The characteristics of novel aerobic ultra high temperature fermented compost

（新規な好気性超高温発酵堆肥の特性に関する研究）

Chunxiang PIAO

朴　春香

2012

TABLE OF CONTENTS

General Introduction

Organic matter is matter that has come from a once-living organism; is capable of decay, or the product of decay; or is composed of organic compounds. Organic matter is broken down organic matter that comes from plants and animals in the environment. Basic structures are created from cellulose, tannin, cutin, and lignin, along with other various proteins, lipids, and sugars. The amount of the total organic matter waste discharged approximately about 280 million tons a year in Japan, deals with approximately 60% of the total waste gross weight, including 94 million tons domestic animal feces and urine, 14 million tons farm products residual substance, 20 million tons garbage and 86 million tons sewage and sludge (Table. GI-1) [Chino, 2000].

Livestock and poultry manures are a traditional source of organic nutrients in agriculture. Under proper management, animal manure is a valuable source of plant nutrients that can reduce or eliminate the use of commercial fertilizer and also provides organic carbon that enhances soil physical properties.

However, excess nutrient application to soil is a major environmental issue in USA and Canada [Ludwick and Johnston, 2002], Europe [Brouwer *et al.*, 1999] and other parts of the world with intensive confined animal production such as Brazil [Oliveira, 1993]. There are major concerns regarding the generation of large amounts of manure by concentration of confined animal production units within relatively small geographic areas and the potential to impair ground and surface water quality due to soil leaching or runoff of land applied nutrients. In the USA, land application of manure may be difficult and costly to implement due to current trends indicating that animal operations are declining in number and growing in size. For confined animal operations in the USA, 60% of available nitrogen and 70% of available P were in excess of the amount of manure N and P that could be assimilated on the farms that produced them.

Therefore, substantial amounts of manure N and P need to be moved at least off the farms and that some need to be transported longer distances beyond county limits to solve distribution problems of these nutrients [United States Department of Agriculture -Economic Research Service, 2000].

In European countries serious environmental problem of the groundwater contamination with nitrogen included in the livestock industry waste in the 1970s. Standing on such experience, various measures for environ mental consideration was introduced after the 1980s [Oenema, 2004]. From 2005, the European Commission (EC) prohibited final deposition of municipal waste in landfills without prior treatment. The directive is overall aim is to prevent or reduce as far as possible negative effects on the environment, in particular the pollution of surface water, groundwater, soil and air, and on the global environment, including the greenhouse effect, as well as any resulting risk to human health, from the landfilling of waste, during the whole life-cycle of the landfill. This legislation also has important implications for waste handling and waste disposal.

The number of livestock in Japan is 300 million chickens (180 and 120 million of laying hens and meat chickens, respectively), 9.8 million pigs and 4.5 million cattle (1.6 and 2.7 million of daily and beef cattle, respectively). The discharge of the organic matter reached approximately 90 million tons/year and came to occupy approximately 20% of the whole industrial waste [Ministry of the Environment, Government of Japan, 2002]. The intensity and concentrated activity of the livestock industry generate vast amounts of organic matter, which must be managed under appropriate disposal practices to avoid a negative impact on the environment (odour and gaseous emissions, soil, water pollution etc.) [Burton and Turner, 2003]. The domestic

animal excrement has been used as precious organic resources for production of farm products and forage crop effectively conventionally by stock raising management. However, recently, the use of the domestic animal excrement was becoming difficult, and the influence to give to local environment came to be concerned about backed by sudden scale expansion of the stock raising management, aging. Depending on an area, it is reported that nitrogen derived from domestic animal feces and urine may raise state nitrogen levels of the river water nitrate more than 20 mg/l [Shimura and Tabuchi, 1997]. The organic matter was characterized by very high level in N and P especially that matters and wastewater comes from livestock industry. Based on such a thing, law of concerning the appropriate treatment and promotion of utilization of livestock manure was enforced in 1999. To generate the domestic animal excrement the livestock industry is obliged must to be carried out under the specific facility [Ministry of Agriculture, Forestry and Fisheries of Japan, 1999]. The stock rising farmer is obliged to carry out appropriate management of the domestic animal excrement, and use promotion is demanded by law of domestic animal excrement management. The problems associated with organic matter disposal have become a major problem of the world due to increase in the amount of the total organic matter. In that, livestock manure accounts for a large part of the total waste generated and can cause environmental problems. (e.g., air, water, and soil pollution) [Hall, 1999].

To find out the better ways of waste management system and tackle the waste problem became a very important problem. To decrease the consumption of energy and raw materials is a one way. But the world population increases and living level improvement will be resulting in increases of organic waste. Burning is another way to treatment of the organic matter. Convert the solid organic matter into residue and

gaseous products. The method is common in countries such as Japan where land is scarce, as these facilities generally do not require as much as area as landfills. Although this method of waste treatment is rather progressive, there are harmful side effects. Sulphur and nitrogen oxides, hydrogen chloride, dioxins, heavy metals and other materials are released into the air. Thus burning method is now generally prohibited in some area and countries. Landfills although are intended to minimize the negative impact of waste on the environment, but they have harmful effects on nature also. The greenhouse effect is aggravated as CO_2 and methane are released in the air as by-products of degraded organic waste. Pesticides, organic pollutants, cyanides, nitrates and heavy metals pollute waters, especially underground. Further use and development of land is restricted by the presence of landfills.

Composting is an extremely limited activity in the world except Japan and some Asia countries. Composting is nature's way of recycling organic waste into new soil, which can be used in vegetable and flower gardens, landscaping, horticulture, agriculture and many other applications. Compost can be rich in nutrients. That can be reduce or eliminate the need of chemical fertilizers and promote higher yields of crops. The compost is beneficial for the land in many ways, including as a soil conditioner, fertilizer, addition of vital humus or humic acids, and as a natural pesticide for soil. Suppress plant diseases and pests. In ecosystems, facilitate reforestation, wetlands restoration, and habitat revitalization efforts by amending contaminated, compacted, and marginal soils.

Traditionally, composting was to pile organic materials until the next planting season, at which time the materials would have decayed enough to be ready for use in the soil. The advantage of this method is that little working time or effort is required

from the composter and it fits in naturally with agricultural practices in temperate climates. Disadvantages are that space is used for a whole year, some nutrients might be leached due to exposure to rainfall, and disease producing organisms, some weed, weed seeds and insects may not be adequately controlled. Modern, methodical composting is a multi-step, closely monitored process with measured inputs of water, air and carbon and nitrogen-rich materials. Composting process aims to turn excrement into manageable high-quality compost by letting aerobic bacteria remove the organic matter, which is produced during the decomposition process, under aerobic conditions, while evaporating moisture in the excrement. The process of composting requires simply piling up waste outdoors and waiting a year or more. The decomposition process is aided by shredding the plant matter, adding water and ensuring proper aeration by regularly turning the mixture. Aerobic bacteria manage the chemical process by converting the inputs into heat, carbon dioxide and ammonium. The ammonium is further converted by bacteria into plant-nourishing nitrites and nitrates through the process of nitrification.

The advantages of composting animal manures compared with direct application can be summarized in: Elimination of pathogens and weeds; microbial stabilization; reduction of volume and moisture; removal and control of odours; ease of storage, transport and use; production of good quality fertilizer or substrate. Amongst the waste management strategies, composting is gaining interest as a suitable option for manures with economic and environmental profits, since this process eliminates or reduces the risk of spreading of pathogens, parasites and weed seeds associated with direct land application of manure and leads to a final stabilized product which can be used to improve and maintain soil quality and fertility [Larney and Hao, 2007].

Recently, increasing public interests have been concerned on safety foods and the environmental aspects, especially on the so-called organic foods, which are made from crops grown with only organic fertilizers or compost but no pesticide nor chemical fertilizers. The law about the promotion of the introduction of a sustained high agricultural production method and promotion such as the recycling of food circulation resources has enforced [Foodstuff Recycling Law, 1999 and 2000, respectively] came to be enforced in Japan. In addition, Japan the cabinet decision about broad strategic view of the biomass accomplished [2002], and the profit inflection of organic resources (bio-mass) is promoted strongly. Exploitation of resources including composting such as the domestic animal feces and urine are promoted.

The composting of animal manures has been demonstrated to be an effective method for producing end-products which are stabilized and sanitized, ensuring their maximum benefit for agriculture [Bernal *et al*., 1995]. The compost should be of high quality in order to guarantee its marketability. The domestic animal dunghill manure performed application of by farmland must be over being safe for the growth of the crops. When compost is unripe, in addition to nitrogen starvation, abnormal reduction of the soil, it cause germination inhibition and growth restraint to the crops [Harada, 1985]. Because the volatility fatty acid which is a growth inhibitor and phenolic acid produce it by the sudden resolution of the fortunetelling degradability organic matter of unripe compost and abnormality reduction to be angry at sequentially [Parr, 1975; Golueke 1975; Matsuzaki, 1976]. It is concerned about causing increase of the unripe compost and adds the sudden increase in production of the compost to the outbreak of the soil obstacle after the application, and a hygienic problem is worried about. The food poisoning occurred when polluted compost with disease-causing germs was used for

fruits and vegetables in Europan and American countries [Cieslak *et al*., 1993; Chapman *et al*., 1997; Itoh *et al*., 1998; Little *et al*., 1999]. In disease-causing germs for humans included in the compost, infection of *Escherichia coli* (*E. coli*) particularly enterohemorrhagic *E. coli* O157 is the most serious menace [Coia *et al.*, 1998]. In USA, such bacteria poisoning more than 20,000 people every year by food and are estimated killed 250 [Finelli *et al*., 1995; Kourkia *et al*., 1997]. In Japan, it was caused by lunch using food polluted by the bacteria, and approximately 10,000 people poisoned, and 8were killed [Ministry of Health, Labour and Welfare, 2005]. As the polluter, the cow's dung and cow intestinal tract contents was concluded. In USA, it was reported that a infection example of *E. coli* O157 through the crop of the private vegetable garden that performed application of unripe compost derived from polluted cow dung [Cieslak *et al.*, 1993]. The compost which assumed domestic animal feces including the cow dung raw materials, that it is an urgent problem to raise food safety to protect the infection of disease-causing germs. The seriousness of hygiene management of the compost increases more and more. During a composting process, it takes 1-2 weeks for the disappearance of the coliform bacterium [Watabe *et al*., 1998; Deportes *et al*., 1998]. Furthermore, it takes 6 weeks [Honta *es al*., 1999] or stay in the product without disappearing [Sciancalepore *et al*., 1996].

Biological decomposition processes are classified as aerobic and anaerobic methods, and hybrids methods, of the so methods. Traditionally, composting was to pile organic materials for half on one year until the next planting season, at that time the materials were decayed enough to be ready for use in the soil. The advantage of the traditional method little working time and effort is required for farmers. The traditional method fits in naturally with agricultural practices in temperate climates. Disadvantages

of it are that large space is used for a whole year, long time is needed, some nutrients are leached due to exposure to rainfall, and remaining of disease producing organisms, some weed, weed seeds, insects etc. which are not adequately controlled. Methods composting are multi-step, closely monitored process with measurment inputs of water and air and carbon and nitrogen-rich materials. Methods of biological decomposition are differentiated as being aerobic or anaerobic methods, though hybrids of the two methods also exist. The composting process can be divided into two phases: the bio-oxidative phase and the maturing phase also called the curing phase [Bernal *et al*., 1996; Chen and Inbar, 1993]. The bio-oxidative phase is developed in three steps [Keener *et al*., 2000]: (i) An initial mesophilic phase lasting 1-3 days, where mesophilic bacteria and fungi degrade simple compounds such as sugars, amino acids, proteins, etc., increasing quickly the temperature. (ii) Thermophilic phase, where thermophilic microorganisms degrade fats, cellulose, hemicellulose and some lignins. During the phase the maximum degradation of the organic matters occurs together with the destruction of pathogens. (iii) Cooling phase, characterized by a decrease of the temperature due to the reduction of the microbial activity associated with the depletion of degradable organic substrates, the composting mass is recolonized by mesophilic microorganisms which are able to degrade the remaining sugars, cellulose and hemicellulose. During the different steps of the bio-degradation phase, the organic compounds are degraded to CO_2 and NH_3 with the consumption of O_2. During the maturation phase, stabilization and humification of the orgenic matter occur, producing the mature compost with humic characteristics in its orgenic matter. Compost can be defined as the stabilized and sanitized product of composting, which has undergone, rapid decomposition, beneficial characteristics for plant growth and certain humic

characteristics, making the composting of waste which is a key issue for sustainable agriculture and resource management [Zucconi and de Bertoldi, 1987; Jakobsen, 1995; Gajalakshmi and Abbasi, 2008].

However, unfortunately, a number of problems have appeared in the composting process in many cases [Romantschuk *et al*., 2005]. Due to insufficient aeration of the composting, the start-up of the composting process is very slow which causes the delay reaching the thermophilic phase of the process. The resulting immature material emerging from the composting requires a prolonged maturation. Immature compost has a health-risk for workers handling the compost mass. Malodorous emissions from these windrows have in some cases been extensive [Romantschuk *et al*., 2000]. There is a fault that fermentation temperature is hard to rise during winter season of cold districts. Recently, the purpose of the composting of domestic animal feces is keep the safety for the crops, and deaden *E. coli* other pathogen and the seeds of weed by the craze for fermentation, and to secure hygienic safety. The decline of the fermentation temperature produces hygienic unsafely. Thus, the study of effective treatment method on the feces and urine using the aerobic ultra high temperature fermentation bacteria was carried out in the animal resource science center, graduate school of agricultural and life sciences, The University of Tokyo (Fig. GI-1). This novel fermentation system is very superior in fermentation temperature, more than 110 °C (maximum 117 °C), organic resolution speed and reduction of volume, less than 1/10 (Fig. GI-2). Aerobic fermentation processes microorganisms emitting heat causes the inside of compost piles to become high temperature, creating an ideal environment for isolating thermophiles [Carlyle and Norman, 1941]. According to Zinder [1986] and Saiki [1978] reports, the inside temperature reaches up to 75-80 °C. In comparison with

it, the aerobic ultra high temperature fermentation system maintained the inside temperature of the compost 110 ℃ (ultra high temperature) about one month and more. This aerobic ultra high temperature fermentation system significantly hastened the fermentation speed. From three months of general aerobic fermentation system to six weeks and less. And implies the volume reduction of the organic wastes, be reduction in volume is approximately 1/10. Over the last decades, research has been focused on the study of the complex interaction amongst physical, chemical and biological factors that occurs during composting. Therefore, the control of parameters such as bulk density, porosity, nutrient content, C/N ratio, temperature, pH, moisture and oxygen supply have demonstrated to be key for composting optimization since they determine the optimal conditions for microbial development and organic matter degradation [Agnew and Leonard, 2003; Das and Keener, 1997; de Bertoldi *et al*., 1983; Haug, 1993; Miller, 1992; Richard *et al*., 2002].

In the present investigation, I decided the optimized and fermentation condition and revealed changes in the bacterial community and in the compost during aerobic ultra high temperature fermentation. I firstly examined characteristics of aerobic ultra high temperature fermentation process, compared temperature, moisture, pH and C/N changes under the different conditions of aeration systems. In Chapter 2, the structure of bacterial communities changes present in different stage of aerobic ultra high temperature fermentation process is described. The structure of the bacterial community was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) system using universal eubacterial primers. Finally, Chapter 3, I described about end-product of compost safety. The bacteriological examination, especially end-product *E. coli* testing was carried out. Use cattle carried

out litter test, mouse for feeding test and several vegetable for fertilization test. The toxicity of aerobic ultra high temperature compost has been evaluated in subchronic toxicity and contact toxicity.

Table GI-1. Amount of the biological waste and ingredient content in Japan

	Amount (Ten thousand ton)	Ingredient content (Ten thousand ton)		
		N	P[1]	K
Straw	1,172	6.9	2.4	11.7
Chaff	232	1.4	0.5	1.2
Domestic animal feces	9,430	74.9	27.4	51.9
Farm products residual substance	167	8.4	11.9	6.2
Bark	95	0.5	0.1	0.3
Sawdust	50	0.1	0.0	0.1
Wood chips	402	0.6	0.1	0.6
Plants and animals residual substance	248	1.0	0.4	0.4
Food industry sludge	1,504	5.3	3.0	0.6
Wood waste from construction industry	632	1.0	0.2	0.9
Kitchen refuse (from family and business)	2,028	8.0	3.0	3.2
Tree and bamboo	247	1.9	0.5	0.9
Sewage sludge	8,550	8.9	9.2	0.6
Night soil	1,995	12.0	2.0	6.0
Septic tank sludge	1,359	1.4	1.5	0.1
Drainage facilities for agricultural communities sludge	32	0.0	0.0	0.0
Total	28,143	132.1	62.1	84.6

[1] Phosphoric acid
[According to Chino, 2000]

Figure. GI-1. (a):Production scenery of high temperature composting; (b): Mixing scenery; (c): Materials; (d): High temperature compost.

