage sludge with potential bacterial pathogens (salmonellae, shigellae, fecal coliforms, fecal streptococci ....etc.,) worm eggs, viruses and protozoa has increased (1). In Thailand, salmonellae are very hazardous pathogenic bacteria and the contamination of salmonellae in sludges from hotels, food industries and Huay Kwang community were 80.70, 51.85 and 68.00 percents, respectively (2). Therefore in order to cut the possible infection chain sewage sludge-plants-animals-man, the disinfection of sewage sludge is demanded.

Various methods of sludge disinfection are known to be used. Heat treament , although effective, has a number of disadvantages. It is an energy intensive and expensive process. Other treatments, such as chlorination have been reported to be inefficient , as viruses associated with solids have been found to be protected aga ,st ina`tipation. Composting of sewage sludges is routinely used as a mean of reducing organisms to very low counts. However, repopulation of certain bacteria is a serious drawback of this treatment process. A very effective method for rendering sewage sludge free of pathogens is irradiation with ionizing radiation (3). The scope of this project was to study the effects of irradiation on some pathogens and on inactivation of microorganisms in sludge treated under larger-scale conditions. Data received from this study may be a preliminary guidance to future pilot plant.

2. Materials and Methods

2.1 Sewage sludge

Raw sludge and dewatered sludge cake from Vajira Hospital and anaerobic digested sludge from Huay Kwang Sewage Treatment Plant were collected. Dewatered sludge cake and anaerobic digested sludge were diluted with distilled water to obtained less than 10% solid content for mixing purpose. Solid content of raw sludge and diluted sludge cake from Vajira Hospital were 2.80-4.30% and 6.05-8.43%, respectively. For diluted anaerobic digested sludge from Huay Kwang Sewage Treatment Plant was 5.44-6.80% solid content.

2.2 Irradiation

A multipurpose irradiator, carrier type Model JS 8900 with activity of 388075-378241 curies at Thai Irradiation Center was used for irradiation treatment. Approximately 200 kilograms of each sample were put in a cylindrical plastic container (120 cm depth, 60 cm diameter, 300 litre capacity) which comprised of mixer and aerator inside. Each sample was irradiated with or without aeration condition at the dosages of 0, 2, 3, 4 and 5 kGy. The dose rate distribution used in this experiment was 6.23 Gy/min.

2.3 Enumeration and detection of bacteria

2.3.1 Enumeration of total bacterial count and indicator organisms were determined mainly as described in the Standard Methods for Examination of Water and Wastewater (4).

2.3.1.1 For total bacterial count, each sludge treatment was diluted serially in sterilized 0.067 M phosphate buffer. Then 0.1 ml of each appropriate dilution was plated in duplicate on the surface of Difco-tryptone glucose extract agar and the plates were incubated at $35 \pm 0.5^{\circ}C$ for 48 ± 3 hr.

2.3.1.2 Coliforms in sludge were enumerated by the multiple-tube fermentation procedure with a series of five tubes per dilution as a Most Probable Number (MPN) index. Lauryl tryptose broth was used for the primary fermentation and brilliant green lactose bile broth fermentation tubes were used for the confirmed test. Incubated the inoculated brilliant green lactose bile broth tube at $35 \pm 0.5^{\circ}C$ for 48 ± 3 hr.

2.3.1.3 Fecal coliforms were determined by the multiple-tube fermentation procedure. All positive presumptive tubes from the total coliform MPN test were transferred to EC medium. Inoculated tubes were incubated in a water bath at $44.5 \pm 0.2^{\circ}C$ for 24 ± 2 hr.

2.3.1.4 The MPN for *E. coli* was determined by tranferring the numbers of all positive tubes from fecal coliforms to Eosin methylene blue agar plate to isolate colonies. Incubation was done at $37^{\circ}C$ for 18-24 hr and a suspicious colony was subsequently picked to test for biochemical characteristics.

2.3.2 Detection of some pathogens

2.3.2.1 Detection of salmonellae

Isolation of salmonellae was carried out by a modified method of the Sta` dapdMethods for Examination of Water and Wastewater (4) and FAO/WHO methods (5).

