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SLUDGE PASTEURIZATION AND UPGRADING BY RADIATION
—BILATERAL RESEARCH COOPERATION BETWEEN OAEP AND JAERI—

July 1995

Department of Radiation Research for
Environment and Resources

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

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Sludge Pasteurization and Upgrading by Radiation
－Bilateral Research Cooperation between OAEP and JAERI －

Department of Radiation Research for Environment and Resources

Takasaki Radiation Chemistry Research Establishment
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(Received June 5, 1995)

The research cooperation between office of Atomic Energy for Peace, Thailand (OAEP) and Japan Atomic Energy Research Institute (JAERI) on "Sludge Pasteurization and Upgrading by Radiation" was carried out for 4 years starting from March 1990. This cooperation was performed through information exchange meetings (Steering Committee Meeting), held in Takasaki and Bangkok, and experiments and discussions by scientist exchange, Many useful results were obtained on radiation inactivation effect of pathogen and parasites, upgrading of irradiated sludges to fertilizer, animal feeds and biological pesticides. This report includes the main results of the research cooperation reported at the First to Fifth Steering Committee Meetings as the progress reports.

Keywords:Sludge, Electron-beam, Gamma-ray, Pasteurization, Disinfection, Pathogen, Parasite, Radiation, Fertilizer, Animal Feed, Biological Pesticide

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This is the report of the research by Bilateral Cooperation Program with the Office of Atomic Energy for Peace, Thailand (OAEP) on "Sludge Pasteurization and Upgrading by Radiation"

放射線による汚泥の殺菌と有効利用
－タイ原子力庁と日本原子力研究所との研究協力－

日本原子力研究所
環境・資源利用研究部

（1995年6月5日受理）

放射線による汚泥の殺菌と有効利用に関するタイ原子力庁（ＯＡＥＰ）と日本原子力研究所（原研）との二国間研究協力を，1990年3月より1994年3月までの4年間実施した。研究協力は，高崎あるいはバンコクで開催される運営委員会での情報交換，双方の研究員交流による共同実験並びに結果の討議により行った。この研究協力を通じて，放射線による汚泥中の病原菌の殺菌並びに寄生虫の不活化，照射汚泥の肥料や飼料，植物病抑制用資材としての有効利用等について多くの成果が得られた。本報告書は，上記研究テーマに関する研究協力終了に当たり，5回にわたる運営委員会で報告されたプログレスレポートを中心に研究協力の成果をまとめたものである。

本報告書は，「放射線による汚泥の殺菌と有効利用」に関するタイ原子力庁（ＯＡＥＰ）との二国間研究協力の成果をまとめたものである。

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1. Introduction

The research cooperation between OAEP and JAERI on radiation treatment of sludge was continued for 4 years starting from March 1990 based on "The Implementing Agreement between the Office of Atomic Energy for Peace and the Japan Atomic Energy Research Institute on the Research Cooperation in the Field of Radiation Processing". This cooperation included

a) exchange of scientific and technical experts
b) joint research program decided by Steering Committee Meeting
c) joint utilization of equipments, laboratories and facilities.

1.1 Background and Major Items of the Research

The rapid expansion of population in cities and increase of industrial activities have brought environmental pollution by waste water or sludges.

Even though sludges contain pathogens and parasites, they have high values as fertilizers or animal feeds if they are properly pasteurized.

Radiation treatment is a useful tool for pasteurization of pathogens and parasites.

This research program is aimed to investigate the radiation pasteurization of sludges and upgrading for fertilizers and animal feeds. This program also includes the development of irradiation technology related to this program.

Major items of the work is as follow;

Radiation disinfection effect of pathogen and parasites

The effect of irradiation on total bacteria, pathogens such as salmonellae, parasites and etc. in sludge from household and industries are to be investigated. Combination effect of radiation and heating and residual microorganisms after treatment are to be clarified.

Upgrading of disinfected sludges

Upgrading of irradiated sludges by useful microorganisms for agricultural uses and animal feeds or fish feeding are to be investigated.

Evaluation of components in sludges

Components useful for fertilizers and animal feed are to be analyzed. Contents of toxic materials such as heavy metals and residual pesticides are also to be clarified.

Utilization test of the products

Utilization test of the products such as irradiated sludges and fermented products to farm land, animal feeds and fish feeding are to be investigated.

Irradiation engineering and feasibility study

Irradiation techniques and plant design are to be investigated. Cost estimation of plant and operation is to be carried out.

1.2 Time Schedule

Item (place)	1990	1991	1992	1993	1994
Radiation disinfection effect on pathogen and parasites (OAEP, JAERI)	———	———	———	———	———
Upgrading of irradiated sludges (JAERI, OAEP)	———	———	———	———	———
Evaluation of components in sludges (OAEP, JAERI)	———	———	———	———	———
Utilization tests of the products (OAEP)			———	———	———
Irradiation engineering and feasibility study (JAERI)			———	———	———

(Calendar year)

1.3 Main Research Staffs

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2. General Views

[Abstract]

2.1 R & D on Sludge Treatment
Research and development of radiation treatment of sewage sludge in the world were introduced.

2.2 Present status of research in OAEP
Present status of research in OAEP on radiation treatment of sludge was introduced.

2.3 Present status of research on sludge treatment in JAERI
Present status of research in JAERI on radiation treatment of sewage sludge was introduced together with the result of pilot plant test.

2.1 R & D on Sludge Treatment

S. Hashimoto

Biological treatments of waste water including activated sludge treatment are common in many cities of the world. However, such treatments generate a large amount of sludge and the amount increases with expansion of sewage services. Therefore, disposal of the sludge is now a serious problem in the world.

Land application of sludge as fertilizer and soil conditioner seems the most valuable disposal method, because the sludge has a nutritive value for plant growth. There are, however, some problems associated with the land application. The sludges are generally contaminated with pathogens, parasites and organic or inorganic toxics. From the viewpoint of public health, at least the pathogens remaining in sludge should be eliminated prior to land application.

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 such as sludge. Chlorination have been reported to be ineffective to viruses associated with solid by the reason of protection effects. Heat treatment of sludge in slurry state is also effective. However, this treatment is energy intensive process and tends to be costly. Ionizing radiation requires much less energy and incurs smaller change in components of sludge compared with heat pasteurization. Moreover, the operation procedures for irradiation are simple and the sludge can be continuously and completely disinfected.

Lowe and Ridenour (1956) and Armbruster (1956) have first reported their studies on sewage and sewage sludge disinfection. Since then, many results on radiation treatment of sludge have been reported (Watanabe et al., 1984, Hashimoto et al., 1986, etc.). It is shown in these reports that doses over 10 kGy are necessary to kill all of bacteria. But, to eliminate all of coliforms, a dose less than 5 kGy is sufficient.

The first application of irradiation technology to sewage sludge was realized at a demonstration facility constructed in 1973 at Geiselbullach, West Germany, where cobalt-60 was used (Lessel and Suess, 1984). The facility is still operating as a commercial plant. In this plant, sludge is irradiated to 2 kGy at the capacity of 270 ton/day.

In the United State, an eight ton/day irradiator was constructed in 1978 by Sandia National Laboratories in Albuquerque, New Mexico (Sivinski and Ahlstrom, 1984). At this facility, cesium-137 was used as the radiation source.

In 1976, an electron beam irradiation system was put on-line at

Boston's Metropolitan District Commission Wastewater Treatment Plant at Deer Island (Trump et al., 1976). The facility handled up to 15.8 m^3/hr of liquid sludge, delivered a dose of 4 kGy, and was primarily used for research purposes. Electrons were generated by a 750 kV, 50 kW commercial electron accelerator.

Based on the research at Deer Island, the Miami-Dade Water and Sewer Authority in Florida decided to locate a sludge irradiator using accelerated electrons at its Virginia Key Wastewater Treatment Plant. This irradiator began operation in September 1984. The electrons are accelerated to an energy of 1.5 MeV and are directed to a maximum beam width of 1.2 m. The applied dose is 3.5 to 4 kGy. This plant allows a flow of 27 m^3/hr.

In Japan, since 1979, disinfection of dewatered sewage sludge by radiation and efficient composting of the disinfected sludge have been studied by TRCRE, JAERI. Disinfection of the sludge was successfully performed not only by gamma ray but also by electron beam. In the case of electron beam irradiation, uniform and continuous irradiation was proved to be possible by spreading sludge cake into a thin layer on a stainless steel conveyor through a flat nozzle. Optimum composting conditions for irradiated sludge was also studied by a small experimental apparatus and it was shown that composting almost completed within 2 or 3 days under the optimum fermentation condition.

In 1987, JAERI constructed a pilot plant for irradiated sludge composting. About 500 kg/batch of dewatered sludge with 80 % water content was used after irradiation. A 2 MeV, 30 mA electron accelerator was used to irradiate the raw sludge (Hashimoto et al, 1990). After this test, another composting test was performed by a fermentor of different type. In this case, 1 ton/batch of dewatered sludge was irradiated by the electron accelerator and composted. The produced compost is now under utilization test for vegetable growth as fertilizer.

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Sivinsky,J. and Ahlstrom,S.(1984); Radiat. Phys. Chem., 24, 191.
Watanabe,H. and Takahisa,M.(1984); Radiat. Phys. Chem., 24, 41.
Hashimoto,S. et al(1986); J. Ferment. Technol., 64, 299.
Hashimoto,S. et al.(1990); IAWPRC 25TH ANNIVERSARY CONFERENCE AND EXHIBITION held in Kyoto, Japan.

2.2 Present status of research in OAEP

C. Banditsing

The studies on radiation disinfection of waste waters were conducted during 1981-1987. The irradiated and non-irradiated waste waters and sludges used as fertilizer for planting morning glory were also carried out in 1983.

During 1987 to 1989 the survey of salmonellae and parasites was carried out. In addition, the application of radiation microbiology and effects data on sludge treatment under larger scale have been also conducted from July 1989 to February 1990 under the IAEA research contract.

At present, the radiosensitivity of salmonellae in both phosphate buffer and sludge is being observed. Moreover, the radiosensitivity of parasites will be conducted this year.

2.3 Present status of research on sludge treatment in JAERI

S. Hashimoto and S. Sato

ABSTRACT

Disinfection of dewatered sewage sludge by electron irradiation has been studied in combination with efficient composting of the disinfected sludge. Uniform and continuous irradiation was possible by spreading sludge cake into a thin layer on a stainless steel conveyor through a flat nozzle. Optimum composting conditions for irradiated sludge was also studied by a small experimental apparatus. Under these conditions, composting almost completed within 2 or 3 days. A pilot plant with a treatment capacity of 500 kg sludge/batch was built to get information on a large scale composting plant of electron-beam disinfected sludge cake. Efficient composting could be realized by the pilot plant, by controlling fermentation temperature in the optimum range, by controlling air flow rate and by frequent mixing in the fermentor. Capital cost and treatment cost were also calculated according to the results of the pilot plant test.

1. Background and outline of the system

1.1 Sludge treatment in Japan

Disposal of sewage sludge has been one of the most serious problems in Japan. The percentage of population who can receive the sewage service was 39% at the end of march in 1987 and amount of sludge is increasing every year. Total amount of sludge is about 2.2 million ton/year in dehydrated form. 76 % of the sludge is disposed in land and off coast. The ratio of reutilization is 15 %. Recently it becomes difficult to ensure sites for disposal because of pollution problems. It is known that land application of sludge benefits plant growth, crop production and soil conditioning. But, there are several problems in the direct application of raw sludge.

One of the important problems related to the land application of sludge is contamination by heavy metals. This should be solved by the legal control on the effluents. Another problem is contamination by pathogenic bacteria. Heating is effective for disinfection. Other problems are bad odor, insects and harmful to plants caused by decomposition of organic materials after land application. Composting is necessary to stabilize easily decomposable organic materials in sludge, such as glucose, organic acid and protein. Because the urbanization of rural areas has been in progress and agriculture is traditionally intensive in Japan.

It is well known that ionizing radiation are also effective to kill pathogenic bacteria in sludge [1]. Recently, an electron accelerator has been evaluated as a practical and economical radiation source. We have been studying on electron-beam disinfection of sewage sludge cake and efficient composting of the irradiated sludge to develop a new treatment system of sewage sludge.

1.2 Outline of the system

Fig. 1 shows the sludge treatment system combined with electron-beam disinfection and composting. At first, sludge cake is disinfected by electron-beam. The penetration range of electron beam is small compared with gamma ray. So, when we irradiate sludge continuously, it is necessary to spread sludge cake into thin layer on a conveyor through a flat nozzle. Then the irradiated sludge is mixed with seed compost to supply seed bacteria to start composting and also mixed with bulking agent to make the sludge aerobic because aerobic bacteria can quickly decompose organic materials without producing bad odor. Composting temperature is controlled at the optimal range, from 40 to 50 °C. After 2 or 3days, evolution of CO_2 ceases and compost is obtained.

Fig. 2 shows difference between conventional composting method and combined method with electron-beam disinfection and composting. In conventional composting, sludge is disinfected by heat which is generated during composting. As the composting is induced by microorganisms, such high temperature also give damage to composting bacteria and it takes long time to finish composting. In our system, disinfection is separated from composting. At first, we kill pathogenic bacteria by electron beam irradiation, then make it into compost under the optimum conditions.

2. Electron-beam disinfection and composting

As shown in Fig. 1, the system consists of two main steps. One is electron-beam disinfection and the other is composting.

2.1 Electron-beam disinfection

Fig. 3 shows an apparatus for electron-beam disinfection. Sludge cake is spread on a stainless steel conveyor through a flat nozzle and disinfected by electron-beam which comes from upside of the apparatus. The width of the nozzle is 20 cm and sludge thickness is variable from 1 to 10 mm. The maximum feed rate of sludge is 300 kg/hr. The electron accelerator used for irradiation is the Cockcroft-Walton type (Nisshin Highvoltage Co.). The maximum accelerating voltage and beam current are 2 MV and 30 mA, respectively.

Fig. 4 shows surviving fraction of total bacteria in sludge irradiated by 1 and 2 MV electron-beam at various sludge

thickness [2]. To kill bacteria effectively, sludge thickness must be less than 6 or 7 mm for accelerating voltage of 2 MV and 3 mm for 1MV.

Fig. 5 shows surviving curves of total bacteria and coliforms at various beam energies and dose rates for 1 mm thickness of sludge [3]. No effects of beam energy or dose rate can be seen on the surviving curves of total bacteria or coliforms. Results for gamma ray are also plotted in this figure by star marks and it shows that the effects of gamma ray are almost the same as those of electron beam irradiation.

In electron-beam disinfection, the time necessary for disinfection is very short. Coliforms become undetectable within 0.2 seconds at dose rate of 10 kGy/sec. But it takes more than 20 days by heat disinfection of conventional composting and it was reported that conventional composts contain 10^2 to 10^4 count/g of total coliforms [4]. But the compost made from irradiated sludge does not contain coliforms at all.

2.2 Composting

Fig. 6 shows the experimental apparatus for composting of irradiated sludge. This apparatus was used to examine optimum conditions for fermentation. The volume of fermentor is 50 ml, and 10 g of sludge cake is used. The range of moisture content of the sludge is from 75 to 80 %. According to the experimental results, the composting rate is seriously affected by temperature and the optimum range is from 40 to 50 °C [6]. The optimum pH is found to be ranged from 7 to 8. It is necessary to supply oxygen into sludge particles for aerobic fermentation and particle size of sludge must be less than 5 mm [7]. But, too small particle made it difficult to send air because of large pressure drop. It is necessary to add seed bacteria at first because irradiated sludge does not start composting. But, produced compost is effective as seed.

Fig. 7 shows rate of fermentation in batch composting of irradiated sludge under optimum conditions. A rate in conventional composting is also shown in this figure. The rate of fermentation is expressed by CO_2 evolution. The time to give maximum rate of fermentation is only 6 hours and the peak value is ten times larger in irradiated sludge composting. CO_2 evolution almost ceases within 2 or 3 days in irradiated sludge composting. But, it takes more than 10 days in conventional composting. According to the recommendation of the Association for Utilization of Sewage Sludge in Japan, Composting must be performed during 10 to 14 days [5].

3. Pilot plant test

A pilot plant was constructed to study on scale-up effects of composting process of irradiated sludge. The amount of sludge treatment is about 500 kg/batch. The plant consists of mixer to prepare mixture of irradiated sludge and seed compost, granu-

lating machine to make pellets, fermentor with three screw-type mixing blades, and conveyor system to transport raw materials and products. The electron accelerator of TRCRE, JAERI is used for sludge irradiation.

Continuous disinfection of sludge was possible by combination of an electron accelerator and the machine to make thin layer of sludge. Efficient composting could be realized also by a pilot plant, by controlling fermentation temperature in the optimum range, by controlling aeration rate and by frequent mixing in the fermentor. The compost made from irradiated sludge does not contain coliforms at all.

4. Plant design

Fig. 8 shows a flow sheet of a plant with treatment capacity of 50 ton/day. Dewatered sludge is spread to a rolling drum through a flat nozzle and disinfected by electron-beam irradiation with a dose of 5 kGy. The irradiated sludge is mixed with dried sludge and produced compost to control initial moisture content around 50 % and is made into small pellets. Composting of the mixed sludge is made at 50 °C for 3 days. The accelerating voltage and capacity of the accelerator are 1.5 MV and 15 kw, respectively.

Bird's view of the plant is shown in Fig. 9. The site of the facility is about 40 m X 50 m. The costs for construction and operation in this system are lower than those in conventional one when the amount of sludge treatment exceeds 25-50 ton/day.

Acknowledgment

This work is indebted to the strong supports by Dr. S. Machi, Director General of TRCRE, JAERI. We wish to express our thanks to Mr. Nishimura and members of Irradiation Service Division of TRCRE, for their useful discussion and supports. We also wish to express our thanks to Mr. H. Iwabu and K. Shinabe, staffs of KUBOTA Ltd., for their kind support to the pilot plant test. Especially, we must express our special thanks to Dr. M. Sago, Emeritus Professor of Tokyo Metropolitan University, Dr. M. Kashiwaya, Professor of Science University of tokyo, and other members of the committee, which was organized by JAERI to evaluate feasibility of the technology of application of electron-beam for sewage treatment, for their useful discussion and advice.

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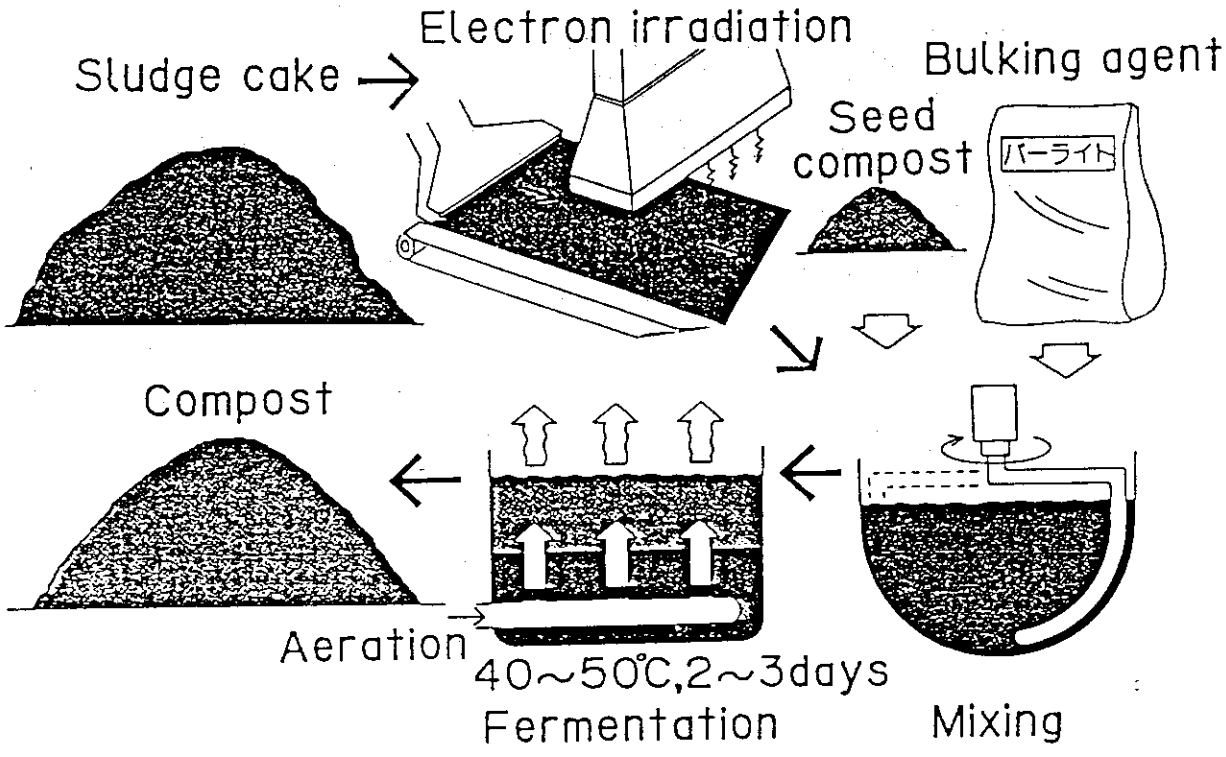


Fig. 1 Sludge treatment system by electron-beam irradiation and composting

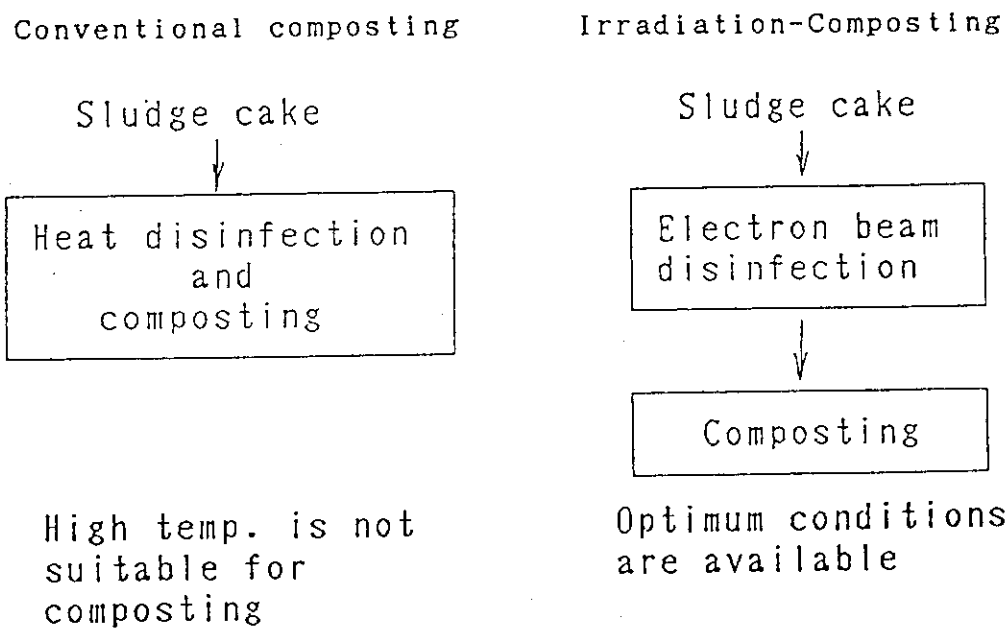


Fig. 2 Difference between conventional composting and Irradiation-composting process

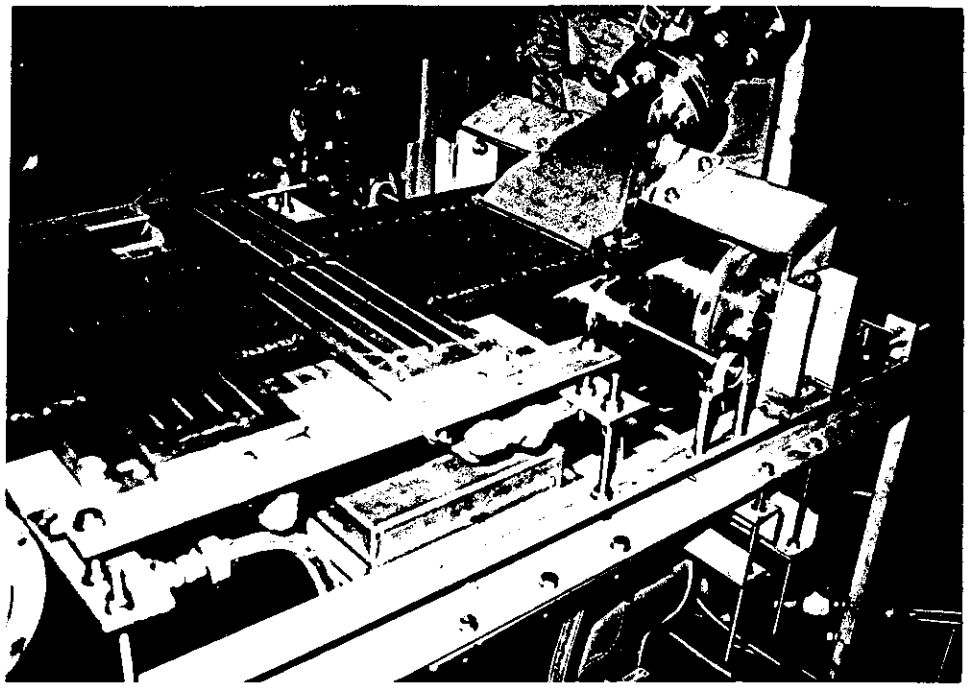


Fig. 3 Apparatus for electron-beam disinfection

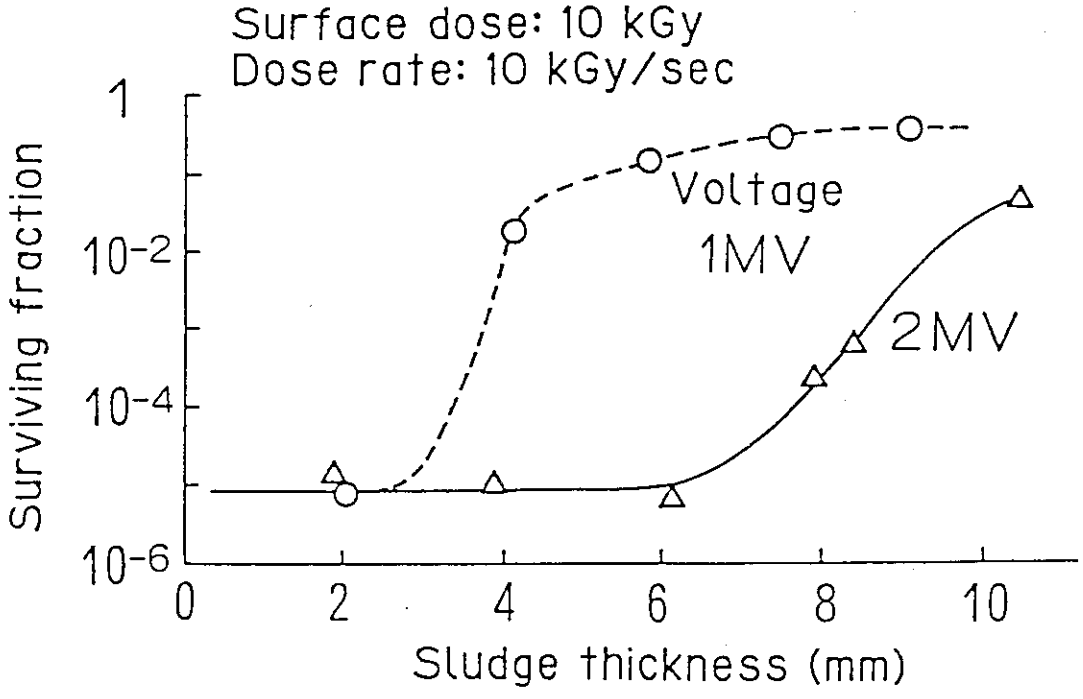
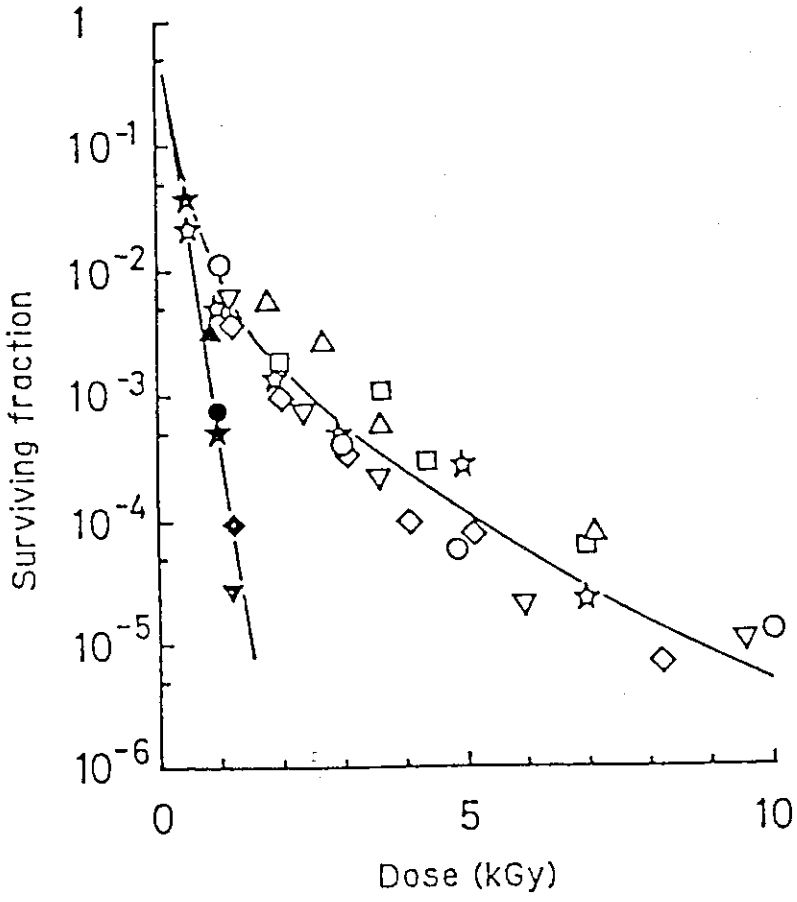


Fig. 4 Surviving fraction vs. sludge thickness



Energy and dose rate (MeV, MGy/h): 2, 0.02 (○), 2, 18 (△), 2, 36 (◇), 2, 65 (□), 0.5, 25 (▽). Sludge thickness: 1 mm. The result of γ-ray irradiation is shown by ☆. Open symbols represent total bacteria, and solid ones total coliforms.

Fig. 5 Surviving curves for total bacteria and coliforms

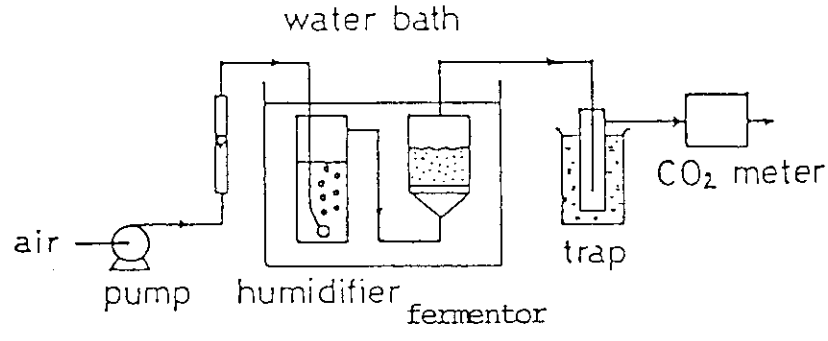


Fig. 6 Experimental apparatus for composting of irradiated sludge

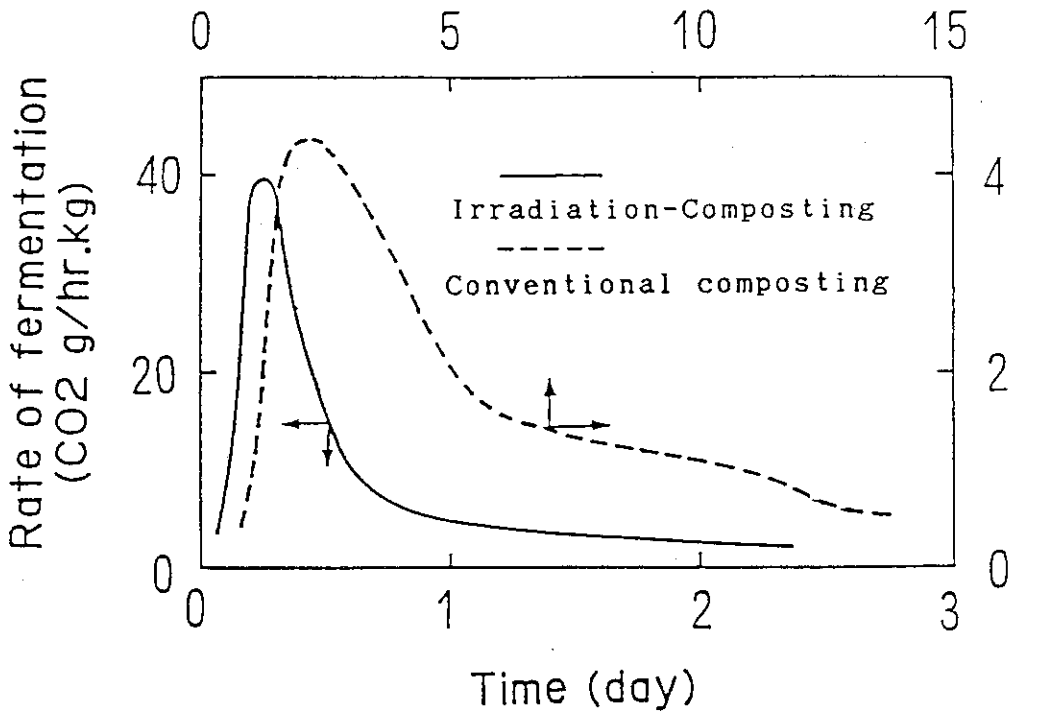


Fig. 7 Rate of fermentation under optimum conditions

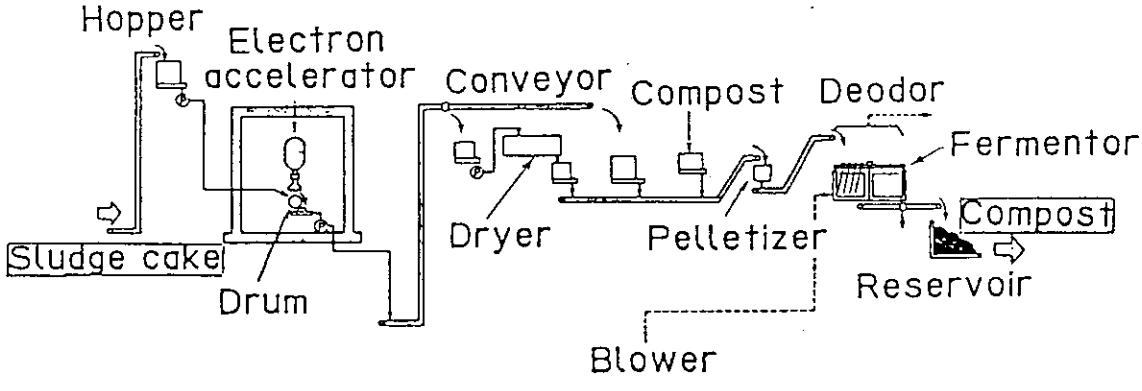


Fig. 8 Flow sheet of a commercial-size plant

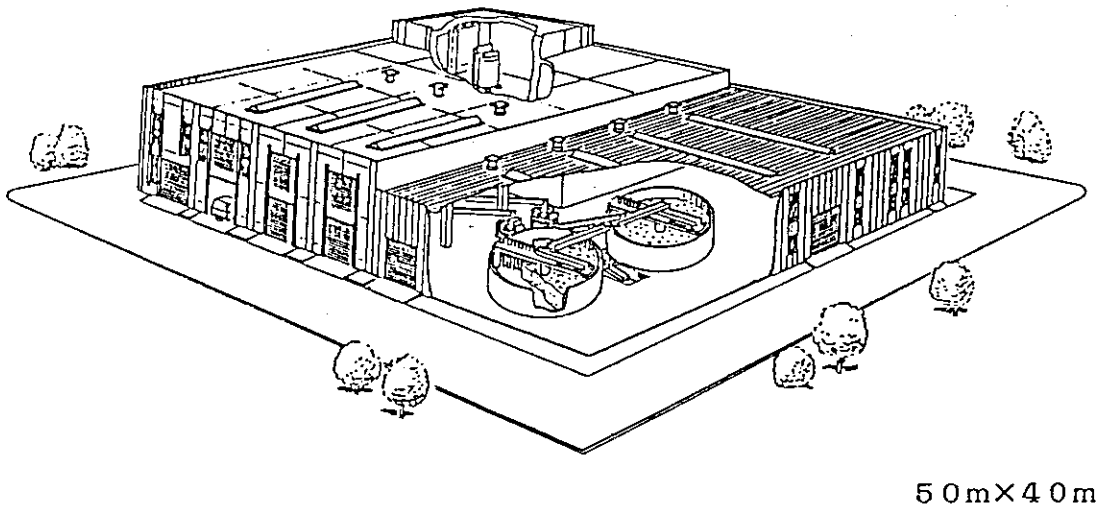


Fig. 9 Bird's view of a commercial-size plant

3. Radiation inactivation of pathogens and parasites

3.1 Effect of radiation on pathogenic bacteria

[Abstract]

3.1.1 A microbiological study on sewage sludge treatment

Results of study on isolation and identification of salmonellae from sewage sludge cake in Japan were shown. Radiation sensitivities of the isolated strains in phosphate buffer and in sewage sludge were also conducted. Furthermore, irradiation dose to decrease salmonellae below 6 log cycles was also described.

3.1.2 Effect of gamma radiation on salmonellae in sewage sludge

The experimental results on the effect of gamma radiation on salmonellae in phosphate buffer were shown. Twenty serotypes of salmonellae were studied for radiosensitivity. The results showed that strain S.I.8,20:y:- is the most resistant to radiation while S. poona is the most sensitive. D_{10} values of strain S.I.8,20:y:- and S. poona were 0.21 and 0.08 kGy, respectively.

3.1.3 Radiation disinfection of salmonellae and other microorganisms in sludge

Radiation disinfection of salmonellae and other microorganisms in sludge was conducted. It was found that the range of radiosensitivities (D_{10} Values) of salmonellae in phosphate buffer were from 0.06 to 0.25 kGy. In addition, total coliforms and fecal coliforms in sludge sample except in dried sludge, E. coli and S. aureus were sufficiently inactivated at 6 kGy. It was also found that irradiation at 20 kGy was enough to inactivate total bacteria and C. perfringens in sludge from Thai Pure Drinks Ltd.

3.1.4 Effect of irradiation on salmonellae in sludge

The Most Probable Number(MPN) of salmonellae contaminated in various kinds of sludge was $<2\text{-}1.4\times10^4$ per 100 g of sample. D_{10} values of 14 serotypes of salmonellae in phosphate buffer were ranged from 0.11-0.21 kGy. Among 47 serotypes (including our previous study), S.paratyphi B. var java was the most resistance to radiation in phosphate buffer. Thus this serotype was selected to study for radiation sensitivity in sludge and D_{10} values of this strain in liquid sludge, sludge cake and dried sludge were obtained to be 0.35, 0.40 and 0.45 kGy, respectively. Hence, necessary dose for sterilization of salmonellae in liquid sludge, sludge cake and dried sludge were 2, 3 and 4 kGy, respectively.

3.1.1 A microbiological study on sewage sludge treatment

N. Sermkiattipong, H. Ito and S. Hashimoto

Introduction

The amount of sewage sludge increases rapidly every year due to the expansion of population in the cities and increase of industrial activities. Utilization of sludge as a fertilizer or soil conditioner and for land filling are common method of resource conservation practiced worldwide. However, most of sludges contain pathogenic viruses, bacteria, protozoa and parasites. In Thailand, salmonellae are very hazardous pathogenic bacteria and the contamination of salmonellae in sludges from hospitals, food industries and Huay-Kwang community were 80.75, 51.85 and 68.00 percents, respectively. Therefore in order to cut the possible infection chain sewage sludge-plants-animals-man, the disinfection of sewage sludge is demanded. A very effective method for rendering sludge free of pathogens is irradiation with ionizing radiation.