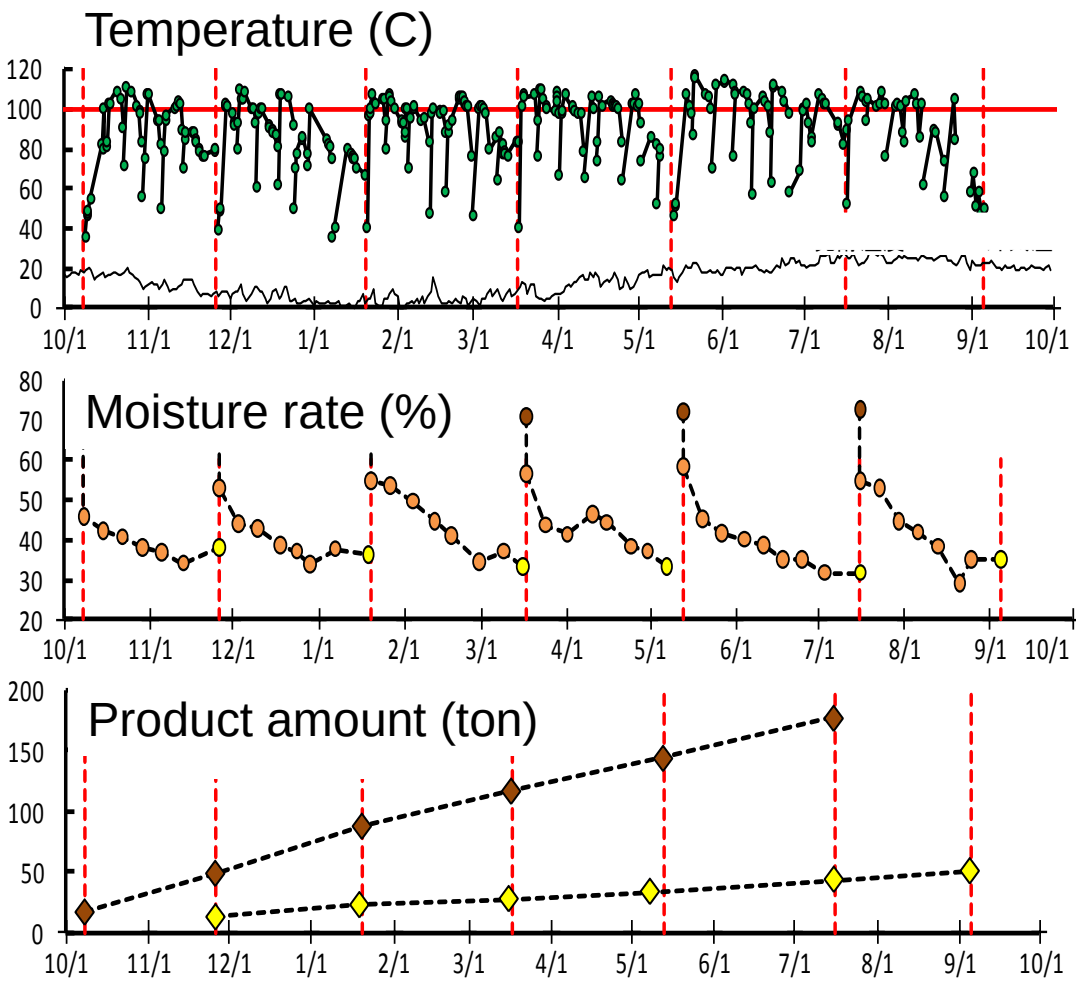


Figure. GI-2. Changes in temperature, moisture rate and product amount on high temperature composting process. During one year, ultra high fermentation temperature could be maintained.

Chapter 1

Characteristics of Aerobic Ultra High Temperature Fermentation Process

Abstract

Each type of manure has its own physical, chemical, and biological characteristics. In Chapter 1, I examined spatial characteristics in aerobic ultra high temperature fermentation process about physical, chemical. The temperature and the moisture rate became heterogeneous spatially in the fermentation process because of influence of the aeration pipe. The highest temperature (117 ℃) was recorded about 100 cm from the bottom. The temperature at 10 cm above the floor and at 10 cm below the face of compost was 70 ℃, suggesting that the waste pile is a good thermal insulator and little heat can escape from the inside of the waste pile. The pH was slightly alkaline in the range of 7.7-8.7 and did not change significantly.

I examined the influence of fermentation temperature and decomposition of organic matter in oxygen demand (aeration), to the optimal conditions for aerobic ultra high temperature fermentation. The present study of the spatial distribution of the temperature of the aerobic ultra high temperature fermentation compost provided the evidence that fermentation of the compost should better do crosscut including low, high and regulated aeration, even in anaerobic fermentation .The fermentation temperature was affected by the aeration considerably. Because of the fermentation temperature is deferent every area and the temperature is depend on the area. Comparing the four kinds of treatment of aerations the low aeration system are better than others in keep temperature, pH around in 7.0-8.0, moisture and organic matter degradation. Low aeration rates was saved to fermentation temperature , but maintained high temperature (more than 110 ℃ , maximum 117 ℃) in regulate aeration rates considerably . As for the evaporation of the water, regulate aeration rates was the top. C/N ratio showed that organic carbon compounds were most actively degraded by the regulate aeration rates.

The high aeration fermentation is also good for organic matter degradation and pH condition. But, because of moisture evaporate the temperature is instability. The pH in the high and anaerobic fermentation show little bit higher, pH 8.0 to 9.0. The C/N ratio showed that organic carbon compounds were most actively degraded by regulated aeration treatment and then low aeration, high aeration and anaerobic fermentation. When zero aeration rates for composting process, considered to anaerobic microbial process. As above mentioned result demonstrated that regulate aeration rates best condition for temperature for temperature, water decrease and the organic matter resolution.

Aerobic ultra high temperature fermentation with pig manure as raw materials, fermentation temperature rose to 105 °C and was always more than 80 °C, showed that aerobic ultra high temperature fermentation with pig feces was also possible .

Introduction

In the general introduction I described that in the nature compost can be rich in nutrients and can be reduce or eliminate the need of chemical fertilizers and promote higher yields of agricultural crops. Suppress plant diseases and pests. In ecosystems, facilitate reforestation, wetlands restoration, and habitat revitalization efforts by amending contaminated, compacted, and marginal soils. But traditional composting system to decay enough organic matter uses much long time even this method requires little working time or effort and it fits in naturally with agricultural practices in temperate climates. The space is used for a whole year, some nutrients might be leached due to exposure to rainfall, and disease producing organisms, some weed, weed seeds and insects may not be adequately controlled. Comparison composting, the ways of waste management system, with burning and landfill methods it is more practices. According to Zinder [1986] and Saiki [1978] reports, aerobic fermentation processes causes the inside of compost temperature reaches up to 75-80 ℃. The aerobic ultra high temperature fermentation system could maintain the inside temperature of the compost 100-110 ℃ about one month and more by regular crosscut. This could reduce fermentation time from three months to six weeks and less. And implies the volume reduction of the organic wastes, be reduction in volume is maximum 1/10.

Composting is a microbial, aerobic, self-heating, solid-phase controlled process bringing about the mineralization and the stabilization of the organic fraction of household waste [de Bertoldi *et al.*, 1983; Finstein and Morris, 1975; Miller, 1996]. Conditionally there are five factors affecting the aerobic composting progress.

(i) Aliment (organic matter): To promote composting, organic materials to be decomposed as energy source for bacteria is essential. (ii) Temperature: Bacteria need

certain temperature to be active. The temperature pattern shows the microbial activity and the occurrence of the composting process. The optimum temperature range for composting is 40-65 °C (de Bertoldi *et al.*, 1983), temperatures above 55 °C are required to kill pathogenic microorganisms. Keeping this temperature also helps to evaporate the moisture and dry the excrement. But if the temperature achieved exceeds the tolerance range of the thermophilic decomposers, the effect is damaging for composting. At temperatures above 63 °C, microbial activity declines rapidly as the optimum for various thermophiles is surpassed, with activity approaching low values at 72 °C. The range of 52-60 °C is the most favorable for decomposition [Miller, 1992]. (iii) Aeration: Aeration is a key factor for composting, providing sufficient air evenly to excrement is essential. Proper aeration controls the temperature, removes excess moisture and CO_2 and provides O_2 for the biological processes. The optimum O_2 concentration is between 15% and 20% [Miller, 1992]. Controlled aeration should maintain high temperatures below 60-65 °C, which ensures enough O_2 is supplied [Finstein and Miller, 1985]. (iv) Moisture: Aerobic composting requires an adequate moisture level. At a high moisture level, air flow is disturbed and air can hardly be supplied. Therefore adequate moisture and air-flow control are necessary. This agitation is also necessary for the evaporation of moisture. When the moisture content exceeds 60% O_2 movement is inhibited and the process tends to become anaerobic [Das and Keener, 1997]. The optimum water content for composting varies with the waste to be composted, but generally the mixture should be at 50-60% [Gajalakshmi and Abbasi, 2008]. During composting a large quantity of water can evaporate, to control temperature, and as water content diminishes the rate of decomposition decreases, then rewetting should be required in order to maintain the optimum moisture content for the

microbial activity. (v) pH: While excrement is generally acidic (around pH 6.0), pH 8.0 to 9.0 is ideal for bacterial activity. As aerobic composting is processed, due to the large amount of NH_3 generation, it turns into alkali, then ideal environment for aerobes are set in place. On the other hand, in the anaerobic environment, as a result of large generation of lower fatty acid, the acidity level gets higher and therefore the pH level gets lower (pH5.0 to 6.0). In this environment, aerobes cannot grow, therefore aerobic composting is stopped. However it can turn back to normal when the pH is adjusted and air is supplied. A pH of 6.7-9.0 supports good microbial activity during composting. Optimum values are between 5.5 and 8.0 [de Bertoldi *et al.*, 1983; Miller, 1992]. Usually pH is not a key factor for composting since most materials are within this pH range. However, this factor is very relevant for controlling N-losses by ammonia volatilization, which can be particularly high at pH >7.5.

I examined the changes of the temperature, moisture and pH under the deferent aeration conditions on aerobic ultra high temperature fermentation comparing with anaerobic condition. Made a judgment for all of the conditions added the completion of the fermentation by carbon-nitrogen ratio (C/N). In addition, I used the best conditions that described above examined possibility of the aerobic ultra high temperature fermentation when using high moisture pig manure. I examined the fermentation that failed of aerobic ultra high temperature fermentation of different fermenter (same system).

Materials and Methods

Samples

The livestock waste (compost) was fermented in the 420 cm wide, 700 cm depth and 220 cm high fermenter (Picture. 1-1). Air was continuously supplied from the bottom floor using a compressor through two pipes buried under the floor (Picture. 1-2 and 1-3). Have a hole (1cm diameter) every 38 cm interval (Picture. 1-2 and 1-3) on the pipe supply air to the inside of the compost. Preparation of the compost; The livestock waste, 65% of cattle manure, 20% goat manure and hey waste and 15% of horse manure and hey waste, premixed with fully fermented compost by same ratio before fermentation. The moisture of compost was adjusted to the 60% before composting for the initiation of the self-heating phase. The compost were crosscutted every 7 days intervals by wheel loader in order to enhance the composting quality and progress and avoid the formation of anaerobic compartments. The sample, use for moisture, pH and C/N ratio study, put them into the plastic bag and bring it to the laboratory analyzed. The moisture analysis was carried out soon.

The spatial distribution of the temperature of the aerobic ultra high temperature fermentation compost

When the compost was crosscutting at the day 21 the temperatures were measured at 55 points for making the spatial diagrams. The detail measure points illustrated at Figure. 1-a and 1-b.

Influence of the aeration strength on the pace of the fermentation progress

In the present study, I used four different treatments of the aeration strength for fermenting to study the pace of the fermentation progress. The crosscutting was carried out 7 days intervals for all of the experiment. ① Zero aeration strength; There is no air supply to the compost during the fermentation. Like anaerobic fermentation. ② Low aeration strength; The compost supplied air by 0.4-0.6 m^3/h. ③ High aeration strength; The compost supplied air by 1.2-1.8 m^3/h. ④ Regulated aeration strength; The compost supplied air by high day 1-2, low at day 3-4, day 5-6 is zero and day 7 high again.

When the crosscutting was carried out every 7 days intervals, after 7, 14, 21, 28, 35 and 42 days fermentation, the temperatures inside of the compost were measured. The measuring point is illustrated at Figure. 1-c and 1-d. The thermometer insert to 100 cm depth from the surface of the section. At same places, 100 cm depth from the surface of the section, the samples were taken and analyzed chemically for the pace judgment of the fermentation progress.

Attempt of composting for other kind of animal manure (pig manure)

Usually the pig manure is difficult to ferment by aerobic. Because it is hold high level of moisture rate and contains some antibiotics inhibits the growth of other microorganisms. According to the results described above, the present study attempt ferment the pig manure. The pig manure was premixed by same rate with the competed compost. The compost were crosscutting every 7 days intervals by wheel loader in order to enhance the composting quality and progress and avoid the formation of anaerobic compartments. The compost supplied regulated aeration by high at day 1-2, low at day 3-4, day 5-6 is zero and day 7 high again.

Attempt of composting for another kind of organic matter (sewage sludge)

Usually the sewage sludge is most difficult fermenting subject, in the organic matter, by aerobic. Because it is hold highest level of moisture rate and contains some antibiotics, chemicals Inhibits the growth of other microorganisms. According to the results described above, the present study attempt ferment the sewage sludge. The sewage sludge was premixed with competed compost by 1:5 rates respectively. The compost was crosscutted after 11 15, 19, 23 and 30 days of fermentation by wheel loader in order to enhance the composting quality and progress and avoid the formation of anaerobic compartments. The samples were taken same with above time. The compost supplied regulated aeration by high at day1-2, low at day 3-4, day 5-6 is zero and day 7 high again.

Measurement analyses

Measurement of temperatures: The temperature was measured at the same time and same places described above. Measurement of moisture rate (loss on drying): In this technique a sample of material is weighed, heated in an oven (105 ℃) for 24 hour, then cooled in the dry atmosphere of a desiccator, and then reweighed. Measurements were carried out in triplicate and the average was taken. Measurement of pH: Add ten times distilled water to the sample, and the solution was stirred for 60 min. Allowing the suspension to stand for a while, the supernatant obtained was subjected to the pH measurement. Measurements were carried out in triplicate and the average was taken. Measurement of C/N ratio: Total carbon content and total nitrogen content were determined using a carbon and nitrogen content analyzer (CN coder MT-700; Yanaco, Tokyo, Japan). Measurement of Electric current (EC): Add five times distilled water to

the sample, and the solution was stirred for 60 min. Allowing the suspension to stand for a while, the supernatant obtained was subjected to the EC measurement. Measurements were carried out in triplicate and the average was taken.

Results

The spatial distribution of the temperature of the aerobic ultra high temperature fermentation compost

The temperature changed from 86 to 92 °C at the depth of 50 cm, the maximum temperature was 92 °C. In the depth of 100 cm, the temperature changed from 91 to 107 °C, the maximum temperature was 107 °C. The temperature of depth of 100 cm was higher than 50 cm. As showing in the Figure. 1-c, the temperatures lowest at the pipe and bottom area, 20-40 °C. And out-side lower than inside. The temperature gradually rises up from the bottom to the upper and on the central area reached maximum temperature (91-100 °C); then gradually down to the surface of the top, 60-90 °C. The spatial distribution of the moisture rate is shown in Fig 1-c-1. The highest moisture rate (around 57.6%) was on the central of the bottom. The minimum moisture rate (35.7%) was on the upper part of the aeration pipe. The pH was slightly alkaline in the range of 7.7-8.7 and did not change significantly.