Identification of salmonellae, various biochemical characteristics of isolated strains were examined according to "Bergey's Manual of Determinative Bacteriology" (6).

Serological test by using Salmonella polyvalent O antiserum and O group antisera were then performed. Identification of Salmonella serovars (serotypes) required determination of H antigens and phase of the organisms as described by Edwards and Ewing (7).

2.3.2.2 Detection of *Clostridium perfringens*

Isolation and confirmation procedures of *C. perfringens* were conducted using the method described in SEAMIC Publication No.12 (8) as below.

Appropriate serial dilution was prepared using 0.067 M phosphate buffer as dilution blanks and subsequently transferred each of dilution to cooked meat broth And nce@ated at 37°C for 24 hr. Each tube was streaked on Eiken-FM-CW egg yolk agar and incubated at 37°C for 18-24 hr. The typical colonies were selected and then tested for Nagler reaction. FM-CW egg yolk agar plate was prepared, dried on the surface of the plate and then divided into two parts. Half of the plate was flooded with antitoxin of *C. perfringens* type A. After the antitoxin was absorbed into the medium, inoculation was done by drawing a line with a needle from the side without antitoxin flooded to the side with antitoxin. It was then incubated at 37°C in an anaerobic jar for 24 hr.

2.3.2.3 Detection of shigellae

Isolation of shigellae was done in accordance with Edwards and Ewing (7) and Riley (9). For identification of shigellae was carried out mainly by the method described in SEAMIC Publication No.6 (10).

3. Results

3.1 Irradiation effect for inactivation of microorganisms in sludge

Inactivation curves of total bacterial count in sludge from Huay Kwang Sewage Treatment Plant are shown in Fig.1. Total bacterial count was determined to be 2.88×10^6 cfu/g in non-irradiated sludge (5.44-6.80% solid content). After irradiation without aeration at the dosage of 5 kGy, total bacterial counts was reduced to be 1.14×10^5 cfu/g. The initial load of total bacterial count in non-irradiated sludge (5.85-7.44% solid content) of Huay Kwang Sewage Treatment Plant was 5.60×10^6 cfu/g. Total bacterial count diminished rather rapidly with an increase in the radiation dose and 2.12×10^4 cfu/g still survived after 5 kGy irradiation with aeration.

Total bacterial counts were reduced to 2 -3 log cycles and 1 - 2 log cycles at 5 kGy in irradiated sludge with and without aeration, respectively. In other report, a reduction of total bacterial count was 2 log by irradiation at the dosage of 260 krad (11).

Fig.2 shows inactivation curves of total bacterial count in sludge from Vajira Hospital. Total bacterial counts in non-irradiated sludges containing 3.73-4.30% and 6.05-8.43% solid content were 1.02×10^{6} and 8.50×10^{6} cfu/g, respectively. After 5 kGy irradiation without aeration, total bacterial counts were reduced to be 5.7×10^{4} and 1.18×10^{5} cfu/g, respectively. The initial loads of total bacterial count in non-irradiated sludges containing 2.80-3.03% and 6.44-6.78% solid content were 3.10×10^{6} and 1.40×10^{7} cfu/g , respectively. After 5 kGy irradiation with aeration, total bacterial counts were reduced to be 4.5×10^{4} and 1.26×10^{5} cfu/g, respectively.

For Vajira Hospital, total bacterial counts were reduced 2 log cycles and 1-2 log cycles at 5 kGy in irradiated sludge with and without aeration, respectively. Sinskey et al. also reported that a dose of 600 krad using electron beam resulted in approximately a 5 log cycle reduction in the total plate count (12).

As shown in Fig.3, total coliforms were 4.95×10^{6}/100 ml in non-irradiated sludge (5.44-6.80% solid content) of Huay Kwang Sewage Treatment Plant. After 5 kGy irradiation without aeration, total coliforms were reduced to be < 2/100 ml. The initial load of total coliforms in non-irradiated sludge (5.85-7.44% solid content) was 1.76×10^{6}/100 ml. After 4 kGy irradiation with aeration, total coliforms were reduced to be < 2/100 ml.