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 as shown in Fig.1

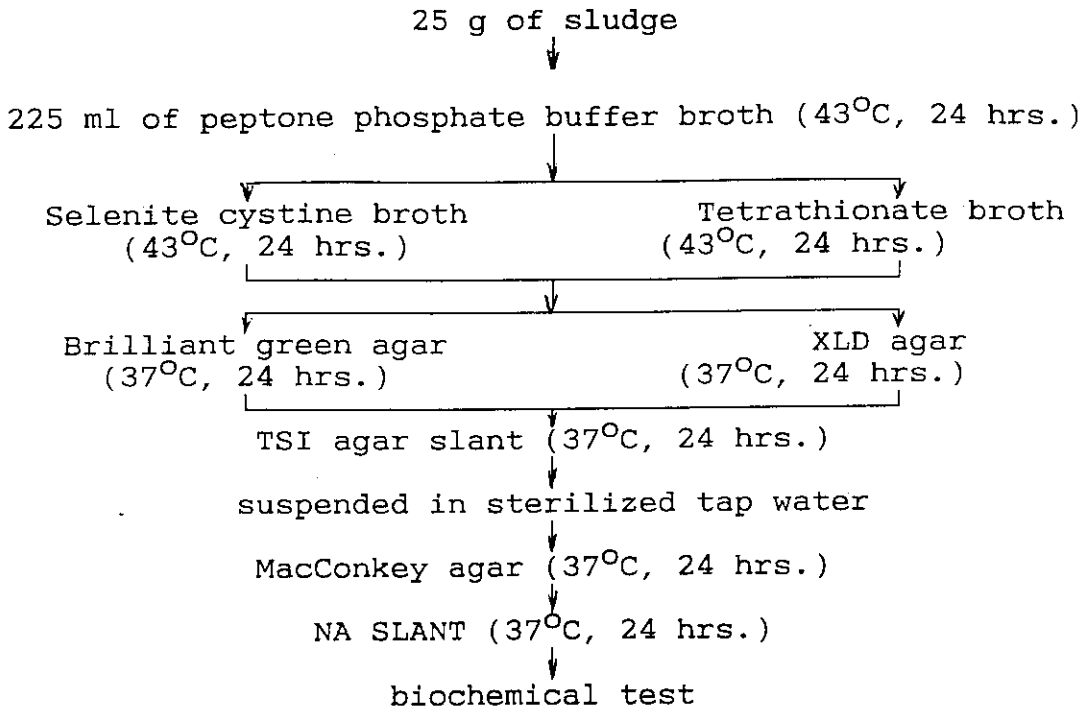


Fig.1 Isolation method of salmonellae in sludge cakes

1.3 Identification of salmonellae

Various biochemical characteristics of isolated strains were examined according to "Bergey's Manual of Determinative Bacteriology". 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.

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 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 poly-ethylene 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.

2. Results and Discussion

2.1 Isolation and identification of salmonellae from sewage sludges

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 1. 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 1.

Table1 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

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 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 2. 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

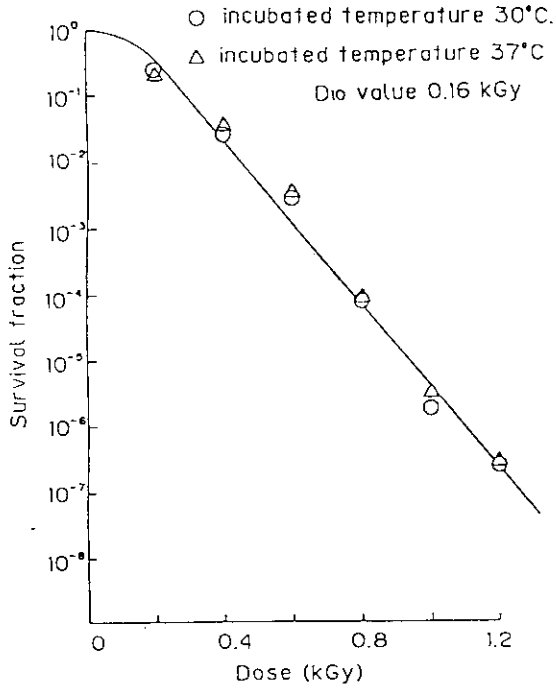


Fig.2 Survival curve of S. typhimurium irradiated in phosphate buffer

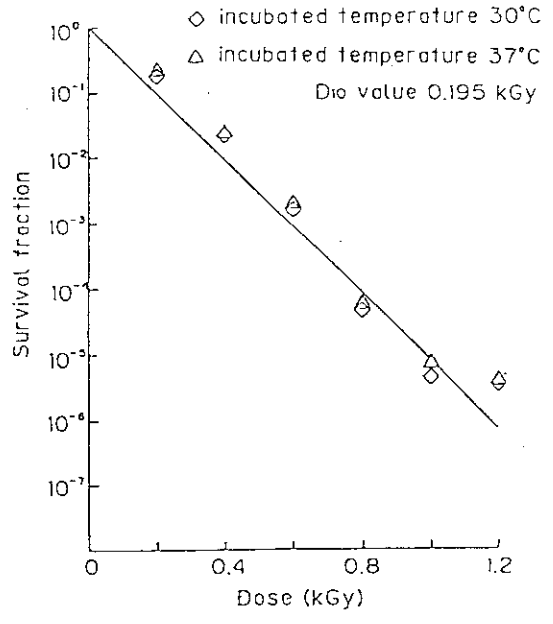


Fig.3 Survival curve of S. oranienburg irradiated in phosphate buffer

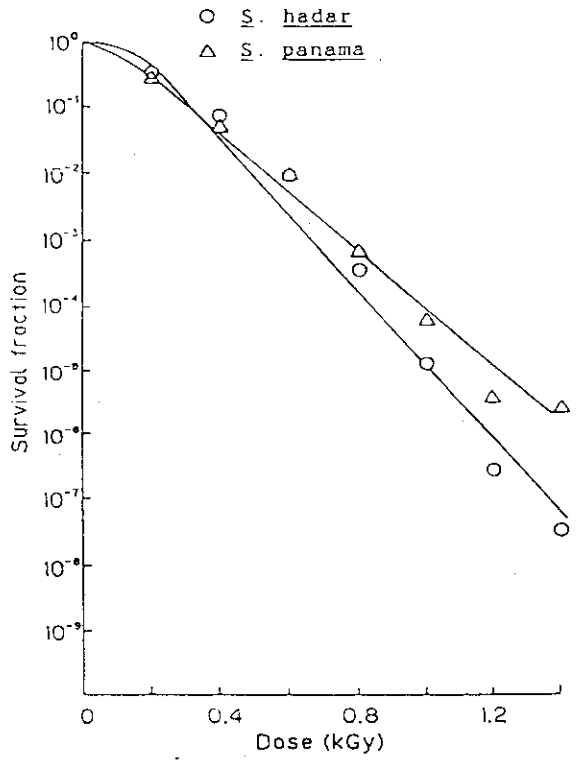


Fig.4 Survival curves of S. hadar and S. panama irradiated in phosphate buffer

Table 2 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 3 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.

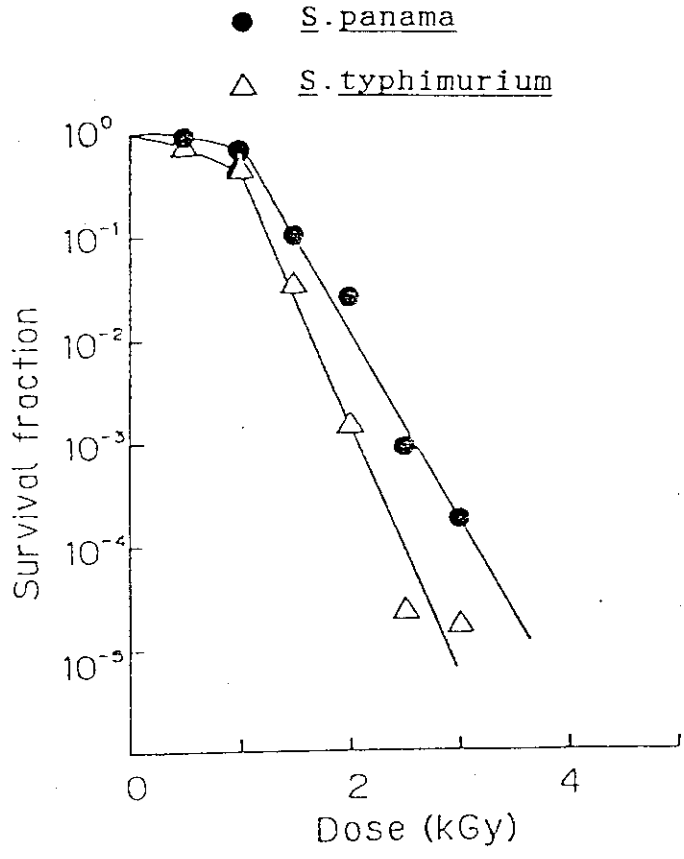


Fig.5 Survival curves of S. panama and S. typhimurium irradiated in sewage sludge

Table 3 D_{10} values of salmonellae in sewage sludge

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

3. Conclusions

According to the results of this study, 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 ranged from 0.41 to 0.55 kGy.

3.1.2 Effect of gamma radiation on salmonellae in sewage sludge

N. Sermkiattipong and S. Pongpat

1. Introduction

In view of its increasing quantity, the use of sewage sludge as fertilizer, soil conditioner and animal feed becomes more and more important. Numerous authors have demonstrated that the number of pathogens and ova of parasites present in sewage sludge is not sufficiently reduced by the mechanical-biological process in a sewage plant. Among the bacteria, salmonellae are of major importance in this respect. In Thailand, the contamination of salmonellae in sludges from hospitals, food industries and the National Housing Authority residential area at Huay-Kwang were 80.7, 51.9 and 68.0 percents, respectively. Hess and Breer (1975) also reported that three kinds of sludges in Switzerland contained salmonellae in the range from 78.1 to 91.8 percents and salmonellae might survive up to 72 weeks. Thus sewage sludge should be disinfected before it can be applied to beneficial purposes. Many investigations have shown that the disinfection of sewage sludge by irradiation is a very effective and preferable method.

2. Experimental Procedures

2.1 Bacterial strains

50 serotypes of salmonellae isolated from hospitals, food factories and a National Housing Authority residential area at Huay Kwang were studied for radiation sensitivities. Radiation sensitivity of each Salmonella serotype was conducted in triplicate.

2.2 Preparation of cell suspension in phosphate buffer

Pure culture of each serotype was grown for 16 hr in 100 ml of nutrient broth under aeration at 35°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. Cell concentration in phosphate buffer was adjusted to be 10^8 cells/ml.

2.3 Irradiation

Bacterial suspension in phosphate buffer solution was irradiated at the dosages of 0, 0.2, 0.4, 0.6 and 0.8 kGy using Co-60 source. The dose rate distribution used in this experiment was 10.9 Gy/min.

2.4 Bacterial count

Total count of salmonellae was conducted using 0.067 M phosphate buffer solution for serial dilutions and subsequently plated on the surface of Difco nutrient agar. The plates were incubated at 37°C for 1 day.

2.5 Calculation of D_{10}value

$$y = a + bx$$

$$b = \frac{\sum xy - \sum x \cdot \sum y/n}{\sum x^2 - (\sum x)^2/n}$$

$$D_{10} = \frac{1}{b}$$

when b = slope, a = intercept
x = irradiation dose (kGy)
y = number of survival bacteria in logarithm

Table 1 D_{10} values of salmonellae in phosphate buffer

Serotypes	D_{10} value (kGy)
S. poona	0.0818
S. virchow	0.1034
S. liverpool	0.1123
S. emex	0.1205
S. langensalza	0.1219
S. blockley	0.1223
S. 16:-:-	0.1263
S. muenchen	0.1275
S. halmstad	0.1288
S. senftenberg	0.1332
S. ardwick	0.1340
S. muenster	0.1357
S. london	0.1397
S. bovismorbificans	0.1420
S. rissen	0.1476
S. regent	0.1519
S. tennessee	0.1557
S. anatum	0.1595
S. agona	0.1665
S. I.8, 20:y:-	0.2089

3. Results and Discussion

Table 1 shows D_{10} values of salmonellae in phosphate buffer. D_{10} values calculated from the slope of lines ranged from 0.0818 to 0.2089. Among 20 serotypes of salmonellae, S.I.8, 20:y:- is the most resistant to radiation in comparison with other serotypes. On the contrary, S. poona is the most sensitive to radiation. In this experiment, radiation sensitivities of the remained 30 Salmonella serotypes are being conducted. Further study on radiation sensitivities of salmonellae in sewage sludge will be performed.

3.1.3 Radiation disinfection of salmonellae and other microorganisms in sludge

N. Sermkiattipong and S. Pongpat

1. Introduction

The increasing quantity of sewage sludges from wastewater treatment plants is one of the major problems for many municipalities. sewage sludges contain, besides organic and inorganic chemicals, pathogenic viruses, bacteria, protozoa and parasite ova. these can prove to be harmful to soil, crops, grazing animals and public health. In a survey, Dudley and his colleagues were able to recover Pseudomonas, Staphylococcus, Mycobacterium, Clostridium, Klebsiella, Salmonella and Shigella species from anaerobic digested sludge. Hess and Breer found salmonellae in more than 90% of the sludges. Greer observed densities of up to 10^7 Clostridium perfringens organisms per gram in sludge. thus sewage sludge should be disinfected before it can be applied to beneficial purposes. One of the most useful methods for disinfection of sludge is ionizing radiation.

The research described in this paper deals with radiation sensitivities of salmonellae and inactivation of other microorganisms in sludge.

2. Experimental procedures

2.1 Sludge

Sludges using for determination of various microorganisms were obtained from Central Chest Hospital, a food factory and Huay Kwang Sewage Treatment Plant. Moisture content of the sludge used in this study was ranged from 9.69 to 97.72 and pH of the sludge was ranged from 7.0 to 7.5

2.2 Irradiation

A Co-60 irradiator No.2 at Office of Atomic Energy for Peace was used for this study. The dose rate distribution used for determination of total bacterial count and Clostridium perfringens was 42.37 Gy/min. For radiation sensitivities of salmonellae, the dose rate distribution was 9.54 Gy/min. And the dose rate distribution used for determination of other microorganisms was 61.20 Gy/min.

2.3 Enumeration of bacteria

2.3.1 Radiation sensitivities of salmonellae

Radiation sensitivities of salmonellae in phosphate buffer was the same method as mentioned in the previous paper.

2.3.2 Enumeration of total bacteria and indicator organisms

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

2.3.3 Enumeration of Clostridium perfringens

Enumeration of C. perfringens organisms were conducted mainly by the method described in SEAMIC Publication No.12.

2.3.4 Enumeration of Staphylococcus aureus

Enumeration of S. aureus were determined by modified method described in Difco Manual 10^{th} edition and by thatcher and clark.

3. Results and Discussion

3.1 Radiation sensitivities of salmonellae in phosphate buffer

As shown in Table 1, D_{10} values of salmonellae in phosphate buffer calculated from the slope of lines ranged from 0.0577 to 0.2512. Among 13 serotypes of salmonellae, S paratyphi B. var java is the most resistant to radiation in comparison with other serotypes. On the contrary, S brunei is the most sensitive to radiation.

3.2 Inactivation of total bacteria by gamma irradiation

As shown in Table 2, the initial loads of total bacterial count of sludge samples were 3.36×10^{5}-9.35×10^{8} cfu/g. After irradiation, total bacterial count decreased to be 0-5×10^{3} cfu/g at dosage of 20 kgy. although irradiation at high dose, total bacterial count of dried sludge from Central Chest Hospital is rather high compared with the others. This may be attributable that this sample is contaminated by radiation resistant bacteria and very low water content.

3.3 Inactivation of indicator organisms

Table 3 shows inactivation of total coliforms by gamma irradiation. Total coliforms are determined to be 7.0×10^{3} - 2.3×10^{9} MPN/100 g before irradiation. After irradiation at 6 kGy total coliforms decrease to be <20-3.5×10^{4} MPN/100 g. Irradiation dose of 6 kGy is not enough to inactivate total coliforms in dried sludge. Total coliforms in most of sludge samples are inactivated by the dose ranged from 1.2 to 6.0 kGy. However, the dosage for inactivation of coliforms depends on tpe of sludge and source of sample.

Inactivation of fecal coliforms by gamma irradiation is shown in Table 4. The initial loads of fecal coliforms in sludge sample are 4.6×10^{3} - 9.2×10^{8} MPN/100 g. After irradiation at 6 kGy, fecal coliforms decrease to be <20-5.4×10^{3} MPN/100 g. Fecal coliforms in sludge samples are inactivated by the dose ranged from 1.2 to 6.0 kGy except in dried sludge.

Table 5 shows inactivation of Escherichia coli by gamma irradiation. The amount of E. coli is determined to be 1.4×10^{3} - 1.1×10^{8} MPN/100 g in non-irradiated sludge. After irradiation at 6 kGy, the number of E. coli organisms decrease to be <20 MPN/100

g. The dosage of 6 kGy is enough to inactivate E. coli in all sludge samples.

3.4 Inactivation of C. perfringens by gamma irradiation

As shown in Table 6, the number of C. perfringens organisms contaminated in sludge samples is 2.0×10^{4}-4.6×10^{8} MPN/100 g. Irradiation at 20 kGy, the number of C. perfringens organisms decrease to be <30-4.6×10^{5} MPN/100 g. The number of these organisms are rather high in sample from Huay Kwang Sewage Treatment Plant in spite of high dose irradiation. However, the initial load of these organisms is effective factor concerned.

3.5 Inactivation of S. aureus by gamma irradiation

As shown in Table 7, Raw sludge and dried sludge from Central Chest Hospital contain S. aureus organisms 2.1×10^{3} and 1.1×10^{4} MPN/100 g, respectively. After irradiation, S. aureus organisms in raw sludge are almost inactivated at dosage of 2.4 kGy. Irradiation dose of 6.0 kGy is enough to inactivate S. aureus organisms in dried sludge. However, the number of S. aureus organisms in other sources are almost below detectable level in spite of non-irradiated sludge.

In this study, sludges from Central Chest Hospital were the most contaminated with pathogens, indicator organisms and total bacteria in comparison with sludges from other sources. On the contrary, sludges from the food factory were the least contaminated with the bacteria mentioned above.

Table 1 D_{10} values of salmonellae in phosphate buffer

Serotype	D_{10} value (kGy)
S. brunei	0.0577
S. westampton	0.0826
S. hvittingfoss	0.0867
S. meleagridis	0.0872
S. gera	0.0879
S. infantis	0.0992
S. worthington	0.0998
S. derby	0.1081
S. krefeld	0.1121
S. ohio	0.1328
S. binza	0.1359
S. panama	0.2071
S. paratyphi B. var java	0.2512

Table 2 Inactivation of total bacteria by gamma irradiation

Sample source	Type of sludge	Total bacterial count (cfu/g) Dose (kGy) 0	5	10	15	20	Moisture content (%)	pH
Central Chest Hospital	Raw sludge	5.60×10^{6}	1.34×10^{3}	8.80×10^{2}	13	4	97.27	7.0-7.5
	Dried sludge	9.35×10^{8}	2.06×10^{7}	1.12×10^{6}	1.42×10^{5}	5.0×10^{3}	9.69	-
Food factory	Anaerobic digested sludge	3.36×10^{5}	3.48×10^{2}	45	1	0	97.72	7.0-7.5
	Sludge cake	4.45×10^{5}	9.20×10^{2}	28	6	0	83.07	7.0-7.5
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	2.20×10^{6}	2.76×10^{5}	1.74×10^{4}	6.0×10^{2}	2	87.61	7.5
	Sludge cake	7.60×10^{6}	5.50×10^{4}	4.40×10^{3}	2.38×10^{2}	40	84.66	7.5

Table 3 Inactivation of total coliforms by gamma irradiation

Sample source	Type of sludge	Total coliforms (MPN/100 g)					
		Dose (kGy)					
		0	1.2	2.4	3.6	4.8	6.0
Central Chest Hospital	Raw sludge	1.3×10^{7}	2.3×10^{4}	20	<20	<20	<20
	Dried sludge	2.3×10^{9}	7.9×10^{6}	1.7×10^{6}	5.4×10^{5}	1.6×10^{5}	3.5×10^{4}
Food factory	Anaerobic digested sludge	7.0×10^{3}	<20	<20	<20	<20	<20
	Sludge cake	2.2×10^{5}	80	50	<20	<20	<20
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	3.5×10^{5}	7.0×10^{3}	2.1×10^{2}	1.2×10^{2}	20	<20
	Sludge cake	1.6×10^{7}	9.2×10^{3}	3.3×10^{2}	2.7×10^{2}	80	<20

Table 4 Inactivation of fecal coliforms by gamma irradiation

Sample source	Type of sludge	Fecal coliforms (MPN/100 g)					
		Dose (kGy)					
		0	1.2	2.4	3.6	4.8	6.0
Central Chest Hospital	Raw sludge	1.3×10^{7}	2.3×10^{4}	20	<20	<20	<20
	Dried sludge	9.2×10^{8}	7.9×10^{6}	1.1×10^{6}	5.4×10^{5}	5.4×10^{4}	5.4×10^{3}
Food factory	Anaerobic digested sludge	4.6×10^{3}	<20	<20	<20	<20	<20
	Sludge cake	2.2×10^{5}	<20	<20	<20	<20	<20
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	1.7×10^{5}	7.0×10^{3}	1.1×10^{2}	90	<20	<20
	Sludge cake	1.8×10^{6}	3.5×10^{3}	1.4×10^{2}	1.2×10^{2}	20	<20

Table 5 Inactivation of Escherichia coli by gamma irradiation

Sample source	Type of sludge	E. coli (MPN/100 g)					
		Dose (kGy)					
		0	1.2	2.4	3.6	4.8	6.0
Central Chest Hospital	Raw sludge	3.5×10^{6}	2.3×10^{4}	20	<20	<20	<20
	Dried sludge	1.1×10^{8}	1.7×10^{6}	1.6×10^{5}	1.3×10^{4}	50	<20
Food factory	Anaerobic digested sludge	1.4×10^{3}	<20	<20	<20	<20	<20
	Sludge cake	1.7×10^{5}	<20	<20	<20	<20	<20
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	7.0×10^{4}	2.3×10^{2}	<20	<20	<20	<20
	Sludge cake	2.6×10^{5}	2.3×10^{2}	20	<20	<20	<20

Table 6 Inactivation of Clostridium perfringens by gamma irradiation

Sample source	Type of sludge	Clostridium. perfringens (MPN/100 g)				
		Dose (kGy)				
		0	5	10	15	20
Central Chest Hospital	Raw sludge	2.4×10^{7}	4.4×10^{5}	6.0×10^{4}	4.6×10^{4}	3.0×10^{3}
	Dried sludge	9.3×10^{6}	2.8×10^{4}	3.9×10^{2}	2.3×10^{2}	<30
Food factory	Anaerobic digested sludge	4.6×10^{4}	2.4×10^{4}	2.4×10^{3}	40	<30
	Sludge cake	2.0×10^{4}	9.6×10^{3}	6.0×10^{2}	3.9×10^{2}	<30
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	4.6×10^{8}	2.4×10^{8}	4.6×10^{7}	2.4×10^{6}	4.6×10^{5}
	Sludge cake	4.6×10^{8}	1.1×10^{8}	4.6×10^{7}	9.3×10^{6}	4.6×10^{5}

Table 7 Inactivation of Staphylococcus aureus by gamma irradiation

Sample source	Type of sludge	Staphylococcus aureus (MPN/100 g)					
		Dose (kGy)					
		0	1.2	2.4	3.6	4.8	6.0
Central Chest Hospital	Raw sludge	2.1×10^3	40	<30	<30	<30	<30
	Dried sludge	1.1×10^4	2.0×10^3	4.3×10^2	1.4×10^2	90	<30
Food factory	Anaerobic digested sludge	<30	<30	<30	<30	<30	<30
	Sludge cake	<30	<30	<30	<30	<30	<30
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	<30	<30	<30	<30	<30	<30
	Sludge cake	<30	<30	<30	<30	<30	<30

3.1.4 Effect of irradiation on salmonellae in sludge

N. Sermkiattipong

1. Introduction

Parallel with the increasing quantity of sludge, contamination of the sewage sludge with potential bacterial pathogens has increased. The salmonellae are very hazardous pathogenic bacteria which often contaminate in sludge and can survive up to 72 weeks. Therefore, in order to cut the possible infection chain, the disinfection of sewage sludge is demanded. The scope of this project is to study enumeration of salmonellae contaminated in various kinds of sludge and radiation sensitivities of salmonellae isolated from sludge. Radiation sensitivities of salmonellae 33 serotypes in phosphate buffer was reported previously.

2. Experimental procedures

2.1 Sludge

Sludge samples collected from Huay Kwang Sewage Treatment Plant, Vajira Hospital, Central Chest Hospital, Food Factory (A) and Food Factory (B) were used for enumeration of salmonellae. Moreover, sludges using for radiation sensitivities of salmonellae were obtained from Thai Pure Drinks LTD and Central Chest Hospital. Moisture content of the sludges used in this study was ranged from 11.42 % to 99.62 % and pH of the sludge was ranged from 6.5 to 8.0.

2.2 Irradiation

A Co-60 irradiator No.2 at Office of Atomic Energy for Peace was used for study radiation sensitivities of salmonellae in phosphate buffer and the dose rate distribution was 9.54 Gy/min. In case of radiation sensitivities of salmonellae in sludge, a Gamma cell 220 was used as an irradiator and the dose rate distribution was 4.16 Gy/sec.

2.3 Enumeration of salmonellae

Enumeration of salmonellae were determined by using a quantitative MPN procedure as described by Standard Methods for Examination of Water and Wastewater and FAO/WHO method.

2.4 Radiation sensitivities of salmonellae

2.4.1 Radiation sensitivities of salmonellae in phosphate buffer

Materials and methods were the same as mentioned in the minutes of the third steering committee meeting.

2.4.2 Radiation sensitivities of salmonellae in sludge

Two serotypes of salmonellae were selected as the representative for study radiation sensitivities of salmonellae in sludge i.e. Salmonella paratyphi B. var java (most resistance to radiation in phosphate buffer) and S. brunei (most sensitive to radiation in phosphate buffer). Pure culture of each strain was grown for 16 hr in 100 ml of nutrient broth under aeration at 30°C. Cells at the stationary phase were harvested by centrifugation and mixed well in 30 ml of 1% peptone aqueous solution. The suspension was poured into the flask which contained 50 g or 80 g of sterilized sludge and mixed well. The mixed sludge were dried until the moisture content became the same as before inoculation. Five grams of each sludge was put into a sterilized test tube and irradiated by gamma ray at the dosages of 0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 kGy. After irradiation, each test tube was appropriately diluted in 0.067 M phosphate buffer and subsequently plated 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.

3. Results and Discussion

3.1 Enumeration of salmonellae

Table 1 shows the amount of salmonellae contaminated in sludge sample from different sources. The Most Probable Number (MPN) of salmonellae is <2-1.4×10^4 per 100 g of sludge. The most contamination of salmonellae is dried sludge from Central Chest Hospital due to the source of sample and very low moisture content. However, the least contamination of salmonellae is sludge from Thai Pure Drinks LTD.

3.2 Radiation sensitivities of salmonellae

3.2.1 Radiation sensitivities of salmonellae in phosphate buffer

As shown in Table 2, D_{10} values of salmonellae in phosphate buffer are 0.1049 to 0.2115 kGy. Among 14 serotypes of salmonellae, S. isangi is the most resistance to radiation in comparison with other serotypes. On the contrary, S.weltevreden is the most sensitive to radiation.

3.2.2 Radiation sensitivities of salmonellae in sludge

Survival curves of S.brunei irradiated in two kinds of sludge are shown in Fig.1. As shown in Table 3, D_{10} values of this serotype were 0.14 and 0.18 kGy. The curve showed sigmoid shape only in sludge cake and induction dose was 0.14 kGy.

Fig.2 shows survival curves of S.paratyphi B. var java irradiated in different kinds of sludge. D_{10} values calculated from the slope of curves are 0.35-0.45 kGy as shown in Table 3. Induction doses of this serotype in sludge cake and dried sludge were 0.75 and 0.35 kGy, respectively.

From these results, radiation resistance of the salmonellae were higher in sludge than in phosphate buffer. Moreover, in low moisture content of sludge, these bacteria became more resistance to radiation. Lastly, necessary dose for sterilization of salmonellae in liquid sludge (anaerobic digested sludge), sludge cake and dried sludge were 2, 3 and 4 kGy, respectively.

Table 1 Enumeration of salmonellae in sludges from different sources

Sample source	Type of sludge	Salmonellae (MPN/100 g)	Moisture content (%)	pH
Huay Kwang Sewage Treatment Plant	Anaerobic digested sludge	4	88.96	7.5-8.0
	Sludge cake	13	84.85	7.5-8.0
Vajira Hospital	Raw sludge	2	96.49	7.0
	Sludge cake	2×10^2	83.50	6.5
Food Factory (A)	Raw sludge	2	99.0	7.0-7.5
	Sludge cake	36	82.63	7.5-8.0
Central Chest Hospital	Raw sludge	33	99.62	7.0-7.5
	Dried sludge	1.4×10^4	15.16	-
Food Factory (B)	Anaerobic digested sludge	<2	98.98	7.0-7.5
	Sludge cake	<2	75.77	7.0-7.5

Table 2 D_{10} values of salmonellae in phosphate buffer

Serotypes	D_{10}value (kGy)
S. weltevreden	0.1049
S. stanley	0.1203
S. javiana	0.1264
S. lexington	0.1330
S. havana	0.1346
S. gaminara	0.1393
S. montevideo	0.1435
S. typhimurium	0.1722
S. 42:-:-	0.1759
S. hadar	0.1827
S. saintpaul	0.1849
S. newport	0.1941
S. heidelberg	0.2064
S. isangi	0.2115

Table 3 D_{10} values of salmonellae in sludge

Serotype	Type of sludge	D_{10} value (kGy)	Moisture content(%)
S.brunei	Liquid sludge (Anaerobic digested sludge)	0.14	98.81
	Sludge cake	0.18	85.32
S.paratyphi B. var java	Liquid sludge (Anaerobic digested sludge)	0.35	98.81
	Sludge cake	0.40	85.32
	Dried sludge	0.45	11.42

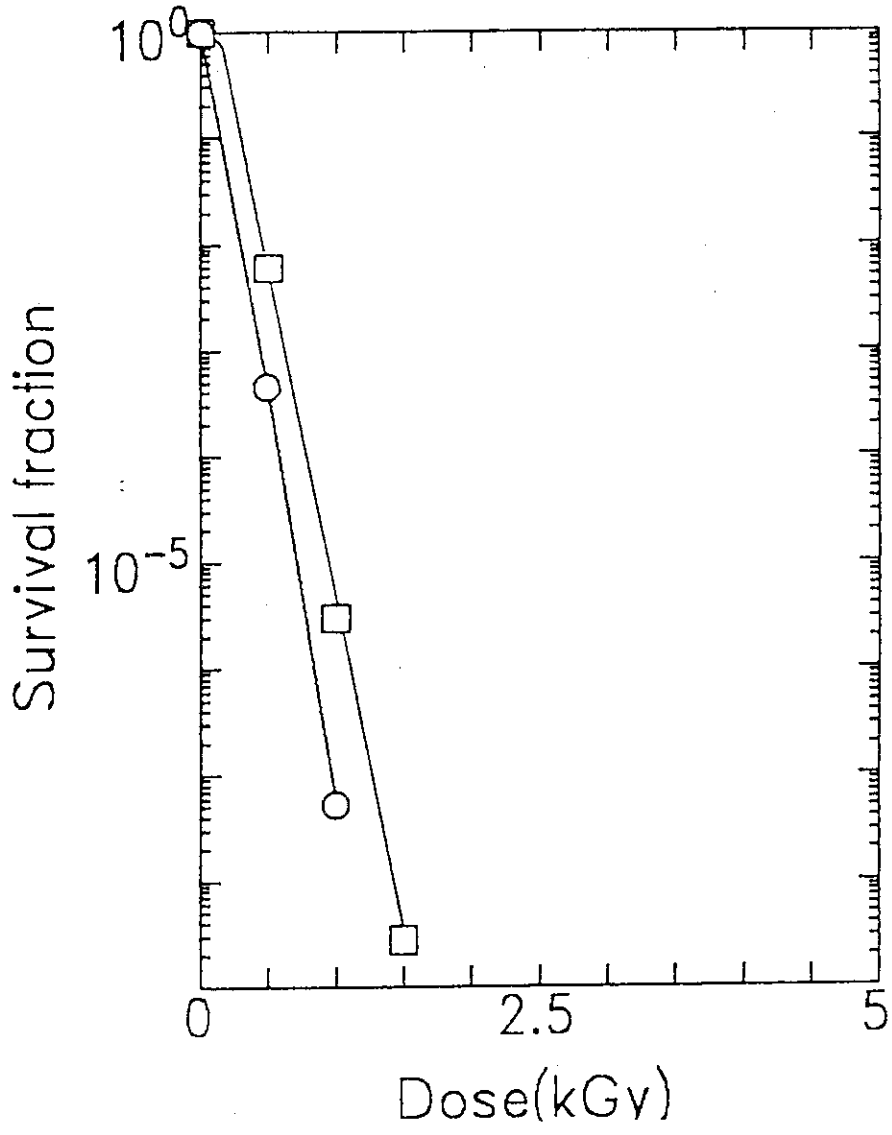


Fig.1 Survival curves of S.brunei irradiated in sludge:
(○)anaerobic digested sludge, (□)sludge cake

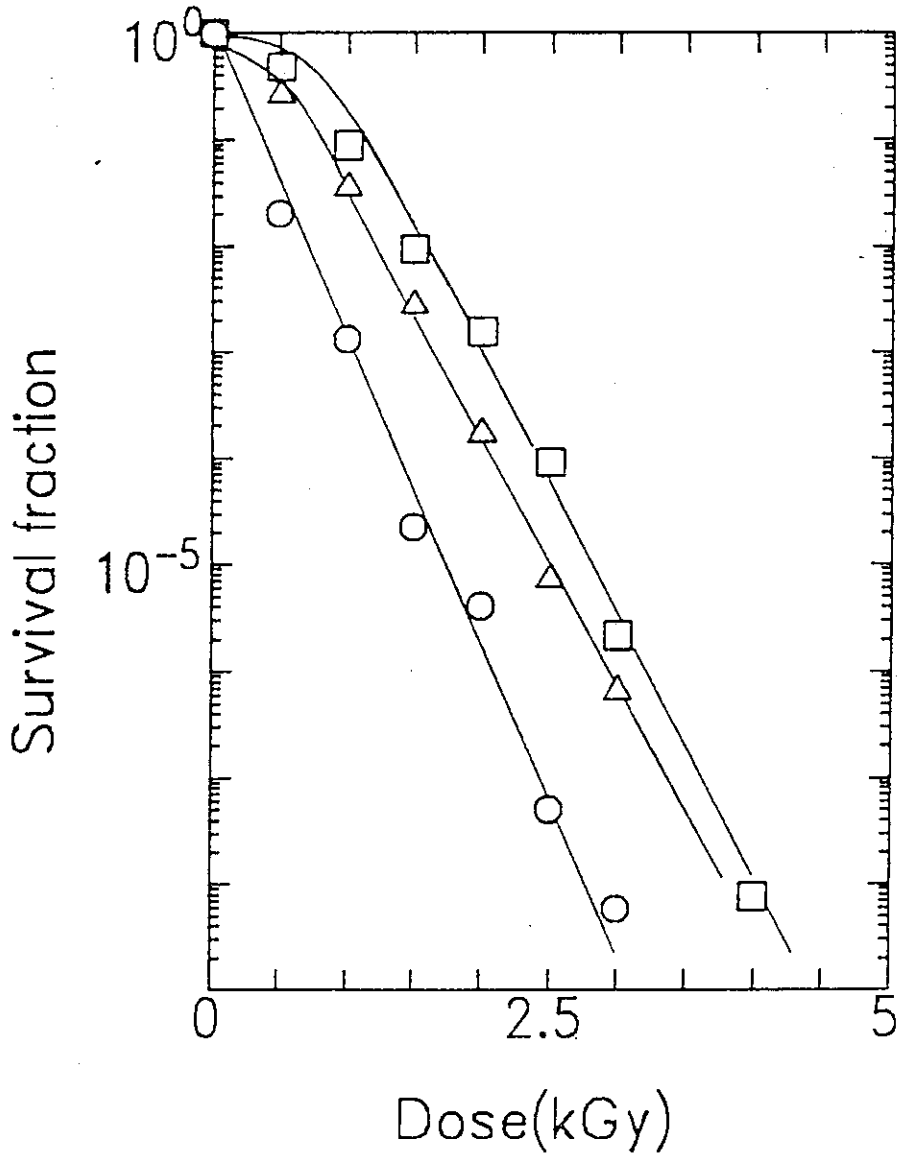


Fig.2 Survival curves of S. paratyphi B. var java irradiated in sludge: (○)anaerobic digested sludge, (□)sludge cake, (△)dried sludge

3.2 Effect of radiation on parasites

[Abstract]

3.2.1 Radiation effect on parasites in sludge

Effect of radiation on Ascaris suum, both 1 cell stage and infective stage, corticated egg, in sludge were studied. For 1 cell stage and corticated egg, showed some inactivation at 2 kGy. Radiation dose up to 10 kGy could not kill infective stage A. suum egg, but caused very slow movement of embryo in egg. Irradiation at 2 kGy caused failing in hatching ability of its egg after 9 weeks.

3.2.2 Radiosensitivity of gamma radiation on Ascaris suum eggs in sludge

Radiosensitivities (D_{10} values) of 1 cell-corticated A. suum egg in 4 % and 90 % solid sludge were 0.52 and 0.64 kGy respectively. D_{10} value of infective, corticated egg in 4 % solid was 0.75 kGy. And those for infective decorticated eggs in 4 % and 90 % solid sludge were 0.71 and 0.75 kGy respectively.

3.2.1 Radiation effect of parasites in sludge

S. Piadang and A. Keittivuti*

Abstract

Ascaris suum, both 1 cell stage and infective stage, corticated egg, in sterile sludge (4% solid) from food industry were studied for radiation effect. Number of its egg killed, viability and hatching abiligy were conducted up to 9 weeks. After treatment of gamma radiation of the range of 1-10 kGy, 10^4 eggs per 1 g sterile sludge were killed. A suum, 1 cell stage egg, showed some inactivation after treatment by gamma radiation at the dose of 2 kGy. Viability of its egg decreased according to the culturing time. Radiation dose up to 10 kGy could not kill infective stage A. suum egg, but caused the movement of embryo in egg from very slow to no movement. Radiation dose at 2 kGy caused failing in hatching ability of its egg after 9 weeks.

Introduction

Survey on the prevalence of intestinal parasites in sewage sludge from hospitals and communities was studied (Piadang et al, 1990). Total number of 162 sludge samples from the provincial hospitals and Huay-Kwang Community of National Housing Authority were examined for the intestinal parasites by using modified magnesium sulfate flatation technique. The mositure content of sludges range from 5.23% to 94.56%. It was found that the prevalence of the intestinal parasites was 55.5%. The predominant parasite was Ascaris lumbricoides (40.12%) the lesser ones found were Hookworm (19.1%), Trichuris trichiura (17.9%). Taenia species (3.70%), Hymenolepis nana (3.08%) and Opisthoorchis viverini (0.61%). Two sources of sludge samples came from hospital and Huay-Kwang community. They were Ascaris lumbricoides (35.8%), Hookworm (18.5%), Trichuris trichiura (15.9%), Taenia species (3.97%), Hymenolepis nana (3.31) and Opisthorchis viverini (0.66%). Huay-Kwang community sludge of 11 samples were found intestinal parasites at 100.00%. They were Ascaris lumbricoides (100.0%), Trichuris trichiura (45.5%) and Hookworm (27.27%). The high frequency area was the southern part of Thailand (88.9%). The predominant intestinal parasite found was Hookworm (55.55%) the lesser species were Ascaris lumbricoides (44.4%), Trichuris trichiura (22.2%), Hymenolepis nana (11.1%). The lesser area was the northern part of Thailand found the intestinal parasites at 63.41%. The high frequencies parasite found was Ascaris lumbricoides (51.2%). The other species were Trichuris trichiura (22.0%), Hookworm (19.5%), Taenia species (7.31%), Hymenolepis nana (4.87%) and Opisthorchis viverini (2.43%). Far North-East are found the parasites at 54.00%. The

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predominant one was Ascaris lumbricoides (36.0%). The other was Trichuris trichiura (16.0%), Hookworm (10.0%), Taenia species (6.00%) and Hymenolepis nana (4.00%). The least area of parasite found was the central at 46.77%. The predominant intestinal parasite found was Ascaris lumbricoides (35.5%). The lesser parasite was Hookworm at 20.96% and Trichuris trichiura (16.1%).

The ova and cyst of protozoan and helminthic parasites are generally resistant to inactivation by physical and chemical process (Riemers et. al, 1979) the resistant ova and cysts are far heavier than bacteria and viruses, and almost are well settle into sludge during primary clarification. This ova and cysts must be removed or killed by later sludge treatments if sludge is to be used without risk to individuals exposed to it. Some human and animal parasites that might be found in sewage and sludge are Ascaris sp., Trichuris sp, Toxocara sp., Taenia sp., Echinococcus sp., Entoamoeba histolytica, Giardia lamblia. These parasites cause many diseases to human and animal.

The cysts of the protozoan parasites Giardia lamblia and Entamoeba histolytica are destroyed by anaerobic digestion, but the resistant stages of the other parasites are not and must be eliminated by a Process to Further Reduce Pathogens treatment before unrestricted use of sludge.

The highly resistant ova of Ascaris have been used in most studies of parasite inactivation in sludge. These ova are resitant to chemicals such as chlorine, and are undamaged by physical stresses such as pH extremes and moderate temperature. Ascaris ova are generally found in higher concentration in sludge than are the resistant stages of other parasites (Riemers, et.al., 1979) Ascaris ova are not affected by anaerobic digestion. But recent research indicates that the ova may be inactivated when sludge is dewatered to mositure levels around 20% (Riemers, et.al., 1979).

Objectives

(1) to find out the effect of radiation to embryo of Ascaris suum in sludge.
(2) to establish the optimum dose for the inactivation of A. suum embryo in sludge.