Influence of the aeration strength on the pace of the fermentation progress

Zero aeration strength: Zero aeration strength means anaerobic fermentation. The results are shown in the Figure. 1-d-1. The transition of the temperatures at the central measure point of the fermentation when supply the zero aeration strength are from 40 °C to 60 °C that measured in 10 min interval. The three point data are show in the Figure. 1-d-2. The transitions of the temperature at the point A are higher than point B and point C. The maximum temperature (60 °C) was reached on day 14 at the point A. The moisture rates of the compost at the beginning of the fermentation, day 7, are

deferent. However, the three point moisture rates have closer to each other with fermentation progresses. At the end of the fermentation the moistures of the three points all of them are near 39%. The pH was slightly alkaline in the range of 8.6-9.1 and did not change significantly. From day 7 to day 42, the pH increased just only 0.2-0.5. C/N ratios did not change significantly. From day 7 to day 42, C/N ratio transited from 12.43 to 12.24.

Low aeration strength: The results show in the Figure. 1-e-1. The transition of the temperatures at the central measure point of the fermentation when supply the low aeration strength most of all are over 100 ℃ measured in 10 min interval. The three point data are show in the Figure. 1-e-2. The temperature is highest at the point A (84-106 ℃), then point B (88-97 ℃) and point C (62-88 ℃), respectively. The maximum temperature (106 ℃) was reached on day 42 at point A. The moisture rates of the compost at the beginning of the fermentation, day7, are deferent similar zero aeration strength treatment. However, the three point moisture rates have closer to each other with fermentation progresses. At the end of the fermentation the moistures of the three points all of them are near 39%. The pH was in the range of 6.7-8.3 and did not change significantly. In terms of pH, the point A was pH 6.7-7.0, point B pH 6.8-7.5, and point C was pH 7.0-8.3 respectively. The C/N ratios during day 7 to 42 are decreased from 15.87 to 13.04%.

High aeration strength: The results show in the Figure. 1-f-1. The transition of the temperature at the central measure point of the fermentation when supply the high aeration strength most of all are below 100 ℃ measured in 10 min interval. The three point data are show in the Figure. 1-f-2. The temperature is highest at the point A (54-89 ℃), then point B (46-86 ℃) and point C (21-61 ℃). The maximum temperature (89

℃) was reached on day 7 at point A. The moisture rates of the compost at the beginning of the fermentation, day 7, are different between the point B, with point A and point C. The highest moisture rate (around 58.6%) was on day 7 in point B. From day 14 the moisture decreased from 52.1% to 41.7%. At the end of the fermentation the moistures of the three points all of them are near 42%. The pH was slightly alkaline in the range of 8.0-8.9 and did not change significantly. In terms of pH, the point A, B and C was 8.1-8.9, 8.3-8.7, and 8.1-8.6 respectively. The C/N ratios changed from 13.92-11.46% during day 7 to day42.

Regulate aeration strength: The results are shown in the Figure. 1-g-1. The transition of the temperature at the central measure point of the fermentation when supply the regulated aeration strength most of all are over 100 ℃ measured in 10 min interval. The three point data are shown in the Figure. 1-g-2. The temperature is highest at the point B (93-105 ℃), then point A (80-83 ℃) and point C (40-43 ℃), respectively. The maximum temperature (105 ℃) was reached on day 14 at point B. The moisture rates of the compost at the beginning of the fermentation, day 7, are same between the point A, with point B and point C. From day 7 the moisture decreased from 54.1% to 33.1%. The pH was slightly alkaline in the range of 7.1-8.0 and did not change significantly. In terms of pH, from 14 days to 21 days, pH increased 0.3-0.5. The C/N from day14 to day43, decreased from 15.96 to 11.97.

Attempt of composting for other kind of animal manure (pig manure)

The results show in the Figure. 1-h-1. The transition of the temperature at the central measure point of the fermentation of the pig manure when supply the regulated aeration strength most of all are below 100 ℃ measured in 10 min interval. The

temperature fluctuations are severe don't like other cases that was described above. The three point data are shown in the Figure. 1-h-2. The temperature of the all three points are decreased depend on the fermentation progresses. It is deferent than other animal manures. The moisture rates of the compost at the beginning of the fermentation, day7, are same between the point A, with point B and point C, about 46.5%. From day 7 the moisture decreased from 46.5% to 27.4%. The pH was slightly alkaline in the range of 7.5-8.6 and did not change significantly. It is show the tendency of increasing during the fermentation. The present study use EC instead of C/N ratio to judge the completion of the fermentation because of no C/N ratio to supply. From 7 days to 42 days, EC increased from 2.9-3.6. If the EC bigger than 3.0 it is indicating the fermentation has completed.

Attempt of composting for another kind of organic matter (sewage sludge)

The results are show in the Figure. 1-i. There is no high temperatures was confirmed. All of them are under the 50 ℃. The moisture rate is also didn't shows down. It is increasing except day 19. The pH was slightly alkaline in the beginning, range of 7.5-8.6 and then decreases fermentation progresses dependently. The C/N ratios are low on day 19 and day 30.

Discussion

The present chapter proved strongly that strength of aeration is very important factor on the organic matter fermentation progresses. Composting end product should not contain pathogens or viable seeds, and it should be stable and suitable for use as a soil amendment [Epstein, 1997]. Stentiford [1996] reported, the maximum temperature of 55-65 °C is necessary to destroy pathogens. Pasteurization [1866] also reported the similar way to heat sterilization of foods for 30 minutes at 63 °C (the actual temperature setting is often set to 68 °C from 65 °C). As mentioned in introduction chapter, actually, the food poisoning case that use polluted raw fruit and vegetables, and happened in Europe and America, and it is reported some by the disease-causing germs of compost [Cieslak *et al*, 1993; Chapman *et al*, 1997; Itoh *et al*, 1998; Little *et al*, 1999]. The infection of *E. coli* particularly enterohemorrhagic *E. coli* O157 are the most serious menace [Coia *et al*, 1998; Konuma *et al*, 2000]. Thus the ultra high temperature composting system is essential. Application of compost in agricultural practice could potentially cause contamination of foodstuffs with pathogenic bacteria such as *E. coli* O157:H7. Gong report [2005] was noticeable that some pathogens survived even in uncompleted compost of which temperature was 54-69 °C. The temperature that is higher than this temperature is necessary to kill a pathogen and the seed of the weed. The unripe compost and even the fermentation completed below 60-80 °C there some dangerous source are exist. The temperature higher than 100 °C is necessary to destroy pathogens of the microorganisms, pest, parasites and weed seeds. The compost made by the aerobic ultra high temperature method was confirmed by National Livestock Breeding Center, Japan to be negative of *E. coli*.

The results of the Figure. 1-c-1, even ultra high temperature fermentation could reach about 100 °C degree but this is in the limited area, indicate to complete ripe compost that crosscut technic is also essential.

In the Figure. 1-j, collect the diagrams that present the temperatures at the central measure point of the fermentation when supply the deferent treatment aeration strength measured in 10 min interval. Comparing the deferent treatment of aeration is results various temperature of fermentation. Anaerobic fermentation the temperature was below 60 °C during the experiment. The fermentation will be cost long distance. Furthermore, it is easy make the unripe compost without crosscut. The highest temperature is shown in low aeration treatment could keep compost over 100 ℃ more than 6 weeks. de Bertoldi *et al*., [1983] indicated the optimum temperature range for composting is 40–65 °C, temperatures above 55 °C are required to kill pathogenic microorganisms. But if the temperature achieved exceeds the tolerance range of the thermophilic decomposers, the effect is damaging for composting. At temperatures above 63 °C, microbial activity declines rapidly as the optimum for various thermophiles is surpassed, with activity approaching low values at 72 °C. And Miller [1992], reported the range of 52-60 °C is the most favorable for decomposition. But in my study the highest temperature is show in low aeration treatment keeps compost over 100 °C more than 6 weeks and the low aeration and regulated aeration treatment are better decomposing organic matte other than high and anaerobic aeration .

Evaporation of moisture is positive with the aeration strength. The high and regulated aeration fermentation has been better. As Das and Keener [1997] reported aerobic composting requires an adequate moisture level and agitation is also necessary for the evaporation of moisture. The present study adjusted moisture content between

50-60%. During composting a large quantity of water evaporated in the high and regulated aeration fermentation, however low aeration treatment show the better decompose capacity than others. Gajalakshmi and Abbasi indicated [2008] as water content diminishes the rate of decomposition decreases, then rewetting should be required in order to maintain the optimum moisture content for the microbial activity. This is agrees with my results.

The pH transition data shows that anaerobic and high aeration fermentation is generally around pH8.0 to pH 9.0. It isn't agrees with de Bertoldi *et al*., [1983] and Miller, [1992] reported ideal for bacterial activity is pH 8.0 to 9.0. It is confirmed pH increased by ammonia because of ammonia gas analysis resulted large quantity of ammonia occurred. In my result pH 7.0 to 8.0 is better for bacterial activity that occurred in low and regulated aeration fermentation.

The C/N ratio is an index of compost maturity, decreasing during the composting process. The regulated aeration fermentation is 20.39, low aeration 17.83, high is 17.67 and anaerobic is only 1.53.

The above mentioned result demonstrated that composting low aeration fermentation is the best condition for temperature, moisture decrease and the organic matter resolution.

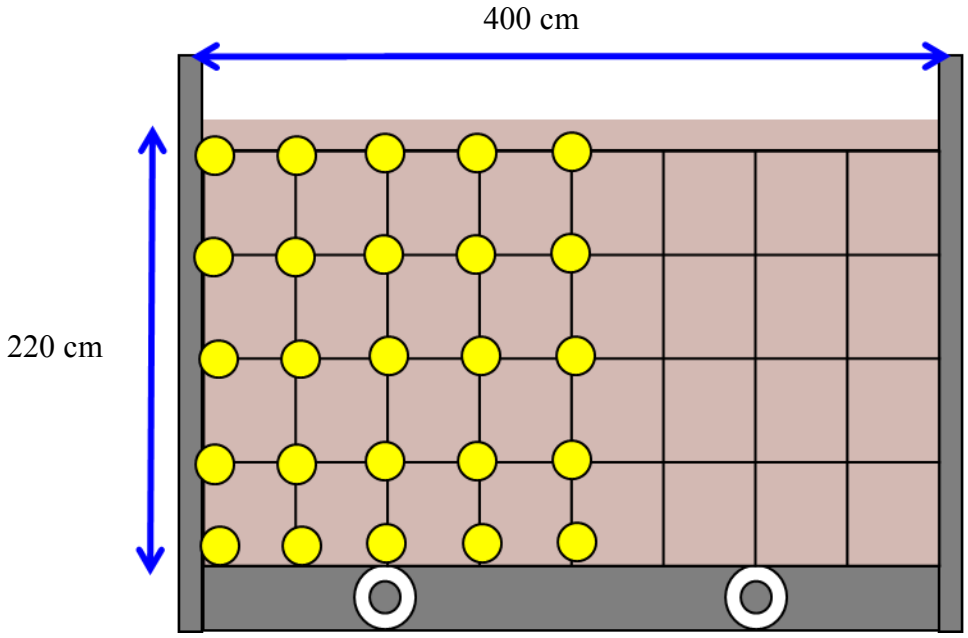


Figure. 1-a. The section diagram of the temperature measured point. The yellow circles are the temperature measure points. The distance between the yellow points have 50 cm. One section have 25 measure points and total have 3 sections.

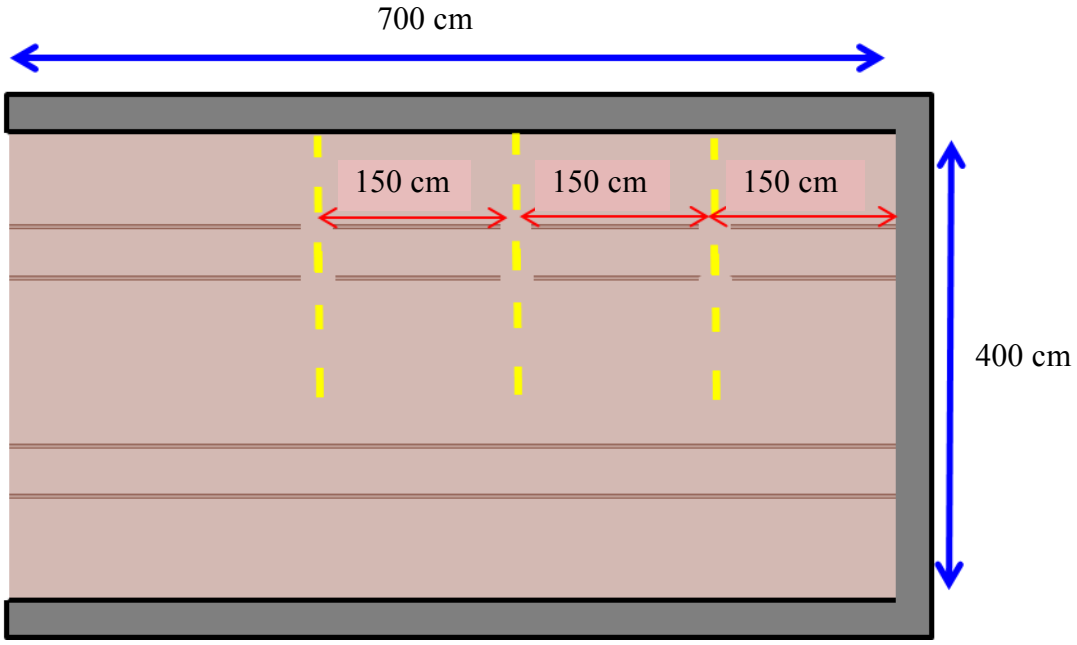


Figure. 1-b. The yellow dotted line is indicating the section positions, and the first section is 150 cm from the back wall.

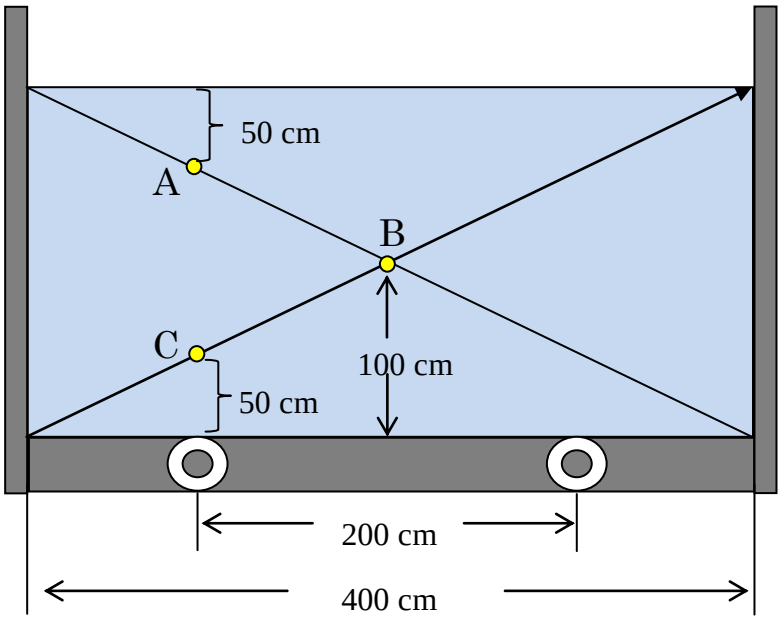


Figure. 1-c. The section diagram of the temperature measured point. The A, B and C of yellow circles are indicating the temperature measure points.