Fig.4 shows inactivation of coliform bacteria in sewage sludge of Vajira Hospital. The initial loads of total coliforms in non-irradiated samples containing 3.73-4.30% and 6.05-8.43% solid content were 7.90×10^{4}/100 ml and 1.60×10^{6}/100 ml, respectively. After 4 and 5 kGy irradiation without aeration, total coliforms reduced to be < 2/100 ml and < 2/100 ml, respectively. Total coliforms in non-irradiated sludge containing 2.80-3.03% and 6.44-6.78% solid content were 1.60×10^{6}/100 ml and 1.60×10^{6}/100 ml , respectively. After 3 and 4 kGy irradiation with aeration, t`tal coliforms were reduced to be < 2/100 ml and < 2/100 ml , respectively.

Inactivation of fecal coliforms in sewage sludge of Huay Kwang Sewage Treatment Plant is shown in Fig.5. Irradiation at 3 kGy without aeration showed reduction of fecal coliform bacteria from 4.0×10^{5}/100 ml to < 2/100 ml. Moreover, irradiation at 2 kGy with aeration showed reduction of fecal coliform bacteria from 1.3×10^{4}/100 ml to < 2/100 ml.

As shown in Fig.6, the initial loads of fecal coliforms in non-irradiated samples of Vajira Hospital containing 3.73-4.30% and 6.05-8.43% solid content were 7.9 $x10^4$/100 ml and 1.3 $x10^6$/100 ml, respectively. After 2 and 3 kGy irradiation without aeration, fecal coliforms reduced to be < 2/100 ml and < 2/100 ml, respectively. Fecal coliforms in non-irradiated sludge containing 2.80-3.03% and 6.44-6.78% solid content were 3.5 x 10^5/100 ml and $1.6x10^6$/100 ml, respectively. After 2 and 3 kGy irradiation with aeration, fecal coliforms reduced to be < 2/100 ml and < 2/100 ml, respectively.

Fig.7 shows inactivation of *E. coli* in sewage sludge of Huay Kwang Sewage Treatment Plant. Irradiation at 3 kGy without aeration showed reduction of *E. coli* from $2.0x10^5$/100 ml to < 2/100 ml. However, irradiation at 2 kGy with aeration showed reduction of *E. coli* from 1.30 x 10^4/100 ml to < 2/100 ml.

Inactivation of *E. coli* in sewage sludge of Vajira Hospital is shown in Fig.8. The initial loads of *E. coli* in non-irradiated samples containing 3.73-4.30% and 6.05-8.43% solid content were $4.9x10^4$/100 ml and $3.40x10^5$/100 ml, respectively. After 2 and 3 kGy irradiation without aeration, these bacteria reduced to be < 2/100 ml and < 2/100 ml, respectively. The numbers of *E.*coli in non-irradiated sludges containing 2.80 - 3.03 % and 6.44 - 6.78 % solid content were $2.20x10^5$/100 ml and $9.20x10^5$/100 ml, respectively. After 2 and 3 kGy irradiation with aeration, these bacteria reduced to be < 2/100 ml and < 2/100 ml , respectively.

3.2 Irradiation effect on some pathogens in sludge

Presence of salmonellae in sludge before and after irradiation treatment is shown in Table 1. The results showed that the frequency of occurrence of salmonellae contaminated in sludge from Huay Kwang Sewage Treatment Plant was 50% and 3 groups of these bacteria namely C, E and K were classified and 4 serovars (serotypes) were identified. In the case of Vajira Hospital, the frequency of occurrence of salmonellae contaminated in sludge was 75% and 4 groups of these bacteria namely B, C, E and K were classified and 5 serovars were identified. A dose of 2 kGy irradiation with or without aeration, salmonellae were undetectable in any of sludge.

In our investigation, *Clostridium perfringens* could be detected in non-irradiated and irradiated sludge from both sources. A dose of 5 kGy was not enough to eliminate this pathogen.