Method

1. sterile sludge at 50 kGy gamma radiation dose.
2. inoculate 10^4 A. suum eggs in 1 g sterile sludge.
3. irradiate each of 10 g inoculated sludge in test tube at the dose range of 0-10 kGy.
4. count the survival egg after irradiation by the following procedure:
   (1) add 20 cc, 15% NaClO solution into each sample
   (2) curtrifuge and leave sample for 1/2 hr.

(3) add 150 ml, sterile water
(4) filter with sterile gauge
(5) wash NaClO from egg for 2-3 times
(6) draw out 0.075 cc, egg suspension and drop on each slide
(7) count survival egg under microscope

5. examine the viability of survival egg after irradiation at 1 week interval as item 4.
6. examine the hatching ability of egg after irradiation by the following procedure:
(1) add 5.25% NaClO solution and 1 N NaOH at 1:1 ratio to wash parasite egg shell.
(2) leave for 1 hr, and mixing
(3) run item (1) and (2) for 2-3 times
(4) add 0.17% HCl and 0.1% pepsin solution
(5) aerate at room temperature for 48 hrs.
(6) count the infective or larval stage egg produced under microscope as hatching number of egg per g sludge.

Result

The inactivation of A. suum egg at 1-cell stage, corticated was shown in table 1.

Table 1 Inactivation of 10^4, 1-cell stage, corticated, A. suum egg in 1 g of sterile sludge from food industry. (4% solid).

dose (kGy)	number of eggs killed/g of sludge				
	immediately after irradiation	1 wk	2 wk	3 wk	4 wk
0	0	0	0	$1x10^2$	$2.0x10^2$
1	0	$7.00x10^2$	$1.95x10^3$	$2.75x10^3$	$4.15x10^3$
2	0	$7.50x10^2$	$1.30x10^3$	$2.30x10^3$	$7.55x10^3$
3	$2.67x10^1$	$1.95x10^3$	$1.45x10^3$	$1.95x10^3$	$7.85x10^3$
4	$3.20x10^1$	$2.05x10^3$	$5.0\ x10^2$	$2.55x10^3$	$6.20x10^3$
5	$6.40x10^1$	$2.35x10^3$	$1.75x10^3$	$2.45x10^3$	$6.80x10^3$

A. suum eggs at 10^4/g without treatment of gamma radiation were hatched after culturing for 6 weeks at 3.5×10^2 eggs/g sludge. For treated A. suum eggs with gamma radiation failed to show hatching ability at any doses applied after 6 weeks period of culturing. The inactivation of infective stage A. suum egg was shown in Table 2.

Table 2 Inactivation of 10^4 infective stage, corticated A. suum egg in 1 g of sterile sludge from food industry (4% solid)

dose (kGy)	1-5 weeks of culturing	
	number of egg killed	movement of embryo in egg
0	0	slow movement
1	0	
2	0	
3	0	
4	0	
5	0	
6	0	
7	0	very slow movement, nearly still
8	0	
9	0	
10	0	

Radiation dose at 10 kGy could not kill the infective stage, A. suum egg. Higher doses at 7-10 kGy caused its movement very slow to nearly still.

The hatching ability of infective stage, corticated A. suum egg were studied after culturing them for 5 weeks. The results were shown in Table 3.

Table 3 The hatching ability of 10^4 infective stage, corticated A. suum egg in 1 g. sterile sludge from food industry (4% solid)

dose (kGy)	number of eggs hatched/g of sludge	
	7 wk	9 wk
0	4.25×10^2	4.00×10^2
1	0	5.00×10^1
2	0	0
3	0	0
4	0	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

The infective stage, corticated A. suum egg failed to hatch at the dose of 1 kGy for 7 weeks, and also at the dose of 2 kGy for 9 weeks.

Summary

A. suum, 1 cell stage and corticated egg, showed some inactivation after treatment by gamma radiation at the dose of 2 kGy. For the infective stage egg, 10 kGy dose could not kill its egg, but caused very slow movement of embryo in egg. Hatching ability of its egg at infective stage failed at 2 kGy dose of gamma radiation for 9 weeks.

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3.2.2 Radiosensitivity of gamma radiation on Ascaris suum eggs in sludge

S. Piadang and A. Keittivuti*

Introduction

Ascaris lumbricoides, one of the most common intestinal parasites of man, is cosmopolitan. Ascariasis are found through out the temperate and tropical areas of the world, especially under condition of poor sanitation.

Morphology: Ascaris is the largest round worm parasitizing the human intestinal tract. The male measures about 12-31 cm in length by 2-4 mm in diameter. It's posterior end is curved ventrally. The female measures 20-40 cm in length by 3-6 mm in diameter. The mouth in both sexes is surrounded by a dorsal and two ventral lips. It is light brown or pink in color when freshly passed from the intestinal tract and gradually changed to white later.

Life cycle: The one cell stage fertilized egg which passed out in the feces takes about 9-13 days to completely develop to be first-stage larva at 22-23^{o}C in moist soil or in water. Another week is required for the first mouit, after the eggs are infective and contain motile second stage larvae. If the infective stage eggs are ingested, the larvae leave the shell in 15 hours and make their way through the intestines to the lungs through the liver and blood vessels. From the lungs, they make their way into the bronchi and coughed up and passed down the esophagus to the ileum. The larvae, on reaching the ileum or small intestine, develop into adult worms and become sexually mature in about 6-10 weeks. The mature female begin to discharge eggs in the stool about two months from the time of infection. The cycle is again repeated.

Characteristics of Ascaris eggs: The fertilized egg measures 65-75 microns in length by 35-50 microns in diameter and consists of the following structure:
(1) A coarsely granular, spherical egg cell which usually does not completely fill in the shell.
(2) A thin inner-most membrane, which is highly impermeable.
(3) A relatively thick, colorless middle layer, which is smooth on both the inner and outer surfaces.
(4) An outermost, coarsely mammillated albuminous layer, laid down in uteri, serving as an auxiliary protective membrane. In this otherwise normal egg, the external, mammillated layer is absent, and the egg is referred as "decorticated egg".

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The unfertilized egg: The eggs are generally more elongate and irregular than fertilized eggs. They are 63-98 microns in length by 40-60 microns in diameter, and have a thin shell and irregular protein coat. The eggs are composed of degenerated granules of variable size and small droplets fill the inside. The eggs are yellowish-brown in color due to the bile pigment in the feces, but when the outer albuminous coat is absent. Diagnosis may be difficult.

The Ascaris eggs were divided into 5 stages according to their development i.e., one cell, morula, tadpole, larval stage and dead egg.

Ascaris lumbricoides compared with Ascaris suum

Many authors had studied the difference between Ascaris lumbricoides and Ascaris suum. Some of them studied the general morphology and came to the conclusion that there was no difference between both human A. lumbricoides and A. suum.

It was also found that there were cross infection to man and pig between both Ascaris. No difference in resistance to various chemicals was experimentally observed between eggs of human and pig Ascaris. However, the former may be replaced by the latter in the screening test for ovicides.

From the above reasons the conclusion can be made that there was no difference between human Ascaris and pig Ascaris.

Radium irradiation (Gamma ray)

Sawada and Oki 1924 using Ascaris eggs radium-irradiated at varying dose, have reported its enhancing effect at the smaller dose and inhibitory one at larger doses upon their development. Using 61.26 mg of radium immature Ascaris eggs were irradiated separately by two difference methods: the one is 1-hour irradiation a day except Sunday and the other is 6-hour irradiation a week. A majority of immature eggs are killed by the irradiation at doses of 735.16 mg. per hour and most of the infective stage are killed at a dose of 802.92 mg per hour.

Cobalt-60 irradiation (Gamma ray)

In an attempt to know the minimum dose of Co-60 irradiation at which larval formation of Ascaris eggs is completely inhibited, Asami et al. 1955 (24) made a series of experiments. The minimum dose of irradiation required for complete inhibition of larval formation is 4.3×10^4 rad, 15.3×10^4 rad, 11.0×10^4 rad and 15.3×10^4 rad as judged from data of 1, 2, 3, 8 weeks and 16 weeks culture respectively. The minimum dose at which complete inhibition of larval formation and 50% in larval formation is brought and maximum dose at which normal development of eggs is permit-

ted, are determined by calculation from the curve as infection of mice with 11.4×10^4 rad, 5.8×10^4 rad and 0.12×10^4 rad respectively.

Abnormal development is seen to occur frequently in irradiated eggs especially at a dose of 11×10^4 rad or more and examined at 4 weeks or more after culture.

Saito 1957 study showed that the minimum dose of irradiation at which complete inhibition of larval development occurs in eggs of varying stages are as follows: 7.0×10^4 - 9.82×10^4 rad in 2 days cultured, 5.79×10^4 - 7.0×10^4 rad in 4 days cultured (at 2-cell stage), 4.32×10^4 - 5.28×10^4 in 7 day (early cleavage stage), 2.1×10^4 - 2.7×10^4 rad in 9 days cultured (at early morula stage), and 12.63×10^4 - 16.85×10^4 rad in 9-10 day cultured (at morula or early tadpole stage) eggs.

Kobayashi et al, 1958, extended the previous works to determine the minimum dose at which the motility and infectivity of Ascaris larval are lost by Co-60 irradiation. The minimum dose varies with the period of postcultivation when those cultured previously for 15 days are used, the minimum dose at which larval motility is lost is 250×10^4 rad, 570×10^4 rad, 350×10^4 rad, 250×10^4 rad and 100×10^4 rad in those postcultured for 0, 1, 2, 4 and 12 weeks. When those cultured for 30 days previously are used, it is 560×10^4 rad, 920×10^4 rad, 340×10^4 rad and 130×10^4 rad in eggs for 0, 4, 7, 8 and 12 weeks. The infectivity of eggs irradiated 16×10^4 rad, 39×10^4 rad, 66×10^4 rad and 100×10^4 rad decreases to 6.8%, 0.8%, 0.04% and 0% of unirradiated control respectively, when eggs pre-incubated for 15 days and postcultured for 4 weeks, are used. In case of those pre-incubated for 30 days and postcultured for 5 days, it decreases to 67.9%, 42.2%, 0% in those irradiated at 16×10^4 rad, 39×10^4 rad and more than 100×10^4 rad respectively. The experiments above indicate that the resistance of infective stage against Co-60 irradiation is much higher than that of unembryonated eggs as well as of developing eggs.

Kadota 1957, 1958 study shows the inhibition of larval formation occurs in 28% of those, pre-incubated for 3 days (1-2 cell stage) and exposed to 24×10^3 r, and in 50% and 7% of those for 5 days (early morula stage) and to 24×10^3 r and 12×10^3 r respectively. The similar inhibition or structural damage occurs in 43% of those for 8 days (late morula and tadpole stages), 15% of those for 10 days (tadpole and larval stages), and 8% of those for 15 days (larval stage) when they are irradiated at 24×10^3r. He made another series of experiments to know the effect of Co-60 irradiation upon infectivity to mice of the larvae which survived previous irradiation of 9.6×10^3 r at their earlier stages. No noticeable difference in infectivity was recognized between irradiated and nonirradiated eggs. But slight retardation of development during the period of 1-6 days after the postculture was observed in those pre-incubated for 3-8 days and exposed to the dose at which normal embryonation was permitted.

Wizigmann, i. Irradiated the sludge mixed with Ascaris suum

eggs at dose 300 krad for 290 minutes and cultured in the petridish with sterile water, kept at 30°C. Examination of irradiated eggs were carried out under the microscope to check development after postcultured. Any noticeable development was not observed in the shell and content inside the eggs but the development of eggs will retard after morula stage and after postculture. More than 3 weeks found that Ascaris eggs were cloudy and inhibited the development of the eggs but the eggs in the control group had developed to embryonated stage and infected the mice.

Sivinski, H.D. Using sludge with A. lumbricoides and irradiated with Co-60 at dose 36 krad/minute at 21°C found that it can decrease the amount of Ascaris eggs 1 log or 90%. Infective stage eggs were irradiated at dose 45-50 krad that can decrease Ascaris eggs to 1 log, radiation dose 65-70 krad can decrease Ascaris eggs to 2 log and at dose 95 krad can completely inhibit the development of Ascaris eggs.

Research at Sandia National Laboratories. Indicated that Ascaris eggs were sensitive to Gamma radiation with D10 values in liquid and composted sludge of about 50 krad.(13) Because of recent concern that Ascaris eggs removed from the uterus of adult ascarids may be more sensitive to irradiation than are eggs hardened by transit through the intestines, a cooperative study was done by Tulane University School of Tropical Medicine and Public Health and Sandia. In this double-blind study three types of Ascaris eggs were seeded into liquid sludge and irradiated to several dose levels. Results of these experiments show that the D10 values of eggs from pig feces is 62 krad, decorticated eggs from pig feces is 45 krad and decorticated eggs from adult female Ascaris is 45 krad.

In Thailand there was no study about the effect of Gamma radiation on Ascaris suum eggs in sludge from wastewater treatment plants. Since the temperature in Thailand differs from other countries, study must be done on those in Thailand.

Methodology

Preparation of fertilized Ascaris suum eggs

Adult female pig Ascaris suum were obtained from the small intestines of slaughtered pigs at Bangkok Metropolis Slaughter House and at Nakornprathom province. After washing the adult female Ascaris with water, the constriction near the anterior third of the body was dissected with a fine scissors. The whole of the exposed bifurcated uteri were removed and one inch of the anterior of the uteri was separated from the rest and crushed with a virtis grinder, filtered the eggs with sterile gauzes. These Ascaris eggs with a small amount of distilled water were kept at 4°C until the sufficient number of eggs were prepared, in order to inhibit their development. At this time, all of them were in one cell stage. Then divided Ascaris eggs to 2 parts, the first part added 10 of Ascaris eggs into 10 g of sludge and

irradiated. The second part cultured Ascaris eggs in petri dish mixed with sterile normal saline and 2% of formalin to prevent the development of fungus and bacteria. Culture Ascaris eggs for 4 weeks, the eggs will develop to embryonated stage.

Preparation of decorticated Ascaris suum eggs. (1 cell and infective stage eggs)

Added 1 N.NaOH and 5.25% NaClO solution to 1 cell stage and infective stage of Ascaris eggs to destroyed egg shell and leave for 2 hours at room temperature. After that, centrifuge the solution with speed of 2,000/minute for 10 minutes and pour off the upper part of solution. Add sterile 0.85% NaCl, mix together and centrifuge again. After that, examine and count by using "Stoll's dilution egg count technic" to prepared 10^5 of Ascaris egg mixed with sludge to be irradiated next time.

Method of sampling the sludge

Using simple random sampling from sludge in the wastewater treatment plant from provincial hospitals and Huay-Kwang community of National Housing Authority, divide sludge to 2 types.

1. solid sludge (90% solid)
2. liquid sludge (4% solid)

Sample size

Simple random sampling from each type of sludge for 10 g and mixed with 10^5 of Ascaris eggs in each test tube.
These, research divided experiment to 2 groups.

1. Experimental group divided to 4 groups as following.
1.1 Inoculated 10 corticated 1 cell of Ascaris suum eggs in 10 g sterile sludge and put it into test tube 25x200 mm. size with cap on. Irradiated each of 10 g inoculated sludge in test tube at the dose range of 0-40 krad with the distance between test tube and source of radiation 81.5 cm.
1.2 Using 1 cell decorticated eggs and done same as 1.1.
1.3 Using infective corticated eggs and done same as 1.1.
1.4 Using infective decorticated eggs and done same as 1.1.

The method of mixing 4 types of Ascaris eggs and liquid sludge were done same as solid sludge as mentioned above.

2. Control group
Using 10 of each types of Ascaris eggs mixed with each type of sludge same as experimental group that were mentioned above, but all of them had not irradiated with gamma ray.

Collecting data from the experiment

After irradiated with different dose of gamma ray, we can analyzed and compared the effect of gamma radiation on destroying Ascaris suum eggs by dividing into 2 types.

1. Counting the survival eggs and examining the development after irradiation and postculture by the following procedure.
   1.1 add 20 cc, 15% NaClO solution into each sample.
   1.2 shake to separate the eggs from sludge and leave sample for 1/2 hour.
   1.3 add 150 cc sterile water.
   1.4 filter with sterile gauze.
   1.5 centrifuge and wash NaClO from eggs 2-3 times.
   1.6 culture in petri dish for 5 weeks (1 cell stage eggs) and for 1 week (infective stage eggs)
   1.7 examine the viability of survival eggs after irradiation at postculture for 1 and 5 weeks depending on type of Ascaris eggs. By using "Stoll's dilution egg count technic" count the survival eggs 3 times and calculated for the average and the percentage of survived eggs.

2. Examined the hatching ability of eggs after irradiation by the following procedure.
   2.1 add 5.25% NaClO and 1 N. NaOH solution at 1:1 ratio to wash albuminous coated of Ascaris eggs.
   2.2 leave for 1 hour, and centrifuge.
   2.3 wash NaClO and 1 N. NaOH solution from eggs for 2-3 times.
   2.4 add 0.17% HCl and 0.1% pepsin solution.
   2.5 aerate at room temperature for 24 hours.
   2.6 count the larvae that hatch from eggs under microscope as hatching number of eggs per gram of sludge by using "Stoll's dilution count technic". Count the larvae 3 times and calculated for the average and percentage of the larvae.

Result

Effect of gamma radiation on 1-cell and infective stage of A. suum eggs. was shown in Table 1.

Table 1 Radiosensitivity of corticated and decorticated A. suum eggs in 4% and 90% solid sludge.

Type of egg	D_{10} value (kGy)	
	4% solid	90% solid
1-cell, corticated	0.52	0.64
1-cell, decorticated	-	-
infective, corticated	0.75	-
infective, decorticated	0.71	0.77

- no data available at the present

Conclusion

D_{10} value of infective stage A. suum egg was higher than 1-cell stage egg.

4. Upgrading of irradiated sludges

4.1 Design of fermentor set for large scale fermentation

[Abstract]

Design of a fermentor system for large scale fermentation and details of the fermentor were shown. The capacities of two fermenters were 5 l, respectively. Each fermentor has water jacket and mixing blade to make uniform aeration and temperature distribution. Sensors for pH and temperature were also installed at the top.

4.1 Design of fermentor set for large scale fermentation

S. Hashimoto and S. Sato

1. Introduction

According to the time schedule of Bilateral Cooperation Program, utilization test will be started from FY 1991. It is necessary to prepare larger amount of fermented products for the utilization test. JAERI is preparing 2 sets of 5 l fermentors for this purpose. In this report, brief introduction of the fermentor system is shown.

2. Flow sheet of the system

Fig. 1 shows the flow sheet of the fermentation system. The system consists of sludge mixers, clean box, fermentors and control box with air compressor, flow meters, circulation water baths, CO_2 meters, pH meters and electronic recorder.

Irradiated sludge is mixed with bulking agent and seed bacteria by sludge mixer. The mixture is put into the fermentor. The fermentation temperature is controlled by jacket attached to the fermentor. The fermentors and sludge mixers are set in a clean box to prevent contamination of other microorganisms. During fermentation, air from a compressor is sent to keep mixture aerobic. CO_2 concentration in exhaust gas from fermentors, pH of the mixture, temperature in the fermentors are continuously monitored to know the state of fermentation. Fig. 2 shows arrangement of the equipments.

3. Details of fermentor

Fig. 3 shows details of the fermentor. The diameter of the fermentor is 160 mm and the height 270 mm. The volume is about 5 l. Normal size autoclave can be used to sterilize the fermentor. Mixing blade is attached inside of the fermentor. Mixing speed can be changed from 6 to 110 rpm. Water chamber is prepared at the bottom to prevent raw mixture from drying. Filtrated air pass through this chamber and get humidity. Flow rate of air can be changed from 0.2 to 2 l/min. Sensors for measurement of pH and temperature can be attached at the top.

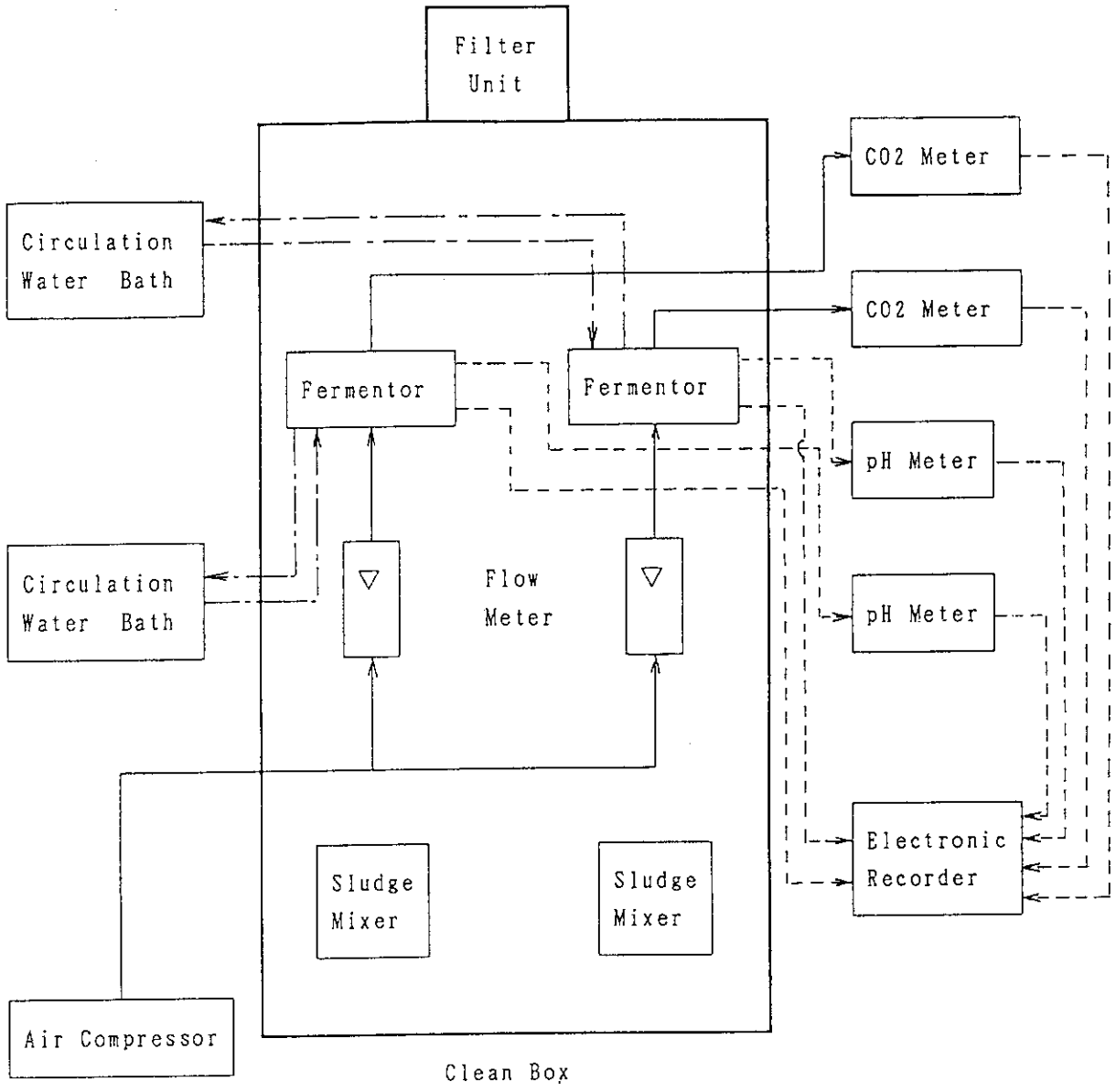

Fig. 1 Flow sheet of fermentor system

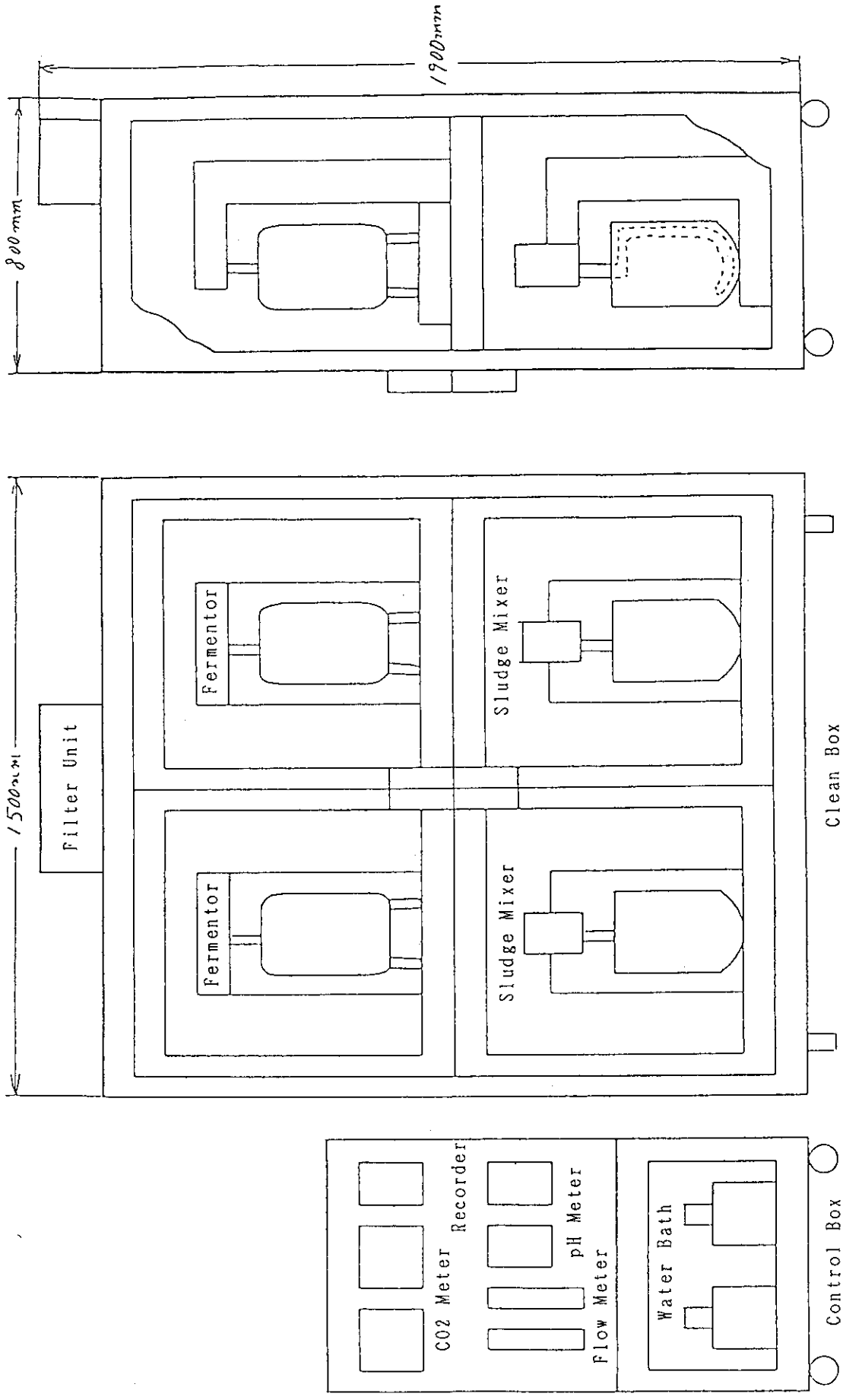

Fig. 2 Arrangement of the equipments

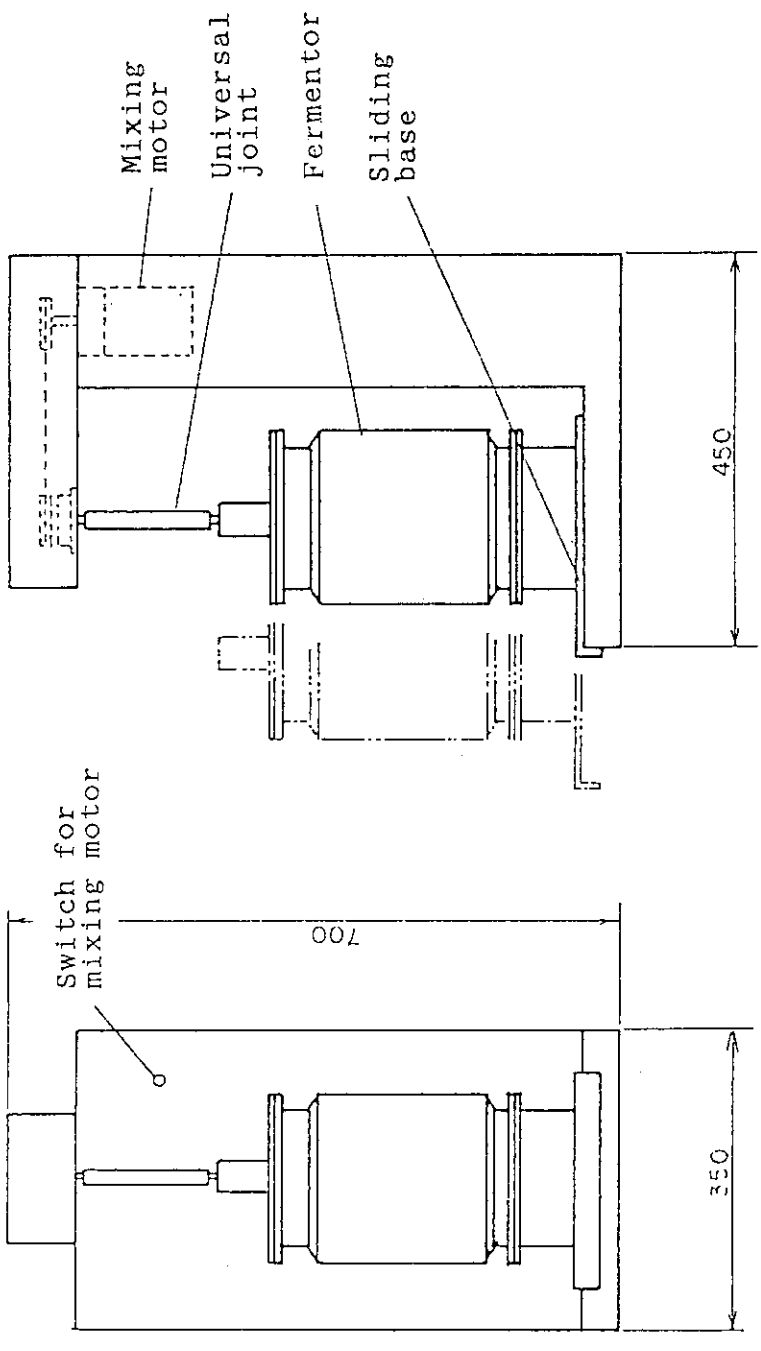

Fig. 3a Details of fermentor

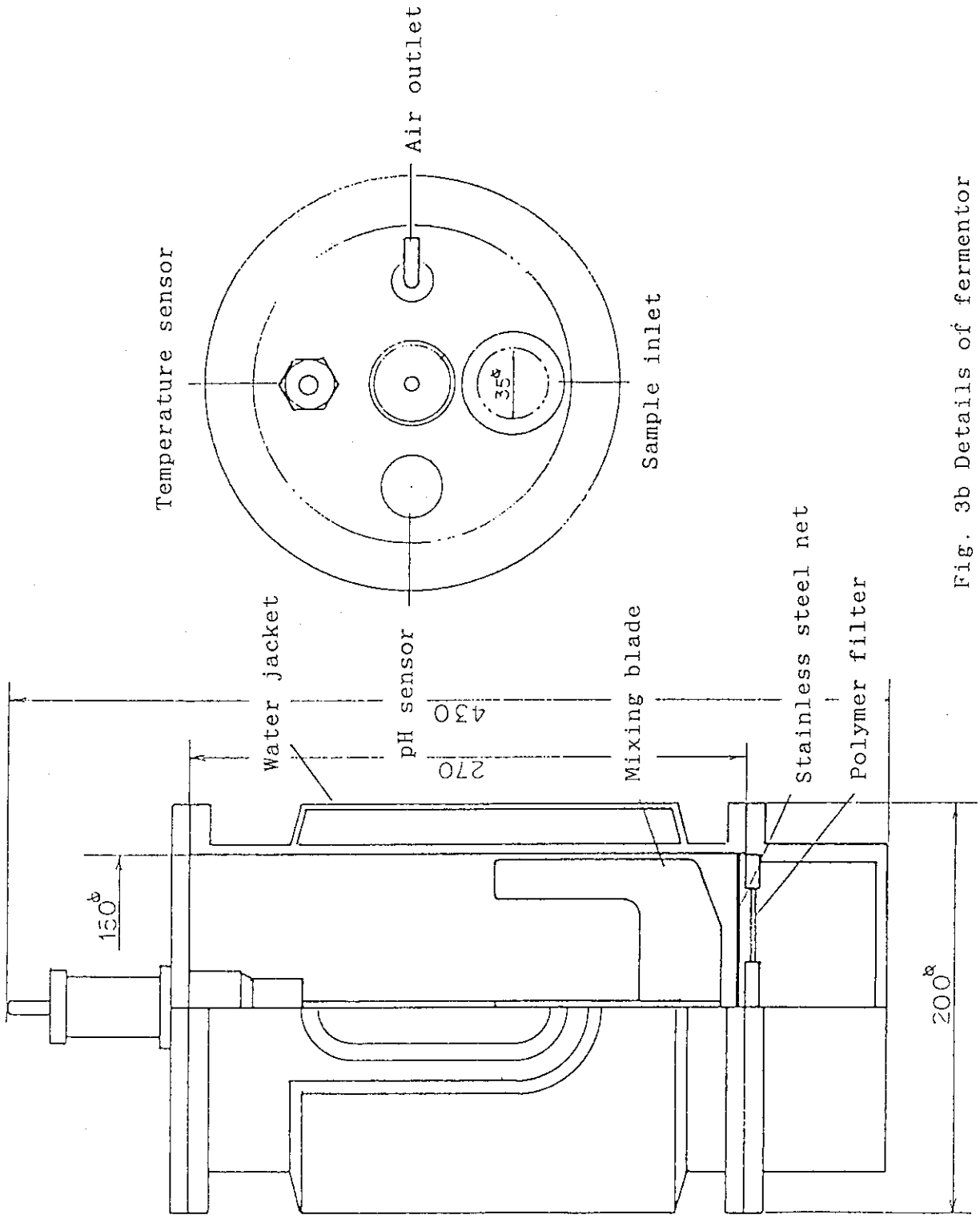

Fig. 3b Details of fermentor

4.2 Upgrading of the irradiated sludge by fermentation

[Abstract]

4.2.1 Microbiological study on irradiated sludge composting

Effect of temperatures on microorganisms in seeds and composts produced from irradiated sewage sludge and the effect of compost on growth suppression of F. oxysporum were shown.

4.2.2 Effect of seeds and temperature on fermentation of irradiated sludge

The experimental results on effect of seed microorganisms and temperature on fermentation of the irradiated sludge were shown. The results showed that 45 °C was the best temperature to decompose sludge and 35 °C was better to get larger amount of biomass.

4.2.3 Decomposition of irradiated sludge in soil

Decomposition of irradiated sludge in soil was reported. The results showed that the irradiated sludge was stabilized more rapidly than the unirradiated sludge and the number of total bacteria in soil increased by the decomposition of irradiated sludge.

4.2.1 Microbiological study on irradiated sludge composting

S. Pongpat and S. Hashimoto

1. Introduction

Composting has recently become widespread as a treatment process of sewage sludge from a viewpoint of recycling of organic materials and other trace elements useful for plant growth. In the process of conventional composting method, high temperature is used to pasteurize pathogenic microorganisms in composting materials. In such ease useful microorganisms for plant growth are also eliminated.

Irradiation is an effective mean of disinfection of sewage sludge as reported by Ahlstrom (1985). The composting of radiation disinfection of sewage sludge was introduced as a new method as reported by Kawakami et al. (1981). By these means, it is possible to make composting at lower temperature compared with conventional method because the sludge is already disinfected. The low temperature composting by using a special seed compost seems to be useful to produce the products contained various kinds of microorganisms which may play the role of growth suppression of plant pathogenic fungi.

In this study the microbiological study on irradiated sewage sludge composting was investigated. The purpose of the study is to investigate the effect of temperatures on microorganisms in seeds and the composts produced from irradiated sewage sludge and also to estimate the quality of the compost products by the test of their effects on growth suppression of F. oxysporum.

2. Experimental procedures

2.1 Apparatus and composting

Dewatered sewage sludge from Akutsu Sewage Treatment Facility was used as a raw material. The sludge was disinfected by gamma irradiation at dose of 10 kGy (dose rate 5 kGy/h.) The composting performed at 30, 40, 50 and 65 C by using six seed composts. The composting materials for each run consisted of 30 g. of irradiated sludge, 3 g. of a seed, 15 g. of sterile perlite as a bulking agent, and 240 mg of sterile sodium carbonate for adjusting pH to around 7. After well mixing, the composting materials were transferred into a sterile glass fermenter, then covered with sterile stopper unit. After embedding the fermenter in the water bath, the operation was started by means of a micro-computer controlling. The operation was stopped when the peak of CO_2 evolution rate had finished as observed from a recorder. CO_2 concentration from exhaust gas of fermenter was continuously measured. The properties of sewage sludge, seeds

and composts, such as moisture content volatile solid and pH were also analyzed. A scheme of experimental apparatus for composting is shown in Fig.1

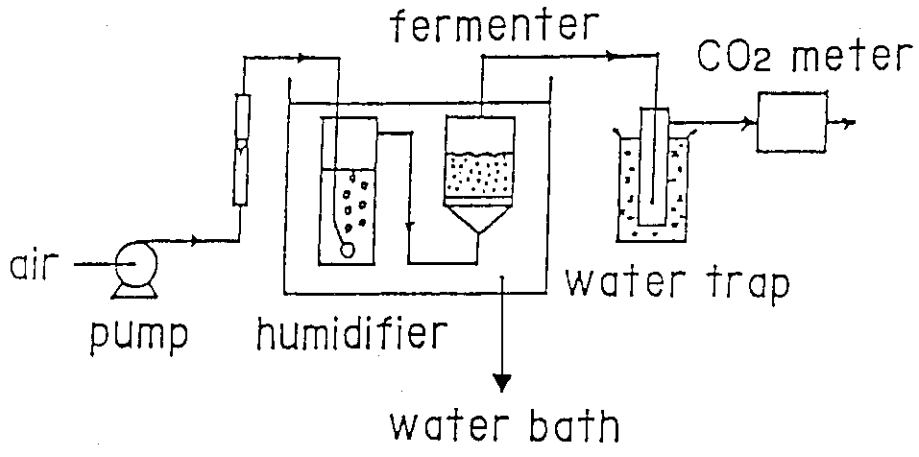

Fig.1 Experimental apparatus for composting

2.2 Isolation and enumeration of microorganisms

Total bacterial counts, coliforms, actinomycetes, fungi and yeasts were enumerated. The technique of spread plate was used in this study. The culture media and incubation conditions for isolation and enumeration of microorganisms are shown in Table 1.

Table 1 Types of media and incubation condition for isolation and enumeration of microorganisms

Isolating media	Microorganisms	Formula ingredients per liter	Incubation temp. and time
Nutrient agar, difco	Bacteria	Beef extract 3 g Peptone 5 g Agar 15 g Distilled water 1 l Final pH 6.8	30°C;48 h
MacConkey agar, difco	Coliforms	Peptone 17 g Proteose peptone 3 g Lactose 10 g Bile salt No.3 1.5 g Sodium chloride 5 g Agar 15 g Neutral red 0.03 g Crystal violet 1 mg Distilled water 1 l Final pH 7.1	37°C; 18-24 h
Soluble starch agar	Actinomycetes	Soluble starch 10 g Ammonium sulfate 0.5 g di-Potassium hydrogen phosphate 0.5 g Agar (Chameleon) 20 g Distilled water 1 l Final pH 7.0	30°C; 4-5 d
Malt yeast extract agar	Fungi and Yeasts	Malt extract 10 g Yeast extract 4 g Glucose 4 g Chloramphenical 20 mg Agar (Chameleon) 20 g Distilled water 1 l Final pH 5.5	30°C; 3 d

2.3 Growth suppression test

The technique of compost inoculation assays was used in this study. A certain amount of compost sample was placed into a sterile vial and inoculated with spore suspension of F.oxysporum. The vial was stopped with a sterile silicone stopper to allow for gas exchange, then incubated at 30°C. The F. oxysporum was enumerated by colony counts on malt-yeast extract agar at inoculation time and 7 days after incubation.

3. Experimental results

3.1 Effect of temperatures on microorganisms in seeds

Table 2 shows the microbial number isolated from seed R. It was found that maximum number of microorganisms was observed at temperatures around 30-40°C. The counts of bacteria, fungi and actinomycetes at 30°C were 3.97×10^{7}, 2.55×10^{4}, and 2.33×10^{7} cfu/g, respectively. As the incubation temperature increased to 65°C the counts of bacteria and actinomycetes reduced to be 1.77×10^{6} and $<1.67\times10^{2}$ cfu/g, respectively and the count of fungi became undetectable level. For all temperatures, yeast counts were found undetectable.