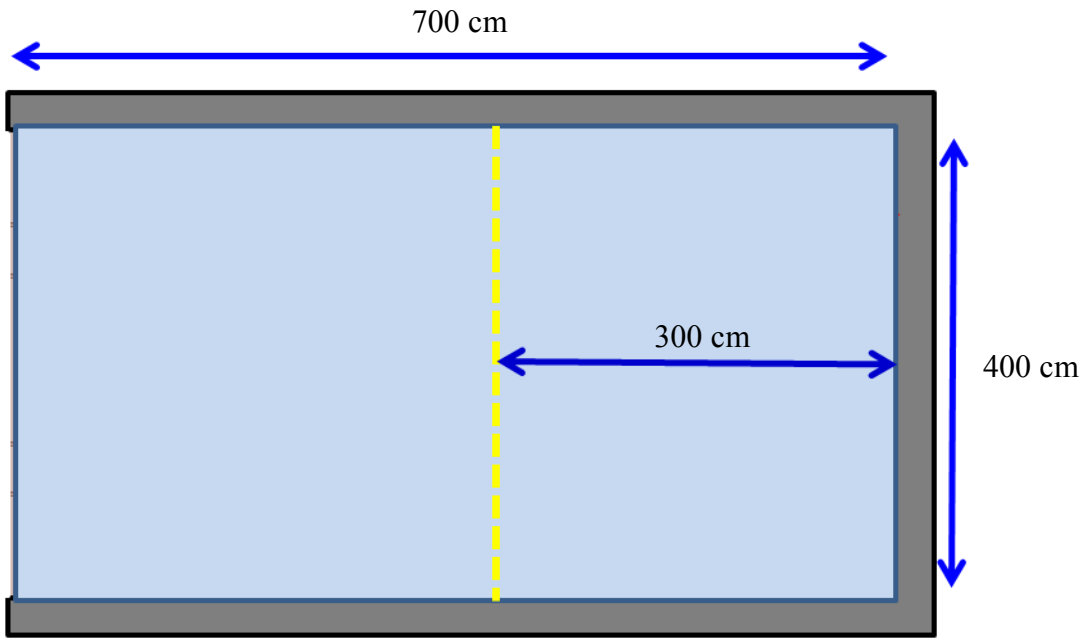
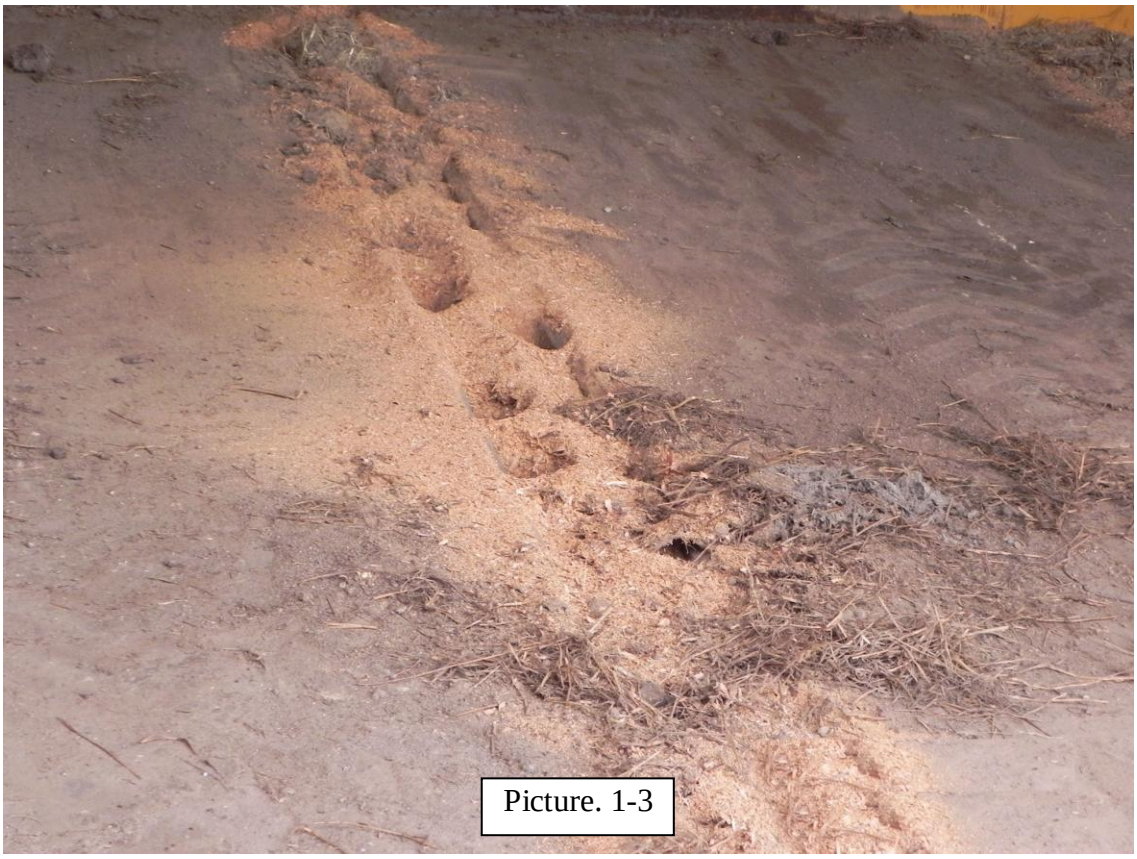


Figure. 1-d. The yellow dotted line is indicating the section positions, and that is 300 cm from the back wall.

Picture. 1-1. The fermenter of aerobic ultra high temperature fermentation for livestock waste. The size is 4.3 m wide, 7.3 m depth and 2.4 m high.

Picture. 1-2. The fermenter of aerobic ultra high temperature germentation for livestock waste. The size is 4.3 m wide, 7.3 m depth and 2.4 m high.



Picture. 1-3. The holes (1cm diameter) positions is located every 38 cm interval on the pipe provide air to the inside of the livestock waste. Air was continuously supplied from the bottom floor using a compressor through two pipes buried under the floor.

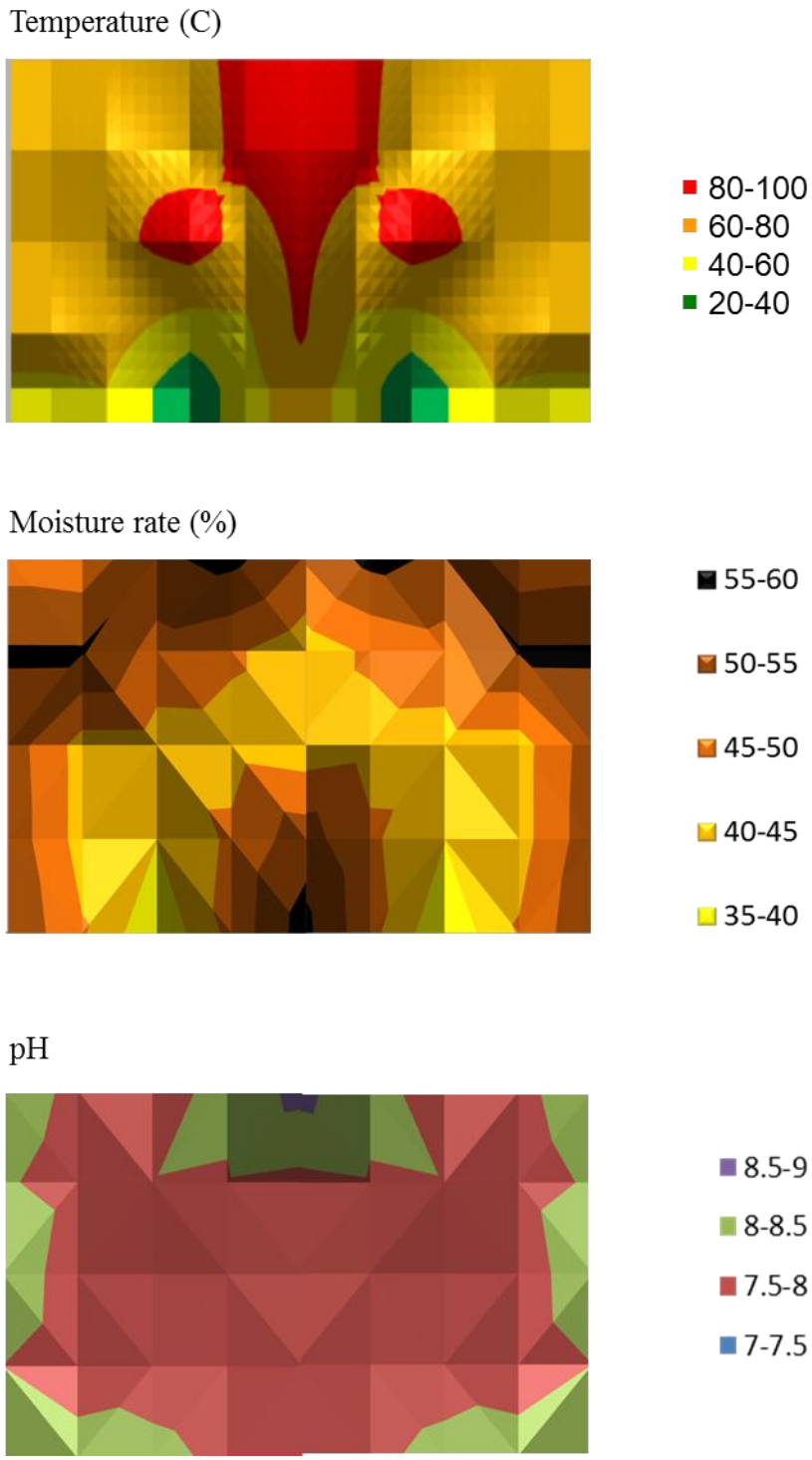


Fig. 1-c-1. The spatial distribution of the temperature, moisture rate and pH of the aerobic ultra high temperature fermentation compost at day 21. Changes in temperature on the vertical section.

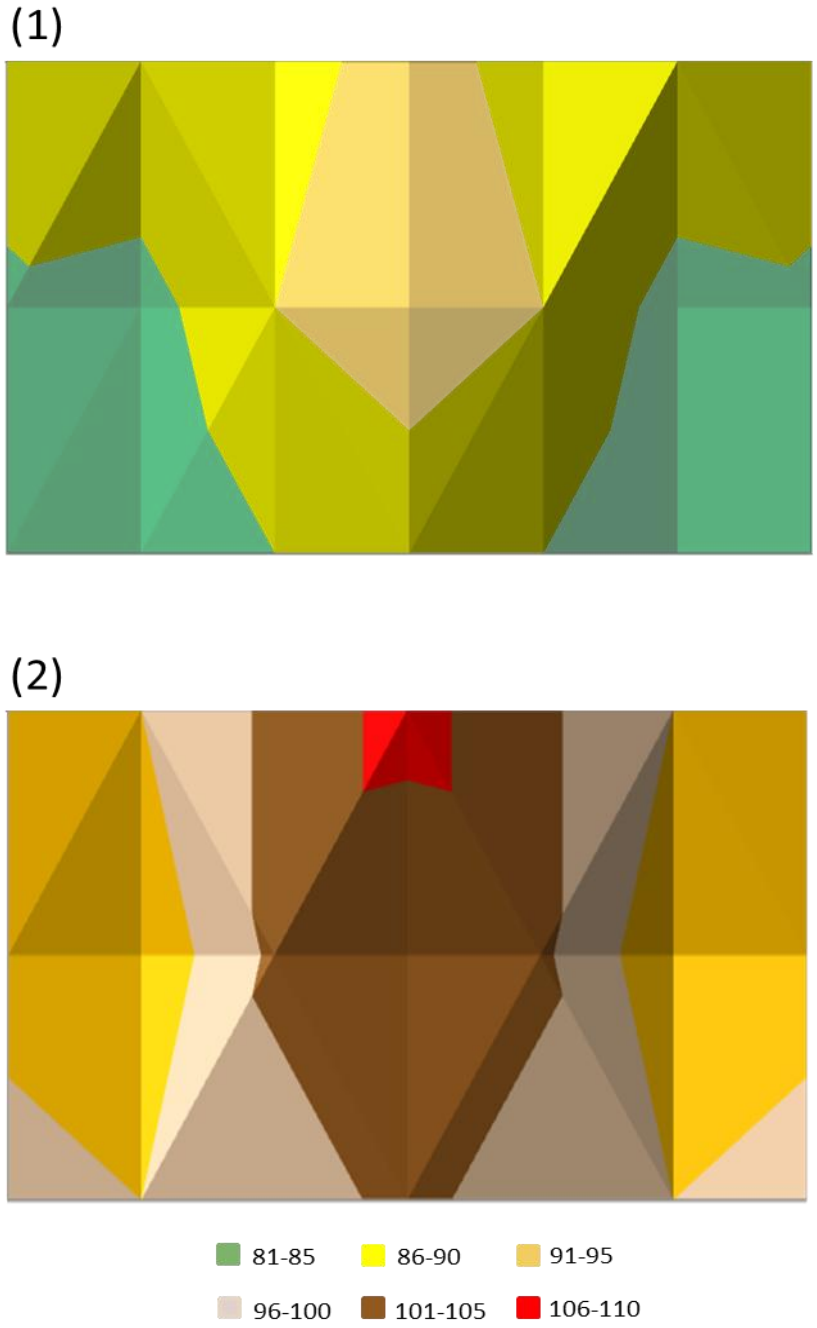


Fig. 1-c-2. The spatial distribution of the temperature, moisture rate and pH of the aerobic ultra high temperature fermentation compost at day 21. Changes in temperature on the horizontal section. (1) Depth of 50 cm (2) depth of 100 cm from the face of the aerobic ultra high temperature fermentation process.

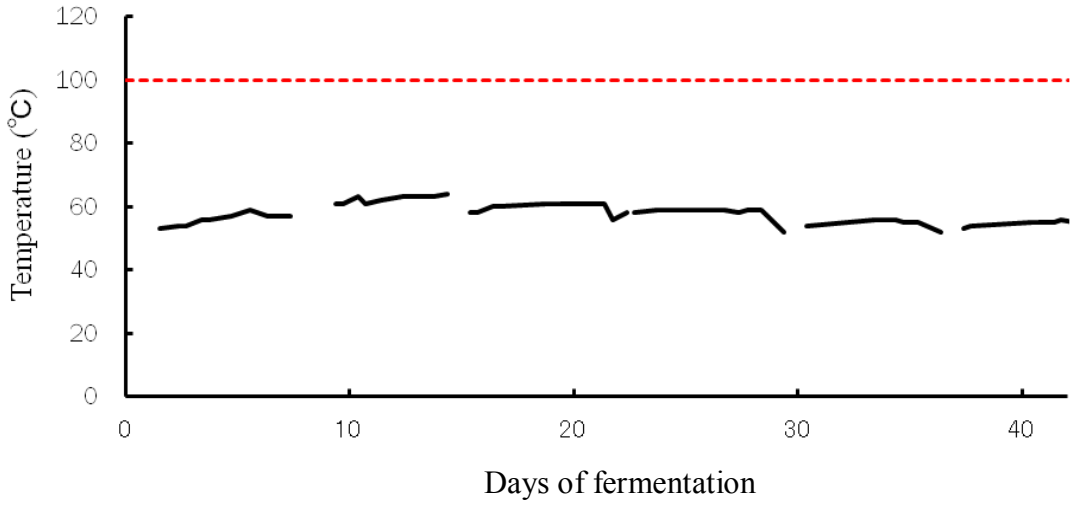


Figure. 1-d-1. The transition of temperature of the fermentation when supply the anaerobic strength fermentation measured in 10 min interval. Most of them under the 60 °C.

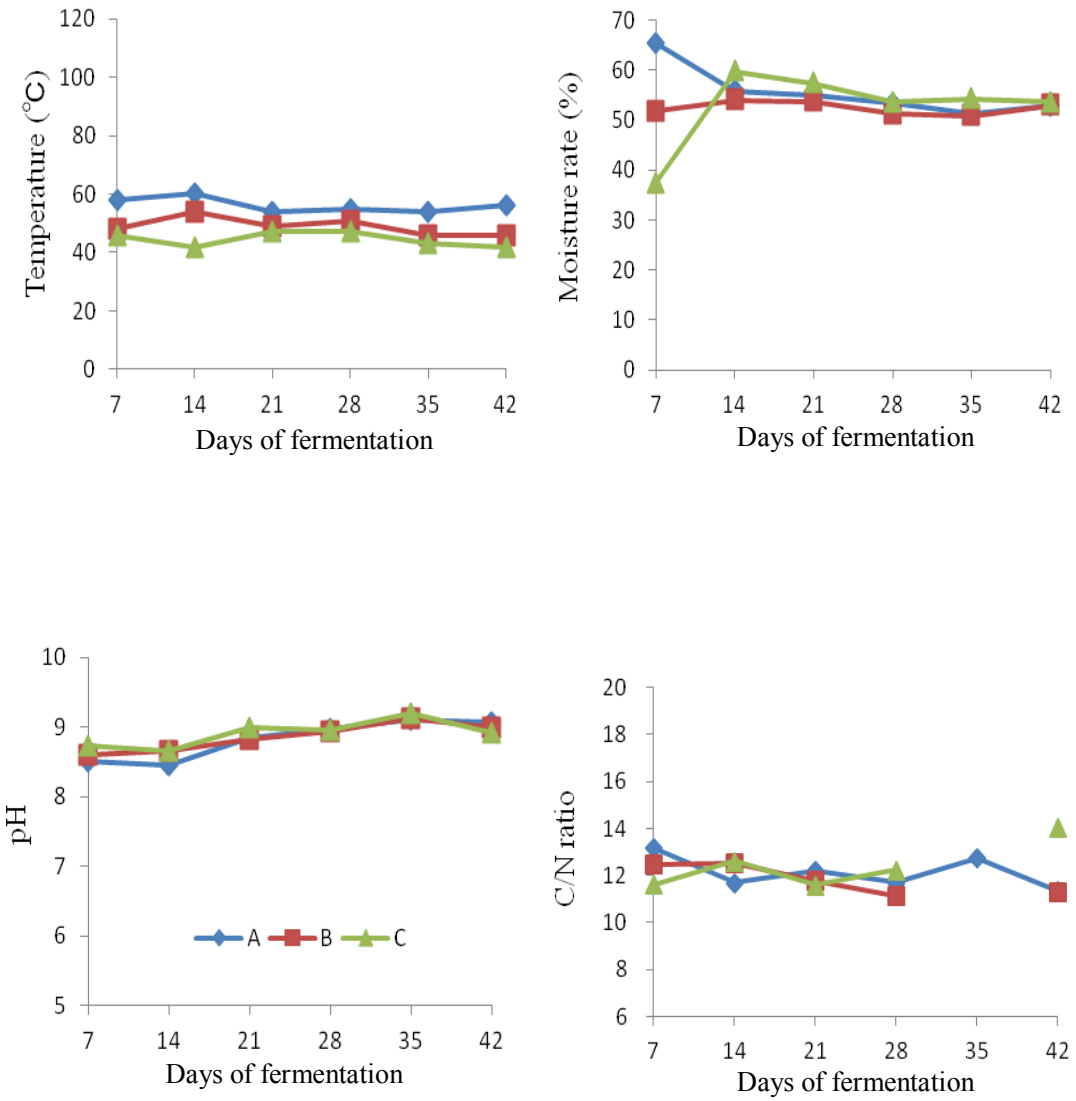


Figure. 1-d-2. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the anaerobic strength measured in 10 min interval.