The shigellae were not detected in either non-irradiated or irradiated sludge from Huay Kwang Sewage Treatment Plant and Vajira Hospital.

4. Discussion and Conclusion

According to the results of this st0dy, irrAdiation with and without aeration at the dosage of 5 kGy reduced total bacterial count by 2-3 log cycles and 1-2 log cycles, respectively. The reduction of total bacteria in irradiated sludge was rather slowly when compared with other experiments. The reason may be due to the dose rate distribution used in this experiment is only 6.23 Gy/min which is rather low, thus the inactivation of total bacteria was not so effective. In addition, the reduction of total bacteria in irradiated sludge with aeration were a little more effective than irradiation without aeration treatment. It can be seen that there was no difference in total bacterial count at various percent solid content in the same treatment.

Inactivation of coliform bacteria in sludge was required irradiation with and without aeration at the dosages of 3 - 4.5 and 4 - 5 kGy, respectively. In our investigation, inactivation of coliform bacteria in irradiated sludge with aeration was required lower dose than irradiated without aeration. Additionally, at various percent solid content in the same irradiation treament, the result showed that the sample with high percent solid content was required higher dose than low percent solid content for inactivation of coliforms. In the case of Atladara sewage sludge, a maximum of 600 krad of gamma radiation was necessary to completely eliminate coliforms (13).

A dose of 2 -3 kGy was sufficient to inactivate fecal coliform bacteria and *E. coli*. The number of fecal coliforms from the samples of Huay Kwang Sewage Treatment Plant and Vajira Hospital diminished exponentia``y wi$h incpeasing dosages. For Huay Kwang Sewage Treatment Plant , inactivation of fecal coliforms in irradiated sludge with aeration required only 2 kGy, however irradiation without aeration required 3 kGy. In the case of Vajira Hospital, it seems that percent solid content of the samples was more effective to the dosage for inactivation of fecal coliforms than aeration or without aeration condition during irradiation. A dose of 2 kGy was enough to inactivate these bacteria in low percent solid content and 3 kGy was sufficient in high percent solid content. Van den Berg et al. reported that a complete kill of fecal coliform bacteria in digested sludge was achieved with radiation alone at a dose of 120 krad and when the water content of the digested sludge was reduced 50 percent , irradiation alone reduced the population of fecal coliform bacteria by only one decade at irradiation dosages to 240 krad (14).

In our investigation, inactivation of *E. coli* in irradiated sludge with aeration of Huay Kwang Sewage Treatment Plant was required 2 kGy, however, irradiated without aeration was required 3 kGy. In the case of Vajira Hospital, the result showed that the dosages for inactivation of *E. coli* were the same as fecal coliform bacteria and the parameter which affected to the dosages was similarly.

The doses used for inactivation all bacteria mentioned above depended on the irradiation condition and percent of solid content. Irradiation with aeration led to an increased microbial inactivation.

The frequency of occurrence of salmonellae contaminated in sludge from Huay Kwang Sewage Treatment Plant and Vajira Hospital was 50% and 75%, respectively. A dose of 2 kGy irradiati%n with or without aeration, salmonellae could not be detected in any of sludge. In other report, a dose of 250 krad resulted in a reduction per gram of eight orders of magnitude of salmonellae in raw primary sludge (15).

C. perfringens was detected in non-irradiated and irradiated sludge. A dose of 5 kGy was not enough to eliminate this pathogen and thus should require higher dose due to its resistance to radiation.

In our investigation, no shigellae were isolated from any treatment of sludge. Ottolenghi and Hamparian also reported that no shigellae were isolated from any of the sludge or stool specimens tested (16). However, only one Shigella organisms was recovered from the attempted Shigella isolations (17). Because of this lack of isolation of *Shigella* sp. from any of sludge, we are unable to reach any conclusion about the presence or absence of this pathogen or about the relative risks involved.