Table 2 Number of microorganisms from seed R at various temperatures

Number of microorganisms (cfu/g)	Temperature (°C)			
	30	40	50	65
TBC	3.97×10^{7}	6.35×10^{7}	2.99×10^{7}	1.77×10^{6}
TF	2.55×10^{4}	4.50×10^{3}	6.33×10^{7}	<D
TY	<D	<D	<D	<D
TA	2.33×10^{7}	2.38×10^{7}	1.60×10^{7}	3.97×10^{2}

TBC: Total bacterial counts; TF: Total fungi; TY: Total yeast;
TA: Total actinomycetes; <D: Undetectable

Table 3 shows the microbial number from seed 0-2. The maximum number of microorganisms in seed 0-2 was observed at temperature 30°C. The number of microorganisms in the seed remarkably decreased as temperature increased. Counts of bacteria, fungi, yeasts and actinomycetes were 1.66×10^{7}, 1.25×10^{2}, 4.39×10^{4} and 1.47×10^{6} cfu/g, respectively at 30°C. The counts of bacteria were 7.83×10^{5} cfu/g while the counts of fungi and actinomycetes were at undetectable level as the temperature increased to 65°C.

Table 3 Number of microorganisms from seed 0-2 at various temperatures

Number of microorganisms (cfu/g)	Temperature (°C)			
	30	40	50	65
TBC	1.66×10^{7}	3.53×10^{7}	1.78×10^{7}	1.77×10^{6}
TF	1.25×10^{2}	6.67×10^{2}	<D	<D
TY	4.39×10^{4}	1.75×10^{3}	<D	<D
TA	1.47×10^{5}	<D	<D	<D

Abbreviations are the same as Table 2.

The results showed that the microorganisms from two seeds were maximum at temperature around 30-40°C. The same results were also obtained from other four seeds. Nakazaki et al. (1985) reported that the effect of increasing seed was clearly reflected in the increase in initial numbers of thermophilic bacteria and thermophilic actinomycetes in the process of thermophilic composting. By using these seeds, therefore, the composting performed at 30-40°C may obtain the products contained high number of various kinds of microorganisms.

3.2 Microbial number in composts

Table 4 shows the number of microorganisms from composts produced from seed R at various temperatures. The number of microorganisms isolated from the composts of these runs remarkably decreased as composting temperatures increased. The counts of bacteria, fungi, yeasts and actinomycetes from the run produced at 30°C were 1.54×10^{9}, 1.52×10^{5}, $<1.67\times10^{2}$ and 3.81×10^{8} cfu/g. respectively. The reduction in number of microorganisms was obtained from the composts produced at higher temperatures. The count of bacteria from compost produced at 65°C became 9.67×10^{5} cfu/g. The counts of fungi, yeasts and actinomycetes from that run became undetectable.

Table 4 Number of microorganisms from composts produced from seed R at various temperatures

Number of microorganisms (cfu/g)	Temperature (°C)			
	30	40	50	65
TBC	1.54×10^{6}	3.22×10^{8}	4.09×10^{6}	1.09×10^{5}
TC	<D	<D	<D	<D
TF	1.52×10^{5}	<D	$<1.67\times10^{2}$	<D
TY	$<1.67\times10^{2}$	3.33×10^{2}	$<1.67\times10^{2}$	<D
TA	3.81×10^{6}	2.32×10^{6}	1.45×10^{5}	<D

TBC: Total bacterial counts; TC: Total coliforms; TF: Total fungi; TY: Total yeasts; TA: Total actinomycetes; <D: Undetectable

Table 5 shows the microbial number from composts produced from seed 0-2 at various temperatures. Reduction of microbial number from the composts produced at higher temperatures was also found. The counts of bacteria, fungi, yeasts and actinomycetes from the compost performed at 30°C were 2.16×10^{9}, 1.57×10^{6}, 1.67×10^{2} and 3.48×10^{8} cfu/g. respectively. The bacterial counts reduced to 9.67×10^{5} and the counts of fungi, yeasts and actinomycetes were at undetectable levels when the composting temperature was increased to 65°C.

Table 5 Number of microorganisms from composts produced from seed 0-2 at various temperatures

Number of microorganisms (cfu/g)	Temperature (^{o}C)			
	30	40	50	65
TBC	2.16×10^{9}	7.25×10^{7}	2.00×10^{6}	9.67×10^{5}
TC	<D	<D	<D	<D
TF	1.57×10^{6}	5.00×10^{2}	$<1.67\times10^{2}$	<D
TY	$<1.67\times10^{2}$	2.67×10^{2}	$<1.67\times10^{2}$	<D
TA	3.48×10^{8}	7.50×10^{5}	<D	<D

Abbreviations are the same as Table 4.

It is noticeable that microorganisms from the composts produced at various temperatures by using seed R and seed 0-2 showed the reduction in the numbers as composting temperatures increased. The maximum number of microorganisms was found from the composts produced at temperatures around 30-40^{o}C. The similar results were obtained from the composting runs by using other four seeds. High temperature composting, therefore, was significantly effective to reduce the growth of microorganisms. The composting at low temperature by using a special seed seems to be very useful to improve the compost quality that can be expected the effect of suppression of plant pathogenic fungi.

Furthermore, it was also observed that composts produced from irradiated sewage sludge did not contain coliforms even the composting was conducted at low temperature, 30^{o}C. The coliform bacteria is generally used as an index for waste water treatment. Since the composts were free from coliform bacteria, therefore, those composts seem to be considered as the microbial safe products.

Results from Table 2 and 4, it was noticeable that number of yeasts, which initially undetected in seed R, was found slightly increasing in composts produced from seed R. These might be resulted by scattered amount of yeast cells in initial seed R.

3.3 Effect of composts on growth suppression of F. oxysporum

Table 6 shows growth suppression of f. oxysporum of composts produced at various temperatures from seed R and seed 0-2. The composts produced at 30 and 40^{o}C from seed 0-2 showed the reduction of F. oxysporum with the order of 10^{2} and 10, respectively. The reduction level in the order of 10^{2} was the highest suppression in this study. On the other hand, growth suppression of F. oxysporum by the composts produced from seed R did not occur. However, at 65^{o}C, the results showed that number of F. oxysporum was reduced from 1.18×10^{5} to 2.52×10^{4} cfu/g, reflecting some scattered data. This could not be considered as growth suppression because the experimental results of both seed R and the compost produced from seed R at lower temperatures obviously did not show any growth effect.

Table 6 Growth suppression of F. oxysporum with composts produced at various temperatures from different seeds

Run	Seed name	Composting condition		Compost property MC(%)	VS(%)	pH	Number of F. oxysporum (cfu/g)	
		T(^{o}C)	Time(h)				0 day	7 days
SU10-2	0-2	30	143	44.4	22.0	8.61	2.27×10^{5}	2.25×10^{3}
SU14-2		40	98	45.4	22.8	8.27	1.13×10^{5}	2.91×10^{4}
SU 8-2		50	92	37.6	17.9	8.34	1.04×10^{6}	4.72×10^{6}
SU13-2		65	95	25.1	20.7	6.53	1.11×10^{5}	1.16×10^{7}
SU10-3	0-2	30	143	44.3	17.6	8.41	2.25×10^{5}	2.63×10^{5}
SU14-3		40	98	45.7	18.5	8.35	1.50×10^{5}	1.69×10^{5}
SU 8-3		50	92	44.0	18.1	8.46	9.47×10^{6}	1.09×10^{6}
SU13-3		65	95	29.8	21.7	6.65	1.18×10^{5}	2.52×10^{4}

T: Temperature; MC: Moisture content; VS: Volatile solid

According to the results of the composts produced from the other four seeds, they also showed reduction of F. oxysporum at the order of 10 at 30^{o}C. Therefore, in general, it can be concluded that the compost product at 30^{o}C showed the higher tendency of growth suppression of F. oxysporum than that of other temperatures.

4. Conclusion

1) Seed composts contained various microorganisms which showed higher growth at low temperature.
2) Composts produced at low temperature contained higher number and varieties of microorganisms.
3) Composts produced from seed 0-2 at 30^{o}C showed the higher tendency of growth suppression.

For better comparison, however, authors suggest that further studies concerning growth suppression by using other methods, isolation and identification of growth suppressive microorganisms should be done.

4.2.2 Effect of seeds and temperature on fermentation of irradiated sludge

S. Hashimoto, S. Pongpat, N. Sermkiattipong
and S. Piadang

1. Introduction

The research work was performed by S. Hashimoto from JAERI, during the stay from 13 January, 1991 to 1 February in OAEP, with the cooperation of OAEP staffs. The purpose of the research work was to know the differences between the sludge in Thailand and Japan. The equipments mainly used in this research were installed in the beginning of January, 1991 by JAERI for the cooperation research.

In this report, the experimental results on effect of seed and temperature on fermentation of the irradiated sludge were shown.

2. Experimental procedures

2.1 Raw materials

a) Sludge

The sludge from waste water treatment facility of Boonrawd Brewery Co., Ltd. was used after irradiation of 30 kGy by gamma ray.

b) Bulking agent

Commercial soil conditioner (Commercial name; Perlite, from Mitsui Kinzoku Kogyo Co. Ltd.) was used as bulking agent.

c) Seed composts for fermentation

Three kinds of seed compost were prepared. Those were F-60 (Commercial seed in Thailand), PD-1 (Commercial seed in Thailand), and Thomaster A (Commercial seed in Japan).

2.2 Experimental procedure

a) Moisture content, volatile solid content and pH

Weight decrease of the sludge was measured after heating by microwave oven until the weight became constant and the moisture content of the sludge was calculated. After drying, the sludge was put into a muffle furnace and heated for more than 4 hrs at 600 °C. Volatile solid content of the sludge was calculated from the weight decrease. pH of the sludge was measured by litmus papers.

b) Effect of seed on fermentation (RUN 1)

Three polyethylene bags, which contain 20 g of sludge each, were mixed with 10 g of bulking agent, 160 mg of sodium carbonate (Na2CO3) and 2 g of different kinds of seed compost. After mixing, the mixtures were put into three glass fermentors. Seed composts F-60, PD-1 and Thomaster A were used for fermentor 1, 2 and 3, respectively. Then the fermentors were put into the water baths and the temperature of the bath was adjusted to be 40°C.

Air from compressor was sent to the fermentors to keep the mixture aerobic. Flow rate of the air was adjusted to be 50 ml/min. CO2 concentration in the exhaust gas from the fermentors was monitored by CO2 meter (Yokogawa Electric Co. Ltd.). The exhaust gas was continuously diluted 2 times by air before measurement.

c) Effect of temperature on fermentation (RUN 2)

Mixtures of irradiated sludge, bulking agent, sodium carbonate and seed compost were also prepared as the same way as b). Only one kind of seed compost, F-60, was used for this experiments. After putting the fermentors into the water baths, the temperatures of the baths were adjusted to be 35, 45 and 50 °C for fermentor 1, 2 and 3, respectively. Concentration of CO2 in the exhaust gas was also monitored by the same way as b).

d) Enumeration of bacteria

Total bacterial counts and total coliforms were measured by using nutrient agar plates and MacConkey agar plates. Five grams of seed or products was mixed with 25 ml of sterilized phosphate buffer solution. Serial tenfold dilution method was used and three plates were used for each dilution. 0.2 ml of diluted solution was spread on each plate. For total counts, the plates was incubated at 30 °C for 3 days and 37 °C for 16 to 18 hrs for total coliforms.

3. Experimental results

3.1 Property of sludge and seed compost

Table 1 shows moisture content, volatile solid content and pH of the sludge. These values are almost same as the sludge from Japanese sewage treatment facility except the volatile solid content was slightly higher. From one plate of nutrient agar, on which original mixture of sludge with sterilized water was spread, two colonies were detected. But, no colony was detected from the first and second dilution. It is not sure that the bacteria, detected from the plate, existed in the irradiated sludge or by contamination during the enumeration procedure.

Table 1 property of sludge

Moisture content (%)	Volatile solid content (%)	pH
79.5	78.4	5.5-6.0

Table 2 shows the property of seed compost. Moisture contents of F-60 and PD-1 are very small but that of Thomaster A is very high. Volatile solid content of PD-1 is low compared with other seed composts. Total bacterial counts of PD-1 is the largest and F-60 is the smallest. But the difference is within 5 times. F-60 and PD-1 contain coliforms but no coliform is contained in Thomaster A.

Table 2 Property of seed compost

Name	Moisture Content (%)	Volatile Solid Content(%)	Total Counts (cfu/g)	Total Coliforms (cfu/g)
F-60	5.4	65.8	4.68×10^{7}	7.89×10^{2}
PD-1	8.2	31.8	2.05×10^{8}	1.10×10^{3}
Thomaster A	60.3	75.4	8.40×10^{7}	0

3.2 Change of bacterial counts by fermentation

Table 3 shows change of bacterial count by fermentation in RUN 1. Moisture content of the product in each fermentor is also shown in this table and the values are almost the same because the fermentation temperature is the same.

The ratios of the total counts for the fermentor 1 to 3 after fermentation to those of before fermentation are 345, 59.5 and 18.9. The largest growth of total bacteria is obtained by the seed compost F-60. Coliforms could not be detected from all the products.

Table 3 Change of bacterial counts by fermentation in RUN 1

Fermentor	Moisture Content (%)	Total Counts(cfu/g) before (calculated)	Total Counts(cfu/g) after	Total Coliforms(cfu/g) before (calculated)	Total Coliforms(cfu/g) after
Fer. 1	56.0	2.93×10^{6}	1.02×10^{9}	49.3	0
Fer. 2	56.6	1.28×10^{7}	7.61×10^{8}	68.8	0
Fer. 3	56.4	5.25×10^{6}	9.91×10^{7}	0	0

Fermentation temperature; 40 °C

Table 4 shows change of moisture content and bacterial counts in RUN 2. The high fermentation temperature gives high moisture content.

The ratios of the total counts for the fermentor 1 and 2 after fermentation to that of before fermentation are 495 and 27.9. No colony appeared from the product in the fermentor 3 because of too much dilution of the sample. According to the calculation, total counts for the fermentor 3 should be less than 9.7×10^{5} cfu/g. It seems that the bacteria in the seed compost will be killed with increase of fermentation temperature. Only thermophilic bacteria can alive at 50 °C.

Table 4 Change of bacterial counts by fermentation in RUN 2

Fermentor	Fermentation Temperature (°C)	Moisture Content (%)	Total Counts(cfu/g) before (calculated)	Total Counts(cfu/g) after
Fer. 1	35	52.2	2.93×10^{6}	1.45×10^{9}
Fer. 2	45	58.3	2.93×10^{6}	8.18×10^{7}
Fer. 3	50	63.0	2.93×10^{6}	$<9.7\times10^{5}$

Seed compost; F-60

3.3 Change of CO2 evolution and C-conversion

Fig. 1 shows Change of CO2 evolution and C-conversion in RUN 1. Peak value of CO2 evolution, time to give peak value of CO2 and C-conversion after 70 hours are shown in Table 5. C-conversion means the ratio of carbon evolved from sludge as CO2 to the

initial value of carbon in the sludge. It is reported that the carbon content in the sludge is about 60 % of the volatile solid. Although this value will change slightly depend on the sludge, this value was used in the calculation of C-conversion.

CO2 evolution is one of the measures to know the state of fermentation. This method is very simple and can monitor the state of fermentation continuously. From the change of CO2 evolution it may be said that the best state of fermentation gives the highest peak of the CO2 evolution in short time and C-conversion should be high. Peak values of CO2 evolution of the fermentor 1 and 2 are slightly higher than the fermentor 3. The peak time is the smallest and the C-conversion is the highest in the fermentor 1. This means that the activity of the seed compost F-60 is the highest. It can also be seen from Table 3 that the total bacterial counts in the product from the seed compost F-60 is the highest compared with other two seed composts.

Table 5 CO2 evolution and C-conversion for RUN 1

Fermentor	Seed compost	Peak value of CO2 evolution (g/hr.kg V.S.)	Peak time of CO2 evolution (hr)	C-conv. after 70 hr (%)
Fer. 1	F-60	29.2	6.5	23.5
Fer. 2	PD-1	29.8	8.8	22.4
Fer. 3	Thomaster A	28.2	8.0	22.9

Fermentation temp.; 40°C

Fig. 2 shows change of CO2 evolution and C-conversion in RUN 2. Peak value of CO2 evolution, time to give peak value of CO2 and C-conversion after 70 hours are shown in Table 6. F-60 was used as the seed compost because of the highest activities.

It can be seen from Tables 5 and 6 that the highest peak value of CO2 evolution and C-conversion and smallest peak time are given at 45 °C. This means that 45 °C is the optimum to decompose the sludge to CO2. Although CO2 evolution is not so small at 50 °C, the total counts after fermentation is very small as can be seen from Table 4. This means that the activity of the bacteria to decompose the sludge is very high. On the other hand, the highest total counts is obtained at 35°C but CO2 evolution is not so large. The seed compost used in this experiments contains various kinds of bacteria. The differences of CO2 evolution and total bacterial counts should be originated from different microflora. It seems that 45 °C is the best tempera-

temperature to decompose sludge and 35 °C is better to get larger amount of biomass. Further experiment is necessary to have more exact conclusion.

Table 6 CO2 evolution and C-conversion for RUN 2

Fermentor	Temp. (°C)	Peak value of CO2 evolution (g/hr.kg V.S.)	Peak time of CO2 evolution (hr)	C-conv. after 70 hr (%)
Fer. 1	35	20.4	8.5	20.9
Fer. 2	45	34.3	5.8	26.9
Fer. 3	50	33.1	7.0	26.4

Seed compost; F-60

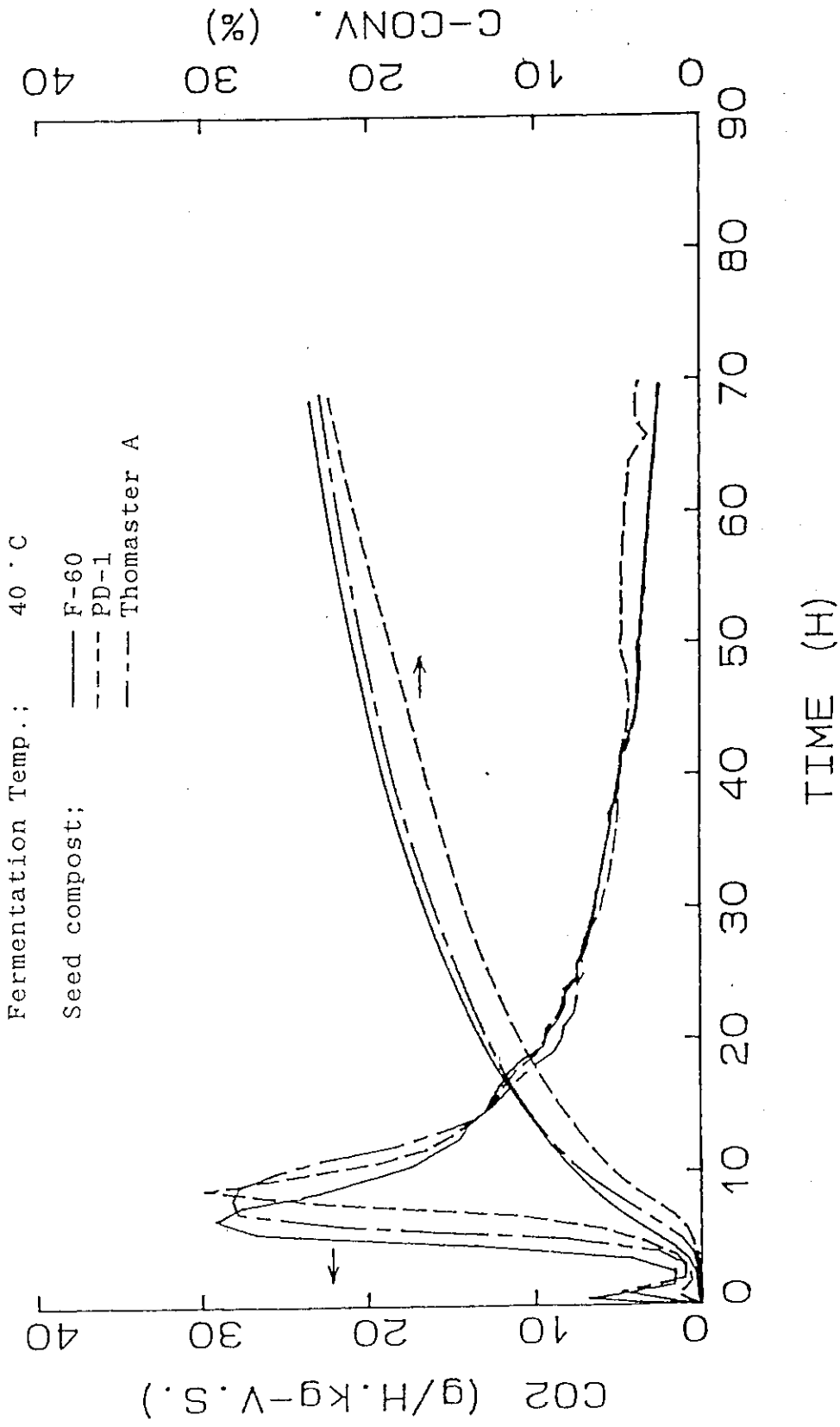

Fig. 1 Change of C02 evolution and C-conversion (RUN 1)

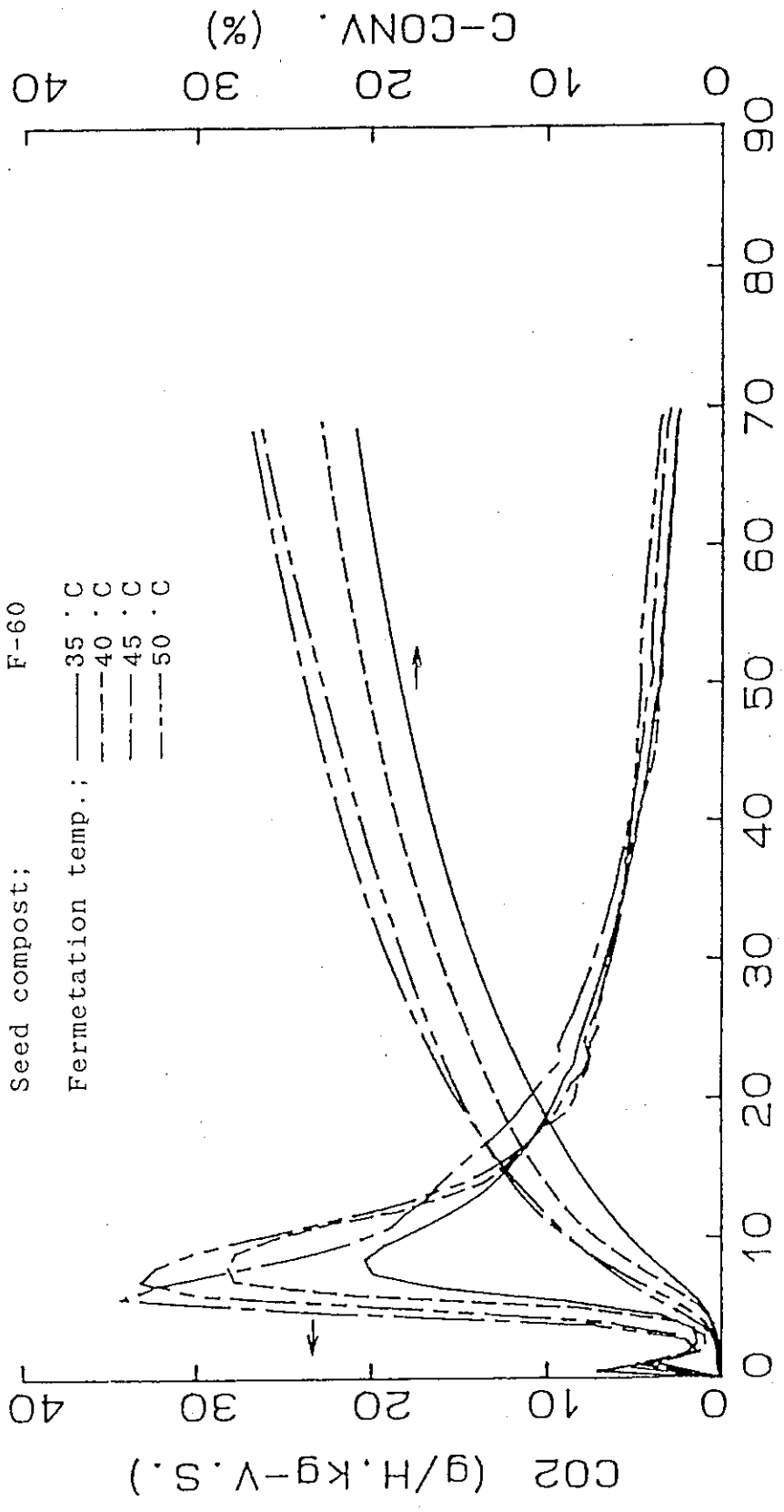

Fig. 2 Change of CO2 evolution and C-conversion (RUN 2)

4.2.3 Decomposition of Irradiated Sludge in Soil

Shoji Hashimoto and Md. M. Hossain*

1. Introduction

Usually, sludge contains various kinds of nutrient for growth of plants and is possible to be used as fertilizer. One of the problems for the application of sewage sludge to farmland is contamination by pathogenic microorganisms. Ionizing radiation is very effective to kill pathogenic microorganisms in sludge. But, even after irradiation, the direct application of sludge is difficult in Japan because of generation of bad odor by anaerobic decomposition of easily decomposable substances in sludge. It is also known that the rapid fermentation of sludge applied directly in soil give a damage to roots of plant.

We have been studying on composting of irradiated sludge by an aerobic fermentation technique for the stabilization of easily decomposable substances in sludge and it was shown that the stabilized compost without pathogenic microorganisms were possible to be obtained in short period by controlling fermentation temperature, pH, aeration and mixing.

But, the direct application of irradiated sludge still has merits to be simple and cost effective and this application method seemed to be also possible if the area and timing of application are properly selected. So, we tried to get data on decomposition of unirradiated and irradiated sludge in soil to know the possibility of direct application to farmland. The results are shown here.

2. Experimental procedure

1) Sludge

Sludge cakes treated by flocculant and dewatered by filter press were collected from sewage treatment facility of the Gunma Prefecture Sewage Works Corporation, Tamamura, Takasaki. Moisture content were ranged from 79.3 to 83.7 % and volatile solid contents were ranged from 83.2 to 84.0 %.

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2) Soil

Soil was collected from the farmland in Takasaki. The soil was mixed well and filtered through mesh No. 12 sieve.

3) Moisture content and Volatile solid content

The moisture contents of sludge, soil and compost products were determined from the loss of the weight after drying at 105 °C until the weight becoming constant. Ratio of the decreased weight to the wet weight of the sludge was calculated in % as moisture content. Determination of volatile solids (organic content) were made by subsequently heating samples to 600 °C for 1 hour. Ratio of the decreased weight to the dry weight of the sludge was calculated in % as volatile solid content.

4) Irradiation

The sludge cakes were put into polyethylene bags. Each bag was sealed and irradiated by cobalt 60 gamma-ray at a dose of 30 kGy.

5) Decomposition of sludge in soil

Both unirradiated and irradiated sludge were used for decomposition tests. Moisture content of the soil used for decomposition was adjusted to be 15 and 20 %. The decomposition temperatures were kept at 20 and 30 °C. Five fermentors, 70 mm in diameter and 60 mm in depth with perforated plate at the bottom to distribute the air from the compressor, were used for the experiment. Each fermentor contained 100 g soil. The amounts of sludge mixed with the soil were 0, 0.3, 0.6, 0.9 and 1.5 g, respectively. These values were calculated on the basis of the application of sludge to land as 1, 2, 3 and 5 tons per 1,000 square meters. The fermentors were embedded in a water bath to control the temperature at 20 and 30 °C during 8 days operation. Aeration was performed with a flow rate of 50 ml/min from the bottom of the fermentor during the fermentation period.

6) Enumeration of microorganisms

For the enumeration of total bacteria and total coliforms, Nutrient agar and MacConkey agar were used as culture media. 5 g of the sample was suspended in 20 ml of sterilized water containing 0.01 % Tween 20 in a sterilized polyethylene bag. The mixture was homogenized with a stomacher Lab-blender 80 for 2 minutes. The slurry was filtered through sterilized four layer gauze. After serial dilution of the filtrate by sterilized water containing 0.01 % Tween 20, 0.2 ml of each liquid was spread in triplicate on the surface of the medium plates. The plates were incubated at 30 °C for 4 days and 37 °C for 20 hours, respectively, for total bacteria and total coliforms.

3. Results and discussion

1) Change of CO_2 evolution

Figures 1 and 2 show the CO_2 evolution rate and peak pattern during the decomposition of unirradiated and irradiated sludge at 30 °C and 15 % moisture content in soil. The main difference between these two figures was in the peak pattern and evolution of CO_2 rate. CO_2 evolution rate for unirradiated sludge was high from the start of decomposition because the unirradiated sludge already contain large population of bacteria. CO_2 evolution for the irradiated sludge increased very rapidly after the start of decomposition because the bacteria in soil can easily grow in irradiated sludge because of no competition.

Microbial activity increased with the addition of increasing amount of sludge to soil and hence maximum CO_2 evolution rate was found in the samples containing sludge at the rate of 1.5 g followed by 0.9, 0.6 and 0.3 g. The peak values of CO_2 evolution during the decomposition were recorded about 57 and 23 g/hr/ton-soil for the mixture of 1.5 g of unirradiated and irradiated sludges with soil. After about 70 hours of decomposition of the irradiated sludge, the value of CO_2 evolution for all the samples were found almost constant and the value became stable after 100 to 150 hours for unirradiated sludge.

The CO_2 evolution at the moisture contents of 15 % and 20 % were almost the same. CO_2 evolutions at 20 °C were very low compared with 30 °C in both cases. The maximum values of peak value of CO_2 evolution at 20 °C were about half of those at 30°C.

2) Change of bacterial counts

Table 1 and 2 show the changes of total bacterial count and total coliforms of the unirradiated and irradiated sludge decomposed for 8 days at 30 °C and 15 % moisture content. The total bacterial counts in unirradiated sludge were almost the same before and after decomposition except the application amount of 1.5 g. But, the increase was large in irradiated sludge.

It was seen from Table 1 that the mixture of unirradiated sludge and soil contains total coliforms and the counts increased one to two orders after decomposition. Coliform was not detected in the mixture of irradiated sludge and soil before and after decomposition as seen from Table 2.

4. Conclusions

1) Irradiated sludge is stabilized more rapidly than unirradiated sludge.
2) Irradiation is very effective to prevent the contamination by pathogenic microorganisms.

3) Population of total bacteria in soil increases by decomposition of irradiated sludge.

Table 1 Change of microorganisms in the mixture of unirradiated sludge and soil

Amount of sludge (g)	Before decomposition (cfu/g)		After decomposition (cfu/g)	
	TB	TC	TB	TC
0	4.1×10^{7}	2.2×10^{3}	2.0×10^{7}	ND
0.3	4.2×10^{7}	3.0×10^{4}	4.2×10^{7}	3.8×10^{5}
0.6	4.3×10^{7}	5.8×10^{4}	6.3×10^{7}	1.2×10^{5}
0.9	4.4×10^{7}	8.6×10^{4}	5.0×10^{7}	1.1×10^{5}
1.5	4.5×10^{7}	1.4×10^{5}	1.4×10^{8}	8.0×10^{5}

TB: Total Bacteria
TC: Total Coliforms
ND: Not detected

Table 2 Change of microorganisms in the mixture of irradiated sludge and soil

Amount of sludge (g)	Before decomposition (cfu/g)		After decomposition (cfu/g)	
	TB	TC	TB	TC
0	5.2×10^{7}	ND	7.4×10^{7}	ND
0.3	5.2×10^{7}	ND	1.0×10^{8}	ND
0.6	5.2×10^{7}	ND	1.1×10^{8}	ND
0.9	5.2×10^{7}	ND	1.3×10^{8}	ND
1.5	5.2×10^{7}	ND	2.1×10^{8}	ND

TB: Total Bacteria
TC: Total Coliforms
ND: Not detected

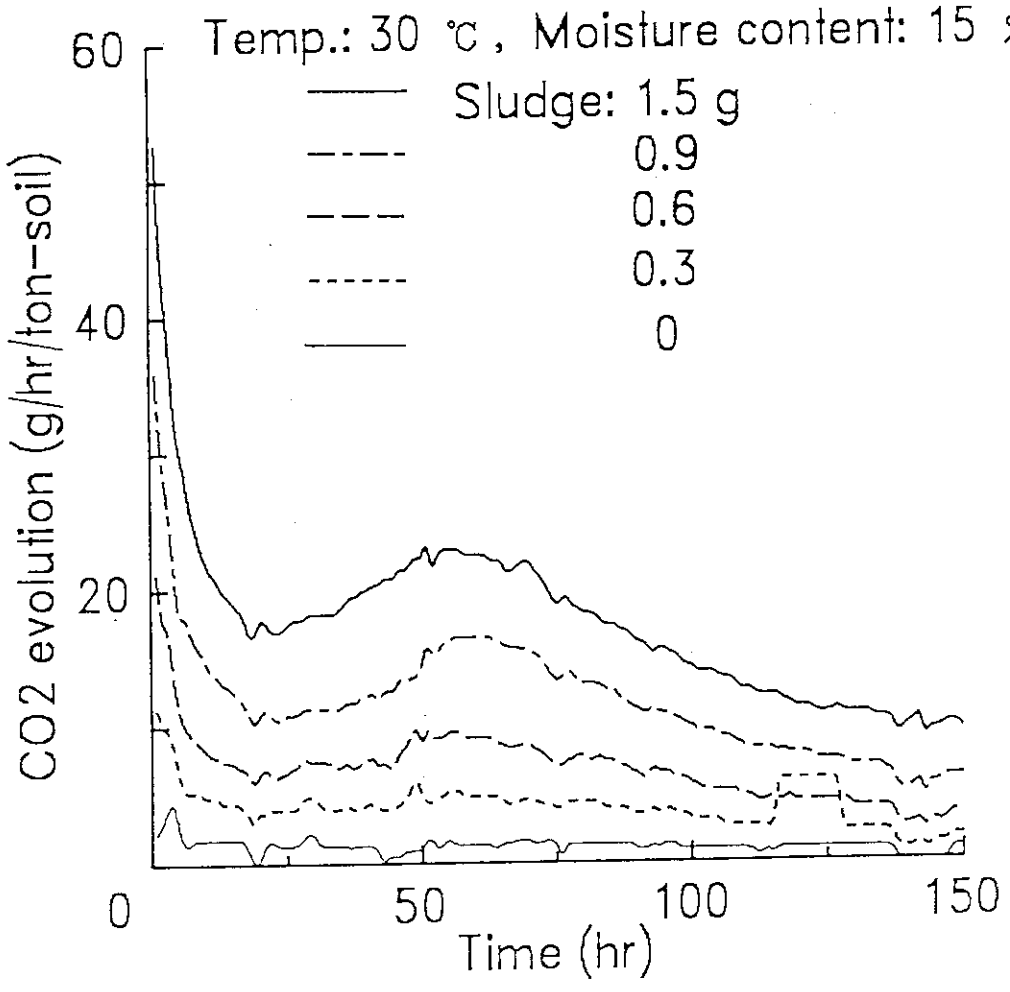

Fig. 1 Decomposition of unirradiated sludge in soil

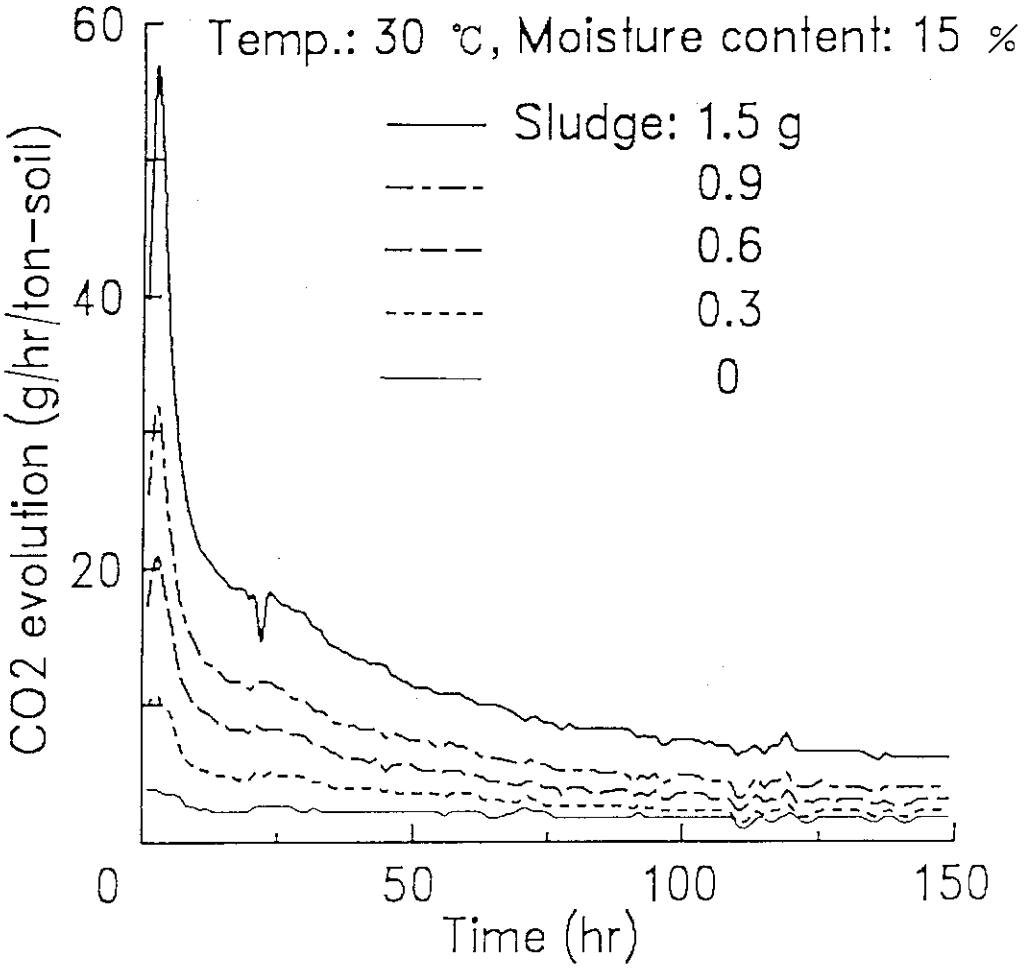

Fig. 2 Decomposition of irradiated sludge in soil

4.3 A preliminary study on upgrading of irradiated sludge to feedstuff

[Abstract]

4.3.1 A preliminary study on upgrading of irradiated sludge for animal feed

Survey of the useful microorganisms for upgrading of the irradiated sludge was performed. Several kinds of molds and yeast were tried to stabilize easily decomposable components in sludge and to increase protein content in the fermented products. optimum condition for the fermentation was also tried to find out in the research.

4.3.2 Upgrading of irradiated sludge to feedstuff

Upgrading of irradiated sludge to feedstuff was reported. It was shown that dried water-hyacinth was suitable as a bulking agent for aerobic fermentation of sludge with fungi. Furthermore, R. oligosporus and A. niger were possible to grow easily in the mixture of sludge and dried water-hyacinth.

4.3.1 A preliminary study on upgrading of irradiated sludge for animal feed

S. Pongpat and S. Hashimoto

1. Introduction

The animal science studies have shown that irradiated sewage sludge is a valuable potential source of nutrients for ruminant animals. Long term (4 years) sludge supplementation of range cattle showed that the positive effects of feed supplementation were about equal with sludge and cottonseed meal. Through almost six years of experiments no significant adverse effects have been noted in laboratory and field studies in which cattle and sheep have been fed with irradiated sludge. In 1975, Miller reported that poultry manure might be upgraded to a high protein feedstuff using fermentation. The purpose of this paper is to make a preliminary investigation of the feasibility on the conversion of irradiated sludge into animal feeds by fungal fermentation.

2. Experimental Procedures

2.1 Apparatus

The fermenter used is a cylindrical glass container (70 mm in diameter, 55 mm in depth) with a perforated plate at the bottom to distribute the air from a compressor. It was covered with silicone stopper directly connected with an exhaust gas carrying unit and a tap water unit. In one set, it was designed to contain six fermenters and each fermenter was separately removable. Those fermenters were embedded in a water bath to control the temperature of fermentation. Air stream from the compressor was sent through two columns which were a calcium chloride and Askarite to remove moisture content and CO_2 in air, respectively. The air was split into seven streams: the first to the sixth supplied through each of fermenter with 50 ml/min of flow rate; the seventh stream for exhaust gas dilution with flow rate of 250 to 500 ml/min.

2.2 Microorganisms and inoculum

Four strains of molds which are commonly employed in industrial fermentation and available in laboratory namely Aspergillus oryzae, Rhizopus oligosporus and 2 strains of A. niger were used as pure culture in fermentation process. The mixed cultures for fermentation, however, were also investigated by using R. oligosporus mixed with a strain of yeasts. Four strains of yeasts to be commonly employed in industrial fermentation and available in the laboratory namely Saccharomyces cereviceae, Candida utilis, C. lipolytica and C. rugosa were studied.