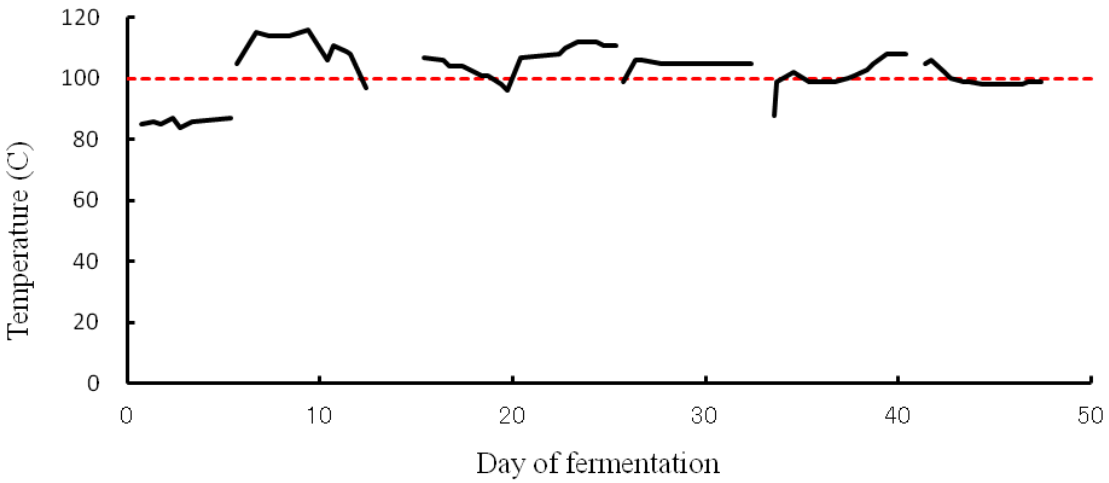


Figure. 1-e-1. The transition of the temperature of the fermentation when supply the low aeration strength. Most of all were over 100 ℃ measured in 10 min interval.

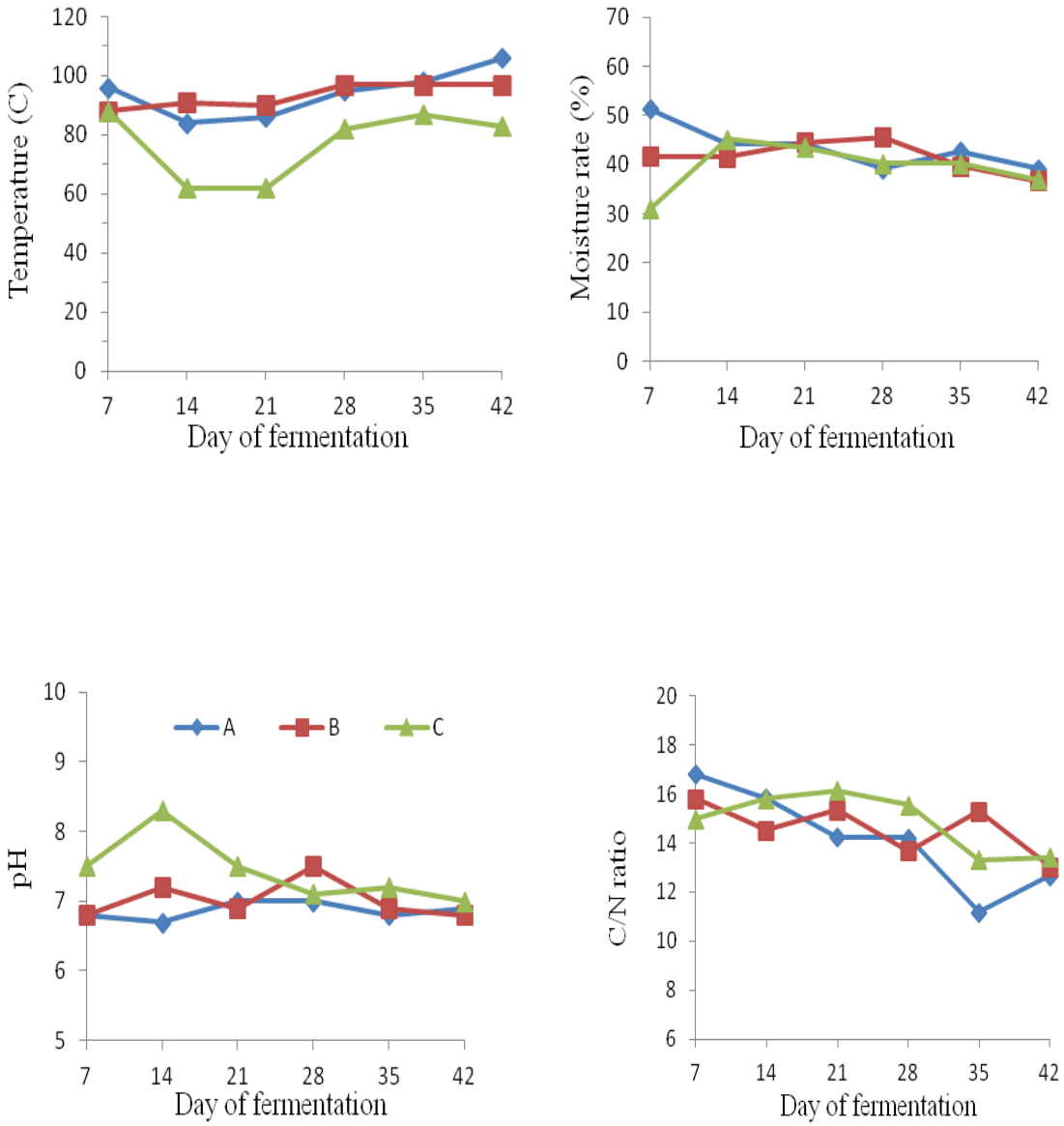


Figure. 1-e-2. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the low aerobic strength measured by 7 days intervals.

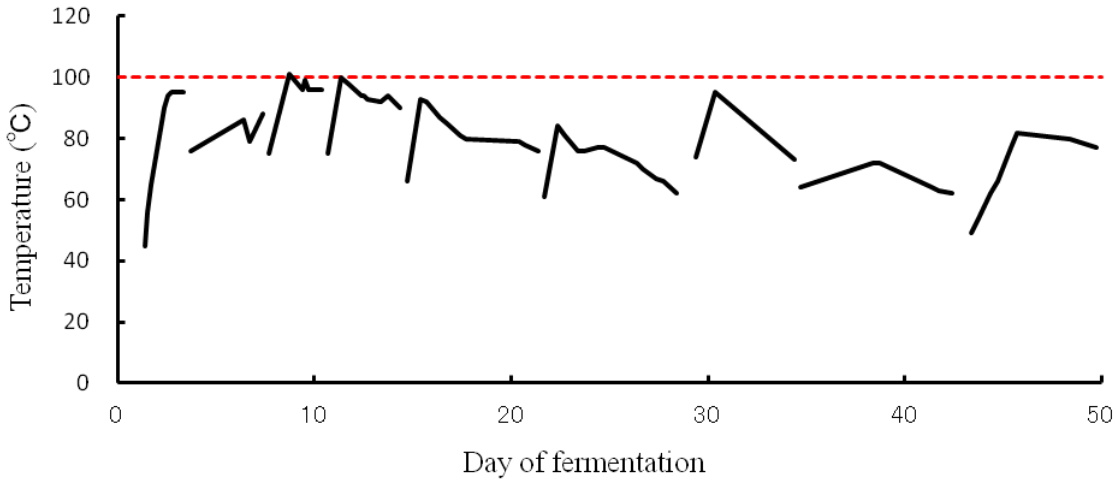


Figure. 1-f-1. The transition of the temperature of the fermentation when supply the high aeration strength. Most of all were below the 100 °C measured in 10 min interval.

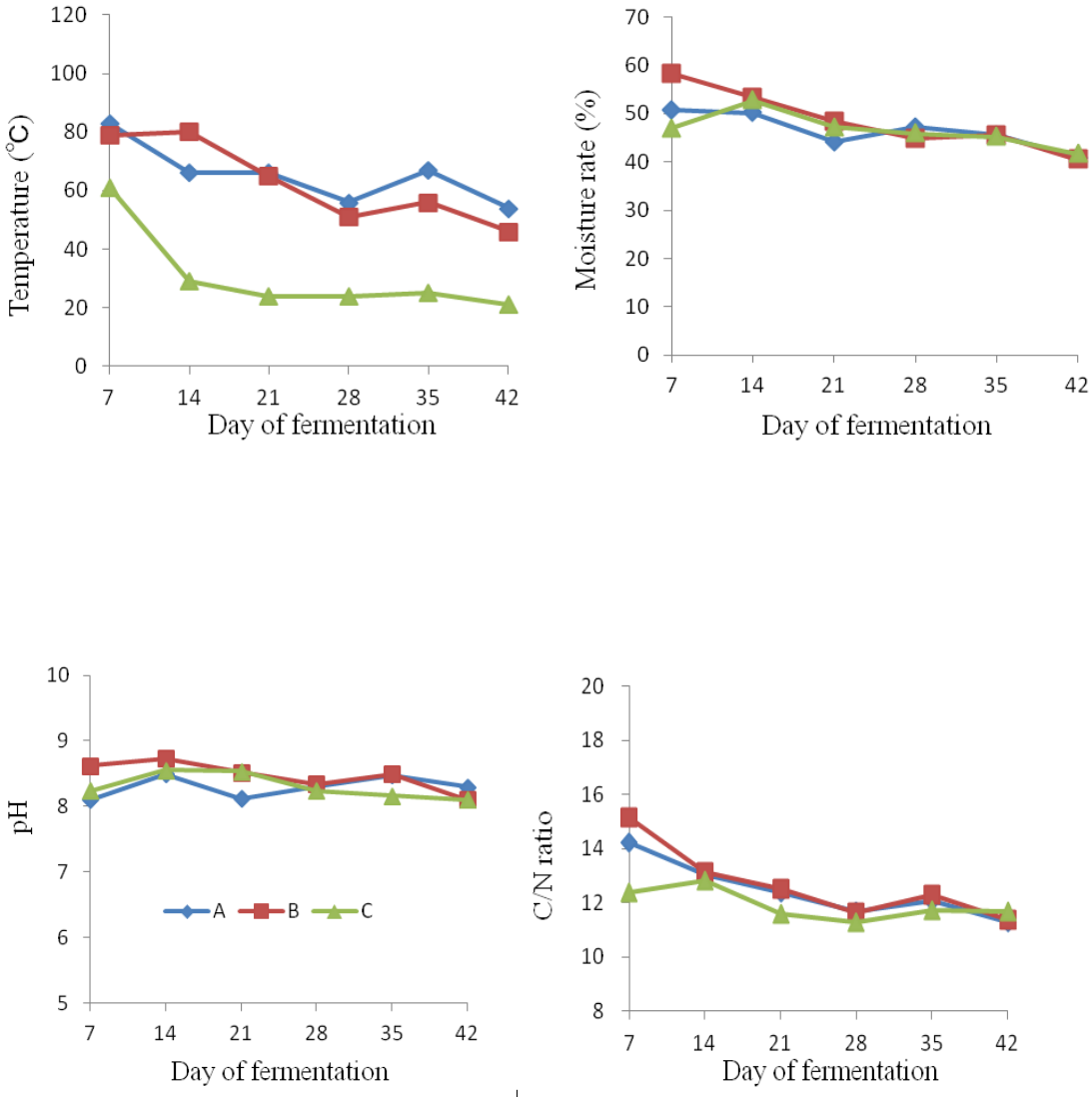


Figure. 1-f-2. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the high aerobic strength measured by 7 days intervals.

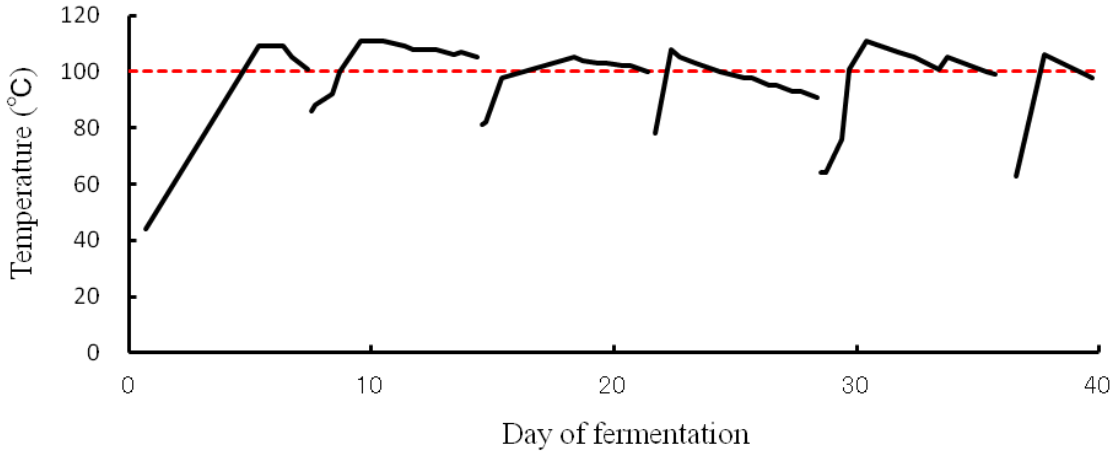


Figure. 1-g-1. The transition of the temperature of the fermentation when supply the regulated aeration strength. Most of all were over the 100 ℃ measured in 10 min interval.