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Table 1. Presence of salmonellae in sludge before and after irradiation treament conditions

Sources of samples	Types of salmonellae in non-irradiated samples		Types of salmonellae in irradiated samples (2 kGy-5kGy)	
	group	serovars	group	serovars
Huay Kwang Sewage Treatment Plant.	C	S. montevideo	none	none
	C	S. emek		
	E	S. weltevreden		
	K	S. cerro		
Vajira Hospital	B	S. agona	none	none
	C	S. blockley		
	E	S. amsterdam		
	E	S. weltevreden		
	K	S. cerro		

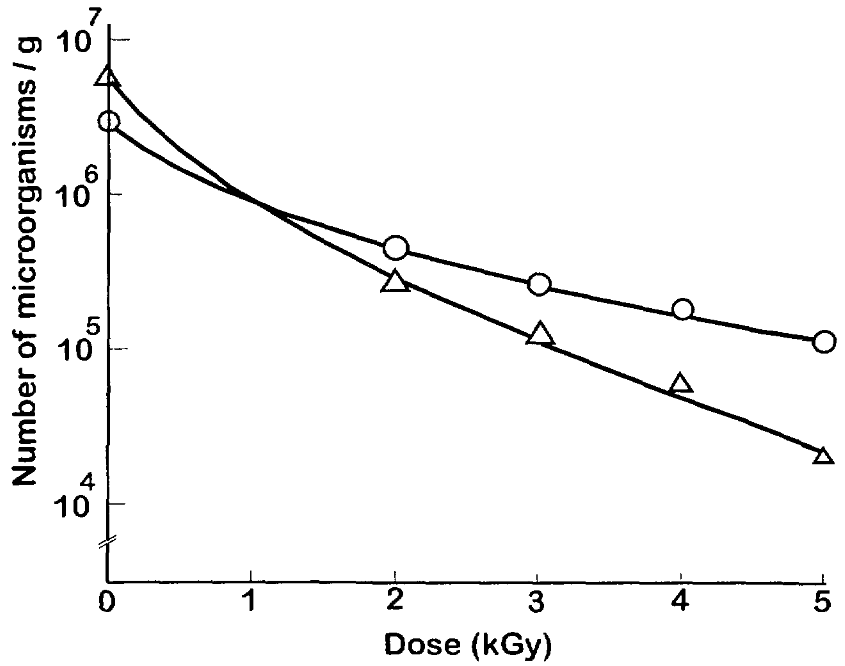

Fig. 1 Inactivation curves of total bacterial count in sludge of Huay Kwang Sewage Treatment Plant.

○ without aeration 5.44 - 6.80 % solid content

△ aeration 5.85 - 7.44 % solid content

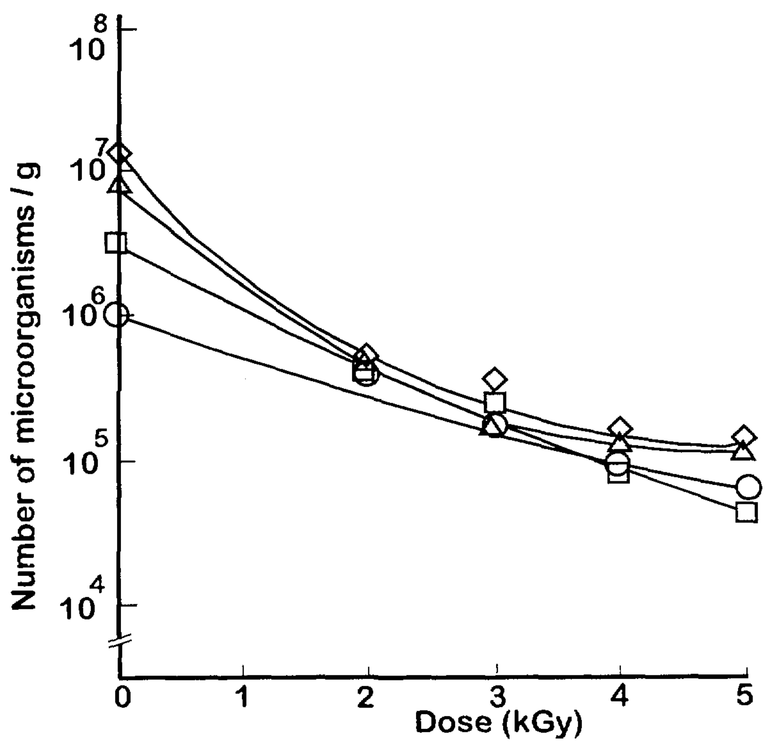

Fig. 2 Inactivation curves of total bacterial count in sewage sludge of Vajira Hospital