A mold culture was grown on malt yeast extract agar (MYA) medium (malt extract, 10 g; yeast extract, 4 g; glucose, 4 g; chloramphenical, 20 mg; agar[Chameleon] 20 g; distilled water, 1 l [pH 5.5]) at 30 C for 3 days. After washing the spores from the surface of agar plate with distilled water containing 0.01 % Tween 20 and filtering through four-layer gauze, the spore suspension was adjusted to be $1x10^7$ spores/ml as counted by a counting chamber. A half ml of the spore suspension was inoculated into fermentation material as an inoculum for pure culture. A culture of yeast was also grown on malt yeast extract agar medium at 30 C for 20 h. After washing the cells from agar slant with distilled water containing 0.01% Tween 20, the cell suspension was adjusted to be $1x10^7$ cells/ml.

A quarter ml of the yeast cell suspension and a quarter ml of spore suspension of R. oligosporus were inoculated into fermentation materials as an inoculum for mixed culture.

2.3 Fermentation

The sludge cake from bean paste company was used as raw material. Rice bran or perlite was used as bulking agent. The properties of sludge and rice bran were shown in Table 1. The sludge and rice bran were disinfected by gamma irradiation at a dose 30 kGy (dose rate 5.0 kGy/h). The perlite was disinfected by autoclave and dried at 60 C over night. The fermentation materials of each run consisted of 0-20 g of sludge, 0-10 g of rice bran, 0-10 g of perlite and 0.5 ml aliquot of spore suspension (pure culture) or yeast cell and spore suspension (mixed culture). After mixing well, the fermentation materials were transferred into a sterile fermenter, then covered with a sterile stopper unit. After embedding the fermenter in a water bath, the fermentation was started by means of a micro-computer controlling. The operation was stopped when the peak of CO_2 evolution rate became constant as observed from a recorder chart. The fermentations were performed at 30 C for each run except the final run which was performed at 40 C according to optimal growth of seed microorganism.

Table 1 Properties of sludge sample and rice bran

Material	pH	M.C. (%)	V.S. (%)	Total Nitrogen(%)	Crude protein(%)	TBC (cfu/ml)
Sludge	5	81.9	77.2	6.96	43.5	-
Rice bran	-	12.8	90.1	2.36	14.8	$5.08x10^6$

M.C.:Moisture content;V.S.:Volatile solid;TBC:Total bacterial count

2.4 Growth condition study

The investigation for optimal growth condition including optimal pH and temperature of R. oligosporus was carried out. For

optimal pH, the study was conducted by using malt-yeast extract broth medium. The pH of medium was adjusted to be 3.0, 4.0, 5.0, 5.5, and 6.0. A hundred ml of the medium were dispensed into each 500 ml erlenmeyer flask then plugged with silicone and sterilized for 15 min at 15 psi. Four flasks of medium were prepared for each pH level. Each flask was inoculated with 0.5 ml of $1x10^7$ spores/ml of the spore suspension. All flasks were shaken with 100 rpm at 30 C for 24, 48, 72, and 96 h. The growth of each flask was measured by determination of mycelial dried weight (MDW) at each period of incubation. The MDW was determined by filtering of medium through tared filter paper. The filtrate was used to measured the final pH of medium.

A study on optimal temperature for growth of R. oligosporus was performed by using MYA medium. The culture was streaked onto the MYA plate and incubation at 30 C for 3 days. A part of agar which was completely covered with mold was cut into small discs (8.0 mm in diameter). The disc was placed onto the surface of MYA in triplicate at each temperature level. The temperatures were ranged 25 to 50 C. The mold growth was observed from enlargement of disc diameter at various time intervals.

2.5 Analytical procedures

Moisture content and volatile solid in raw materials were determined from the loss of the weight after drying at 106 C until the weight becoming constant. The dried solids were heated at 600 C for 4 h. The loss of weight was calculated as volatile solid. The pH of sludge sample was measured by using pH meter (Horiba compact pH meter C-1).

CO_2 concentration from exhaust gas of each fermenter was continuously measured every 1 h after it was diluted with 250-500 ml/min flow rate air stream.

After harvesting, products were freeze dried. Then, the following analyses were carried out. Total nitrogen and crude protein in the samples were determined by the Kjeldahl method. The soluble protein was determined by Bio-Rad protein assay.

Moisture content in the products was determined both before and after freeze dry condition by the mentioned method.

3. Experimental Results

3.1 Growth of molds and yeasts in mixture of irradiated sludge and rice bran

3.1.1 Pure culture

Four strains of molds were tested to cultivate in the mixture of irradiated sludge and rice bran at the ratio of 80/20. In order to select the strain that can easily grow in this mixture, growth and activity of molds during cultivation were measured. Table 2 shows change of mold count after cultivation in the mixture. Peak value of CO_2 evolution, peak time of CO_2 evolution and C-conversion after 80 h were also shown in this table. After cultivation for 4 days, the count of each mold was

found increasing from the order of 10^4 cfu/ml to 10^7-10^8 cfu/ml and A. niger strain 2 showed the lowest count comparing with the other three strains. It was not able to select the mold strain that showed the highest growth among the other three strains because the counts were not remarkably different. The consideration was pointed to the activity of microorganism during degradation of substrate. The run of R. oligosporus showed the highest peak value of CO_2 evolution in the shortest time and C-conversion in a certain period was high. This means R. oligosporus showed the highest activity. R. oligosporus, therefore, seems to be more attractive than the others. It was, then, selected to study for cultivation as mixed culture.

Table 2 Growth of molds in the mixture of irradiated sludge and rice bran

Mold strain	Mold count(cfu/ml) before cultivation	Mold count(cfu/ml) after cultivation	Peak value of CO_2 evol. (g/h.kg.V.S.)	Peak time of CO_2 evol.(h.)	C-conv. after 80 hr
A. niger 1	5.47×10^4	4.57×10^7	43.7	22.3	8.7
A. niger 2	9.75×10^4	1.25×10^8	44.6	21.3	8.7
A. oryzae	3.60×10^4	1.70×10^8	50.3	17.6	8.8
R. oligosporus	1.57×10^4	3.34×10^8	56.6	14.9	11.6

3.1.2 Mixed culture

Mixed cultures of R. oligosporus and yeasts were also attempted to cultivate in the mixture of irradiated sludge and rice bran. Four strains of yeasts were studied in order to select an appropriate seed and product. Table 3 shows growth of R. oligosporus and yeasts in the mixture of irradiated sludge and rice bran. Peak value of CO_2 evolution, peak time of CO_2 evolution and C-conversion after 40 h were also shown in this table. After cultivation for 4 days, it was found that the counts of each run were not remarkably different as same as found in the case of pure culture. However, the run of mixed culture between R. oligosporus and C. lipolytica showed the highest peak value of CO_2 evolution in the shortest time and the highest C-conversion after 40 h. This means the highest activity of the seed in this run was obtained. Although this seed seems to be very interesting, the property of the product made from this seed should be considered in order to make the decision for seed selection.

Table 3 Growth of mold and yeasts in the mixture of irradiated sludge and rice bran

Microorganism	Count(cfu/ml) before cultivation	Count(cfu/ml) after cultivation	Peak value of CO_2 evol. (g./h.kg.V.S.)	Peak time CO_2 evol.(h)	C-conv. after 40hr(%)
R. oligosporus	3.74×10^4	7.60×10^7	50.5	13.5	33.8
and C. utilis	7.63×10^4	3.09×10^8			
R. oligosporus	3.24×10^4	4.56×10^7	51.8	14.3	36.8
and S. cereviceae	3.27×10^4	2.12×10^7			
R. oligosporus	2.32×10^4	2.81×10^7	43.4	14.8	31.6
and C. rugosa	1.02×10^4	1.46×10^7			
R. oligosporus	2.05×10^4	2.31×10^7	59.0	13.3	45.0
and C. lipolytica	4.10×10^4	5.17×10^6			

3.1.3 Comparison of products

Table 4 shows the properties of the products from fermentation of irradiated sludge and rice bran with various microorganisms. Among 8 runs of fermentation, the highest total nitrogen and crude protein were observed in the product from the run of A. niger 2. The product from the run of R. oligosporus also showed total nitrogen and crude protein contents almost the same as that of A. niger 2. However, R. oligosporus showed the highest activity as reported above. This means it was able to grow rapidly and vigorously in the substrate.

In case of mixed culture, it was expected that the protein content in the product should be higher than that of pure culture. From this experiment, however, the protein content in both cases were found approximately the same. These might be the results of the growth competition between R. oligosporus and yeasts during fermentation.

Table 4 Properties of the products from fermentation of irradiated sludge and rice bran by various seeds

Microorganism	Total nitrogen(%)	Crude protein(%)	Soluble protein(%)	M.C. (%)	V.S. (%)
A. niger 1	5.84	36.5	0.76	74.9	80.8
A. niger 2	6.06	37.9	0.77	76.1	79.1
A. oryzae	5.71	35.7	0.65	76.4	80.8
R. oligosporus	5.94	37.1	0.43	75.7	77.8
R. oligosporus and C. utilis	5.73	35.8	0.71	75.1	78.0
R. oligosporus and S. cereviceae	5.67	35.4	0.62	76.0	79.1
R. oligosporus and C. rugosa	5.88	36.8	0.53	75.4	77.8
R. oligosporus and C. lipolytica	5.86	36.6	0.70	77.6	77.7

3.2 Effect of rice bran on fermentation rate

Table 5 shows effect of rice bran on fermentation rate. The amount of rice bran was designed to be in the range 0-50 % and 100 %. The fermentation rate was observed from peak value of CO_2 evolution. It was found that the peak value of CO_2 evolution was increased as the amount of rice bran increased. When the rice bran was used at 100 % for fermentation, no sludge added, the peak value of CO_2 was remarkably decreased. This means that rice bran was useful to the fermentation process. It may be a source of growth factors for R. oligosporus. However, the ratio of 80/20 was selected in this experiment because the sludge was considered as the major material of the process.

Table 5 Effect of rice bran on fermentation rate

Amount of rice bran (%)	Peak value of CO_2 evolution (g./h.kg.V.S.)
0.0	4.0
5.0	13.9
10.0	27.8
20.0	56.6
30.0	94.7
40.0	99.3
50.0	144.2
100.0	25.4

3.3 Effect of moisture content on fermentation rate

The moisture content in the mixture of fermentation materials was adjusted to be in the range 30-70 %. The fermentation rate was also observed from peak value of CO_2 evolution. Table 6 shows effect of moisture content on fermentation rate. The fermentation rate was found remarkably in creasing as moisture content of fermentation materials increased. The ratio of sludge (81.9% M.C.) and rice bran (21.8% M.C.) in fermentation material was designed to be 4:1 which gave 70 % of moisture content in the mixture. This seems to be an appropriate ratio of fermentation materials. The reason was due to the sludge and rice bran were able to mix directly without any adjusting of moisture content. pH of the mixture still remained around 5.

Table 6 Effect of moisture content on fermentation rate

Run No.	Moisture content(%)	Peak value of CO_2 evolution (g./h. kg.V.S.)
1	30	17.4
2	40	30.9
3	50	46.0
4	60	54.8
5	70	58.7

3.4 Optimal growth condition of R. oligosporus

Fig. 1 shows effect of pH on mycelial dried weight of R. oligosporus. As shown in this figure, the optimum pH of R. oligosporus was around 5.0.

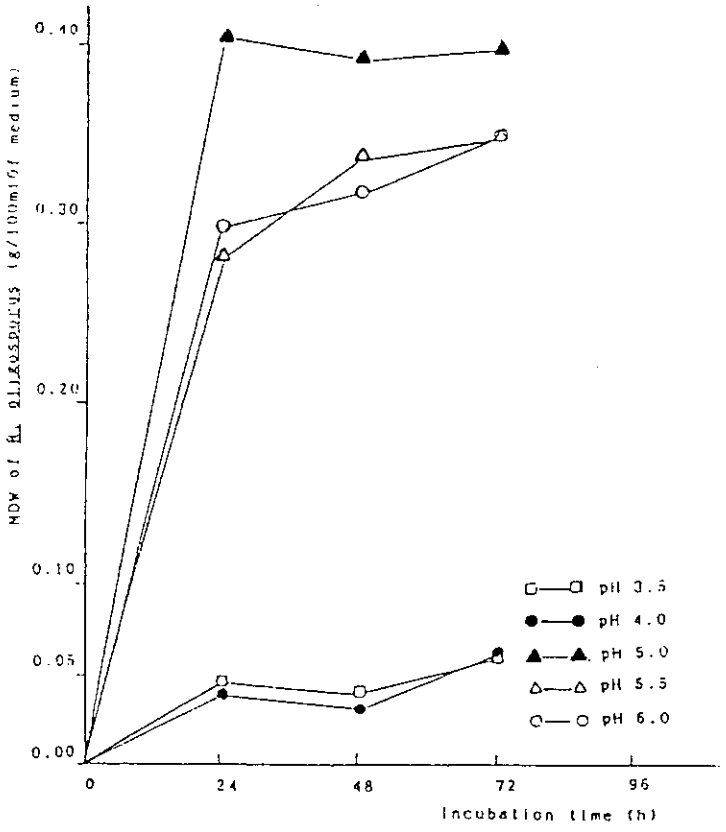

Fig. 1 Effect of pH on mycelial dried weight (MDW) of R. oligosporus

pH 5.0 was then selected to study on effect of temperature on growth of R. oligosporus. Fig. 2 shows effect of temperature on growth of R. oligosporus. The enlargement of mold disc represented the mold growth. It was found that the optimum temperature ranged 35-40 C.

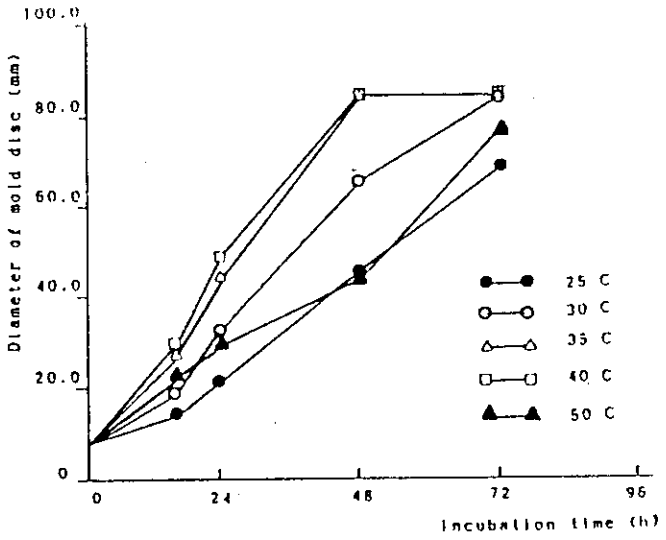

Fig. 2 Effect of temperature on growth of R. oligosporus

3.5. Properties of fermentation material and product

Table 7 shows total nitrogen, crude protein and soluble protein in different materials. The protein content in fermented product was found increasing 9-10 % higher than the mixture before fermentation. However, it was still about 5% lower than that of sludge sample.

Table 7 Property comparison of materials and fermented product

Material	Total nitrogen(%)	Crude protein(%)	Soluble protein(%)	M.C. (%)	V.S. (%)
Sludge	6.96	43.5	-	81.9	77.2
Rice bran	2.36	14.8	0.37	12.8	90.1
Mixture	4.68*	29.3*	-	-	-
Fermented product	6.19	38.7	0.33	73.5	78.9

* Calculated; M.C.: Moisture content; V.S.: Volatile solid

Table 6 Effect of moisture content on fermentation rate

Run No.	Moisture content(%)	Peak value of CO_2 evolution (g./h. kg.V.S.)
1	30	17.4
2	40	30.9
3	50	46.0
4	60	54.8
5	70	58.7

3.4 Optimal growth condition of R. oligosporus

Fig. 1 shows effect of pH on mycelial dried weight of R. oligosporus. As shown in this figure, the optimum pH of R. oligosporus was around 5.0.

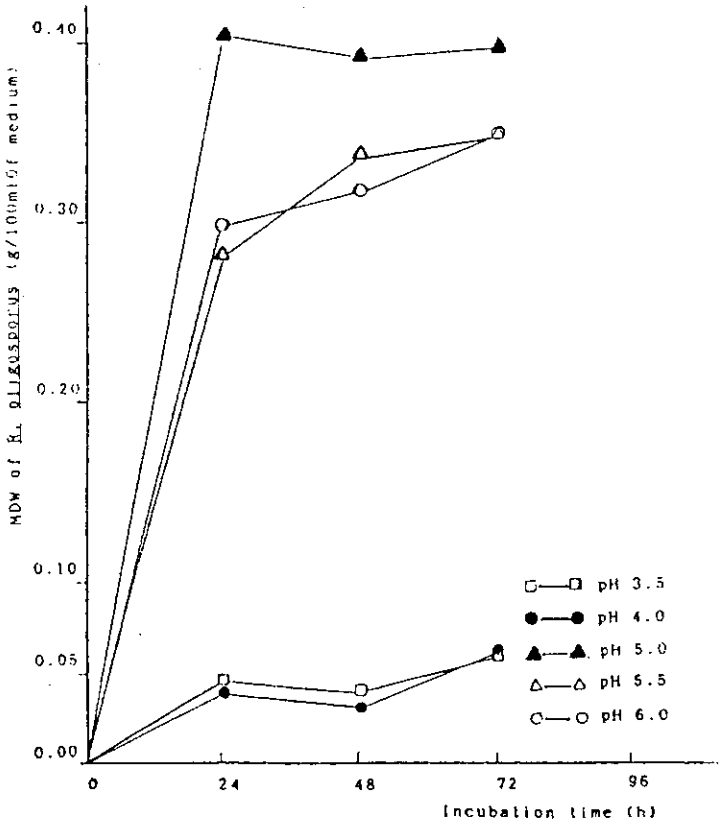

Fig. 1 Effect of pH on mycelial dried weight (MDW) of R. oligosporus

4. Conclusion

According to sludge recycling objectives, the sludge to rice bran ratio of 80/20 was preferably selected in this experiment. It must be also noted that the protein content in original sludge sample is rather high because of collecting from bean paste company. From this experiment, the results showed that protein contents in final product and original sludge were not significantly different. However, it was recognized that the protein content in end product was found increasing 9-10 % higher than that of the mixture before fermentation. Due to the limitation of sludge and strain of microorganism used, at this stage, this method is able to upgrade the sludge by increasing the protein content up to 10 % and stabilizing easily decomposable components in sludge. The author recommended that the further studied should be carried on not only variation among types of sludge, bulking agent and microorganism but also quality of protein in the product. With proper processes, it seems that this technique is able to upgrade the irradiated sludge successfully in the future.

4.3.2 Upgrading of irradiated sludge to feedstuff

N. Sermkiattipong and S. Hashimoto

1. Introduction

Sludge contains various kinds of nutrient not only for growth of plants but also for growth of fishes and animals. Although most of sludges from sewage treatment facility is not suitable as raw material for feedstuff because of high concentration of heavy metals such as mercury, cadmium, chromium, etc. But it is expected that the concentration of heavy metals in sludges from food industries are low. Even in this case, sludges have possibility to cause pathogenic contamination if supplied without any treatment. Ionizing radiation is very effective for disinfection of pathogens in sludges. Moreover, sludge usually contains easily decomposable organic substances and often generate bad odor originating from anaerobic fermentation. So, a stabilization of those substances are also seemed to be necessary. In this paper, results of preliminary study on growth of fungi for the stabilization of sludge by aerobic fermentation using dried water-hyacinth as bulking agent are shown.

2. Experimental procedure

1) Sludge

Activated sludge cake dewatered by filter press was collected from Hanamaruki Foods Incorporation. Moisture content of the sludge was 77.7 %. The value of pH was 5.0 and volatile solid content measured after heating at 600 °C for 2 hours was 78.0 %. Total bacterial count in the sludge was determined to be 9.7×10^6 cfu/g.

2) Dried water-hyacinth

Water-hyacinth collected from Kasetsart University in Thailand was washed with tap water and exposed to sun light and dried. Afterwards, it was ground to be powder less than 9 mesh. Moisture content of dried water-hyacinth was 12 % and volatile solid content was 85.2 %. The total bacteria in dried water-hyacinth was 1.8×10^9 cfu/g.

3) Gamma-ray irradiation

Twenty grams of sludge cake was put into a polyethylene bag and irradiated at 30 kGy. Two and a half grams of dried water-hyacinth was wrapped with aluminum foil and put in a polyethylene bag. Then irradiated at 42 kGy.

4) Optimum growth temperature for seed fungi

Three strains of fungi, namely, Rhizopus oligosporus, Aspergillus niger and Coprinus cinereus, were used as seeds. Pure culture of each strain was grown on Potato Dextrose Agar (PDA) plate for 3 days for R. oligosporus and A. niger and 5 days for C. cinereus at 30 °C. Discs with 8 mm diameter covered with mycelium of R. oligosporus and C. cinereus were cut out from the agar plates and put on the center of PDA plates as seeds. In case of A. niger, spores were transferred by a needle to the center of PDA plate. After the inoculation, all the plates were incubated at various temperatures ranged from 25 to 45 °C. Growth of fungi was observed by measuring the diameter of colonies at various time intervals.

5) Preparation of seed fungi for fermentation

Pure culture of R. oligosporus and A. niger was grown on each PDA plate at 30 °C for 3 to 4 days. After spores were noted, each fungus was transferred to 0.01 % Tween 20 and mixed well. The suspension of mycelium and spores of fungus were filtrated by four sheets of gauze so that the filtrate contained only spores. Then the spore suspension was adjusted by dilution with sterilized water to be about 1.36×10^7 to 1.88×10^7 spores/ml for fermentation seed.

In case of C. cinereus, this mushroom was grown on PDA at 30 °C for 6 days. After the mycelium covered over the plate, 2 discs with a diameter of 6 mm were cut out by sterilized cork borer and the mycelium on the disc was separated from the part of agar. Then the small discs of mycelium were cut into tiny pieces and used as seed for the fermentation.

6) Fermentation

Experimental apparatus for fermentation was the same as reported before. The fermentor used was a cylindrical glass filter (70 mm in diameter, 55 mm in depth) with a perforated plate at the bottom. The irradiated sludge was mixed with dried water hyacinth and perlite. After adjusting moisture content to 65 % with sterilized water, the mixture was put into the fermentor.

Aeration was carried out from the bottom of the fermentor and CO_2 concentration in exhaust gas was continuously monitored during fermentation period.

3. Results and discussion

1) Optimum growth temperature for seed fungi

During incubation, diameter of fungus increased with time. Growth of R. origosporus, A. niger and C. cinereus at various temperatures are shown in Fig. 1, Fig. 2 and Fig. 3. It can be seen from these figures that the temperatures to give the maximum growth of R. origosporus, A. niger and C. cinereus were 40, 35 and 40 °C, respectively. The optimum temperature for each fungus was used in the fermentation study of mixture of sludge and dried water hyacinth.

2) Fermentation of the mixture of sludge and dried water-hyacinth

Fig. 4 shows CO_2 evolution curves for R. oligosporus at various mixing ratio of sludge and dried water-hyacinth. CO_2 evolution increased initially and then decreased in all the cases. The peak values of CO^2 evolution from the first to fourth fermentors were not so different. The times to attain the peak values (peak time) were slightly short for the first and second fermentors compared with those for third and fourth fermentors. The peak value of CO^2 evolution was small in the fermentation without dried water-hyacinth even perlite was used as bulking agent.

Patterns of CO_2 evolution of A. niger for various mixing ratio are shown in Fig. 5. The CO_2 evolution curves of the first and second fermentors showed the largest peak values within short time among the five fermentors. Although the peak value for the third fermentor also showed the almost the same value as these two fermentors, peak time was slightly longer. In this case, also the peak value was small and peak time was long in the fermentation without dried water-hyacinth.

It could be seen from Figures 4 and 5 that R. oligosporus and A. niger grew very well in the mixture of sludge and dried water-hyacinth and were suitable as seed for aerobic fermentation.

Fig. 6 shows CO_2 evolution curves for C. cinereous. It took more than 50 to 100 hours to start CO_2 evolution when the mixture contained sludge. Even without sludge, the peak value was less than half of those for R. origosporus and A. niger and the peak

time was about 4 times longer compared with other seed fungi. It could be seen from this figure that C. cinereus was not suitable as a seed for aerobic fermentation of sludge.

4. Conclusions

1) Dried water-hyacinth is suitable as a bulking agent for aerobic fermentation of sludge with fungi.

2) R. oligosprus and A. niger are possible to grow easily in the mixture of sludge and dried water-hyacinth.

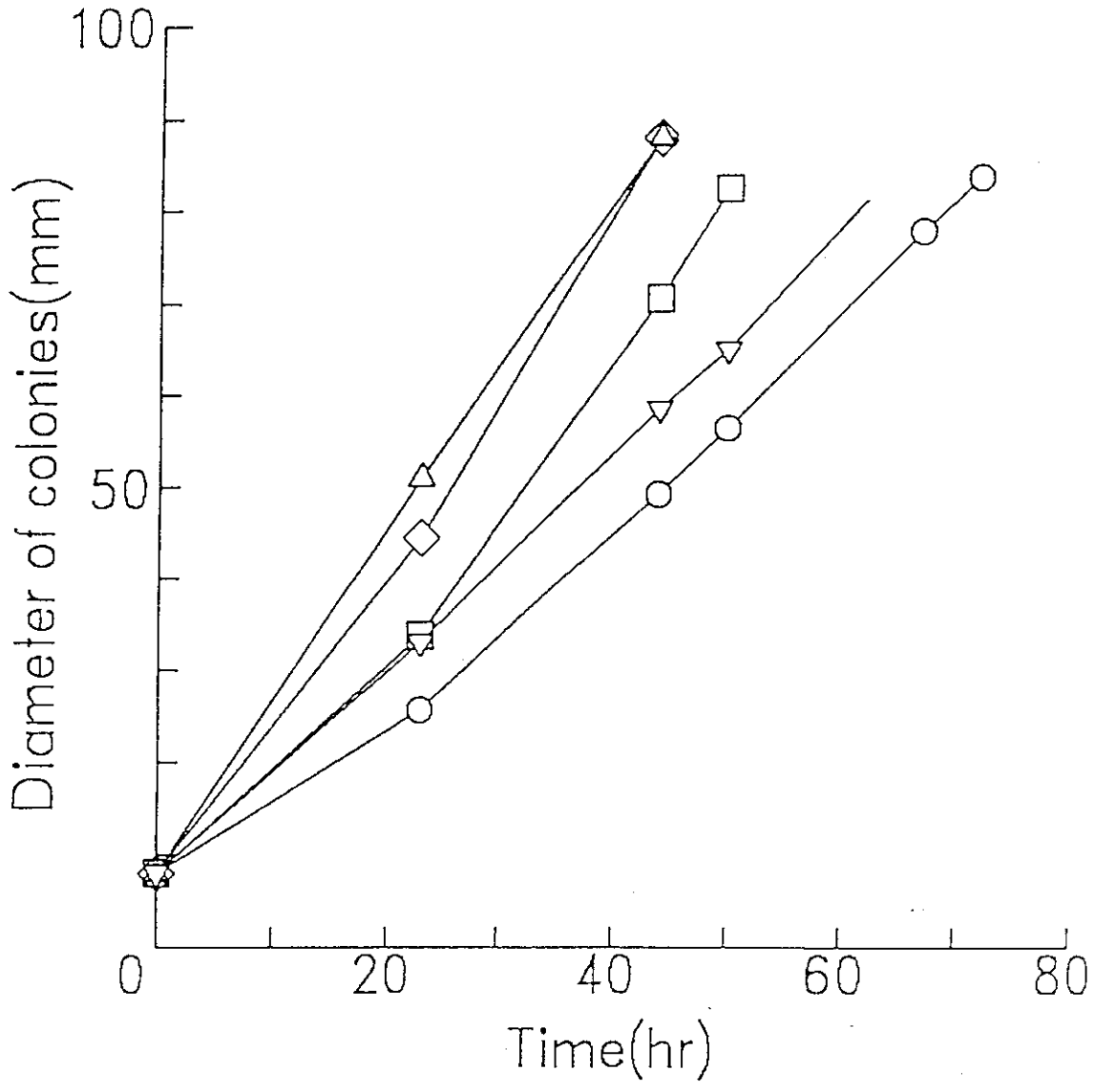

Fig. 1 Growth of R. oligosporus at various temperatures on PDA: (○) 25°C, (□) 30°C, (◇) 35°C, (△) 40°C, (▽) 45°C.

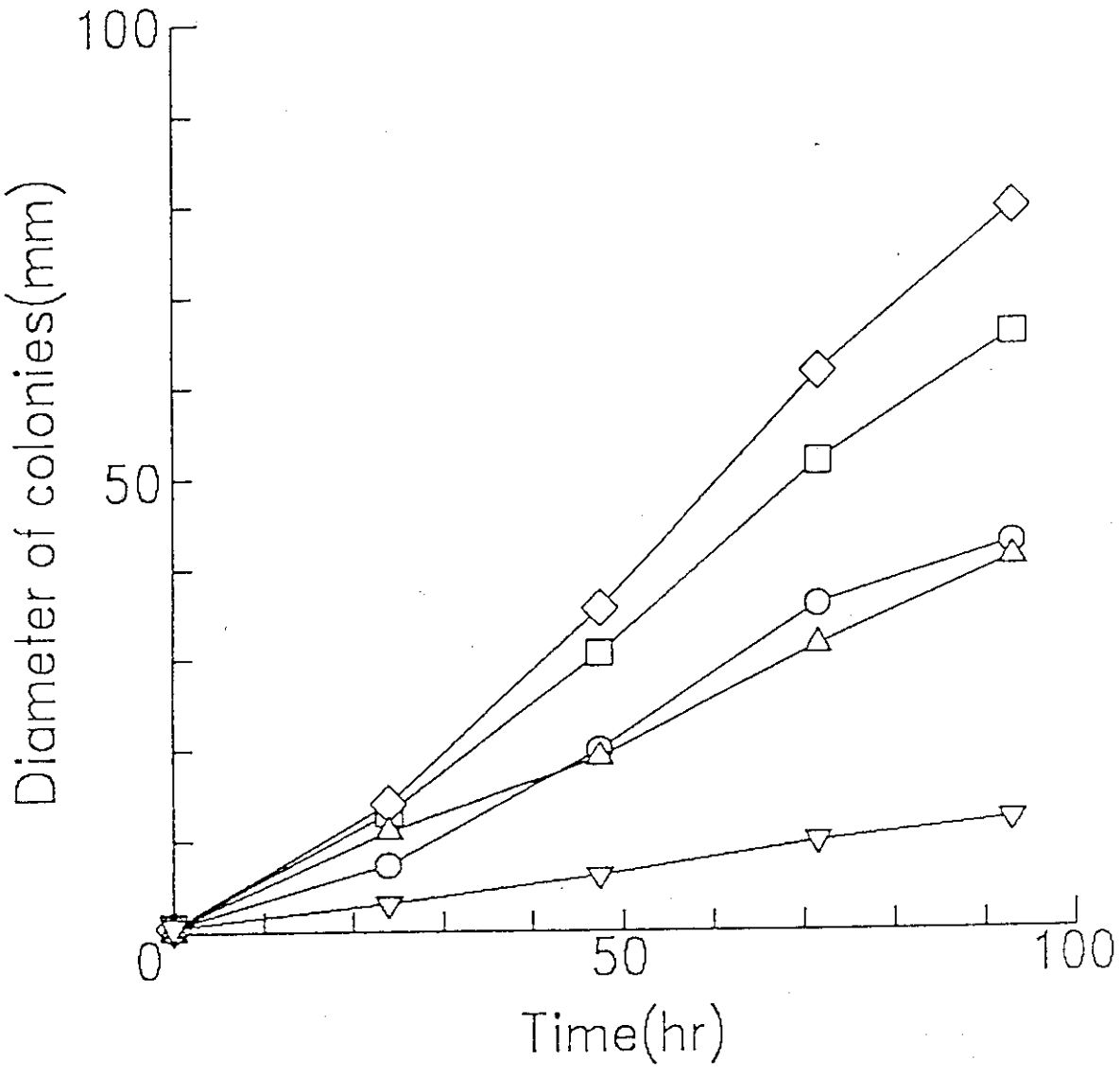

Fig. 2 Growth of A. niger at various temperatures on PDA:
(○) 25°C, (□) 30°C, (◇) 35°C, (△) 40°C, (▽) 45°C.

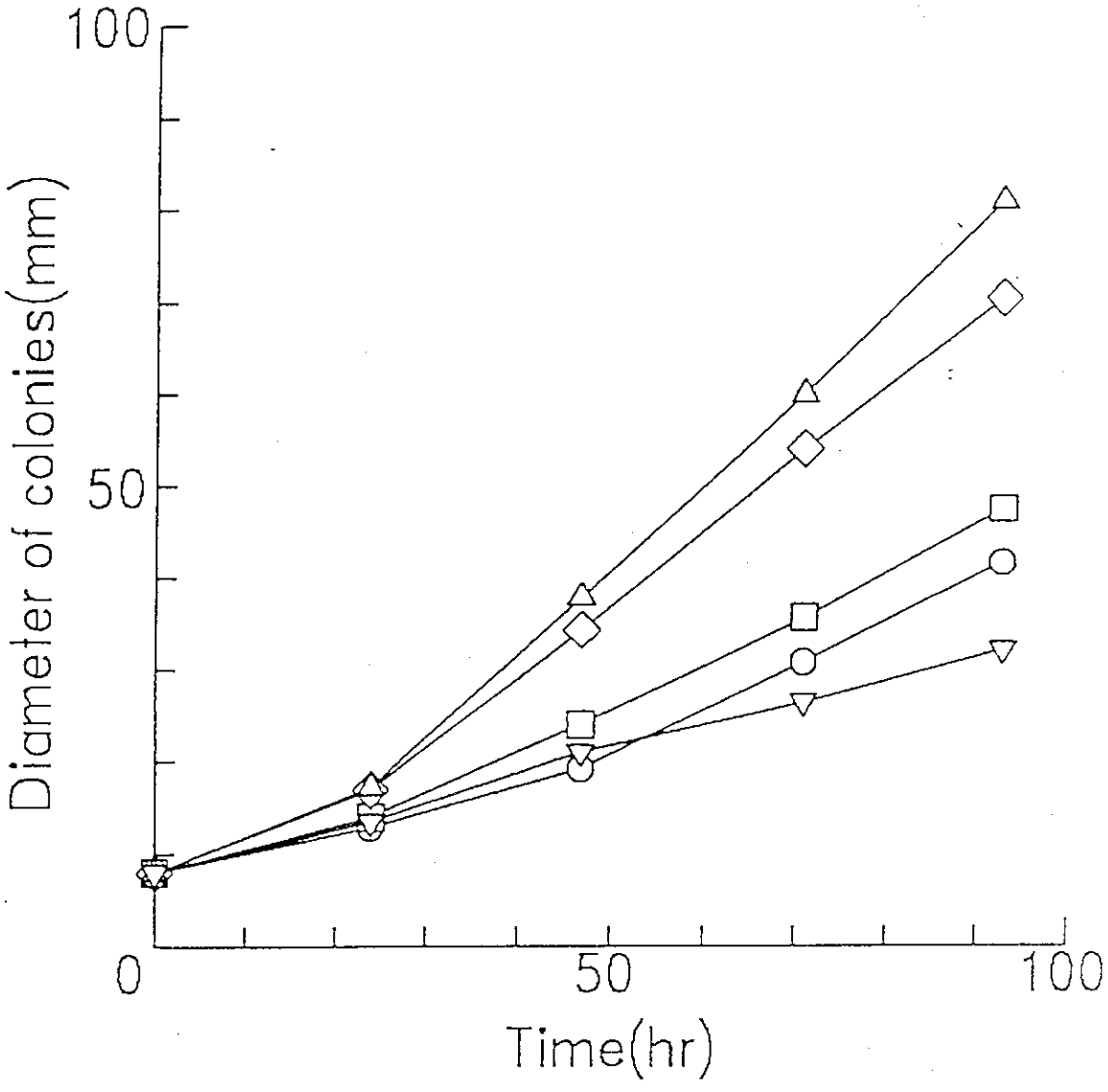

Fig. 3 Growth of C. cinereus at various temperatures on PDA: (○) 25°C, (□) 30°C, (◇) 35°C, (△) 40°C, (▽) 45°C.