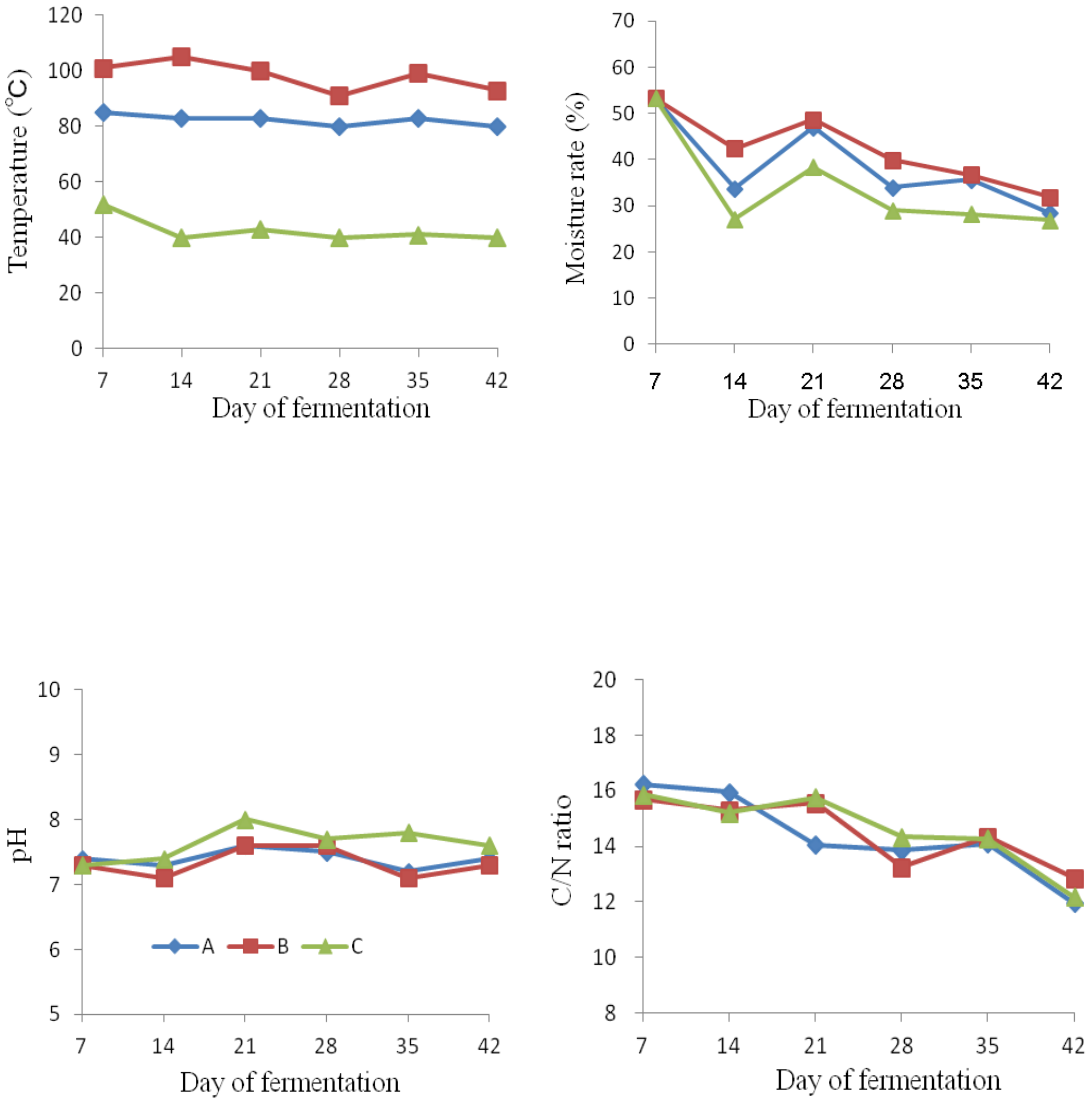


Figure. 1-g-2. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the regulated aerobic strength measured by 7 days intervals.

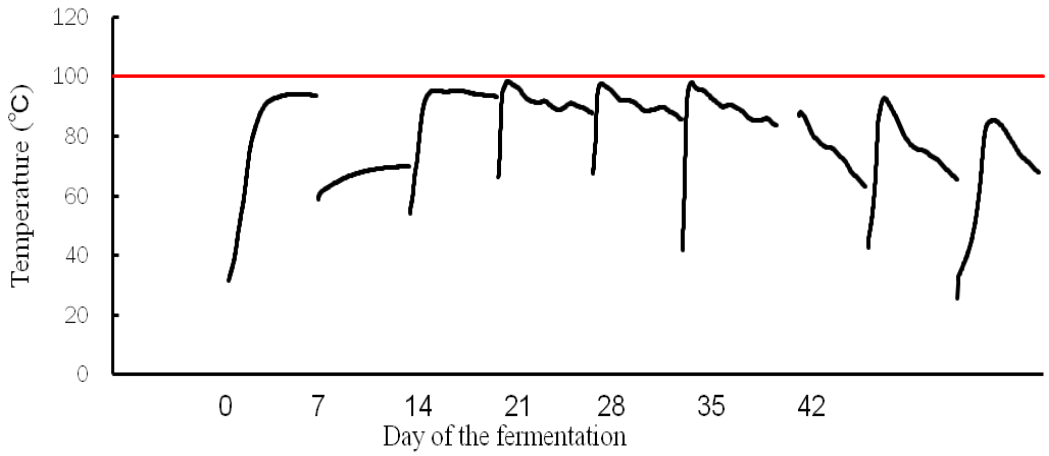


Figure. 1-h-1. The transition of the temperature at the central measure point of the fermentation of the pig manure when supply the regulated aeration strength most of all are below 100 °C measured in 10 min interval. The temperature fluctuations are severe don't like other cases that was described above.

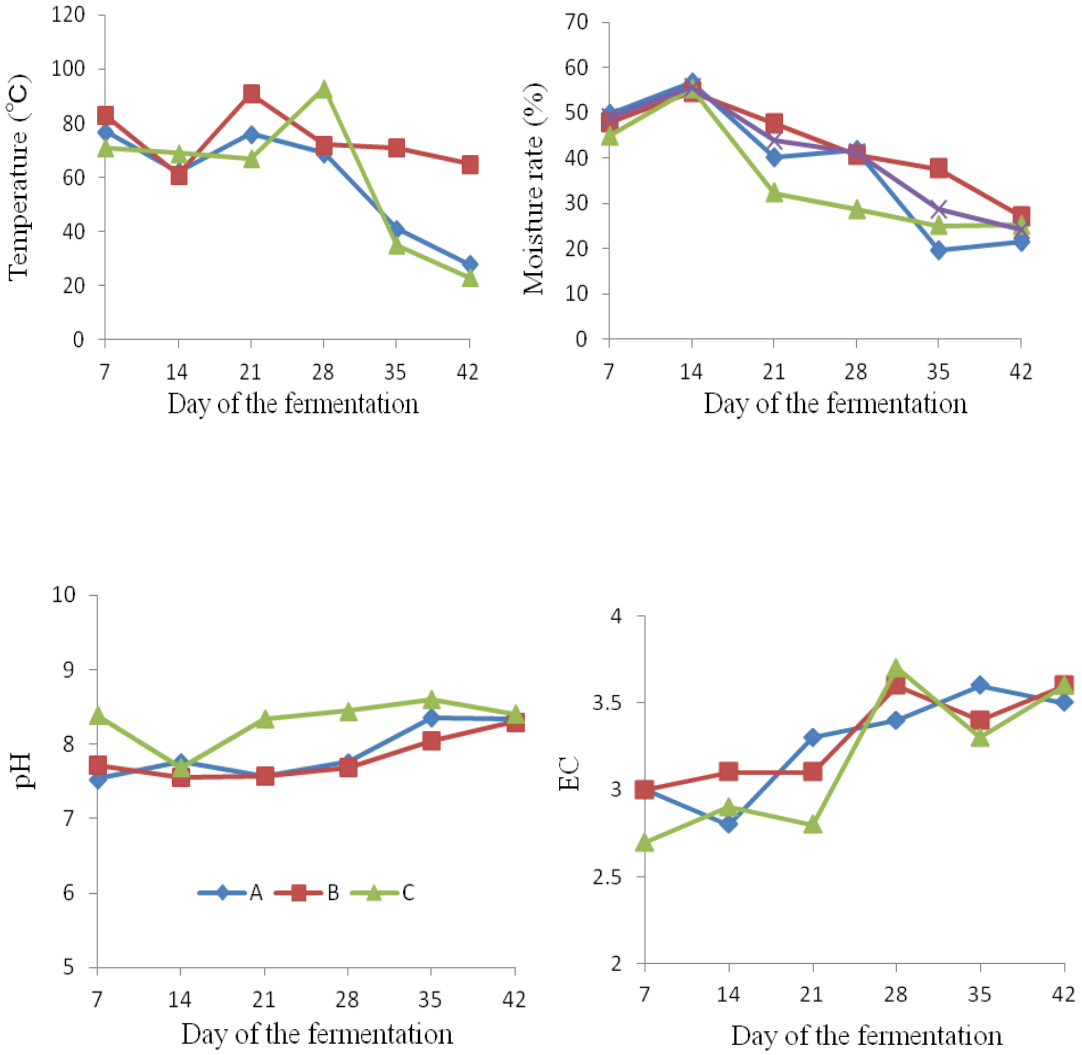


Figure. 1-h-2. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the regulated aerobic strength measured by 7 days intervals.

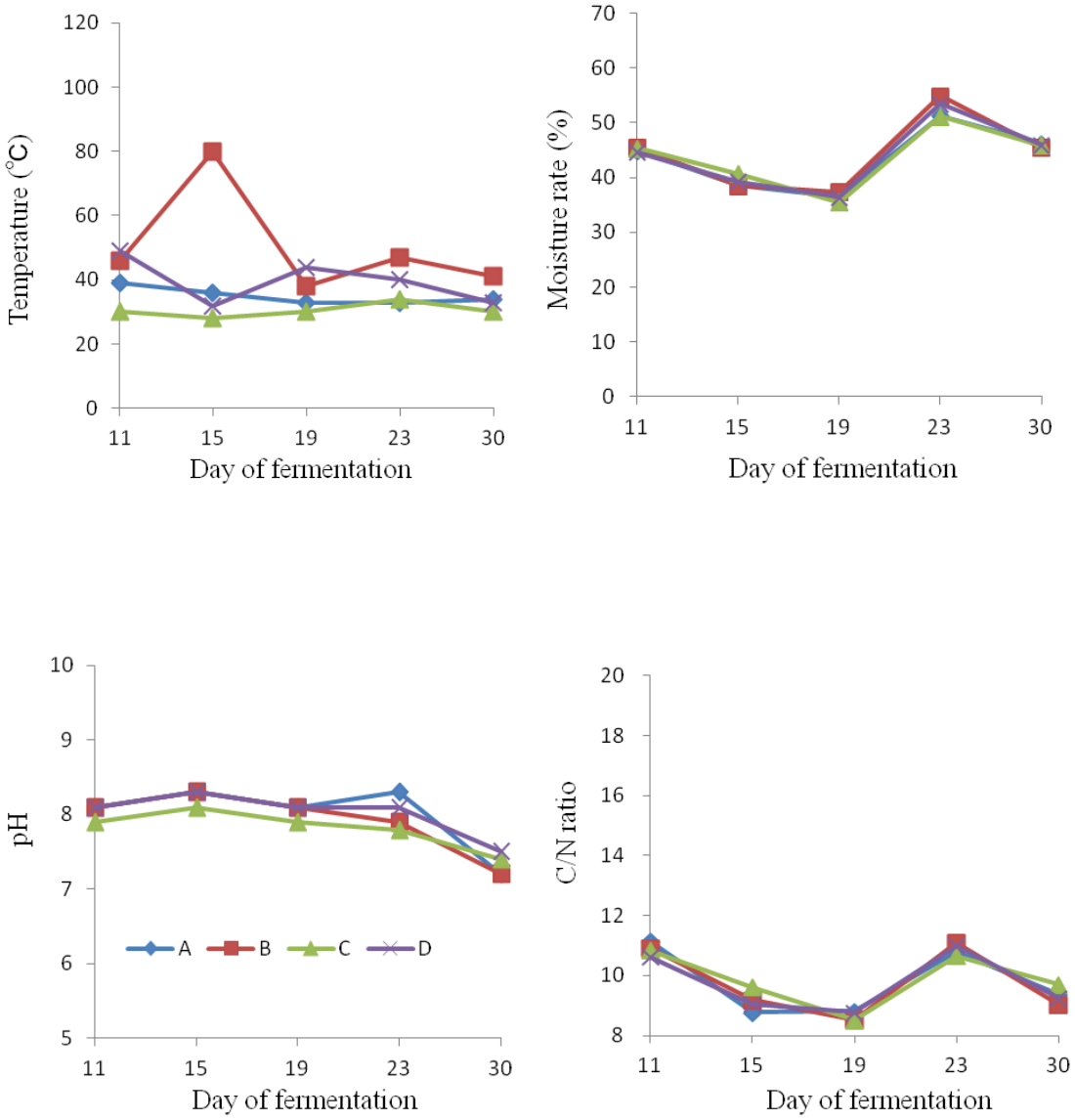


Figure. 1-i. The transition of temperature, moisture rate, carbon to nitrogen ratio (C/N), and pH of the fermentation when supply the regulated aerobic strength measured by 7 days intervals.

Figure. 1-j

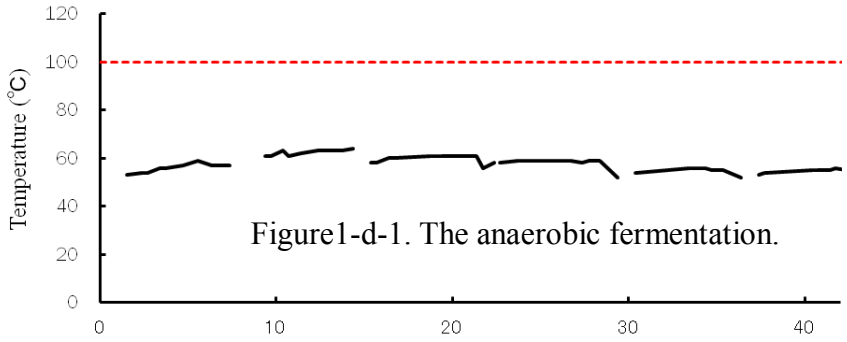


Figure1-d-1. The anaerobic fermentation.

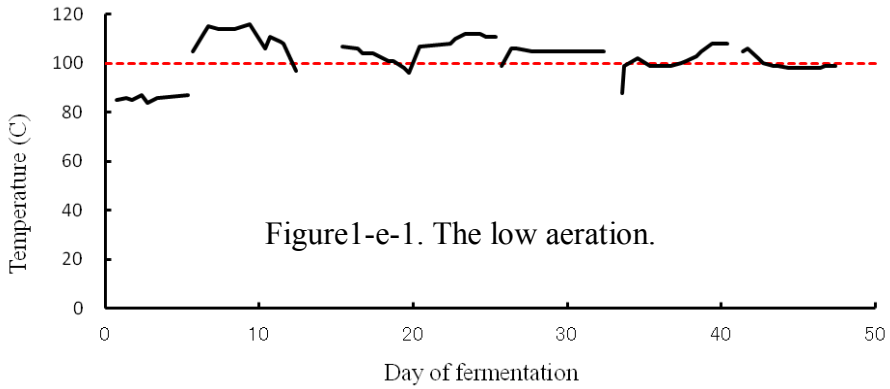


Figure1-e-1. The low aeration.

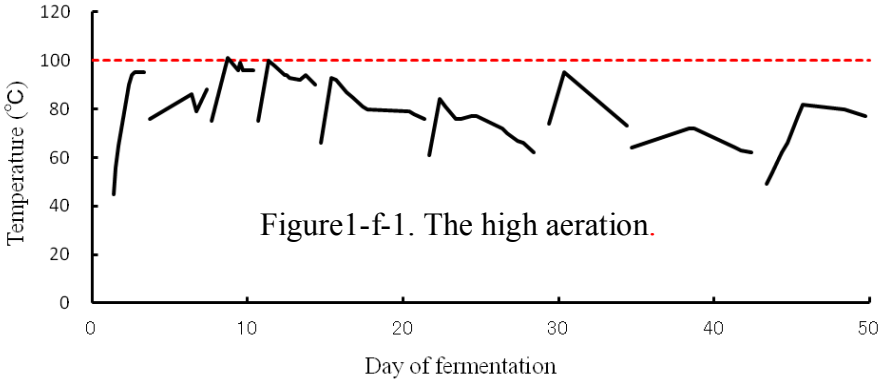


Figure1-f-1. The high aeration.

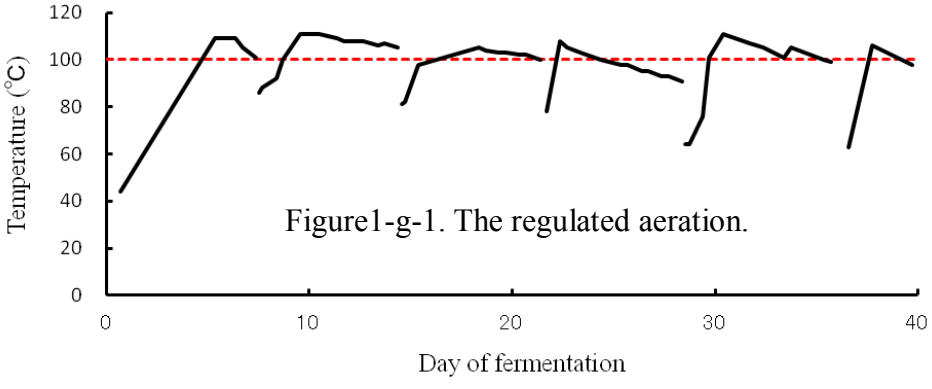


Figure1-g-1. The regulated aeration.