○ without aeration 3.73 - 4.30 % solid content

□ aeration 2.80 - 3.03 % solid content

△ without aeration 6.05 - 8.43 % solid content

◇ aeration 6.44 - 6.78 % solid content

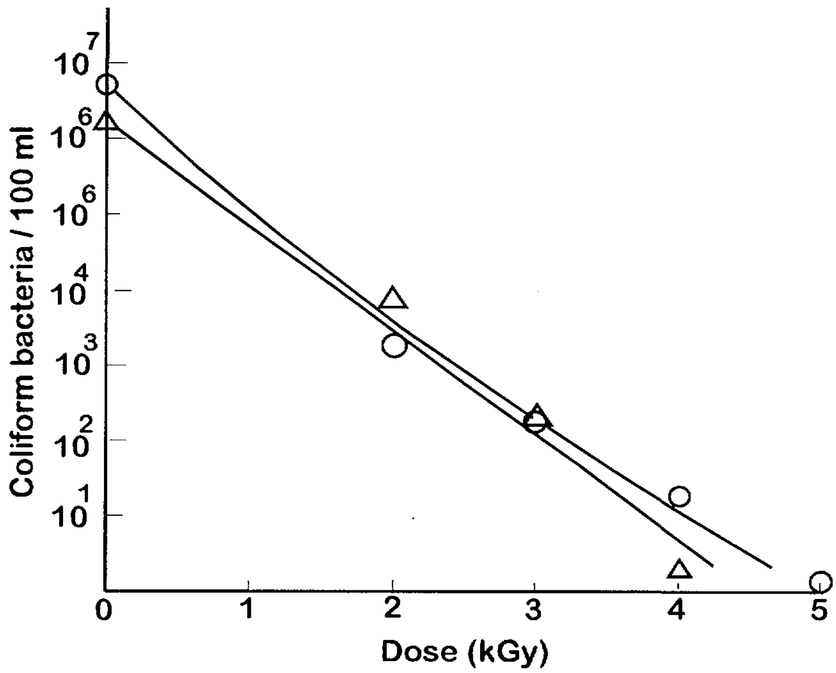

Fig. 3 Inactivation of coliform bacteria in sewage sludge of Huay Kwang Sewage Treatment Plant.