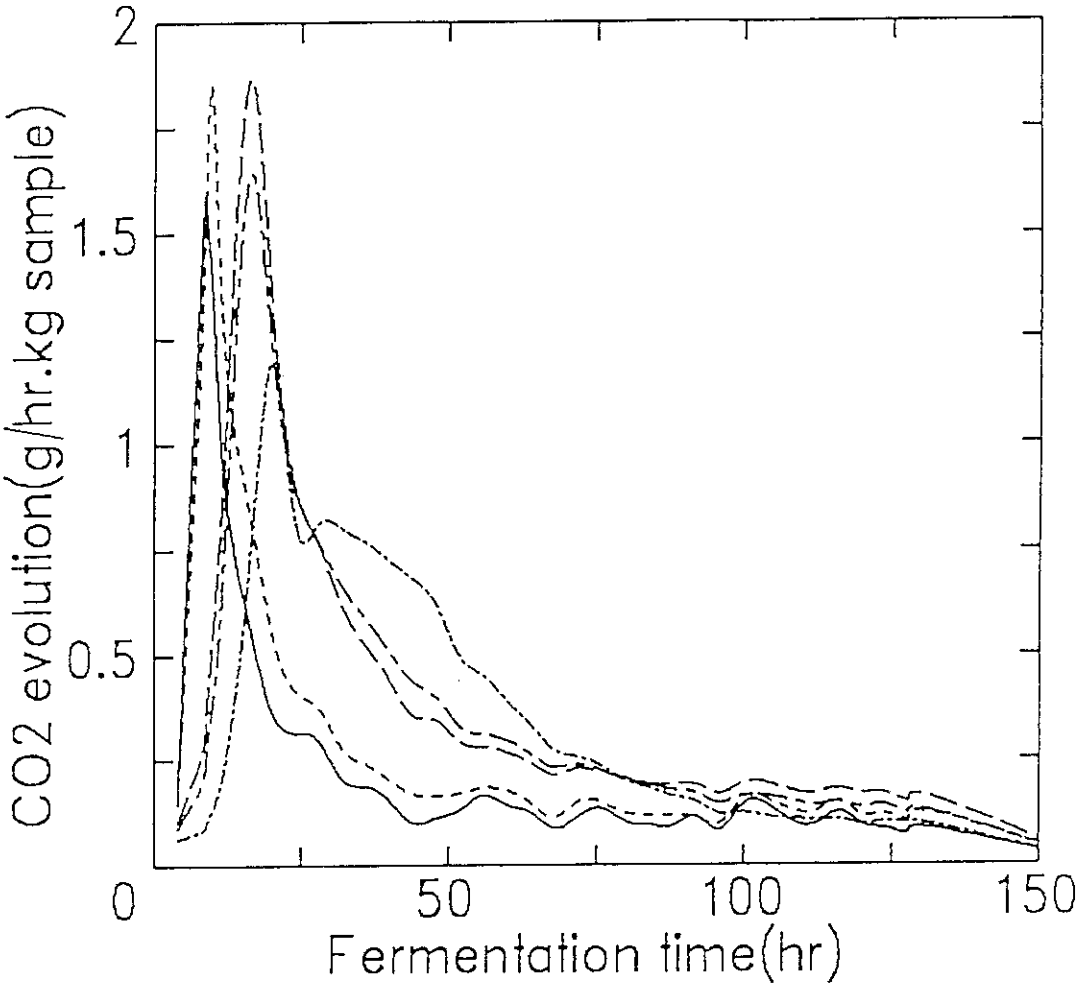

Fig. 4 CO_2 evolution curves of R.oligosporus at various mixing ratio of sludge and dried water hyacinth

——— fermentor 1: sludge 1.67 g + dried water hyacinth 5 g + water 6.97 ml

- - - - fermentor 2: sludge 5 g + dried water hyacinth 5 g + water 5.76 ml

— — fermentor 3: sludge 20 g + dried water hyacinth 5 g

— — — fermentor 4: sludge 20 g + dried water hyacinth 2.5 g + perlite 1.869 g

—·—·— fermentor 5: sludge 20 g + perlite 3.9 g

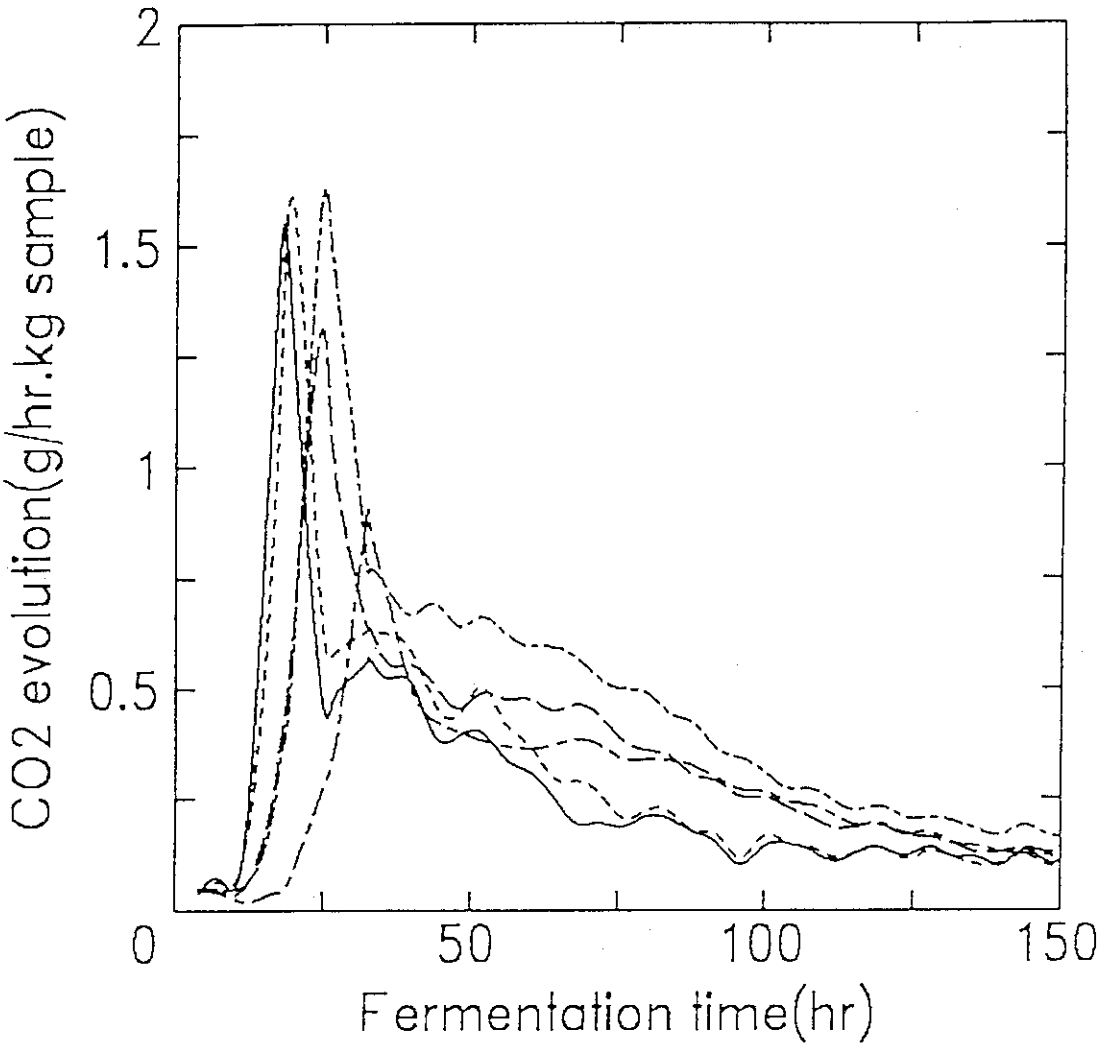

Fig. 5 CO_2 evolution curves of A.niger at various mixing ratio of sludge and dried water hyacinth

———— fermentor 1: sludge 1.67 g + dried water hyacinth 5 g + water 6.97 ml

- - - - - - fermentor 2: sludge 5 g + dried water hyacinth 5 g + water 5.76 ml

- - - - - - - fermentor 3: sludge 20 g + dried water hyacinth 5 g

— — — fermentor 4: sludge 20 g + dried water hyacinth 2.5 g + perlite 1.869 g

- — - — fermentor 5: sludge 20 g + perlite 3.9 g

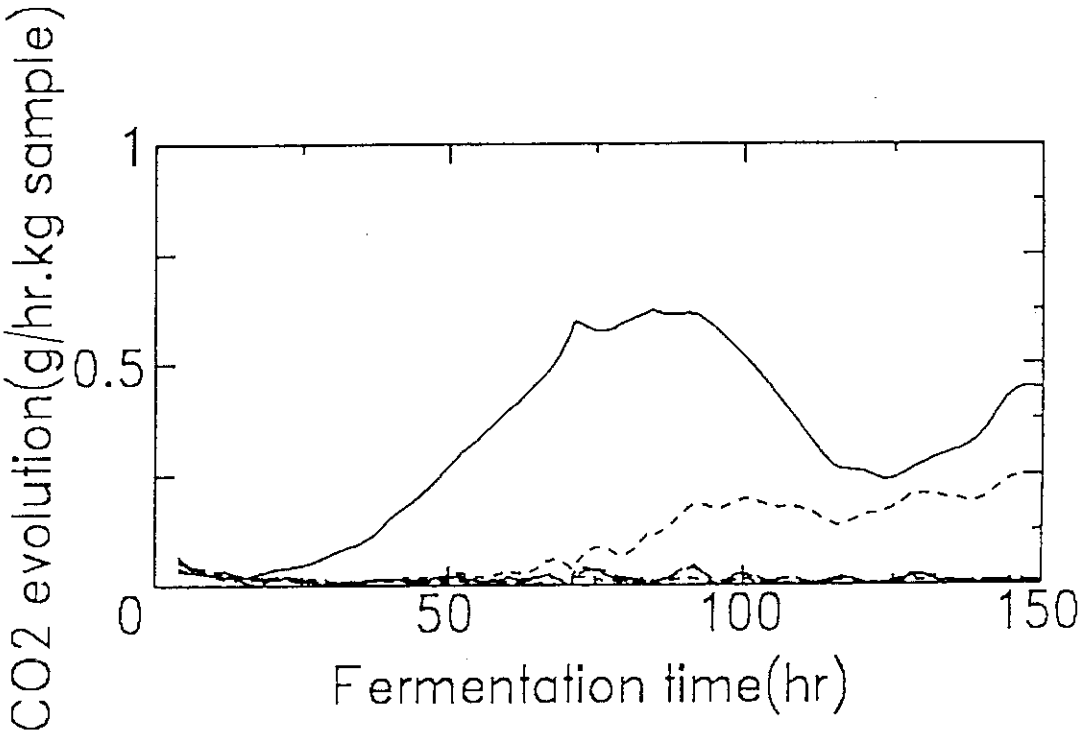

Fig.6 CO_2 evolution curves of C.cinereus at various mixing ratio of sludge and dried water hyacinth

———— fermentor 1: dried water hyacinth 5 g + perlite 5 g + water 16.86 ml

- - - - - - fermentor 2: sludge 1.67 g + dried water hyacinth 5 g + water 6.97 ml

— — — fermentor 3: sludge 5 g + dried water hyacinth 5 g + water 5.76 ml

- — - — fermentor 4: sludge 10 g + dried water hyacinth 2.5 g

..... fermentor 5: sludge 20 g + dried water hyacinth 2.5 g + perlite 1.869 g

4.4 Cultivation of microorganisms for suppression of plant diseases

[Abstract]

4.4.1 A study on suppressive bacteria to phytopathogenic fungi

Isolation and identification of suppressive bacteria from soil and commercial seed composts were conducted. The strain received from Tokyo Institute of Technology and many isolated strains were tested for clear zone inhibitory to phytopathogenic fungi. Optimum conditions for growth of these suppressive bacteria in both culture media and irradiated sludge were also studied. Lastly, effect of inoculum size on growth of suppressive bacteria was carried out.

4.4.2 Cultivation of Bacillus subtilis N4 in grass and sludge

Cultivation of Bacillus subtilis N4 in grass and sludge was reported. Gamma-ray irradiation at 10 kGy seemed to be enough to inactivate bacteria in grass waste and the growth of the bacteria in sludge was very well compared with the grass waste because the sludge contained much nutrients.

4.4.1 A study on suppressive bacteria to phytopathogenic fungi

N. Sermkiattipong, S. Hashimoto and S. Sato

1. Introduction

Sewage sludge contains enough nutrient necessary for growth of bacteria and, especially, some bacteria can grow very well in irradiated sludge because of no competition. Bacteria useful for suppression of Fusarium oxysporum f. sp cucumerinum (pathogenic fungi of cucumber) and Giberella fujikuroi (pathogenic fungi of rice) were selected and used as seed. A study on cultivation condition of the suppressive bacteria in nutrient broth and irradiated sludge was also performed.

2. Experimental Procedures

2.1 Irradiated sludge

Sewage sludge cake was collected from Ken-ou Water Purification Center near TRCRE, JAERI. Moisture content and volatile solid content were 77.3 and 80.8 %, respectively. Twenty grams of sludge cake was put into polyethylene bag. Each bag was sealed and irradiated at 30 kGy by Co-60 gamma ray.

2.2 Isolation of suppressive bacteria

Five grams of soil sample collected from Takasaki Radiation Chemistry Research Establishment was put into a sterilized polyethylene bag. Approximately 18 ml of sterilized 0.01% tween 20 was added to the bag and homogenized with a Stomacker Lab Blender-400 for 1 min. The suspension was diluted serially tenfold dilutions for 2 times in 0.01% tween 20 and then mixed well with 1 ml of spore suspension of Fusarium oxysporum containing 5.75×10^8 spores. Afterwards 0.2 ml of the suspension was spread duplicately on the surface of nutrient agar plate and potato dextrose agar plate. The plates were incubated at 30°C for 7 days and observed for suppressive effect on the agar plates. Bacterial colonies that showed a significant effect on the nutrient agar or potato dextrose agar plates against phytopathogenic fungi (Fusarium oxysporum) were isolated into pure cultures using nutrient agar and potato dextrose agar as the isolating media.

2.3 Identification of suppressive bacteria

Morphological and various biochemical characteristics of suppressive bacteria to phytopathogenic fungi were determined according to Bergy's Manual of Systematic Bacteriology.

2.4 Bacterial strains

Most of the bacterial strains were isolated from soil and commercial seed composts. B. subtilis NB 22 was obtained from Tokyo Institute of Technology.

2.5 Suppressive effect

Amount of 0.1 ml of spore suspension of each mold F.oxysporum and G. fujikuroi (10^7 spores/ml) was spread on potato dextrose agar plate. Cells of each isolated bacterium were spotted by needle on the center of the plates and cultivated at 30°C for 5 days. Diameters of colony and inhibition zone (clear zone) were measured.

2.6 Factor effect on growth of bacteria in nutrient broth

Bacillus subtilis NB22 and strain O 1-1 were used in this study. Optimum pH and temperature for growth of bacteria in nutrient broth were conducted. Spore suspension of each bacterium was adjusted to be 10^4 cfu/ml and 0.5 ml of suspension was transfered to each aeration bottle containing 100 ml of nutrient broth at different pH. The bottles were incubated at 30°C for 18 hr. In the case of study on effect of temperature, the bottles were incubated at different temperatures for 18 hr and pH of nutrient broth was adjusted to be 7. Cell growth was monitored by optical density at 560 nm in a spectrophotometer.

2.7 Effect of temperature on growth of bacteria in irradiated sludge

B. subtilis NB22 and strain O 1-1 were used in this experiment. Twenty grams of irradiated sludge was mixed well with fifteen grams of sterilized perlite. pH of the mixture was adjusted to 7 by adding 0.1 gram of sodium carbonate. Approximately 1 ml of 10^8 of spore suspension of each bacterium was mixed well in the mixture. The mixture was put in the glass fermentor and kept fermenting at various temperatures for 5 or 7 days. Aeration was performed from the bottom of the fermentor during fermentation. The state of fermentation was observed by measuring CO_2 concentration in the exhaust gas using an infrared-type analyser.

2.8 Effect of inoculum size on growth of bacteria in irradiated sludge

The initial inoculum sizes of B. subtilis NB22 and strain O 1-1 were prepared the concentration to about 3.9×10^8 and 1.19×10^9 cfu/ml, respectively. The experiment procedure was almost done in the same as 2.7 but inoculum size of each bacterium was diluted 100 and 10000 times from initial inoculum size and kept fermenting only at temperature of 40°C for 5 or 7 days.

3. Experimental results

3.1 Suppressive effect of bacteria on phytopathogenic fungi

Table 1 shows effect of suppression of various kinds of bacteria on F. oxysporum and G. fujikuroi. Most of bacterial strains listed in this table showed suppressive effects except a strain isolated from a commercial seed compost R. In preliminary study this strain showed suppressive effect on nutrient agar. Bacterial strains that showed suppressive effect against phytopathogenic fungi were identified as follow; O1-1 was B.subtilis, O 1-2 and O-2 were Bacillus spp., EFS 1-1 and EFS 1-2 were B.polymyxa. Strains O1-1 and B. subtilis were selected as seed bacteria because they were able to grow very well and showed strong suppressive effect on both fungi.

Table 1 Suppressive effect of bacteria on phytopathogenic fungi

Bacterial strain	Fusarium oxysporum		Giberella fujikuroi	
	colony	inhibition zone	colony	inhibition zone
O1-1*	7.9	12.5	11.8	16.0
O1-2*	7.1	10.5	10.5	14.5
O-2 *	6.3	10.8	9.8	13.6
R *	0.5	none	none	none
EFS 1-1 **	2.5	7.0	5.5	8.3
EFS 1-2 **	2.8	6.8	2.3	5.5
B. subtilis NB22 ***	5.3 mm	9.5 mm	11.5 mm	16.5 mm

* Isolated from commercial seed composts.
** Isolated from soil in JAERI.
*** Obtained from Tokyo Institute of Technology.

3.2 Growth of bacteria in nutrient broth

Fig. 1 shows absorption vs. pH after cultivation for 18 hours at 30 ˚C in nutrient broth. The initial loads of bacteria in nutrient broth were approximately 10^2 cfu/ml. As shown in this figure, the optimum pH of both bacteria ranged from 6 to 7. The bacterial counts were ranged from 10^7 to 10^8 cfu/ml after cultivation at the optimum temperature.

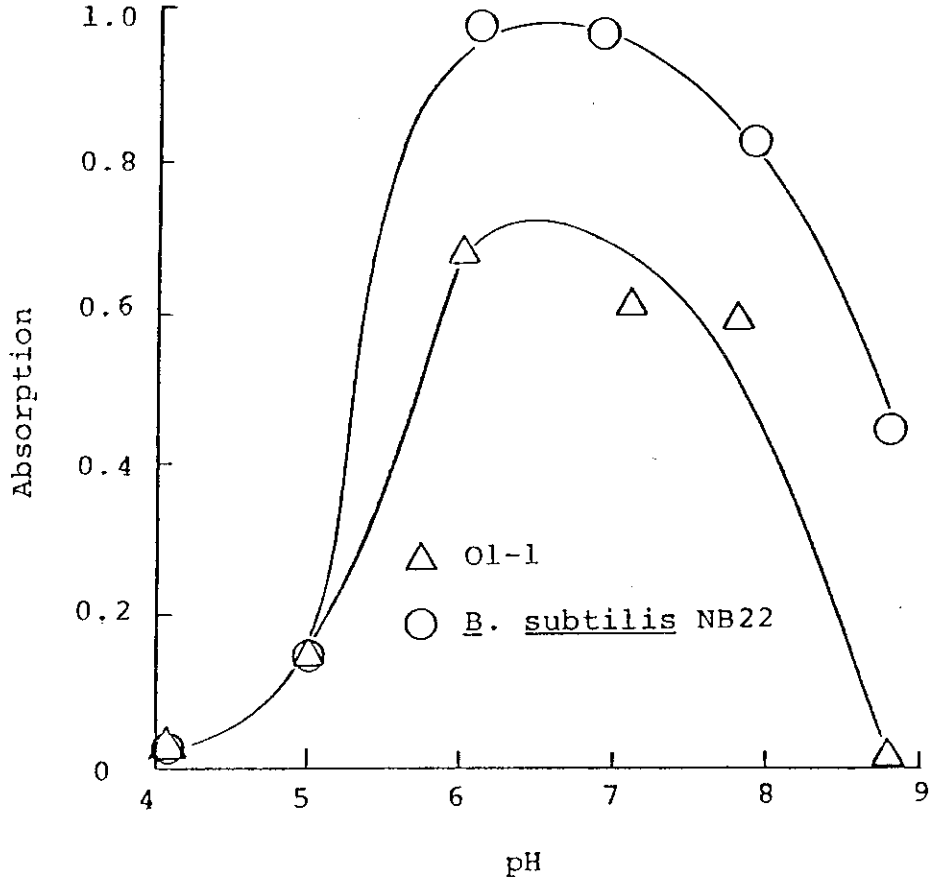

Fig. 1 Absorption vs. pH after cultivation in nutrient broth

pH 7 was selected to study on effect of temperature on growth of both bacteria. Fig. 2 shows absorption vs. temperature after cultivation for about 20 hours in nutrient broth. It can be seen that the optimum temperature is ranged from 35 to 40 ˚C for 01-1 and 40 ˚C for B. subtilis NB22.

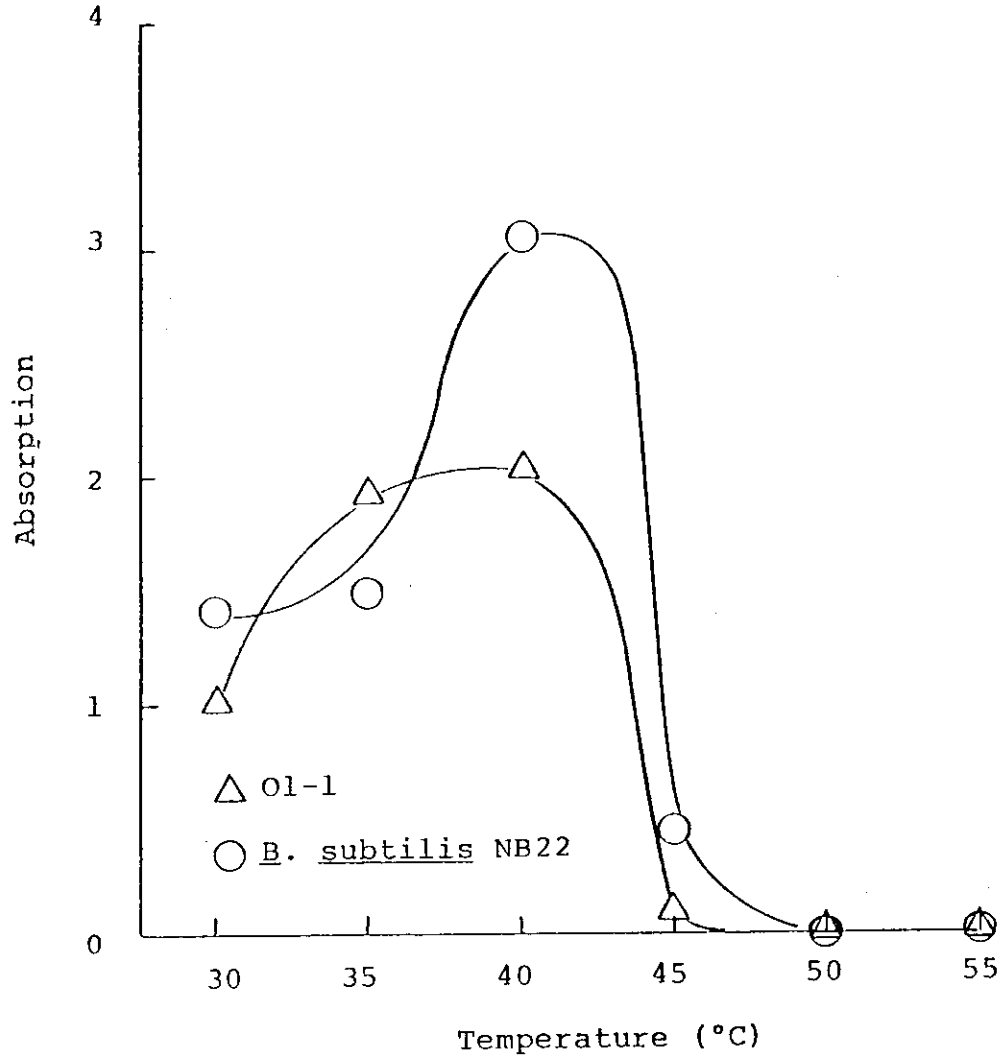

Fig. 2 Absorption vs. temperature after cultivation in nutrient broth

3.3 Growth of bacteria in irradiated sludge

pH of the mixture of irradiated sludge and perlite was about 5.5. To adjust pH to the optimum value, 0.1 gram of sodium carbonate was added. Fig. 3 shows bacterial counts vs. temperature after cultivation in irradiated sludge. Before cultivation, the numbers of bacteria in the mixtures were approximately 10^6 cfu/g in both seed bacteria. The optimum temperature for growth of 01-1 was ranged from 35 to 40 ˚C and about 40 ˚C for B. subtilis NB22. These results are almost same as those in nutrient broth. It should be noted that these bacteria could not grow at high temperature more than 50 ˚C. Total counts after fermentation at optimum temperature were about 10^8 cfu/g in both bacteria.

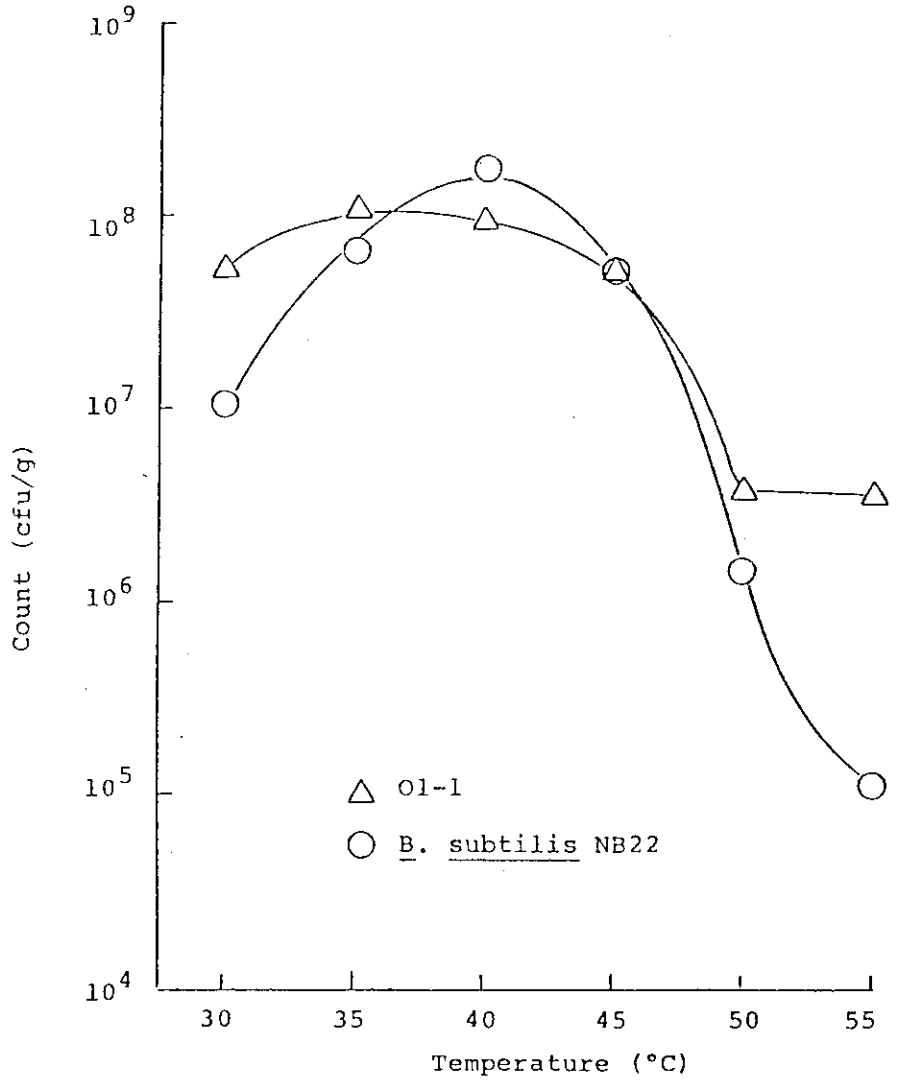

Fig. 3 Bacterial counts vs. temperature after cultivation in irradiated sludge

Table 2 shows effect of inoculum size on growth of bacteria. O1-1 could not grow well when the initial inoculum size was low. But, B. subtilis NB22 could grow very well even low initial inoculum size.

Table 2 Growth of bacteria in irradiated sludge at different inoculum size

Strain	Initial inoculum size (cfu/ml)	Before fermentation (calculated) (cfu/ml)	After fermentation (cfu/ml)	Moisture content (%)
O1-1	1.19×10^{5}	3.3×10^{3}	2.4×10^{7}	42.9
	1.19×10^{7}	3.3×10^{5}	2.3×10^{6}	45.4
	1.19×10^{9}	3.3×10^{7}	1.22×10^{8}	41.9
B. subtilis NB22	3.9×10^{4}	1.08×10^{3}	4.2×10^{8}	51.3
	3.9×10^{6}	1.08×10^{5}	2.3×10^{8}	49.8
	3.9×10^{8}	1.08×10^{7}	1.48×10^{8}	50.1

4. Conclusion

1) Many bacterial strains useful to suppress phytopathogenic fungi were isolated from soil. Suppressive bacteria were identified as Bacillus subtilis, B.polymyxa and Bacillus spp. O1-1 and B. subtilis were most effective in comparison with the others.

2) Optimum pH for growth of O1-1 and B. subtilis NB22 was ranged from 6 to 7.

3) Optimum temperature for growth of O1-1 was ranged from 35 to 40 °C and 40 °C for B. subtilis NB22. At high temperature more than 50 °C, these bacteria could not grow at all.

4.4.2 Cultivation of Bacillus subtilis N4 in grass and sludge

S. Pongpat and S. Hashimoto

1. Introduction

Grass waste after trimming from golf court and sewage sludge are considered as the waste of large cities. The grass and sludge contain enough nutrients for growth of microorganisms. B. subtilis N4 is a bacterial strain which shows antagonistic effect on some plant pathogenic microorganisms. An attempt for the cultivation of B. subtilis N4 in irradiated grass and sludge samples was carried out in this study in order to obtain fermented products for being effective as both fertilizer and biological pesticide.

2. Experimental procedures

2.1 Preparation and Irradiation of grass and sludge

Grass sample obtained from Shizuoka University was used. Sewage sludge cake was collected from sewage treatment facility of the Gunma Prefecture Sewage Works Cooperation, Tamamura, Takasaki. After well mixing, each sample was prepared for irradiation. A 6 g of grass was placed into a plastic bag and irradiated with the dose rate of 5 kGy/hr for the study on inactivation of microorganisms in the grass sample. For the study on cultivation of B. subtilis N4 in irradiated grass and sludge, a 20 g of sample was placed into a plastic bag, then irradiated at 15 kGy at the dose rate of 7.5 kGy/hr.

2.2 Growth condition study of B. subtilis N4

B. subtilis N4 obtained from Shizuoka University was used in the study. Growth of B. subtilis N4 in nutrient broth at different pH and temperatures was investigated. The inoculum was prepared by transfer a small amount of B. subtilis N4 from agar slant to 100 ml of nutrient broth. The pH of the broth was adjusted to be 7 before transfer. The incubation was made with the condition of 160 rpm shaking at 30 °C for 22 hr. Then, 0.5 ml of the inoculum was added to 100 ml of nutrient broth in a 500 ml erlenmeyer flask, then incubated with the condition of 160 rpm shaking at 30 °C for 22 hr for the study on effect of pH. In case of study on effect of temperature, 0.5 ml of inoculum was

added to 100 ml of nutrient broth in a 300 ml aeration bottle. The bottle was incubated in a water bath and aeration was made during incubation for 22 hr. Cell growth was determined by using a spectrophotometer (UV-265FW) at wave length of 560 nm.

2.3 Growth of B. subtilis N4 in irradiated grass and sludge

A 20 g of irradiated grass was mixed well with 0.5 ml of suspension of B. subtilis N4. In case of sludge, a 20 g of irradiated sludge was mixed well with 10 g of perlite, 160 mg of sodium carbonate and 0.5 ml of cell suspension. Then, each sample was transferred into a sterile glass fermentor. The fermentor was incubated in a water bath at 35 °C with aeration rate of 50 ml/min. CO_2 concentration in exhaust gas was continuously measured every 1 hr during fermentation. Viable counts were determined at different incubation times.

2.4 Enumeration of bacteria

Spread plate technique was used for enumeration of bacteria. Nutrient agar medium was used for total bacteria and MacConkey agar medium was used for total coliform. The incubation condition were 30 °C for 2 days for total bacteria and B. subtilis N4 and 37 °C for 20 hr for total coliform.

2.5 Measurement of moisture content and volatile solid content

Moisture contents and volatile solid contents in grass and sludge were determined from the loss of the weight after drying at 105 °C until the weight becoming constant. The dried solids were heated at 600 °C for 4 hr. The loss of weight was calculated as volatile solid.

3. Experimental results

3.1 Inactivation of microorganisms in grass by gamma-ray irradiation

Fig. 1 shows the number of microorganisms in irradiated grass at various doses. The total bacterial count decreased from 10^8 to 60 and 55 cfu/g after irradiation at dose 10 and 15 kGy, respectively. The total coliform which is more sensitive to radiation decreased from 10^7 cfu/g to undetectable level at the dosage of 5 kGy. Therefore, it can be seen that the irradiation dose at 10 kGy was regarded to be enough for inactivation of bacteria in grass sample before being used in this study.

3.2 Optimal growth condition of B. subtilis N4

Fig. 2 shows absorption vs. pH after cultivation at 30 °C for 22 hr in nutrient broth. The value to show the maximum absorption was observed to be about pH 6 and the count of bacteria at this pH increased from the order of 10^5 to of 10^7 cfu/ml after cultivation. This means the optimal pH of B. subtilis N4 is about 6.

pH 6 was selected to study on effect of temperature on growth of B. subtilis N4. Fig. 3 shows the relation between absorption and temperature after cultivation for 22 hr in nutrient broth. It can be seen that the absorption showed remarkable increase at the temperature ranging from 35 to 45 °C. The count of bacteria at maximum absorption after cultivation increased from the order of 10^5 to 10^7 cfu/ml. It should be noted that this bacteria could not grow at temperature higher than 50 °C.

3.3 Growth of B. subtilis N4 in irradiated grass and sludge sample

Fig. 4 shows the viable count of B. subtilis N4 during cultivation in grass. The viable count of bacterial cells rapidly increased from the order of 10^5 to 10^9 cfu/g after 2 days' incubation and gradually increased up to the maximum of about 10^{10} cfu/g after 5 days' incubation.

Fig. 5 shows the viable count of B. subtilis N4 during cultivation in sludge. The count of bacterial cells rapidly increased from the order of 10^5 to 10^{10} cfu/g after incubation for 1 day. Then, the count of bacterial cells gradually decreased to be in the order of 10^8 to 10^9 cfu/g within 7 days'incubation.

The activities of B. subtilis N4 during degradation of grass and sludge were observed from the CO_2 evolution curve as shown in Fig. 6 and Fig. 7. The peak value of CO_2 evolution for grass was about 2.8 g/hr.kg-v.s. after around 39 hr of incubation while, was about 6.5 g/hr.kg-v.s. after around 23 hr of incubation in sludge. It can be seen that the remarkable difference of the peak value and the peak time of CO_2 evolution during degradation of those samples was observed. This means that sludge contains larger amount of nutrients and more suitable for growth of B. subtilis N4 than grass.

Table 1 shows the moisture contents and volatile solid contents of raw materials and products. The moisture contents in both fermented products were rather higher than those in the mixtures before fermentation. This may result from the effect of the humidity from the aeration during fermentation.

On the other hand, the volatile solid contents in both fermented products were slightly lower than those in the

mixtures. However, the difference was unremarkable. Theoretically, organic carbon contained in a substrate for fermentation is a part of the components in volatile solid. During fermentation process, the organic carbon should be evolved as CO_2 and remarkable decrease of organic carbon should be obtained. This should result in remarkably decrease of volatile solid content. But the results showed unremarkable decrease of volatile solid contents in both fermented products. This may be due to the low conversion of carbon and also the heterogeneity of a sample.

4. Conclusions

1)Gamma irradiation at the dose 10 kGy seemed to be enough to inactivate bacteria in grass sample.
2)Optimal pH and temperature for growth of B. subtilis N4 in nutrient broth were at 6 and ranging from 35 to 45 °C, respectively.
3)Growth of B. subtilis N4 in grass sample was observed with the peak value of CO_2 evolution about 2.8 g/hr.kg-v.s. after around 39 hr of incubation and viable count increased from the order of 10^5 to 10^9 cfu/g after 2 d incubation.
4)Growth of B. subtilis N4 in sludge sample was observed with the peak value of Co_2 evolution about 6.5 g/hr.kg-v.s. after around 23 hr of incubation and viable count increased from the order of 10^5 to 10^{10} cfu/g after 1 day incubation.
5)According to the viable count of bacterial cells, the peak value and the peak time of CO_2 evolution during degradation of grass and sludge, it can be concluded that sludge contained larger amount of nutrients and more suitable for growth of B. subtilis N4 than grass.

Table 1. The moisture content and volatile solid content in raw materials and fermented products

	Moisture content (%)	Volatile solid content (%)
Raw grass	69.2	91.2
Mixture, before fermentation	69.4	91.0
Fermented grass	73.6	90.1
Raw sludge	76.0	84.7
Mixture, before fermentation	54.4	28.3
Fermented sludge	57.0	24.5

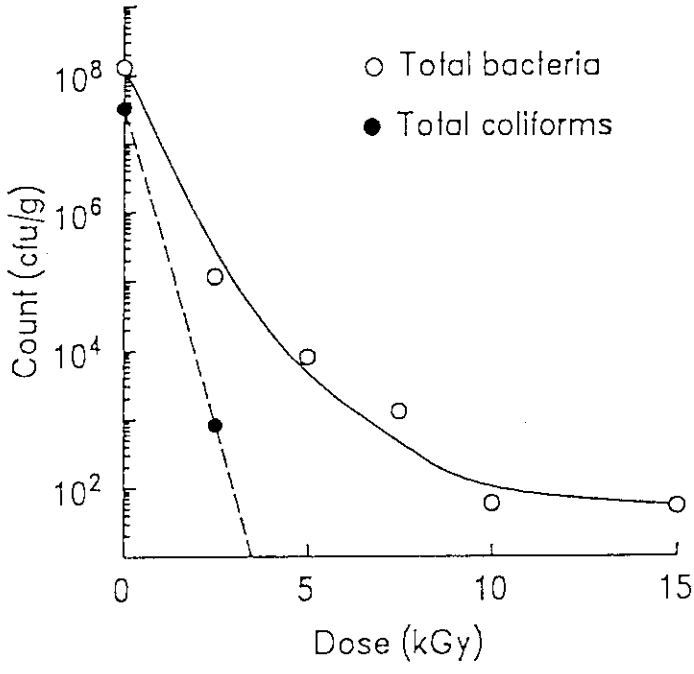

Fig. 1 Number of microorganisms in grass after gamma-ray irradiation at various doses

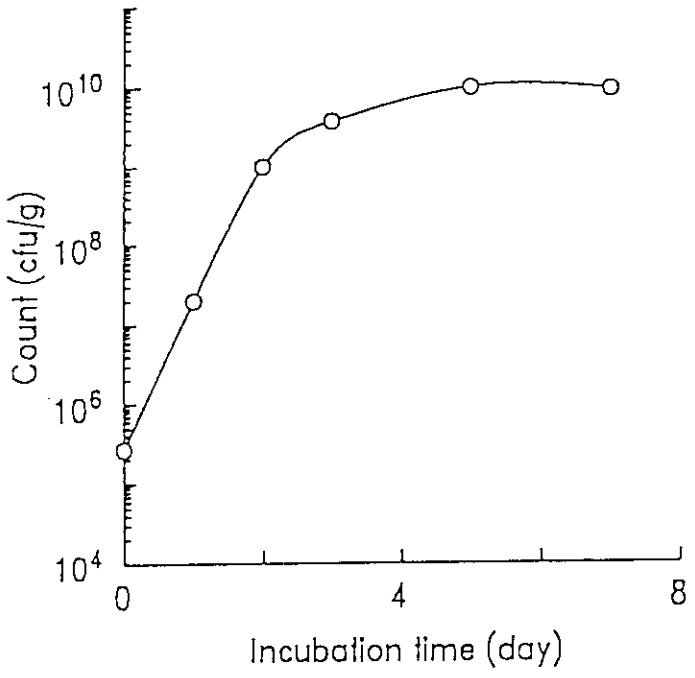

Fig. 4 Viable count of B. subtilis N4 after cultivation in grass

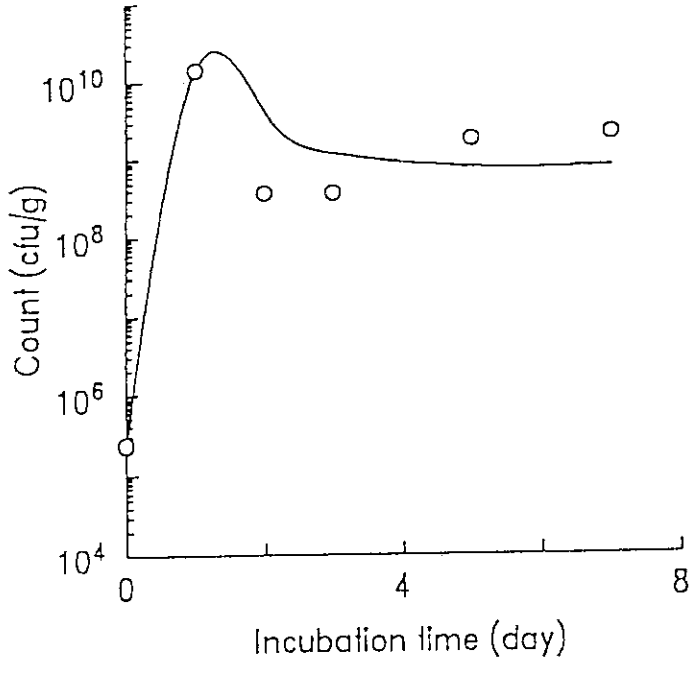

Fig. 5 Viable count of B. subtilis N4 after cultivation in sludge

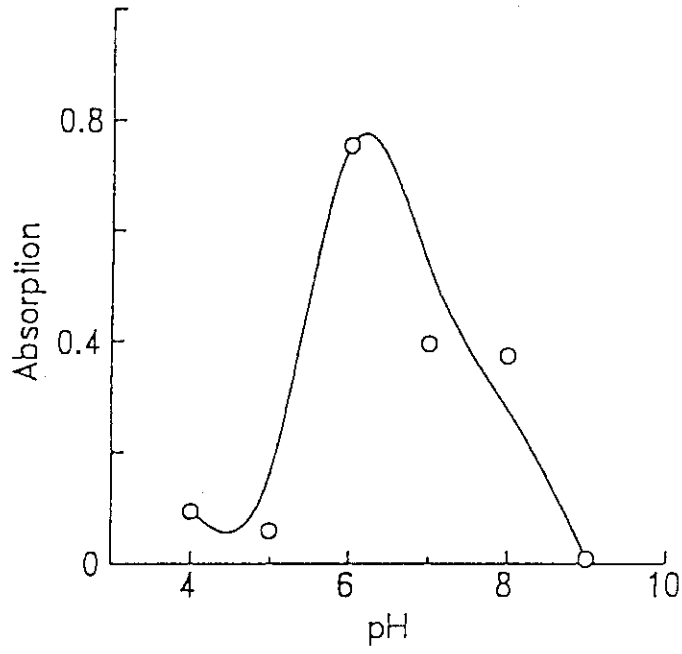

Fig. 2 Absorption vs. pH after cultivation of B. subtilis N4 in nutrient broth

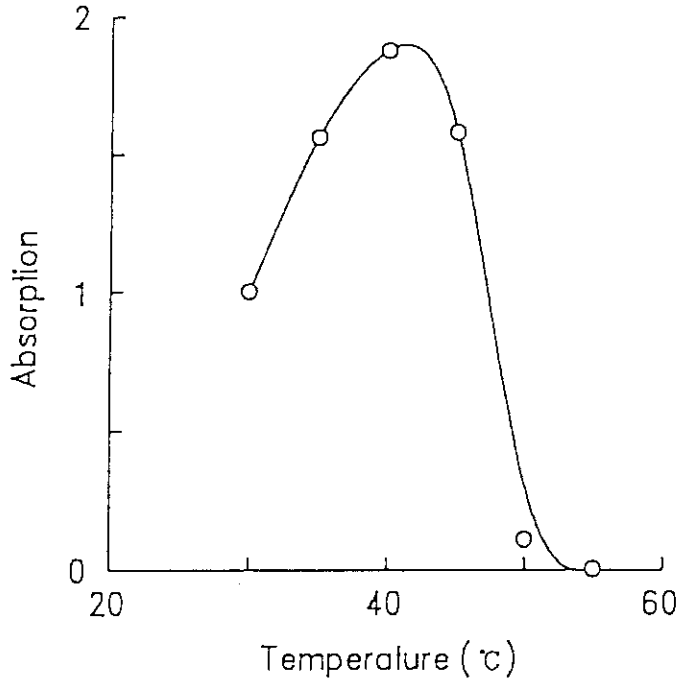

Fig. 3 Absorption vs. temperature after cultivation of B. subtilis N4 in nutrient broth

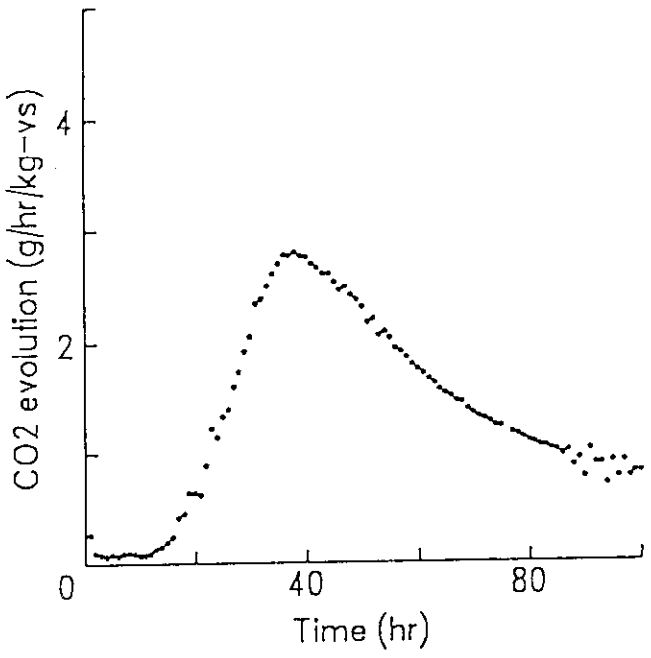

Fig. 6 CO_2 evolution during cultivation of B. subtilis N4 in grass

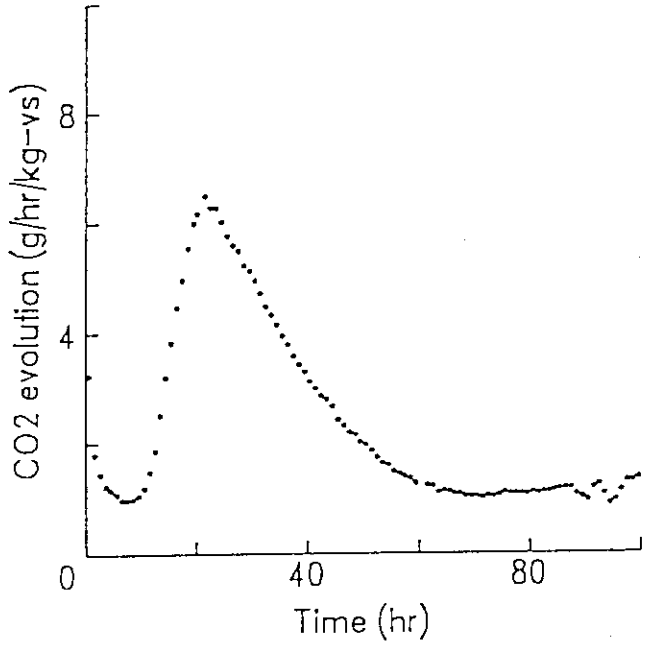

Fig. 7 CO_2 evolution during cultivation of B. subtilis N4 in sludge

5. Analysis of components in sludge and fermented products

[Abstract]

5.1 Evaluation of components in sludge

Major elements, metals and other chemical compositions of sludge samples from industry, hospital and community were studied. It showed that major elements (N,P,K) of samples from industry were at maximum level, which were at the average range of 1.92-5.96 %, 3797-85787 ppm, and 2937-4018 ppm respectively. The concentration of metals(Ca, Mg) in sludges from industry and community, both total and available, were higher than from hospital. But Fe, Zn and Cu in sludges from industry were lower than hospital and community. C/N ratios of samples from industry and hospital were smaller than community. Moisture content of sludge from industry was the highest, while pH was from medium to acid.