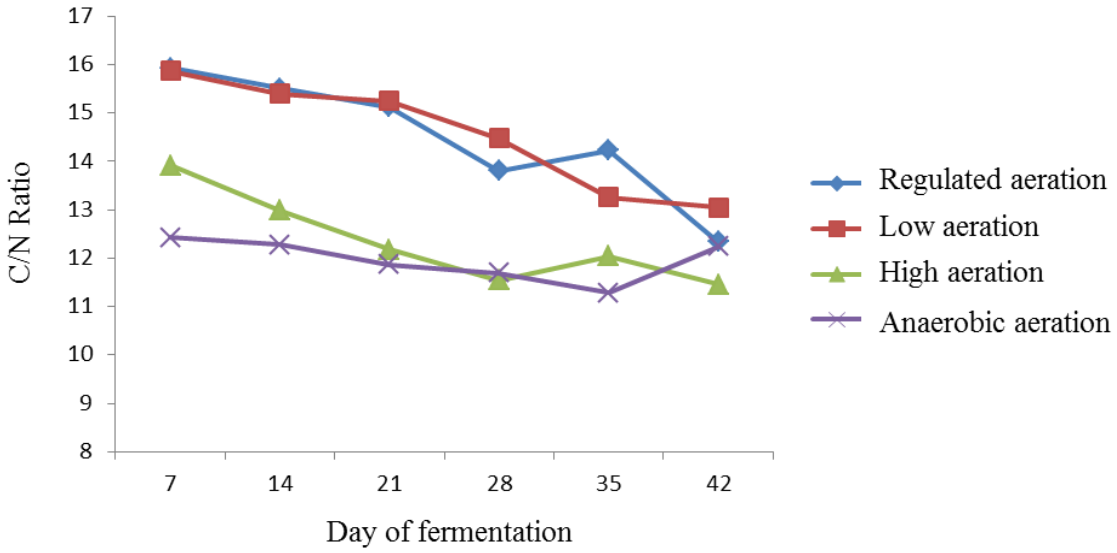


Figure. 1-k. The transitions of C/N ratio of the deferent aeration treatment. The low aeration and regulated aeration treatment are better decomposing organic matte other than high and anaerobic aeration **.**

Chapter 2

Investigation of Bacterial Community for the Aerobic Ultra High Temperature Fermentation Process

Abstract

The present study revealed the structure of bacterial communities present in aerobic high temperature fermentation and evaluated the bacterial succession during the composting progress. The structure of the bacterial community was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) using universal eubacterial primers. I identified the sequences belonged to 17 kind microorganism belonged to six bacterial phyla (*Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Chloroflexi* and *Deinococcus Thermus*) based on a phylogenetic analysis. I confirmed the six bands (*Acidimicrobiaceae bacterium*, *Planifilum fimeticola*, *Planifilum yunnanense*, *Thermaerobacter composti* and *Thermus thermophiles*) have relation with the high temperature expressed during the fermentation. During six main bands, I thought that *Thermus thermophilus* have relation with the ultra high temperature fermentation decreases by the progress.

At the fermentation not to provide air, sequences are grouped to either class *Bacteroidetes* of class *Firmicutes* and that most sequences belong to the members of class *Clostridia*. Because the composting process did not aerated, anaerobic conditions have been created. From these results, I conclude it when process is not contributed to establish ecosystem to easily use oxygen for from the early days to the last time of the composting by only mixing the materials every 7 days.

I compared it differences were detected between the bacterial communities in optimally and suboptimally processes. The DGGE pattern of sample of the sludge fermentation was significantly different from those of the manure mix and pig manure process. There is no detect any any high temperature bacteria such as members of class

Thermus.

Introduction

Composting is a self-heating, aerobic microbial decom-position of organic materials [Dirk *et al.*, 2006]. Organic matter decomposition is carried out by many different groups of microbial populations [Ryckeboer *et al.*, 2003]. The microorganisms involved in composting develop according to the temperature of the mass, which defines the different steps of the process [Keener *et al.*, 2000]. Bacteria predominate early in composting, fungi are present during all the process but predominate at water levels below 35% and are not active at temperatures less than 60 °C. Actinomycetes predominate during stabilisation and curing, and together with fungi are able to degrade resistant polymers.

In present study I planned four kinds of aeration strength to investigate the efficiency of the best fermentation treatment and microorganism populations. I thought that in the anaerobic and aeration fermentation, deferent oxygen environmental, should have varies kinds of microorganism activity. Even in the aeration compost as the temperature changed the microorganism populations should shown some kind of survivals. de Bertoldi *et al.*, [1983] reported the optimum temperature range for composting is 40-65 °C. The temperatures above 55 °C are required to kill pathogenic microorganisms. But, Miller [1992] indicated if the temperature achieved exceeds the tolerance range of the thermophilic decomposers, the effect is damaging for composting. At temperatures above 63 °C, microbial activity declines rapidly as the optimum for various thermophiles is surpassed, with activity approaching low values at 72 °C. The range of 52-60 °C is the most favorable for decomposition. In the Chapter1 I described the low aeration compost is best condition. Under the condition the microorganism

decompose organic matter fastest and it could keep high level temperature, over 100 ℃, for more than 6 weeks. The C/N ratio decreased fastest than other treatment. The study of spatial distribution of the temperature of the aerobic ultra high temperature fermentation compost tell us there are many kinds of conditions existed in the compost, temperature, moisture, pH and nutrient. Thus to understand how the microorganism community changes under the conditions is developing is the subject of the Chapter 2.

Composting is an efficient and cost-effective process for organic waste treatment. To make reliable compost with security is preferable maintaining 60 ℃ or more at fermentation temperature for three weeks [Japan Greenhouse Horticulture Association, 2003]. Because the activity of the microbe decreases when fermentation temperature rises too high, the normal compost does not rise than 80 ℃. As results in Chapter 1 shown, however, in the aerobic ultra high temperature composting process, can maintain the large upper central zone remain at temperatures higher than 100 ℃ for 6 weeks. Shorten fermentation period and complete sterilization of disease-causing germs are characteristic of the novel composting system. As microbes play a key role in the composting, the knowledge of the microbial communities present of the composting process and in differently functioning processes is of value. Understanding the microbiology of composting is critical for understanding the process itself, and for finding new methods to boost the process and improve the final product. I became interested in describing the bacterial community causing in the high temperature compost.

As the traditional, cultivation based methods have many recognized limitations regarding the coverage of the identification of microbes in a sample, methods based on molecular techniques have become popular. DNA analyses, including

16S rRNA based approaches, have been employed for analyzing the diversities of environmental bacteria present in wastes, rivers and soils [Zwart *et al*., 1998; Philips and Verstraete, 2001; Herbert *et al*., 2002; Salles *et al*., 2002; Callia *et al*., 2006]. Fingerprinting methods, such as denaturing gradient gel electrophoresis (DGGE), phospholipid fatty acid analysis (PLFA), restriction fragment length polymorphism (RFLP) and single strand conformation polymorphism (SSCP) [Herrmann and Shann, 1997; Klamer and Baath, 2004; Peters *et al.,* 2000; Ishii *et al.,* 2000; Schloss *et al.,* 2003; Steger *et al.,* 2007] have been found to focus on the most abundant groups, while deep characterisation of compost microbes through cloning and sequencing has not been carried out. PCR-DGGE might have some biases. However, these techniques are useful to determine the dominant microbial population even in complex microbial systems such as composts [Gonzalez *et al*., 2003].

In the chapter 2, I examined bacterial community structures in composting process were examined by 16S rDNA PCR-DGGE, to clarified regarding microorganisms in the aerobic ultra high temperature fermented processing. Comparative analyses of bacterial community successions in the composting materials were done for different fermentation state by the air adjustment of the composting process, fermentation using different materials and optimally and suboptimally functioning processes. I examined that about change of the bacterial quantity by the composting process by RT-PCR analysis.

Materials and Methods

*Sample*s

The samples take as described in Chapter1. The sample use for DNA and RNA analysis put into the liquid nitrogen and then keeps in -80 °C freezer for later analysis.

Quantitative real time reverse transcription-polymerase chain reaction (RT-PCR) and/or PCR analyses

Total RNA in compost samples was extracted using a RNA PowerSoil® Tatal RNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA), and then cDNA was synthesized from 1 μg of total RNA using a Ready to go QuantiTect® Reverse Transcription Kit (Qiagen, Valencia, CA. USA). DNA was extracted from each compost sample using a DNA extraction kit (ISOIL; Nippon Gene, Tokyo, Japan).

DNAs were quantified using a Light-Cycler system (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions. Briefly, PCRs were performed in a reaction mixture (Light-Cycler DNA master SYBR green I; Roche) containing 0.5 μM of each primer, nucleotides, Taq DNA polymerase and 3 mM $MgCl_2$. The primers used for reactions were as follows: 5'- CCT ACG GGA GG C AGC AG-3' (forward) and 5'- ATT ACC GCG GCT GCT GG -3' (reverse). The conditions of amplification were as follows: Denaturation at 95 °C for 10 min and 40 cycles of denaturation at 95 °C for 15 sec, annealing at 64 °C for 4 sec and extension at 72 °C for 9 sec. Quantification was performed using the Light-Cycler analysis software (Roche) on an IBM computer.

PCR-DGGE analysis

Portions of the 16S rDNA V3 region were amplified from the extracted DNA samples using primer sets that target eubacteria [Muyzer *et al*., 1993]. Both the DGGE primers GC-341F and 907R were used for direct amplification from the DNA samples. PCR primer sequences were as follows: 357F-GC, 5'-CGC CCG CCG CGC GCG GGC GGG GCG GGG GCA CGG GGG GCC TACGGG AGG CAG CAG-3'; and 907R, 5'-CCG TCA ATT CMT TTR AGT TT-3' [Green *et al*., 2004]. PCR was performed with a GeneAmp® PCR system 9700 (PE Applied Biosystem). The PCR mixture consisted of 10×Ex Taq™ buffer, 2.5 mM of dNTP mixture, 0.2 µM of each primer, 1.25 U of Taq DNA polymerase (TaKaRa Bio, Ohtsu, Japan), and 1 µl of template DNA in 25 µl of PCR reaction mixture. The PCR conditions were as follows: initial denaturation at 95 °C for 10min, followed by 30 cycles of 30 sec denaturation at 93 °C and 30 sec annealing at 65 °C(first ten cycles), then at 60 °C (second ten cycles), and finally at 55 °C (last cycles). Each annealing step was followed by a 1 min extension at 72 °C except for the final extension (at 72 °C for 5min). The products of PCR amplification were examined by electrophoresis on 1% (w/v) agarose gel plates prior to DGGE analysis.

DGGE analyses of the PCR products were evaluated by the method reported by Muyzer *et al*. [1993] with the DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA). Denaturing gradient gel, 1 mm thickness and 160 x 160 mm, was prepared in 20 mM Tris buffer (pH 7.4) containing 0.5 mM ethylenediamine tetraacetic acid (EDTA; Wako Pure Chemicals, Osaka, Japan) and 10 mM sodium acetate (Wako). The concentration gradient used was 8-12% polyacrylamide and 1.4-4.9 M urea (Wako) with a denaturant gradient 30 to 70%. Electrophoresis was carried out at

100 V for 850 min in 20 mM Tris buffer (pH 8.0) at 60 °C. After electrophoresis, the gel was stained with SYBR Green I (Invitrogen, Carlsbad, CA, USA) for 30 min, visualized and photographed using an image analyser (LAS-1000Fujifilm, Tokyo, Japan).

Sequence analysis

The DGGE bands were sequenced according to the following protocol: a band in the DGGE gel was carefully excised with a cutter blade under UV illumination and then placed in 50 µl of distilled water. The DNA from the cut-out gel was subsequently used as the template DNA in a reamplification PCR performed with primers GC-357F and 907R. The resulting amplicons were electrophoresed again on a DGGE gel to verify the position of the original band. These steps were carried out until a single band appeared. Them a plified PCR products were purified by a Wizard® SV Gel and PCR Clean-Up System (Promega). Sequences were determined by TaKaRa Bio. The sequence data were phylogenetically analyzed using the DDBJ-BLAST program provided by DNA Data Bank (Center for Information Biology, Mishima, Japan).

Results

Chenges in the expression levels of DNA of bacterial in composting estimated by Quantitative PCR

I examined the changes in the expression levels of DNA of bacteria community in composting by Quantitative PCR (RT-PCR). The compost treated with regulated aeration. The results shown in Figure. 2-a. In the point C there are no temperature data displayed. Because of the point C is too deep to measure. The point A and point B reduce the amount of DNA due to temperature rise. Usually the point B is the place of most hot so the expression levels of DNA are low. However, there was not difference for the day 52 of the point A, B and C.

PCR-DGGE analysis of bacterial communities

DGGE profiles from all compost samples are shownn in Figure. 2-b. At the diagram of DNA, the band pattern of three places (A, B and C) was differed by cluster analysis. The band pattern of point C was significantly differed that of point A and B. The temperature of each point is go down by fermentation. The band of the three points (A, B and C) are similar at the day 58th of the fermentation. The point of high temperature is less number of band, especially the light bands are disappeared at high temperature point. There is no change of the number of bands at the point C.

At the RNA diagram, there is no any band at the high temperature point on the day 22, 34 and 44 respectively. There is no changes of the number of band at the point C. The samples of point B, there are no band on day 22nd and 34th. The banding pattern of day 44-58 with point A and C were similar by cluster analysis. Most of the 16S rDNA

were same as 16S rRNA of DGGE pattern. In these bands, six bands expressed change over time by fermentation process. It is j (*Planifilum fimeticola*), k (*Planifilum yunnanense*), l (*Rhodothermus marinus*), m (*Rhodothermus clarus*), o (*Thermaerobacter composti*) and q (*Thermus thermophiles*). The band of j, k and l increased and band q decreased by fermentation. The band o increased first and decreased afterwards.

The detail identification of sequences and cluster analyzed results of all samples are shown in the Table. 2-a. The sequences of six bacterial phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Deinococcus-Thermus*) based on a phylogenetic analysis. Since sequences representing the Firmicutes were by far the largest group, this phylum was further divide into the classes *Bacillales* and *Clostridiales* in order to study the community composition (Figure. 2-b and Table. 2-a). Based on the observed frequencies of similar sequence types, bacterial sequences were thus divided into four main groups: *Deinococci*, *Bacillus*, *Actinobacteria* and *Clostridia*. Sequences included in the above mentioned groups were those classified up to the genus or species level. The uncultured-group included sequences that are reported as uncultured bacteria in the DDBJ database and the unclassified-group represent sequences with no close similarity to sequences in the nucleotide database (Figure. 2-b).

When using low aeration strength no band was confirmed other than the day14. The band k, o and q was confirmed at point C sample of day 14 (Figure. 2-c). It might be due to the high temperature on. The high aeration strength treatment, the DGGE banding pattern did not change significantly from 7 days to 42 day. The band of i (*Acidimicrobiaceae bacterium IC-180*), j (*Planifilum fimeticola*), k (*Planifilum*

yunnanense), o (*Thermaerobacter composti*) and q (*Thermus thermophiles*) was confirmed at all of the samples from day 7 to day 42. Some *Ureibacillus* such as s appeared in place C (Figure. 2-d). The sample of regulated aeration strength treatment no band was confirmed at point A and B of the day 14, 21 and 35. At the point C the band j, k, n, o and q. was confirmed. After day 35 same *Geobacillus* was confirmed (Figure. 2-e). The DGGE band pattern profile of the anaerobic fermentation is shown in Figure. 2-f. The pattern of the band is completely different with aerobic fermentation results. The detail information of analyzed sequences of all samples is shown in Table. 2-a.

The sequence results belonged to two bacterial phyla (*Bacteroidetes*, *Firmicutes*) based on a phylogenetic analysis. Since sequences representing the *Firmicutes* were by far the largest group, this phylum was the classes *Clostridiales* predominantly in the community composition (Figure. 2-f and Table. 2-a). There was no change of the bacteria glass between place A and B and C in fermentation.

The DGGE profile of the sludge form day 11 to day 30 is presented in Figure. 2-h. The each red letter indicates the band sequenced. This is a represent of the bad example for the aerobic ultra high temperature fermentation. It was failed to increase the temperature because of high moisture.

The sequences belonged to two bacterial phyla (*Actinobacteria* and *Firmicutes*) based on a phylogenetic analysis. Since sequences representing the *Firmicutes* were the largest group (Figure. 2-g and Table. 2-a). Based on the observed frequencies of similar sequence types it is mainly by *Bacillus*.

Discussion

Aerobic ultra high temperature composting provides a economic and safety way to decompose organic matter without the production of toxic pollutants such as NOx, SOx and dioxins. Information of the bacterial community composition and predominant genus or species is important to know the composting performance and to develop the effective composting processes. I have investigated microorganisms involving in this process by 16S rDNA PCR-DGGE analysis.