○ without aeration 5.44 - 6.80 % solid content

△ aeration 5.85 - 7.44 % solid content

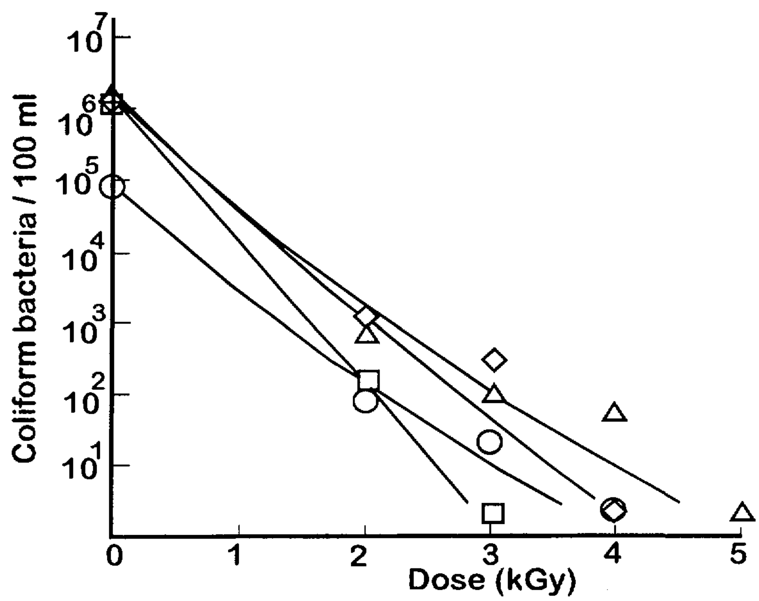

Fig. 4 Inactivation of coliform bacteria in sewage sludge of Vajira Hospital

○ without aeration 3.73 - 4.30 % solid content

□ aeration 2.80 - 3.03 % solid content

△ without aeration 6.05 - 8.43 % solid content

◇ aeration 6.44 - 6.78 % solid content

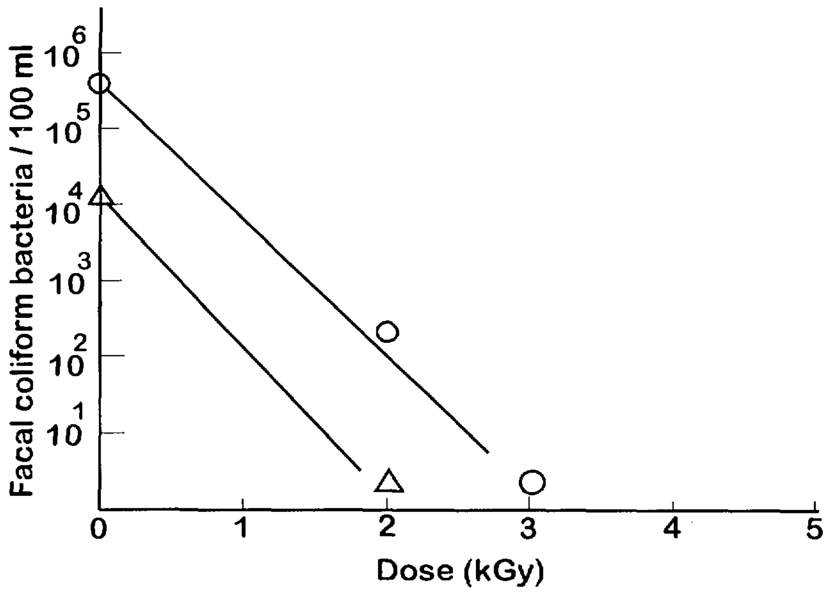

Fig. 5 Inactivation of fecal coliform bacteria in sewage sludge of Huay Kwang Sewage Treatment Plant.

○ without aeration 5.44 - 6.80 % solid content

△ aeration 5.85 - 7.44 % solid content

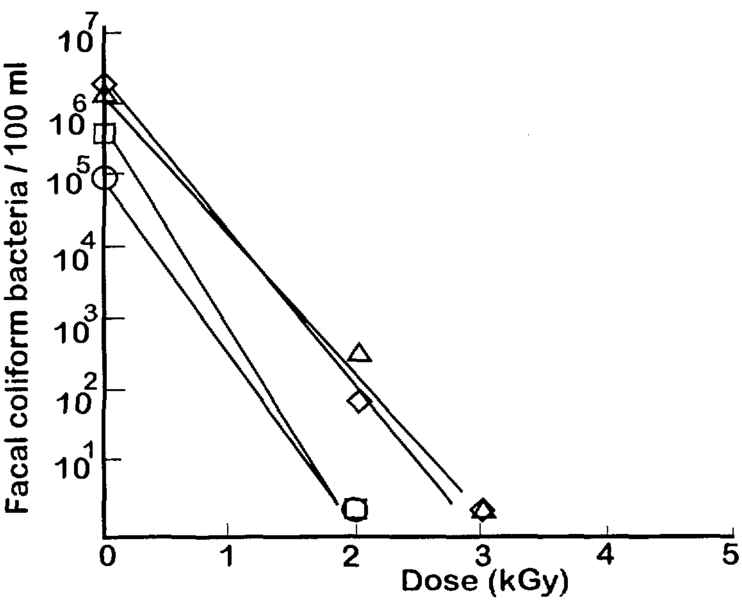

Fig. 6 Inactivation of fecal coliform bacteria in sewage sludge of Vajira Hospital