5.2 Analysis of components in sludge and compost

Components analysis of sludge and compost was reported. Among three sources of sludge samples, the sludge from Vachira Hospital contained relatively higher amount of nutrients effective to plant growth than the others. Radiation dose at 25 kGy does not affect to the nutrient components contained in sludge.

5.1 Evaluation of components in sludge

S. Piadang and N. Suddhapreda*

Abstract

Major, minor elements and other chemical compositions of sludge samples from industry, hospital and community were studied. It showed that major elements (N-P-K) of samples from industry were at maximum level, which were at the average range of 1.92-5.96%, 3,797-85,787 ppm, 2,937-4,018 ppm respectively. The minor elements (Ca-Mg) of sludges from industry and community, both total and available, were higher than from hospital. But Fe, Zn, Cu in sludges from industry were lower than hospital and community. C/N ratio of samples from industry and hospital were narrower than community. It means that sludges from industry and hospital can decompose more rapidly. Moisture content of sludge from industry was at maximum, while pH was at medium to acid.

Introduction

Sewage and industrial sludge from wastewater treatment plant was recognized as waste that must be regarded. When the country has grown up, the wastes become more and more serious problem and need for proper management. Utilization or recycling of sewage sludge as new resource had been widely studied in many countries. Office of Atomic Energy for Peace has interested to study the utilization of sewage sludge as fertilizer, feed, etc. Such studies need to know the chemical compositions of sewage sludge. therefore, Organic matter, N-P-K, C/N ratio, major and minor elements, etc. had been studied.

Objective

To determine the quantity of components of plant nutrients in sludges from industries, hospitals and communities.

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Method

1. sample

The sewage sludges used in this study were sampling from 4 industries which were brewery, dairy, household uses and condiment factories : from 2 communities which were Huay Kwang and Pattaya communities : from 3 hospitals which were from Siriraj, Vachira and Chonlburi hospitals. Each location was sampling 1-3 times.

2. analysis

All samples were analysed for moisture content, pH, organic matter, C/N ratio, total N-P-K content, total Ca, Mg, Fe, Zn, Cu and available Ca, Mg and K.

3. preparation of sample

All samples were dried at $80^{o}C$, then sieved and grinded before analysed.

Moisture content

Weighed 2 g. of sludge in constant weight of crucible. Dried at $100^{o}C$ for 6 h. Weighed the sludge and crucible after drying. Calculate the mositure content of sludge sample in percentage.

pH

pH of sludge sample was determined by mixing with water at the ratio of 1:3 and pH measurement were read by using pH meter.

Organic matter and C/N ratio

Organic matter was doned by using Walkley and Black method and reported in %OM. Organic carbon was obtained by the formular which is :-

$$\% OC = \% OM \times 0.58$$

$$C/N \text{ ratio} = \frac{\% OC}{\text{Total N.}}$$

Total Nitrogen

Total nitrogen of sludge samples were determined by Kjedahl method and reported in percentage of total nitrogen.

Total phosphorus

Total phosphorus of sludge samples were determined by Vanadomolybdo phosphoric acid Colorimetric Method. Absorbance of digested samples were read in ppm (mg/l.) of total phosphorus by comparing with standard curve of phosphorus.

Total K, Ca, Mg, Fe and Zn

0.1 g. sludge sample was added with 10 ml. conc. $HClO_4$, digest until clear. Adjust the volume of solution to 100 ml. Measure the quantities of K, Ca, Mg, Fe and Zn in sample by using Atomic Adsorption Spectrophotometer and reported in mg/l.

(ppm.)

Available phosphorus

2 g. sludge sample was added to 20 ml. standard solution bray 1 (0.03 N NH_4F + 0.025 NHCl). Mixed by shaker for 40 sec., then filtered it. 5 ml. of mixed solution was added with 5 ml. ascorbic acid, then adjust to 25 ml. Stand for 15-30 min. before measurement by Spectrophotometer at 882 nm, and reported in mg/l (ppm).

Available Ca, Mg and K.

0.1 g sludge sample added with 10 ml, 1 N., pH 7, CH_3COO NH_4. shake for 30 min. in shaker, then filter. The extract was read for available K, Ca, Mg by using Atomic Adsorption Spectrophotometer and reported in mg/l (ppm).

Result

Table 1 Shown average and range of average of organic matter, organic carbon, pH, C/N ratio, and moisture content of sludge sample from 4 industries (ppm)

Factories	Replication	% OM	% OC	pH	C/N ratio	% MC
Brewery	1	22.29	12.93	5.4	2.20:1	82.16
	2	22.69	13.16	5.5	2.26:1	82.08
	3	22.45	13.02	5.3	2.18:1	85.15
	Average	22.48	13.04	5.4	2.21:1	83.13
Dairy	1	23.61	13.65	5.9	2.33:1	92.20
	2	27.31	15.84	6.0	2.58:1	91.27
	3	27.24	15.80	6.1	2.65:1	90.77
	Average	26.05	15.09	6.0	2.52:1	91.41
Household uses	1	24.48	14.20	6.5	2.40:1	76.06
	2	24.63	14.29	6.6	2.80:1	75.00
	Average	24.55	14.25	6.55	2.6:1	75.53
Condiment	1	10.76	6.24	3.8	2.40:1	23.19
	2	20.82	12.08	3.8	9.40:1	23.05
	Average	15.79	9.16	3.8	5.9:1	23.12
Range of average		15.79-26.05	9.16-15.09	3.8-6.55	2.21-5.9:1	23.12-91.41

MC : moisture content

Table 2 Average and range of average of organic matter, organic carbon, pH, C/N ratio, and moisture content of sludge sample from communities(ppm)

Communities	Replication	% OM	% OC	pH	C/N ratio	% MC
Huay Kwang	1	20.41	11.84	6.9	4.65:1	88.11
	2	20.93	12.14	6.9	4.47:1	87.39
	Average	20.67	11.99	6.9	4.56:1	87.75
Pattaya	1	30.74	17.83	6.3	8.52:1	5.24
Range of average		20.67-30.74	11.99-17.83	6.3-6.9	4.56-8.52:1	5.24-87.75

Table 3 Average and range of average of organic matter, organic carbon, pH, C/N ratio, and moisture content of sludge sample from hospitals (ppm)

Hospitals	Replication	% OM	% OC	pH	C/N ratio	% MC
Siriraj	1	23.39	13.57	6.4	2.59:1	82.08
	2	22.27	12.92	6.6	2.58:1	84.67
	3	21.72	12.60	6.4	2.54:1	84.31
	Average	22.46	13.03	6.5	2.57:1	83.69
Vachira	1	16.77	9.73	6.3	2.50:1	84.31
	2	17.88	10.37	6.1	2.82:1	79.73
	Average	17.33	10.05	6.2	2.66:1	82.02
Cholburi	1	25.47	14.77	6.0	3.20:1	10.34
Range of average		17.33-25.47	10.05-14.77	6.0-6.5	2.57-3.20:1	10.34-83.69

MC : moisture content

Table 4 Average and range of average of total and available nitrogen (N), phosphorus (P), and potassium (K) in sludge sample from industries (ppm)

Factories	Replication	% N	P (total)	P(extract)	K (total)	K (extract)
Brewery	1	5.86	15783	93.2	2926	869.8
	2	5.82	20341	93.4	3303	843.8
	3	5.97	16308	91.6	2583	858.7
	Average	5.88	17477	92.7	2937	857.4
Dairy	1	5.87	22350	150.2	2700	836.4
	2	6.03	31008	156.6	3336	858.7
	3	5.97	36908	163.0	5760	836.4
	Average	5.96	30089	156.6	3932	843.8
Household uses	1	4.93	82966	236.6	3720	829.0
	2	5.07	88608	208.2	4316	848.8
	Average	5.00	85787	222.4	4018	838.9
Condiment	1	2.53	3085	125.6	4406	350.9
	2	1.30	4508	134.4	3100	392.8
	Average	1.92	3797	130.0	3753	371.9
Range of average		1.92-5.96	3797-85787	92.7-222.4	2937-4018	371.9-857.4

Table 5 Average and range of average of total and available nitrogen (N), phosphorus (P), and potassium (K) in sludge sample from communities (ppm)

Communities	Replication	% N	P (total)	P(extract)	K (total)	K (extract)
Huay Kwang	1	2.53	21441	129.2	3293	453.8
	2	2.73	21550	110.0	2433	856.2
	Average	2.63	21496	119.6	2863	655.0
Pattaya	1	2.09	23508	50.6	1977	566.2
Range of average		2.09-2.63	21496-23508	50.6-119.6	1977-2863	566.2-655.0

Table 6 Average and range of average of total and available nitrogen (N), phosphorus (P), and potassium (K) in sludge sample from hospitals (ppm)

Hospital	Replication	% N	P (total)	P(extract)	K (total)	K (extract)
Siriraj	1	5.24	41216	120.2	3556	867.3
	2	5.00	35741	67.0	2383	845.1
	3	5.15	36600	71.6	2993	859.9
	Average	5.13	37852	82.3	2997	857.4
Vachira	1	3.88	29158	89.4	4537	569.8
	2	3.69	34375	53.8	3683	869.8
	Average	3.79	31767	71.6	4110	719.8
Cholburi	1	4.63	35166	60.0	1364	421.7
Range of average		3.97-5.13	31767-37852	60.0-82.3	1364-4110	421.7-857.4

Table 7 Average and range of average of total Ca, Mg, Fe, Zn, Cu and available Ca, Mg insludge samples from industries (ppm.)

Factories	Replication	Ca(total)	Ca(extract)	Mg(total)	Mg(extract)	Fe(total)	Zn(total)	Cu(total
Brewery	1	7335	561.4	2380	438.2	2894	161	344
	2	7404	557.2	2818	417.6	3150	301	268
	3	8433	555.2	2747	407.4	2200	528	423
	Average	7724	557.9	2648	421.1	2748	330	345
Dairy	1	25306	643.2	2438	402.2	1133	634	269
	2	26050	619.0	2478	384.3	1945	762	206
	3	28406	614.0	3173	414.2	1651	492	235
	Average	26587	625.4	2696	400.2	1576	629	237
Household uses	1	25550	594.0	4098	400.5	9763	884	273
	2	25666	593.4	7886	440.5	8343	571	363
	Average	25608	593.7	5992	420.5	9053	728	318
Condiment	1	14746	518.0	1593	350.9	6216	549	250
	2	14280	551.6	1253	392.8	15003	339	202
	Average	14513	534.8	1423	371.9	10610	444	226
Range of average		7724-26587	534.8-625.4	1423-5992	371.9-421.1	1576-10610	330-728	226-345

Table 8 Average and range of average of total Ca, Mg, Fe, Zn, Cu and available Ca, Mg in sludge samples from communities (ppm.)

Communities	Replication	Ca(total)	Ca(extract)	Mg(total)	Mg(extract)	Fe(total)	Zn(total)	Cu(total)
Huay Kwang	1	24290	587.4	7793	336.4	23700	4147	633
	2	24213	572.8	6753	378.3	20700	3466	549
	Average	24252	577.1	7273	357.4	22200	3807	591
Pattaya	1	19050	658.8	7506	208.8	30633	1783	344
Range of average		19050-24252	577.1-658.8	7273-7506	208.8-357.4	22200-30633	1783-3807	344-591

Table 9 Average and range of average of total Ca, Mg, Fe, Zn, Cu and available Ca, Mg in sludge samples from hospitals (ppm.)

Hospitals	Replication	Ca(total)	Ca(extract)	Mg(total)	Mg(extract)	Fe(total)	Zn(total)	Cu(total)
Siriraj	1	12633	607.8	7113	433.9	16260	2340	687
	2	10663	567.6	3573	427.0	12203	1537	635
	3	11570	571.6	6453	434.8	16507	1774	701
	Average	11622	582.3	5713	431.9	14990	1884	674
Vachira	1	12097	598.4	3798	381.7	22133	1638	459
	2	14150	616.0	3827	368.1	24000	2038	398
	Average	13124	607.2	3813	374.9	23067	1838	429
Cholburi	1	8620	546.4	2728	309.9	11286	1441	297
Range of average		8620-11622	546.4-607.2	2728-5713	309.9-431.9	11286-23067	1441-1884	297-674

Table 10 Shown the evaluation of range of average of components of sludge samples from industries, hospital and communities comparing to standard table 1 and 2 in Annex

Characteristics	Industries		Hospitals		Communities	
	Range of average	Evaluation	Range of average	Evaluation	Range of average	evaluation
pH	3.8-6.5	medium-low	6.0-6.5	medium	6.3-6.9	medium
% MC	23.12-91.41	-	10.34-83.69	-	5.24-87.75	-
C/N ratio	2.21-5.9:1	-	2.57-3.20:1	-	4.56-8.52:1	-
% OM	15.79-26.05	very high	17.33-25.47	very high	20.67-30.74	very high
% OC	9.16-15.09	-	10.05-14.77	-	11.99-17.83	-
% N	1.92-5.96	very high	3.79-5.13	very high	2.09-2.63	very high
P (total)	3797-85787	"	31767-37852	"	21496-23508	"
P (extract)	92.7-222.4	"	60.0-82.3	"	50.6-119.6	"
K (total)	2937-4018	"	1364-4110	"	1977-2863	"
K (extract)	371.9-857.4	"	421.7-857.4	"	566.2-655.0	"
Ca (total)	7724-26587	"	8620-11622	"	19050-24252	"
Ca (extract)	534.8-625.4	low	546.4-607.2	low	577.1-658.8	low
Mg (total)	1423-5992	very high	2728-5713	very high	7273-7506	very high
Mg (extract)	371.9-421.1	high	309.9-431.9	high	208.8-357.4	high
Fe (total)	1567-10610	higher than critical	11286-23067	higher than critical	22200-30633	higher than critical
Zn (total)	330-728	"	1441-1884	"	1783-3807	"
Cu (total)	226-345	"	297-674	"	344-591	"

Annex

Table 1 Standard value of general plant nutrients

	Very low	Low	Medium	High	Very high
pH	<4.5	4.5-6.0	6.0-7.5	7.5-8.5	> 8.5
% OM	<0.5	1.0-1.5	1.5-2.5	2.5-3.5	> 4.5
% N	0.025	0.05-0.075	0.075-0.125	0.125-0.175	0.225
P (ppm)	<30	3.0-10.0	10.0-15.0	15.0-45.0	> 45.0
K (ppm)	<300	30.0-60.0	60.0-90.0	90.0-120.0	> 120.0
Ca^{++}(ppm)	<400	400-1000	1000-2000	2000-4000	> 4000
Mg^{++}(ppm)	<37.5	37.5-125	125-375	375-1000	> 1000
Na^{+}(ppm)	<2.3	2.3-6.9	6.9-16.1	16.1-46	> 40

Source: Hunger Sign (1-2)

Table 2 Critical level of minor element to plant

Minor element	Critical level (ppm)
Cu	0.2
Fe	2.5-4.5
Zn	1.5

Source: De Datta, 1981

Critical level means maximum level of plant nutrient at mormal condition. If the level is higher than critical it will be toxic to plants.

Conclusion

Sludge samples from industries, hospitals and communities in this study were composed of the following plant nutrients :-

- pH of sludges from industry, hospital, and community were at the average range of 3.8 - 6.5, 6.0 - 6.5 and 6.3 - 6.9 respectively.
- % MC of sludges from industry, hospital and community were at the average range of 23.12 - 91.41, 10.34 - 83.69 and 5.24 - 87.75% respectively.
- % OC and % OM of sludges from industry were at the average range of 9.16 - 15.09 and 15.79 - 26.05% : from hospital at 10.05 - 14.77 and 17.33 - 25.47% : community at 11.99 - 17.83 and 20.67 - 30.74% respectively.
- C/N ratio from industry, hospital and community were at the average range of 2.21 - 5.9:1, 2.57 - 3.20 : 1 and 4.56 - 8.52 : 1 respectively.
- % total nitrogen of sludge from industry, hospital and community were at the range of 1.92 - 5.96, 3.79 - 5.13 and 2.09 - 2.63 respectively.
- total and available phosphorus of sludge from industry were at the range of 3,797 - 85, 787 and 92.7 - 222.4 ppm; hospital at 31,767 - 37,852 and 60.0 - 82.3 ppm; community at 21,496 - 23,508 and 50.6 - 119.6 ppm. respectively.
- total and available potassium of sludge from industry were at the range of 2,937 - 4,018 and 371.9 857.4 ppm; hospital at 1,364 - 4,110 and 421.7 - 857.4 ppm; community at 1,977 - 2,863 and 566.2 - 655.0 ppm respectively.
- total and available calcium of sludge from industry were at 7,724 - 26,587 and 534.8 - 625.4 ppm ; hospital at 8,620 - 11,622 and 546.4 - 607.2 ppm; community at 19,050 - 24,252 and 577.1 - 658.8 ppm respectively.
- total and available magnesium of sludge from industry were at the range of 1,423 - 5,992 and 371.9 - 421.1 ppm; hospital at 2,728 - 5,713 and 309.9 - 431.9 ppm; community at 7,273 - 7,506 and 208.8 - 357.4 ppm respectively.
- total ferrous, zinc and copper of sludge from industry were at the average range of 1,567 - 10,610, 330 - 728 and 226 - 345 ppm; hospital at 11,286 - 23,067, 1,441 - 1,884 and 297 - 674 ppm; community at 22,200 - 30,633, 1,783 - 3,807 and 344 - 591 ppm respectively.

Discussion

From table 1, pH of sludge sample from community was higher than industry and hospital and was at the medium level comparing to standard level. The moisture content of sample from industry was higher than community and hospital. C/N ratio of the sample from industry and hospital was narrower than community. It means that sludge from industry and hospital can more rapidly decompose than from community in soil. Organic matter of samples from three sources were very high. It means that high OM was from decomposition.

Average range of major elements (N-P-K) of sludge samples from industry were higher than from hospital and community.

Minor elements (Ca, Mg) of samples from three sources were very high comparing to standard level (table 1 in Annex). But available Ca of samples from 3 sources were low, which opposit to available Mg was high. Comparing to the source of sludges, samples from industry and community have the average range of total and available Ca and Mg higher than from hospital. For Fe, Zn, Cu, low quantity needed plant nutrients were higher than critical level comparing to standard level table 2 (Annex). Fe, Zn, Cu were at maximum in samples from hospital and community, which opposit to low level from industry.

Considering to major and minor elements of sludge samples, it showed that sludge from wastewater treatment plant from industry can use as fertilizer better than from hospital and community. The utilization of sludge as fertilizer should study the effect of sludge to plant in many factors such as limitation, disadvantage and ratio of fertilizer, etd, to prevent the toxicity of sludge occuring to plant.

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5.2 Analysis of components in sludge and compost

S. Pongpat and C. Banditsing

1. Introduction

Composting is a useful method to convert easily decomposable organic materials in sludge into a stage in which it can be applied to the land without adversely affecting to the environment. In the conventional composting process, however, high temperature during composting process is necessary for pasteurization of pathogenic microorganisms in composting material. Such high temperature also gives damage to composting microorganisms, so that long time to complete composting is needed. According to the recommendation on conventional composting process by Association for Utilization of Sewage Sludge in Japan, composting must be performed during a period from 10 to 14 days and the temperature should be kept higher than 65 °C for more than 2 days during the composting period.[1] The new method for composting of sludge was reported since 1981. In this process, disinfection is separated from composting. After pasteurization of sludge by electron beam irradiation, composting is performed by fermentation at the condition with the optimal temperature in the range of 40 to 50 °C, optimal pH in the range of 7 to 8. In addition, particle size of raw material should be adjusted less than 5 mm for aerobic fermentation and the addition of seed bacteria is also required. The fermented product is also useful as seed.[2),3)] In 1991, it was reported that efficiency of composting for this process could be also realized at a pilot plant by controlling fermentation temperature in the optimal range, by controlling aeration rate and by frequent mixing in the fermentor. From the economic feasibility study based on the results of pilot plant test, capital cost and treatment cost for the process was expected to be lower than those for conventional one, when the treatment capacity exceeded 50 ton/day.[4)]

However, there has been not so much information associated with the nutrient composition of compost from irradiated sludge to plant. In this paper, the nutrient compositions of unirradiated and irradiated sludge and compost product were reported.

2. Experimental procedures

2.1 Preparation and irradiation of samples

Sludge samples collected from the waste water treatment plants of Huay Kwang Community, Vachira Hospital and Thai Pure Drink Co., Ltd. were used to study in this experiment. After collecting, a sludge sample was mixed well and divided into 3 parts. Each part was kept in plastic bag. The first part was used as control or unirradiated sample. Another two parts were irradiated 25 kGy at the dose rate of 0.7-1.0 kGy/hr. One part of irradiated sludge was used for composting, while the other was used as an irradiated sample. The moisture content and pH of sludge samples are shown in Table 1.

The irradiated and unirradiated sludge samples were separately mixed with perlite, the mixing ratio of sludge and perlite was 2:1. Five replications were made for each sample.

2.2 Composting

Composting material for each run consisted of 30 g of irradiated sludge, 15 g of sterile perlite and 3 g of commercial seed. After well mixing, the material was transferred into a sterile glass fermentor and covered with sterile stopper unit. The fermentor was embedded into the water bath at 40 °C. The operation was started by means of micro-computer controlling and stopped when the peak of CO_2 evolution rate had become constant as observed from recorder. CO_2 evolution in exhaust gas during fermentation process was continuously measured by CO_2 infrared gas analyzer unit in order to know the fermentation stage. A scheme of experimental apparatus for composting is shown in Fig 1.

2.3 Analytical procedures

2.3.1 Moisture content

Moisture content was determined from the loss of the weight after drying at 105 °C until the weight becoming constant and reported in percentage of moisture constant.

2.3.2 Chemical components

Chemical components were analyzed by using the procedure as described in The Manual for Soil and Plant Analysis.[5)]

3. Experimental results

3.1 Nutrient components in unirradiated and irradiated sludge

The nutrient components in unirradiated and irradiated sludges from different waste water treatment plants are shown in Table 2. The nutrient components in commercial seed and perlite

are shown in Table 3. It can be seen that sludge contains varying amount of nutrient components depending on the source of sludge. The sludge from Vachira Hospital contains the highest quantities of N, P and OC. The sludge from Huay Kwang Community contains relatively high content of K and Mg, while the sludge from Thai Pure Drink Co., Ltd. contains the highest quantity of Ca. In general, N, P, and K are used in large quantities by plants and are therefore called "major (or primary) nutrients". Ca, Mg and S are required in smaller but appreciable quantities and are now classified as "major" rather than "secondary" nutrients.[6] According to the classification of those components, sludge from Vachira Hospital seems to be more beneficial for plant growth than the others. However, all samples in this experiment can be used as a source of N, P, K and other nutrients for plants.

In order to kill the pathogens in sludge cake samples used in this experiment, the samples were irradiated at the dose of 25 kGy. The comparison of nutrient compositions of unirradiated and irradiated samples is observed. The result shows that although the range of each component in unirradiated and irradiated sludge samples is slightly different, the mean value of those shows no significant difference. This indicates that the radiation dose even at 25 kGy does not affect to the nutrient composition of sludge sample at all. On the other hand, it was reported that the radiation dose at 20 kGy was able to reduce the total bacterial counts in sludge samples from Huay Kwang Community and Thai Pure Drink Co., Ltd. from the order of 10^5-10^6 to be 0-40 cfu/g. The total coliforms were also reduced from the order of 10^5-10^7 to <20-80 MPN/100 g at the dose 6 kGy.[7] Radiation disinfection of sludge, therefore, is very effective and preferable method before being applied to beneficial purpose.

3.2 Comparison of nutrient components in mixture and compost from irradiated sludge

In order to know the change of the quantities of nutrient components after composting process, the calculation of nutrient components in the mixture before composting was made and the results are shown in Table 4. The mean values of organic carbon and nitrogen contents in mixture of sludge from Huay Kwang Community is rather lower than in compost, while the quantities of those two components in the mixture of sludges from Vachira Hospital and Thai Pure Drink Co., Ltd. show rather higher mean values than in composts. The mean values of C/N ratio in composts are larger than in the mixtures. But it can be seen that there are no remarkable differences between the quantities of the nutrient components in the mixture and compost.

In composting process, organic carbon and nitrogen should

be evolved as CO_2 and NH_3 and remarkable decrease of the quantities of the two components in compost should be obtained. However, from the result of this experiment, those two components do not show remarkable decrease as seen in Table 4. This may be due to the low conversion of carbon and nitrogen and also the heterogeneity of the sludge sample, even within the same lot of sampling.

In addition, only a certain ratio of carbon and nitrogen contained in sludge is taken by microorganisms during the composting process. Therefore, the change of C/N ratio is observed after fermentation. It was also described that the value of CO_2-C/NH_3-N in exhaust gas is smaller than the ratio of C to N contained in sludge. This means that the C/N ratio of the product increase by fermentation.[8)]

4. Conclusions

1. Among three sources of sludge samples, the sludge from Vachira Hospital seems to be more beneficial for plants than the others.
2. Radiation dose at 25 kGy does not affect to the nutrient components contained in sludge. Therefore, irradiation is very effective and preferable method for disinfection of sludge before being applied to beneficial purpose.

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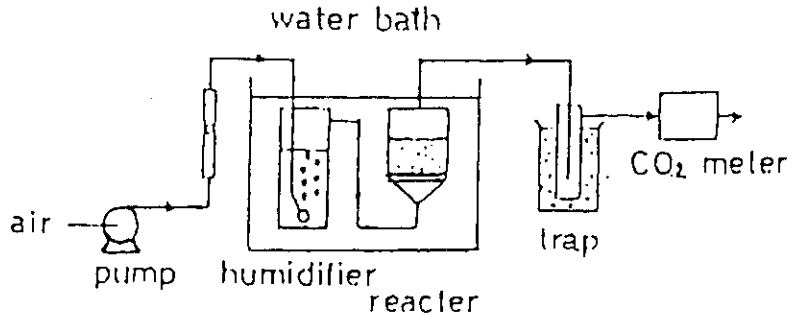

Fig. 1 Experimental apparatus for composting

Table 1 Moisture contents and pH of sludge samples from different waste water treatment plants

Plant	Moisture content (%)		pH
	Mean	Range	
Huay Kwang Community	83.7	82.1-85.4	7.0-7.5
Vachira Hospital	84.1	82.2-86.1	6.5-7.0
Thai Pure Drink Co., Ltd.	83.2	82.1-84.1	6.5-8.0

Table 2 Nutrient components of unirradiated and irradiated sludge

	Unirradiated sludge		Irradiated sludge	
	Mean	Range	Mean	Range
Huay Kwang Community				
OC (%)	24.0	20.1-26.7	25.5	25.2-25.5
N (%)	2.27	2.12-2.48	2.72	2.16-3.03
C/N	10.6	9.5-11.5	9.6	8.4-11.7
P (%)	0.80	0.63-1.04	0.79	0.78-80
K (%)	0.72	0.63-0.79	0.79	0.70-0.81
Ca (%)	0.28	0.20-0.41	0.20	0.17-0.22
Mg (%)	0.36	0.29-0.43	0.37	0.31-0.36
Vachira Hospital				
OC (%)	33.3	31.0-34.3	30.8	29.2-32.9
N (%)	4.17	3.54-4.66	4.59	4.97-4.95
C/N	8.0	7.4-8.8	7.2	6.3-8.4
P (%)	1.53	1.22-1.64	1.68	1.36-1.93
K (%)	0.26	0.25-0.27	0.28	0.25-0.31
Ca (%)	0.34	0.33-0.35	0.34	0.33-0.36
Mg (%)	0.20	0.17-0.22	0.22	0.21-0.25
Thai Pure Drink Co., Ltd.				
OC (%)	27.3	25.2-31.1	26.5	25.6-27.2
N (%)	3.24	2.64-3.82	3.16	2.93-3.58
C/N	8.8	7.4-9.6	8.2	7.5-9.2
P (%)	0.66	0.58-0.80	0.70	0.63-0.79
K (%)	0.70	0.56-0.79	0.7	0.66-0.78
Ca (%)	4.49	4.08-4.79	4.30	3.38-4.86
Mg (%)	0.31	0.28-0.37	0.31	0.27-0.36

Abbreviations: OC= total organic carbon; N= total nitrogen; P= total phosphorus; K= total potassium; Ca= total calcium; Mg= total magnesium
%: Dry weight base

Table 3 Nutrient components of commercial seed and perlite

	Components						
	OC (%)	N (%)	P (%)	K (%)	C/N	Ca (%)	Mg (%)
Cmmercial seed	23.8	1.12	0.03	1.01	23.8	-	0.31
Perlite	0.19	0.03	0.02	0.35	5.6	0.002	0.0003

Abbrevations: The same as Table 2.

Table 4 Nutrient components of composting material and compost from irradiated sludge

	Mixture		Compost	
	Mean*	Range*	Mean	Range
Huay Kwang Community				
OC (%)	8.1	7.6-8.7	9.3	8.6-9.9
N (%)	0.65	0.61-0.69	0.69	0.65-0.79
C/N	12.6	11.6-13.4	13.9	13.2-14.8
P (%)	0.19	0.16-0.23	0.22	0.19-0.27
K (%)	0.51	0.49-0.53	0.42	0.40-0.44
Mg (%)	0.12	0.10-0.14	0.11	0.10-0.12
Vachira Hospital				
OC (%)	9.8	9.2-10.3	9.5	9.0-10.8
N (%)	1.02	0.94-1.04	0.93	0.90-0.99
C/N	9.7	8.9-10.5	10.2	9.1-12.0
P (%)	0.33	0.29-0.37	0.37	0.35-0.41
K (%)	0.41	0.40-0.42	0.42	0.40-0.45
Mg (%)	0.09	0.07-0.08	0.09	0.05-0.10
Thai Pure Drink Co., Ltd.				
OC (%)	9.0	8.4-9.9	8.5	8.0-9.0
N (%)	0.85	0.77-0.89	0.68	0.54-0.98
C/N	10.6	9.4-11.4	12.4	8.6-14.4
P (%)	0.18	0.15-0.21	0.21	0.17-0.24
K (%)	0.51	0.47-0.53	0.44	0.33-0.52
Mg (%)	0.11	0.10-0.12	0.13	0.13-0.14

Abbreviations: The same as Table 2.
* Calculated figures.

6. Utilization test of raw sludges and fermented products

[Abstract]

6.1 Utilization of irradiated sludge as fertilizer

Utilization tests of irradiated and non-irradiated sludge were conducted as alternative fertilizers for morning glory. Plant grown with both irradiated and non-irradiated sludge were larger and heavier than those grown with other fertilizers and without fertilizer. There was no significant difference between the irradiated and non-irradiated sludge for using as fertilizer. It was concluded that the irradiated sludge can be used as an safe alternative source of fertilizer.

6.2 Application of irradiated sludge compost to plants

Utilization test of compost was conducted with cooperation of Gunma Agricultural Research Center. The compost used for the test was produced by a pilot plant. By this test, it was shown that the irradiated sludge compost is very effective on the growth and yield of rice and vegetables, especially on the yield of brown rice.

6.1 Utilization of irradiated sludge as fertilizer

S. Piadang, S. Pongpat and N. Sermkiattipong

ABSTRACT

Study on the utilization of both irradiated and non-irradiated sludges and waste waters as alternative fertilizer for morning glory was conducted. Korat Soil and Kui-Pai soil sets were used. This experiment was conducted at the Office of Atomic Energy for Peace from July to August 1983 by Completely Randomized Design. The total of 6 treatments were consisted of 1 check without fertilizer and 5 fertilizers which are urea (46%N), irradiated and non-irradiated waste waters and irradiated and non-irradiated sludges. One liter of irradiated and non-irradiated waste waters, urea solution (16 grams in 10 liters of water), irradiated and non-irradiated sludges (sludge:soil = 1:7) were added to the soil.

The height of morning glory at 7, 12, 17, 22, 27 and 32 days after planting were recorded. Plants grown on both irradiated and non-irradiated sludges were taller than those grown on soil with other fertilizers and check. Fresh and dry top weight of morning glory grown on irradiated and non-irradiated sludges were heavier than those grown on other fertilizers and check. Furthermore, it was also found that the growth rate of the top was greater than the root. In contrast, there is no significant difference between irradiated and non-irradiated sludges for using as fertilizer. It is concluded that irradiated sludge can be used as an alternative source of fertilizer.

Introduction

Effluent from conventional waste water treatment of National Housing Authority is discharged to the river but the sludges are dried by the sun and used as fertilizer. Those sludge might be the source of pathogens namely; bacteria, parasites and virus which are dangerous to environment including men and animals. At present, there is waste water treatment and pasteurization of the sludge by irradiation plant at commercial in West−Germany. The irradiated sludge are distributed to the farmer for using as fertilizer. The radiation waste water treatment was studied at the Office of Atomic Energy fo Peace. The implementation of irradiated sludge as fertilizer for growing the morning glory were studied.

Materials and Methods

1. Materials

Five kinds of fertilizers were consisted of urea (46%N), irradiated and non-irradiated waste waters, irradiated and non-irradiated sludges and 1 check without fertilizer were used. Korat soil and Kui Pai soil sets mixed with different kinds of fertilizers were used for planting in 10 inches diameter pots. Morning glory was testing plant.

2. Methods

The experimental design used in this study was a completely randomized design. Each treatment was replication 3 times. The total of 6 treatments were consisted of 1 check without fertilizer and 5 fertilizers which are urea (46%N), irradiated and non-irradiated waste waters and irradiated and non-irradiated sludges.

Soil preparation and planting

Soil preparation

Soil was dried and grinded. Then mixed with five fertilizer formulars. The rates of fertilizers used are as follow: 1 liter/pot of

irradiated and non-irradiated waste waters, 1 liter/pot of urea (16 gm/10 liter) and 1:7 of irradiated and non-irradiated sludges with soil sets.

Planting

The morning glory seeds were mixed with captan, then soaked in the water over night. Growing seeds were planted in 2 cm. deep with 2.5 cm. in row and 2.5 cm. between stem, and 10 plants/pot. Harvesting was made at 32 days after planting. All parameters were made after the plant had been at 7, 12, 17, 22, 27 and 32 days of age. These included plant height, fresh and dry top weights and fresh and dry root weights.

Results

The average plant height of 10 plants/pot from 3 pots at 7, 12, 17, 22, 27 and 32 days after planting was shown in Table 1 and 2. It was shown that 2 kinds of soil sets did not significantly different. Plants grown on both irradiated and non-irradiated sludges were taller than those grown on soil with other fertilizers and check. The average of fresh and dry top weights and root weights of plants grown on 2 soil sets harvested at 32 days was shown in Table 5 and 6. It was found that fresh and dry top weights and root weights were not significantly affected by irradiated and non-irradiated sludge mixed with Kui Pai soil. The fresh and dry root weights of plants grown on 5 fertilizers and check were not significantly different but the weights of morning glory grown on irradiated and non-irradiated sludges with Kui Pai soil set were significantly ($P<0.05$) heavier than those grown on other fertilizers and check.

The influence of fertilizer formular upon the fresh and dry weights of the top and root was studies. It was found that the irradiated and non-irradiated sludges mixed with Korat soil set were not significantly different in the fresh and dry top weight of the plants. But the irradiated and non-irradiated sludges were highly significantly ($P<0.01$) different to other fertilizer formular. And all fertilizer formular did

not make the fresh and dry top and root weights of the plants grown on Korat soil set significantly different.

Table 1 The effect of fertilizer formular on the average height of the morning glory at 7, 12, 17, 22, 27 and 32 days after planting on Korat soil set

Fertilizer formular	Average of plant heights (cm) at different ages after planting (day)					
	7	12	17	22	27	32
Check	4.0	5.3	6.9	7.9	9.8	9.9
Urea	4.9	4.8	6.4	6.9	7.2	7.8
Non-irradiated Waste Water	4.4	6.3	7.4	8.2	9.1	10.2
Irradiated Waste Water	4.8	6.2	6.8	7.9	9.2	9.1
Non-irradiated Sludge	3.7	5.7	7.9	10.4	14.0	18.9
Irradiated Sludge	4.6	6.4	9.7	11.6	14.5	18.7

Table 2 The effect of fertilizer formular on the average height of the morning glories at 7, 12, 17, 22, 27 and 32 days after planting on Kui Pai soil set

Fertilizer formular	Average of plant height (cm) at different ages after planting (day)					
	7	12	17	22	27	32
Check	3.9	5.3	7.0	9.1	10.0	9.5
Urea	3.8	5.2	6.7	6.9	9.8	11.3
Non-irradiated Waste Water	3.9	5.3	6.8	7.9	9.6	11.8
Irradiated Waste Water	4.2	5.5	6.8	7.3	8.9	10.9
Non-irradiated Sludge	3.5	5.5	8.0	13.0	15.3	19.3
Irradiated Sludge	3.9	5.3	9.0	12.5	15.4	20.0

Table 3 The chemical analysis of soil set mixed with different fertilizer formular in pot before planting

Samples	pH	%Organic matter	Phosphorus ppm	Potassium ppm	%Moisture
K.P.	6.0	5.56	23	172	9.98
K.P.+ N.W.	6.0	5.93	11	102	12.36
K.P.+ I.W.	6.0	5.42	9	102	11.23
K.P.+ N.S.	6.1	7.73	230	137	9.77
K.P.+ I.S.	6.1	7.19	165	137	9.27
K.P.+ U	6.2	9.22	11	126	10.86
Kr	3.7	0.77	2	36	5.93
Kr + N.W.	3.4	0.75	2	36	14.94
Kr + I.W.	3.5	0.81	1	35	14.42
Kr + N.S.	4.5	1.36	120	48	6.04
Kr + I.S.	4.2	1.22	40	50	6.04
Kr + U	3.7	0.68	1	42	14.81

Remark : K.P. = Kui Pai soil set
Kr = Korat soil set
N.W. = Non-irradiated Waste Water
U = Urea
I.W. = Irradiated Waste Water
N.S. = Non-irradiated Sludge
I.S. = Irradiated Sludge

Table 4 The chemical analysis of waste water and sludge used as fertilizer

Samples	pH	% Moisture	%Total Nitrogen	%Total P_2O_5	%K_2O Total	%K_2O Water soluble	% Organic matter	% C/N
waste water	8.27	-	0.20	0.50	-	0.002	-	-
sludge	6.13	1.44	3.70	2.17	0.20	-	50.24	13.7:1

Table 5 The effect of the fertilizer formular on the average fresh top and root weights of the morning glory at harvesting. (gm)

Fertilizer formular	Korat soil set		Kui Pai soil set	
	top	root	top	root
check	35.53	10.00	53.30	23.4
urea	28.83	15.03	59.37	26.5
non-irradiated waste water	34.27	15.43	41.73	32.17
irradiated waste water	27.80	11.40	43.13	35.83
non-irradiated sludges	117.50	14.70	130.37	28.37
irradiated sludges	129.33	14.13	143.43	33.63

Table 6 The effect of fertilizer formular on the average of dry top and root weights of the morning glory at harvesting. (gm)

Fertilizer formular	Korat soil set		Kui Pai soil set	
	top	root	top	root
check	5.75	2.59	9.06	5.88
urea	4.66	2.78	8.69	5.53
non-irradiated waste water	6.23	3.23	5.79	5.20
irradiated waste water	5.48	2.94	6.28	6.75
non-irradiated sludge	14.15	3.67	18.90	7.15
irradiated sludge	15.97	2.92	20.50	8.45

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6.2 Application of irradiated sludge compost to plants

S. Hashimoto

Effect of application of the compost on growth and yield of plant was examined using rice, vegetables, mulberry and flower. This application test was performed with the cooperation by Gunma Agricultural research Center, Japan. The compost was produced by the pilot plant which was constructed in a sewage treatment facility in Takasaki. Some properties of the compost is shown in Table 1.