○ without aeration 3.73 - 4.30 % solid content

□ aeration 2.80 - 3.03 % solid content

△ without aeration 6.05 - 8.43 % solid content

◇ aeration 6.44 - 6.78 % solid content

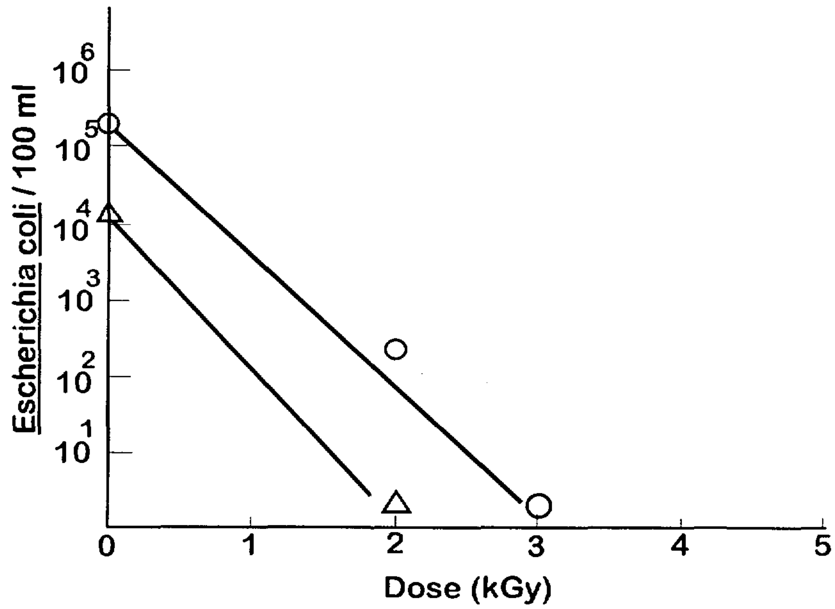

Fig. 7 Inactivation of Escherichai coli in sewage sludge of Huay Kwang Sewage Treatment Plant.

○ without aeration 5.44 - 6.80 % solid content

△ aeration 5.85 - 7.44 % solid content

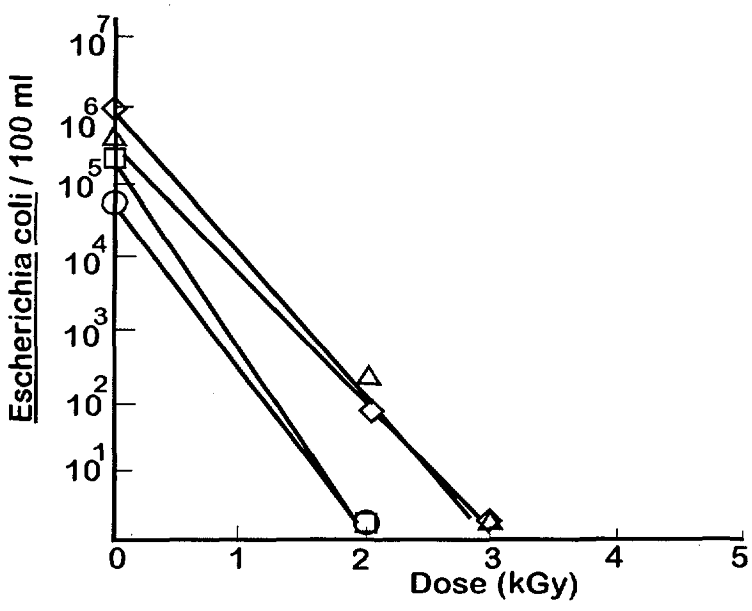

Fig. 8 Inactivation of Escherichia coli in sewage sludge of Vajira Hospital

○ without aeration 3.73 - 4.30 % solid content

□ aeration 2.80 - 3.03 % solid content

△ without aeration 6.05 - 8.43 % solid content

◇ aeration 6.44 - 6.78 % solid content