1. Rice

The irradiated sludge compost was mixed with soil and was put in pots. Three pots with 30 cm diameter and 50 cm height were used for each test. Three plants were used for one pot and the same pots were used for two years. The growth and yield of rice is shown in Table 2.

Application of compost brought the increase of number of ear and yields of rice grain increased 1.2 and 1.8 times in No. 3 and 4 in the first year. Remarkable increases of the yields of brown rice were also observed in the second year. It seems that these effects were mainly attributable to nitrogen and available phosphorus contained in the compost.

2. Vegetables

Four pots with 25.0 cm diameter and 30.0 cm height were used for each test and five plants were used for one pot. The same test was continued for two years. Table 3 shows length of leaves and yields of spinach in the first year. The yields increased by application of compost. It can be seen that the application of 2.5 tons/10a gives the maximum yields and too much application is not necessary. Effects of compost on three kinds of komatsuna (leaf vegetable) were also examined. The results were almost similar to the effects on spinach. Remarkable increase of spinach was not observed in the second year because of the change of pH in the soil by the application of compost.

3. Mulberry

Irradiated sludge compost was applied on march in a field of mulberry with 80 m^2 wide. The leaves were harvested two times in July and September. Results are shown in Table 4. No difference was observed between with and without application of compost. It seems, as a reason, that the amount of application is too small. The same application test was continued for two years and the result in the second year was almost the same as the first year.

4. Flower

A kind of aster was used for test. Number and length of branches were examined. The result is shown in Table 5. It can be seen that the application of compost yields longer branches and larger weight of plants. This means that flowers with higher grade for decoration are obtained.

Table 1 Some properties of raw sludge and irradiated sludge compost

	Raw sludge	Compost
Moisture content	25.9 %	75.2 %
Volatile solid content	57.0 %	75.7 %
Total carbon	27.4 %	38.8 %
Total nitrogen	3.4 %	4.1 %
BOD	24.3 mg/g	250 mg/g
pH	8.6	6.1
NO_3-N	Not detected	Not detected

Table 2 Effect of compost on growth and yield of rice

(First year)

No.	Amount of Compost (tons/10a)	Culm Length (cm)	Number of Ear	Total Weight (g)	Weight of Brown rice (g)	Relative Weight of Brown rice (%)
1	0	66.6	14.0	187.3	74.7	100
2	1	65.4	15.3	200.0	75.7	101
3	2	71.9	17.7	190.0	90.3	121
4	4	73.4	25.0	310.0	135.0	181

(Second year)

No.	Amount of Compost (tons/10a)	Culm Length (cm)	Number of Ear	Total Weight (g)	Weight of Brown rice (g)	Relative Weight of Brown rice (%)
1	0	58.4	12.8	158.3	58.9	100
2	1	63.9	18.8	239.7	97.8	166
3	2	63.4	24.2	287.7	120.0	204
4	4	65.0	34.0	374.7	155.3	264

Table 3 Effect of compost on growth and yield of spinach

No.	Amount of Compost (tons/10a)	Plant Height (cm)	Top Weight (g)	Relative Weight (%)
1	0	15.9	65.0	100
2	2.5	20.0	100.0	154
3	5.0	19.3	85.5	132
4	7.5	18.8	78.0	120
5	10.0	18.6	79.5	122

Table 4 Effect of compost on yield of mulberry leaves

No.	Amount of Compost (tons/10a)	First harvest (kg)	Second harvest (kg)	Total Weight (kg)	Relative Weight (%)
1	0	2504	1233	3737	100
2	1	2654	1175	3829	102
3	2	2683	1228	3911	105

Table 5 Effect of compost on growth and yield of aster

No.	Amount of Compost (tons/10a)	Number of Cut Flower	Ratio(%) length of cut flower(cm) 50-60	61-70	71-80	>81	>71	Weight /Plant (g)
1	0	4.3	69.2	30.8	0	0	0	25.3
2	2	4.2	64.3	9.5	19.5	7.2	26.2	25.4
3	4	4.4	31.8	25.0	38.6	4.5	43.1	31.7
4	8	4.4	47.7	22.7	20.5	9.1	29.6	44.0

7. Cost analysis of radiation treatment of sludge

[Abstract]

7.1 Cost analysis of radiation treatment of sludge

Rough calculation of costs for sludge treatment by gamma-ray and electron-beam were carried out. It was shown that electron-beam treatment seemed to be more economically feasible than gamma-ray treatment at larger treatment capacity.

7.1 Cost analysis of radiation treatment of sludge

S. Pongpat and S. Hashimoto

1. Introduction

Sludge is continuously and increasingly generated in Thailand due to the expansion of metropolitan societies and industrial activities. According to the project for wastewater treatment in metropolitan area, the amount of sludge is expected to be generated more than 100 tons/day in the near future. The city will be seriously faced to the problem of disposal of sludge. Although sludge has high potential applications for both agronomy as a soil conditioner and fertilizer, it has a potential hazard to human health and environment as it contains pathogens. Various methods are known for elimination of pathogenic microorganisms and parasites. Ionizing radiation, however, is a well known effective method for the decontamination of sludge. Gamma-ray from cobalt-60 has been mainly used as a radiation source. Recently, electron beam irradiation has been evaluated as a practical and economical method. In this report, results of rough cost estimation of sludge decontamination by using gamma-ray from cobalt-60 and electron beam from electron accelerator are shown.

2. Sludge pasteurization system

Fig. 1-a shows an example of sludge pasteurizing system by electron accelerator. This figure shows a normal size irradiation room for 50 tons/day of sludge treatment capacity. Sludge cake with the moisture content about 80 % is spread on a stainless steel conveyer through a flat nozzle and disinfected by electron beam which comes from the electron accelerator through scan horn upside of the apparatus. Irradiated sludge is moved out from the chamber by conveyer system. A schematic diagram of apparatus for preparation of sludge film is shown in Fig. 1-b.

Fig. 2 shows an example of sludge pasteurizing system by cobalt-60 irradiator developed by Nordion Co., Ltd. The irradiation is performed by lifted cobalt-60 source rack to the designed position and the containers of sludge are moving around within a designed period by roller conveyer. Irradiated sludge is moved out from the irradiation room by conveyer system.

3. Cost analysis

Table 1 shows the treatment cost of sewage sludge by an electron accelerator. These figures were based on the following conditions and assumptions.

The irradiation facility was designed to be installed to a sewage treatment plant. It was designed to irradiate dewatered sewage sludge with the treatment capacity ranging from 10 to 100 tons/day. The beam energy was selected to be 2 MeV. Irradiation dose was set at 5 kGy with 60 % efficiency. According to these conditions and assumptions, the price of electron accelerator was estimated to be in the range of 40-60 million bath (MB). The installed price including building & shielding and other equipments was 25 MB. The estimation of capital cost was obtained to be in the range of 65-85 MB.

The capital cost was amortized 15 years for equipments and 25 years for building & shielding at 12 % interest. The annual maintenance was assumed to be 10 % of equipment price. An operation time was assumed to be 8 hrs/day. The labor cost considered as government service was ranged 0.31-0.37 MB/y. Utility cost was ranged from 1.0-2.6 MB/y. The estimation of annual cost and unit cost were obtained to be in the range of 19.2-25.5 MB and 5,272-699 B, respectively.

Table 2 shows the treatment cost by a cobalt-60 irradiator. The estimation of these figures were based on the following conditions and assumptions. The irradiation facility was designed to be installed to a sewage treatment plant as the same as electron accelerator. It was designed for sludge irradiation with the treatment capacity at 10-100 tons/day. The irradiation dose was set at 5 kGy with 25 % efficiency. The unit cost of cobalt-60 source was assumed to be 46 B/Ci and the loading ranged from 452-4640 kCi was about 20.8-213.4 MB. The capital cost was estimated to be in the range of 45.8-273.4 MB.

The capital cost was also amortized 15 years for equipments and 25 years for building & shielding at 12 % interest as the same as electron accelerator. The decay rate of cobalt-60 source was assumed 12 %/y, therefore, the annual replenishment cost was ranged from 2.5-25.6 MB/y. Its annual maintenance was assumed to be 5 % of equipment price. An operation time was supposed to be 8 hrs/day. The utility cost was assumed to be about 0.25 MB/y. The labor cost considered as government service was ranged from 0.31-0.37 MB/y. The annual cost and unit cost were estimated to be in the range of 7.6-37.2 MB and 2,095-1,020 MB, respectively.

4. Discussion

Since the penetrating quality of electron beam is less than that of gamma-ray, so the dose distribution along the pass in the sample to be irradiated is different. Fig. 3 shows dose distribution of electron beam in water. Dose in water increases at first with increase of thickness and then decreases. Maximum penetration range (mass thickness, g/cm^2) is different depending on beam energy and the value is 1.1 cm for 2 MeV. The thickness to give the same dose as that at the surface is called "Effective penetration range".[1)]

In order to kill microorganisms effectively in case of electron accelerator, sludge must be prepared as a film and the thickness must be less than 6 mm for beam energy of 2 MeV and 3 mm for beam energy of 1 MeV as seen in Fig. 5. These value correspond to the effective penetration ranges for those energies.[2)] The sludge film preparation needs both experience and technology. The thicker film seems to be easier for preparation but irradiation needs higher beam energy of electron accelerator. The price of electron accelerator, however, remarkably depends on the beam energy. According to these factors, 2 MeV electron accelerator is selected for the treatment facility in this study.

On the other hand, the penetrating quality of gamma ray is higher compared with electron beam. Fig. 4 shows dose distribution of gamma-ray in water. Relative dose decreases exponentially with thickness of water. Half-value layer in water is about 11 cm. The sludge to be irradiated is, therefore, possible to contain in a bucket for irradiation.

In comparison between 2 systems of treatment cost, the annual cost and unit cost by cobalt-60 irradiation treatment are lower than those by electron beam treatment when the treatment capacity is in the range of 10-50 tons/day. On the other hand, the annual cost and unit cost of electron beam treatment becomes lower than those by cobalt-60 irradiation treatment when the treatment capacity is up to 100 tons/day. This result is affected by the remarkable increase of the source loading and building & shielding prices. Moreover, the enlargement of irradiation room for cobalt-60 irradiator treatment is necessary when the treatment capacity increases in order to bring up the residence time of sample for irradiation.

Conclusively, at higher treatment capacity, electron accelerator treatment seems to be more economically feasible than cobalt-60 irradiator treatment. However, the technology of electron accelerator treatment requires highly skilled personnel.

References

1. S. Hashimoto, "Electron Beam Treatment of Wastewater and Sewage Sludge", JAERI-M 93-160 (1993).
2. S. Hashimoto et al., "Economic Feasibility of Irradiation Composting Plant of Sewage Sludge, Radiat. Phys. Chem., Vol. 31, p. 109 (1988).

Acknowledgment

The authors wish to thank Dr. Keiichi Yotsumoto for his useful advises and discussions.

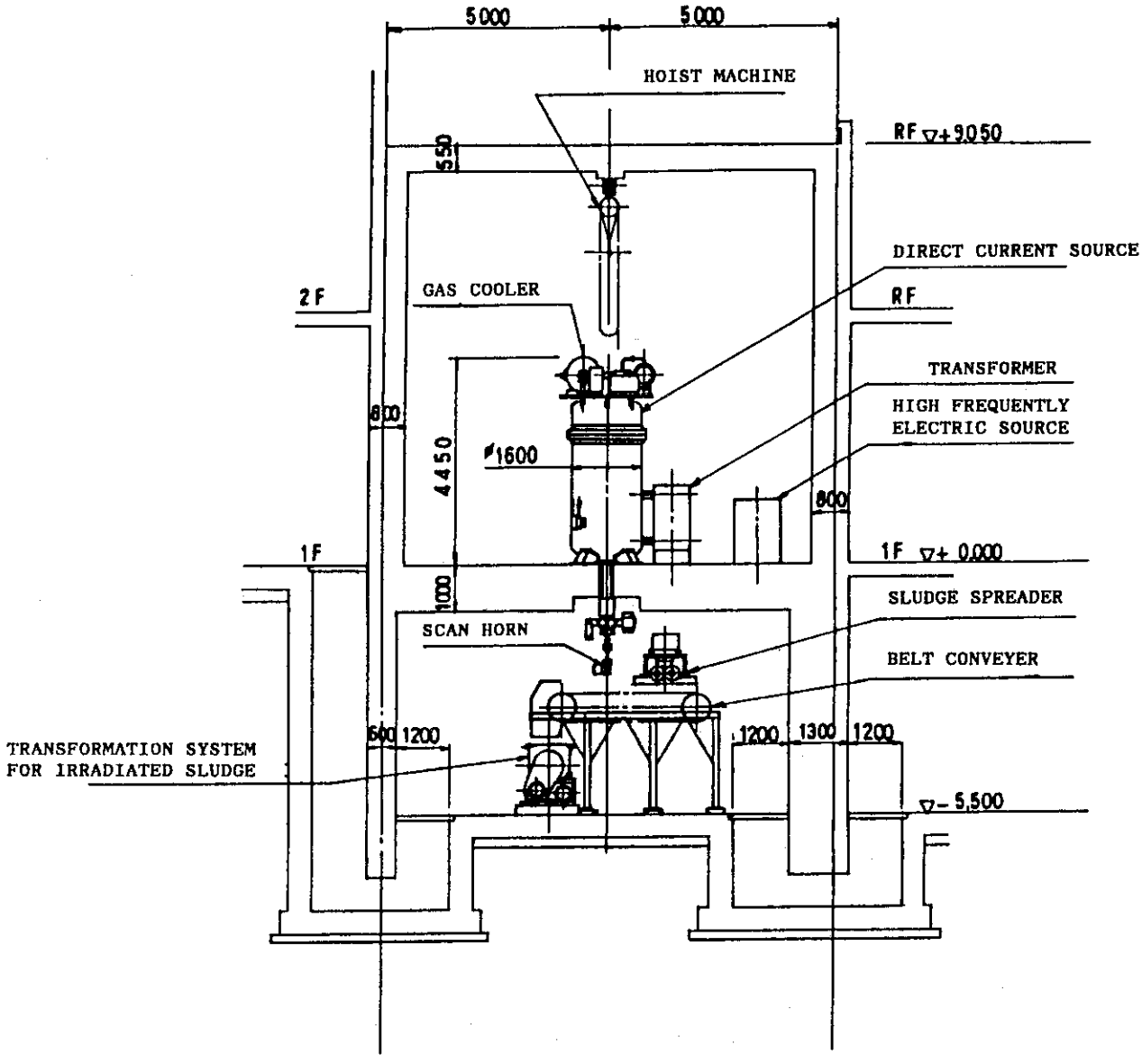

Fig.1-a An example of sludge pasteurization system by electron accelerator

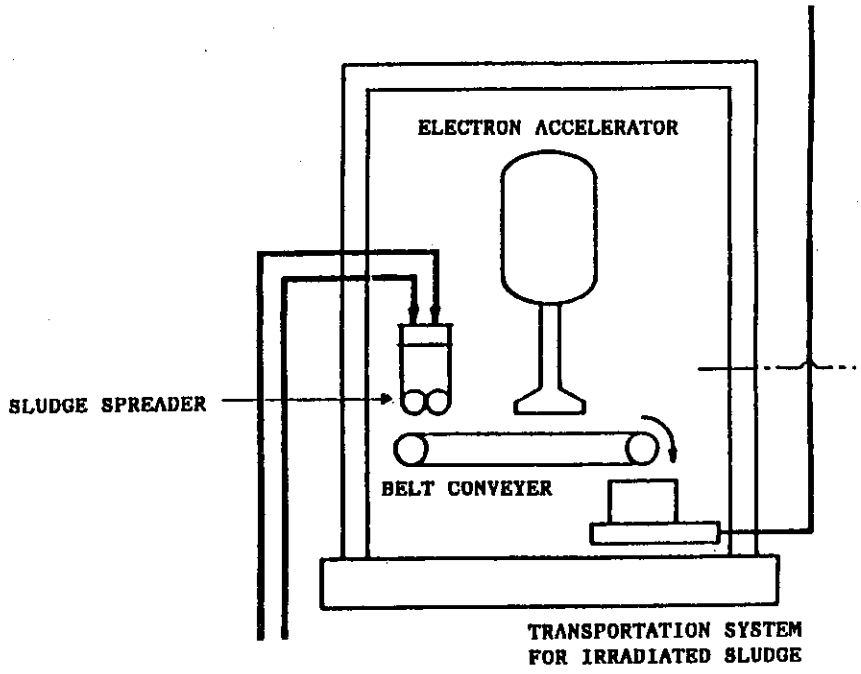

Fig. 1-b A schematic diagram of an apparatus for preparation of sludge film

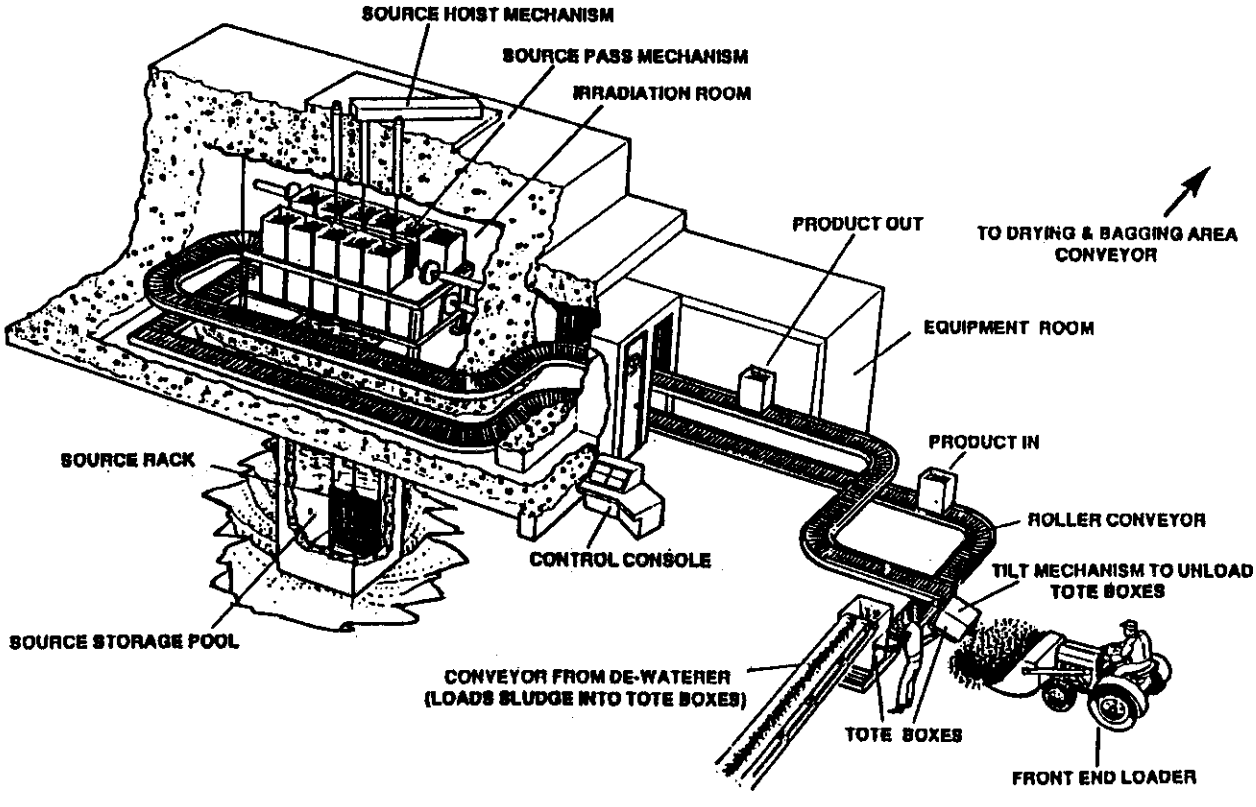

Fig. 2 An example of sludge pasteurization system facility by cobalt-60

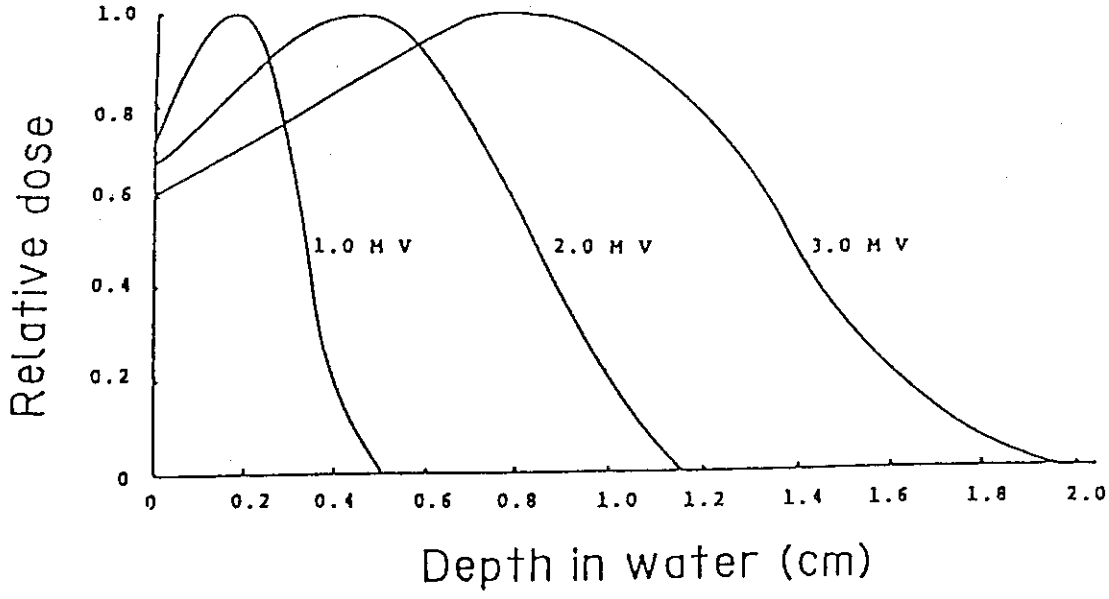

Fig. 3 Dose distribution of electron beams in water

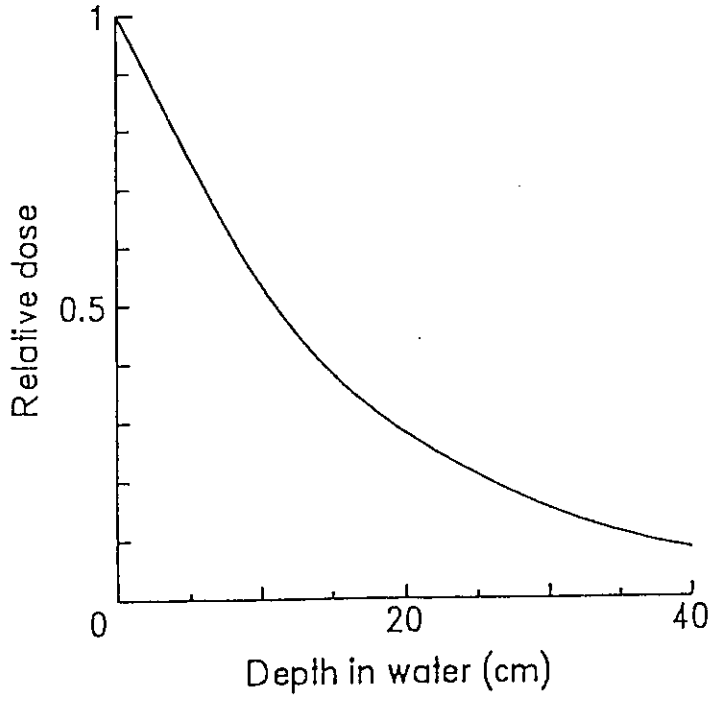

Fig. 4 Dose distribution of gamma ray in water

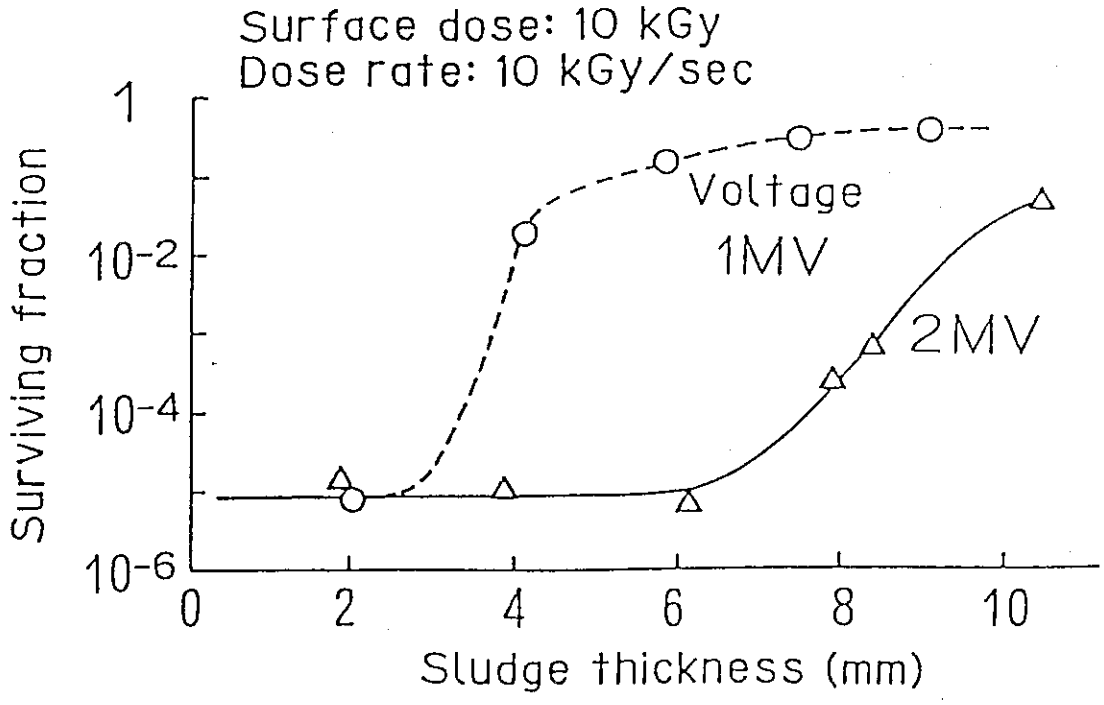

Fig. 5 Surviving fraction of bacteria in sludge irradiated with various thickness

Table 1 Treatment cost by an electron accelerator

	Treatment capacity (ton/day)		
	10	50	100
Energy (Mev)	2	2	2
Calculated output power (kw)	1.7	8.7	17.4
Output power (kw)	3	15	30
Beam current (mA)	1.5	7.5	15
Total power (kw)	53	65	80
Capital Cost			
Building and Shielding (B)	10,000,000	10,000,000	10,000,000
Accelerator(B)	40,000,000	50,000,000	60,000,000
Others (B)	15,000,000	15,000,000	15,000,000
Total cost	65,000,000	75,000,000	85,000,000
Annual Cost			
Building & shielding (25 y)	400,000	400,000	400,000
Equipments (15 y)	3,680,000	3,680,000	3,680,000
Total interest payment (12 %/y)	8,310,000	9,640,000	10,970,000
Maintenance (10 % of equipment)	5,500,000	6,500,000	7,500,000
Utilities	1,045,000	1,733,000	2,611,000
Electricity (B/y)	159,400	722,000	1,444,000
(kwh/d)	(424)	(1920)	(3840)
Water (B/y)	85,400	211,000	367,000
(m^3/d)	(36)	(89)	(155)
Active carbon (B/y)	800,000	800,000	800,000
(l/d)	(13.7)	(13.7)	(13.7)
Labor cost	309,600	366,960	366,960
Manager (B/y)	151,920	151,920	151,920
(persons)	(1)	(1)	(1)
Engineers (B/y)	81,120	81,120	81,120
(persons)	(1)	(1)	(1)
Technicians (B/y)	38,160	76,320	76,320
(persons)	(1)	(2)	(2)
Laborers (B/y)	38,400	57,600	57,600
(persons)	(2)	(3)	(3)
Total annual cost	19,244,600	22,319,960	25,527,960
Hours of operation (h/y)	2,920	2,920	2,920
Treated amount (ton/y)	3,650	18,250	36,500
Utilities (B/ton)	286	95	72
Cost (B/ton)	5,272	1,223	699

Efficiency of irradiation: 60 %
Operation: 8 hrs/day
Electricity: 1.03 (B/kwh)

Table 2 Treatment cost by Cobalt-60

	Treatment capacity (ton/day)		
	10	50	100
Calculated output power (kw)	1.7	8.7	17.4
Amount of cobalt-60 (kCi)	113	580	1160
Total amount of cobalt-60 (kCi)	452	2320	4640
Capital Cost			
Building and Shielding (B)	15,000,000	28,000,000	36,000,000
Source loading (B)	20,792,000	106,720,000	213,440,000
Others (B)	10,000,000	18,000,000	24,000,000
Total cost	45,792,000	152,720,000	273,440,000
Annual Cost			
Building & shielding (25 y)	600,000	1,120,000	1,440,000
Equipments (15 y)	667,000	1,200,000	1,600,000
Total interest payment (12 %/y)	2,820,000	5,170,000	6,760,000
Cobalt-60 supply (12 %/y)	2,500,000	12,810,000	25,610,000
Maintenance (5 % of equipment)	500,000	900,000	1,200,000
Utilities	250,000	250,000	250,000
Electricity (B/y)	60,000	60,000	60,000
(kwh/d)	(160)	(160)	(160)
Water (B/y)	190,000	190,000	190,000
(m^3/d)	(80)	(80)	(80)
Labor cost	309,600	366,960	366,960
Manager (B/y)	151,920	151,920	151,920
(persons)	(1)	(1)	(1)
Engineers (B/y)	81,120	81,120	81,120
(persons)	(1)	(1)	(1)
Technicians (B/y)	38,160	76,320	76,320
(persons)	(1)	(2)	(2)
Laborers (B/y)	38,400	57,600	57,600
(persons)	(2)	(3)	(3)
Total annual cost	7,646,600	21,816,960	37,226,960
Hours of operation (h/y)	2,920	2,920	2,920
Treated amount (ton/y)	3,650	18,250	36,500
Utilities (B/ton)	69	14	7
Cost (B/ton)	2,095	1,195	1,020

Efficiency of irradiation: 25 %
Operation: 8 hrs/day
Cobalt-60 source: 46 (B/Ci)
Electricity: 1.03 (B/kwh)
Water: 6.50 (B/m^3)
Active carbon: 160 (B/l)

8. Conclusion

Radiation inactivation effect of pathogens and parasites

S.paratyphi B. var java was selected to study for radiation sensitivity in sludge because this serotype was the most resistant, among 47 serotypes, in phosphate buffer solution. D_{10} values of this strain in liquid sludge, sludge cake and dried sludge were obtained to be 0.35, 0.40 and 0.45 kGy, respectively. Hence, necessary dose for sterilization of salmonellae in liquid sludge, sludge cake and dried sludge were 2, 3 and 4 kGy, respectively.

Total coliforms and fecal coliforms in sludge sample except in dried sludge, E. Coli and S. aureus were sufficiently inactivated at 6 kGy. It was also found that irradiation at 20 kGy was almost enough to inactivate total bacteria in sludge sample except in dried sludge.

Irradiation on Ascaris suum at 2 kGy caused failing in hatching ability of its egg after 9 weeks. D_{10} value of 1 cell-corticated A. suum egg in 4 % and 90 % solid sludge were 0.52 and 0.64 kGy respectively. D_{10} value of infective, corticated egg in 4 % solid was 0.75 kGy. And those for infective decorticated eggs in 4 % and 90 % solid sludge were 0.71 and 0.75 kGy respectively.

Upgrading of disinfected sludges

Design of a fermentor system for large scale fermentation were carried out. The fermentors were installed in OAEP for the experiment of upgrading of irradiated sludge.

The effect of temperature and seed compost on composting of irradiated sludge by microbiological fermentation was investigated. The results showed that 45 °C was the best temperature to decompose sludge and 35 °C was better to get larger amount of biomass. Decomposition of irradiated sludge in soil proceed more rapidly than the unirradiated sludge and the number of total bacteria in soil increased by the decomposition of irradiated sludge.

Survey of the useful microorganisms for upgrading of the irradiated sludge to feedstuff was performed. It was found out that R. oligosporus and A. niger were possible to grow easily in the mixture of sludge and dried water-hyacinth.

Isolation and identification of bacteria, which has effect to suppress phytopathogenic fungi, from soil and commercial seed composts were conducted. By the inhibition zone test, it was found that several isolated strains has effect to suppress F. oxysporum, one of the phytopathogenic fungi. Optimum conditions for growth of these bacteria in both culture media, irradiated sludge and grass waste were also obtained.

Evaluation of components in sludges

Major, minor elements and other chemical compositions of sludge samples from industry, hospital and community were studied. It showed that major elements (N,P,K) of samples from industry were at maximum level, which were at the average range

of 1.92-5.96 %, 3800-85800 ppm, and 2940-4020 ppm respectively. The minor elements (Ca, Mg) of sludges from industry and community, both total and available, were higher than from hospital. But Fe, Zn and Cu in sludges from industry were lower than hospital and community. C/N ratios of samples from industry and hospital were smaller than community.

Utilization test of the products

Utilization tests of raw sludge, irradiated sludge and irradiated sludge compost were conducted. It was shown that raw sludge and irradiated sludge are effective for growth of morning glory. It was also shown that the irradiated sludge compost is very effective on the growth and yield of rice and vegetables, especially on the yield of brown rice. From the results of the test carried out in Thailand and Japan, it was concluded that irradiated sludge and irradiated sludge compost can be used as safe and effective fertilizer.

Irradiation engineering and feasibility study

Rough calculation of costs for sludge treatment by gamma-ray and electron-beam were carried out. It was shown that electron-beam treatment seemed to be more economically feasible than gamma-ray treatment at larger treatment capacity.

ACKNOWLEDGEMENT

The bilateral cooperation between OAEP and JAERI on "Sludge Pasteurization and Upgrading by Radiation" was supported by many persons.

The authors wish to express our thanks to Dr. Suchat Mongkolphantha, former Secretary-General of OAEP and Dr. S. Sato, former Director General of TRCRE, JAERI for the great effort to make the cooperation fruitful.

For the researches on effect of radiation on parasites and analysis of sludge components, we got a special support by Dept. of Parasitology, Faculty of Public Health, Mahidol University, Dept. of Soil Science, Faculty of Agriculture, Kasetsart University and Dept. of Soil, Agricultural Engineering Training Center.

For the research on upgrading of irradiated sludge, several kinds of effective bacteria and seed composts were kindly presented from Prof. M. Shoda, Tokyo Institute of Technology and Mr. T. Maki, Managing Director, M. I. M., Ltd.

The effect of irradiated sludge compost on growth of plants were tested by Gunma Agricultural Research Center, this test was continued for about three years.

We also wish to thank many people working at the sewage and waste water treatment facilities, Takasaki Water Cleaning Center, Gunma Prefecture Sewage Works Corporation, Hanamaruki Foods Inc., Boonrawd Brewery Co., Ltd., Thai Pure Drinks Ltd., Vajira Hospital, Central Chest Hospital, Huay Kwang Sewage Treatment Plant, etc., for supporting sludge sampling.

Lastly, authors also wish to express our thanks to Dr. H. Arai, Principal Scientist, TRCRE, JAERI for his valuable discussion and advises for the preparation of this report.

APPENDIX 1 Steering Committee Meetings and participants

1. First Steering Committee Meeting
(28-29 March, 1990 at OAEP, Bangkok)

JAERI Dr. Shoichi Sato,
Director General, TRCRE
Dr. Shoji Hashimoto
Head Resources Utilization Technology Lab.,
Dept. of Radiation Research for Environment
and Resources
OAEP Mr. Suchat Mongkolphantha,
Secretary-General
Dr. Chettachai Banditsing, Director,
Biological Science Division
Observers
Mr. Ratana Pumlek
Ms. Sarunya Piadang
Ms. Suchada Pongpat
Ms. Ngamnit Sermkiattipong
Ms. Yoawaluck Leenanupan

2. Second Steering Committee Meeting
(17-18 December, 1990 at OAEP, Bangkok)

JAERI Dr. Shoichi Sato,
Director General, TRCRE
OAEP Dr. Pakit Kiravanich
Secretary-General
Dr. Chettachai Banditsing,
Director, Biological Science Division
Observers
Mr. Ratana Pumlek
Ms. Sarunya Piadang
Ms. Suchada Pongpat
Ms. Ngamnit Sermkiattipong
Ms. Yoawaluck Leenanupan
Ms. Waree Supol

3. Third Steering Committee Meeting
(4-5 July, 1991 at JAERI, Takasaki)

JAERI Dr. Shoichi Sato,
Director General, TRCRE
Dr. Waichiro Kawakami
Director, Dept. of Rad. Research for Environment
and Resources, TRCRE
OAEP Dr. Pakit Kiravanich
Secretary-General
Observers
Dr. Chettachai Banditsing
Ms. Suchada Pongpat

Mr. Hideo Osono
Dr. Shoji Hashimoto
Dr. Hitoshi Ito
Dr. Tamikazu Kume
Dr. Shinpei Matsuhashi
Mr. Akio Toraishi

4. Fourth Steering Committee Meeting
(8 and 10 July, 1992 at OAEP, Bangkok)

JAERI Dr. Shoichi Sato,
Director General, TRCRE
Dr. Shoji Hashimoto
Head Resources Utilization Technology Lab.,
Dept. of Radiation Research for Environment
and Resources, TRCRE
OAEP Mr. Suchat Mongkolphantha,
Secretary-General
Dr. Chettachai Banditsing, Director,
Biological Science Division
Observers
Ms. Sarunya Piadang
Ms. Suchada Pongpat
Ms. Ngamnit Sermkiattipong
Ms. Yoawaluck Leenanupan

5. Fifth Steering Committee Meeting
(24-25 February, 1994 at JAERI, Takasaki)

JAERI Dr. Shoichi Sato,
Director General, TRCRE
Dr. Shoji Hashimoto
Head Resources Utilization Technology Lab.,
Dept. of Radiation Research for Environment
and Resources, TRCRE
OAEP Mr. Suchat Mongkolphantha,
Secretary-General
Dr. Chettachai Banditsing, Director,
Biological Science Division
Observers
Dr. Manoon Aramrattana
Ms. Suchada Pongpat
Dr. Shin-ichi Ohno
Mr. kiyoshi Asai
Dr. Okihiro Tokunaga
Dr. Hitoshi Ito
Dr. Tamikazu Kume
Dr. Shinpei Matsuhashi
Mr. Yoichi Suto

APPENDIX 2 Exchange of scientists

1990

From OAEP to JAERI

Ms. Ngamnit Sermkiattipong
25 June - 22 September
"Upgrading of irradiated sludge"

From JAERI to OAEP

Dr. Shoji Hashimoto
30 March - 7 April
"Discussion on cooperative research schedule"

1991

From OAEP to JAERI

Ms. Suchada Pongpat
17 June - 14 September
"Upgrading of irradiated sludge"

Dr. Chettachai Banditsing
3 July - 12 July
"Discussion on the research schedule of Bilateral Cooperation"

From JAERI to OAEP

Dr. Shoji Hashimoto
13 January - 1 February
"Fermentation test using A Set of Equipments for Incubation Test of Useful Microorganisms"
29 September - 12 November
"Fermentation test using Fermentor Set for Large Scale Fermentation"

Dr. Tamikazu Kume
24 February - 9 March
"Fermentation test using Fermentor Set for Large Scale Fermentation"
18 November - 27 November
"Analysis of sludge and the products"

1992

From OAEP to JAERI

Ms. Ngamnit Sermkiattipong
28 September - 26 December
"Upgrading of irradiated sludge"

From JAERI to OAEP

Dr. Shin-ichi Ohno
22 July - 25 July
"Discussion on the research cooperation"

Dr. Shoji Hashimoto
6 July - 11 July
"Discussion on the research cooperation"

Dr. Tamikazu Kume
18 October - 22 October
"Discussion on the research cooperation"

1993

From JAERI to OAEP

Dr. Shinpei Matsuhashi
14 February - 26 February
"Analysis of components in sludge"

Dr. Tamikazu Kume
5 December - 12 December
"Analysis of components in sludge"

1994

From OAEP to JAERI

Ms. Suchada Pongpat
10 January to 31 March
"Upgrading of irradiated sludge"

From JAERI to OAEP

Dr. Hitoshi Ito
6 February - 19 February
"Discussion on the research plan for the future cooperation"