The sequences belonged to six bacterial phyla (*Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Chloroflexi* and *Deinococcus-Thermus*) based on a phylogenetic analysis. As described previously, the dominant bacteria consisted of the members of the order *Bacillales*. It is consisted that Ishii *et al*., Pedro *et al*., (2001) reported the Bacillus-type spp. were mainly detected at thermophilic stage of composting. Many studies revealed members of *Proteobacteria* dominate the initial stages [Peters *et al*., 1999; Ishii *et al*., 2000; Dees and Ghiorse, 2001; Schloss *et al*., 2003]. *Rhodothermus* detected in hot synthetic compost [Dees and Ghiorse, 2001] it is agree with my result. *Planifilum fimeticola* and P*lanifilum yunnanense* isolated from a high temperature composting process [Hatayama *et al*., 2007]. *Thermus thermophilus* was originally isolated from a thermal vent which place is Mine-hot spring in Izu, Japan by Oshima and Imahori [1974]. The organism has also been found to be important in the degradation of organic materials in the thermogenic phase of composting [Beffa *et al*., 1996]. It is not yet clear whether there are new ultra high temperature bacteria in these 17 kinds of bacteria. But it is more likely to be connected with high temperature to have become that the bacteria of many kinds is aggregate. This is extremely rare.

In chapter1, I found that fermentation temperature and the fermentation situation change by adjustment of the air slightly. Adversely, the DGGE band was not found from the samples of fermentation temperature exceeded 100 ℃ (Figure. 2-c and Figure. 2-e). Because of the fermentation temperature influence the activity of bacteria. It is consisted Miller [1992] indicated the range of 52–60°C is the most favorable for decomposition. Bacteria may be present throughout the composting process as active or dormant cells, or as spores. Only their numbers and level of activity change during the composting process [Gentleman *et al*., 2004]. The band did not appear in DGGE on sample of the fermentation at low aeration, but can found that fermentation advanced (in chapter 1). Therefore, it is thought that the bacteria work on the resolution again when fermentation temperature falls.

The six bands expressed change over time by fermentation progress. In six mainly bands, I presume that *Thermus thermophilus* have relation with the ultra high temperature decreases by the fermentation progress. The DGGE band j (*Planifilum fimeticola*) and k (*Planifilum yunnanense*), was detected from all of the compost from mixed manure, pig manure or sludge. The *Thermus thermophilus* could be saving the ultra high temperature fermentation. The sample of the anaerobic fermentation, sequences are grouped to either class Bacteroidetes or class Firmicutes and that most of sequences belong to the members of class Clostridia. These organisms are known to be anaerobic organisms living in soil, sediments rumen, faces etc. [Wiegel *et al*., 2006]. In fact, the bacteria grouped in class Clostridia are often found in cow rumen fluid [Whitford *et al*., 1998] and cow manure [Ozutsumi *et al*., 2005]. In chapter 1, I found that in composting progress under the anaerobic condition the fermentation maintained low temperature (50-60 ℃)) always. I believe that anaerobes such as members of class

Clostridia present in animal manure survived in the compost material and were responsible for incomplete degradation of organic compounds. From these results, in order to contribute to establish ecosystem aeration fermentation is necessary with crosscut on timely.

In composting process of pig manure, temperature changed dynamically (61-91 °C)) during day14 to 21. In the DGGE profile, same bands appeared and disappeared during the course of composting. This suggests that specific bacterial communities, which could adapt to the conditions in the composting material, were established in each stage of composting. However, the DGGE pattern of sample of the sludge fermentation process was significantly different from those of the mixed manure or pig manure. Additionally, when the compost temperature increased to 80 °C), there was a shift in the band pattern, suggesting that thermophilic bacteria dominate in this stage. In the present study could not detect any high temperature bacteria such as members of class *Thermus*. The aeration fermentation rather than the aerobic ultra high temperature fermentation even offered air a lot for compost the temperature could not increase beyond 50 °C) and high temperature could not be maintained for a long time. Because of the crosscut compost every 2-4 days intervals make water vaporize. The operational characteristics of this method prevented the dominance of thermophilic aerobes such as *Thermus*.

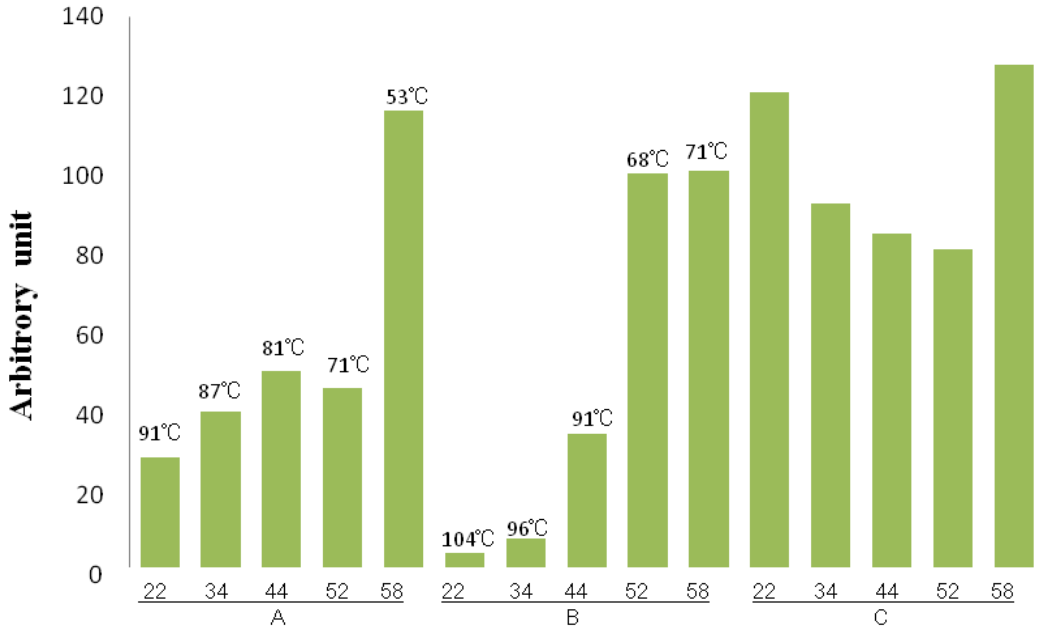


Figure. 2-a. Changes in expression levels of DNA quantity. The A, B and C indicate the sampled points, the number of above them is day of the sampling.

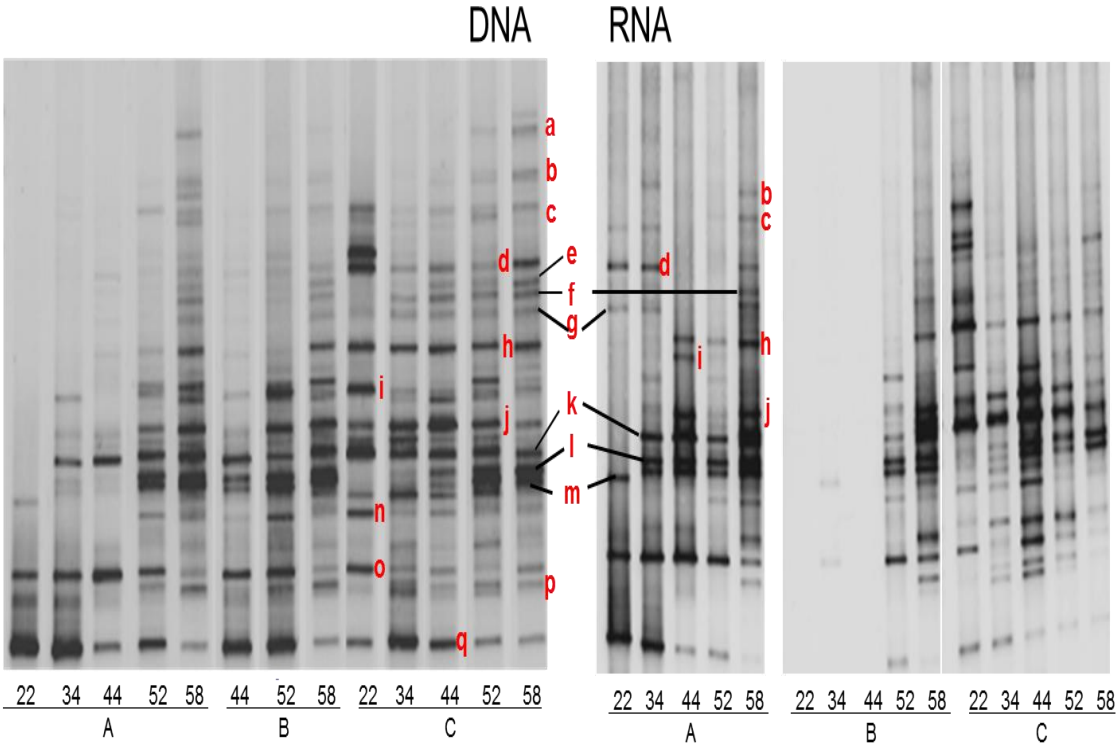


Figure. 2-b. DGGE profile of the samples in the composting processes from day 22 to day 58. The number expressed below DGGE profiles corresponds to each sample. Each letter indicated the bands sequenced. See Table. 2-a for the identification.

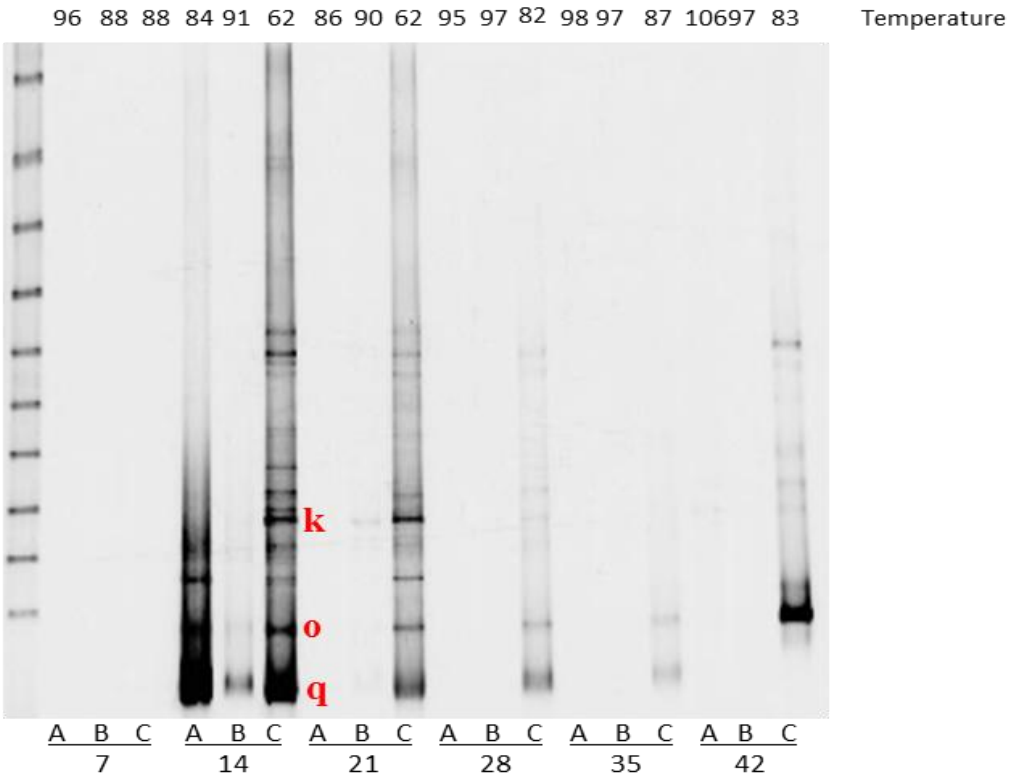


Figure. 2-c. DGGE profile of the low aeration samples from day 7 to day 42.

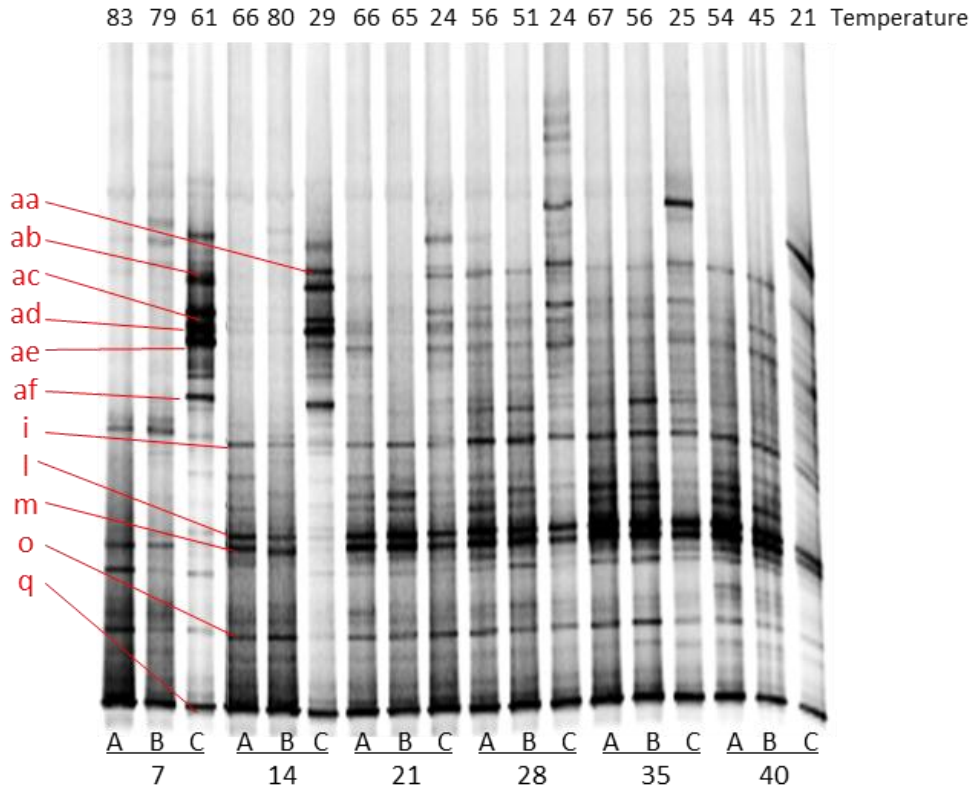


Figure. 2-d. DGGE profile of high aeration samples from day 7 to day 42.

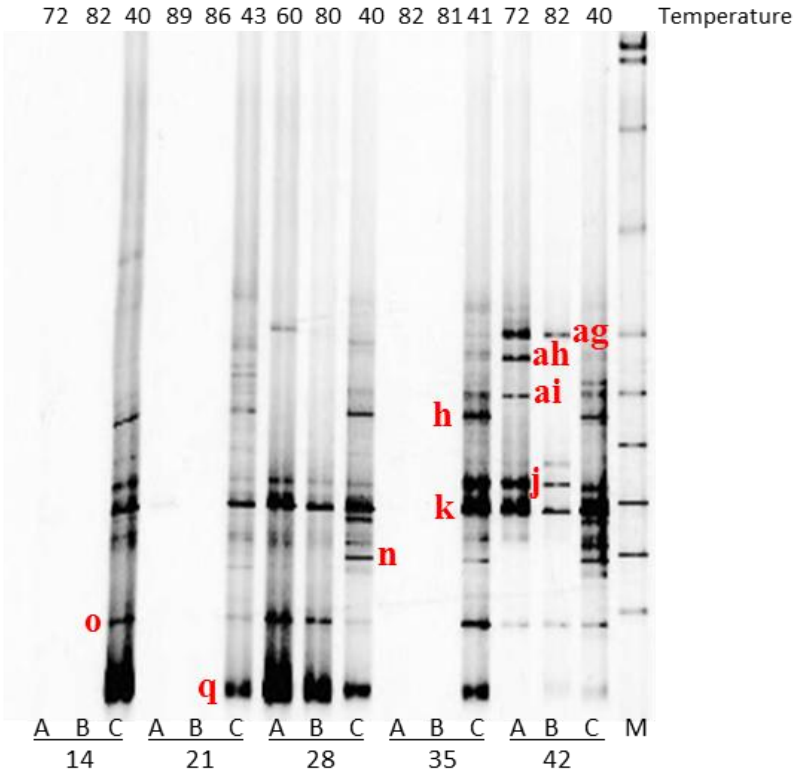


Figure. 2-e. DGGE profile of the regulated aeration samples from day 14 to day 42.

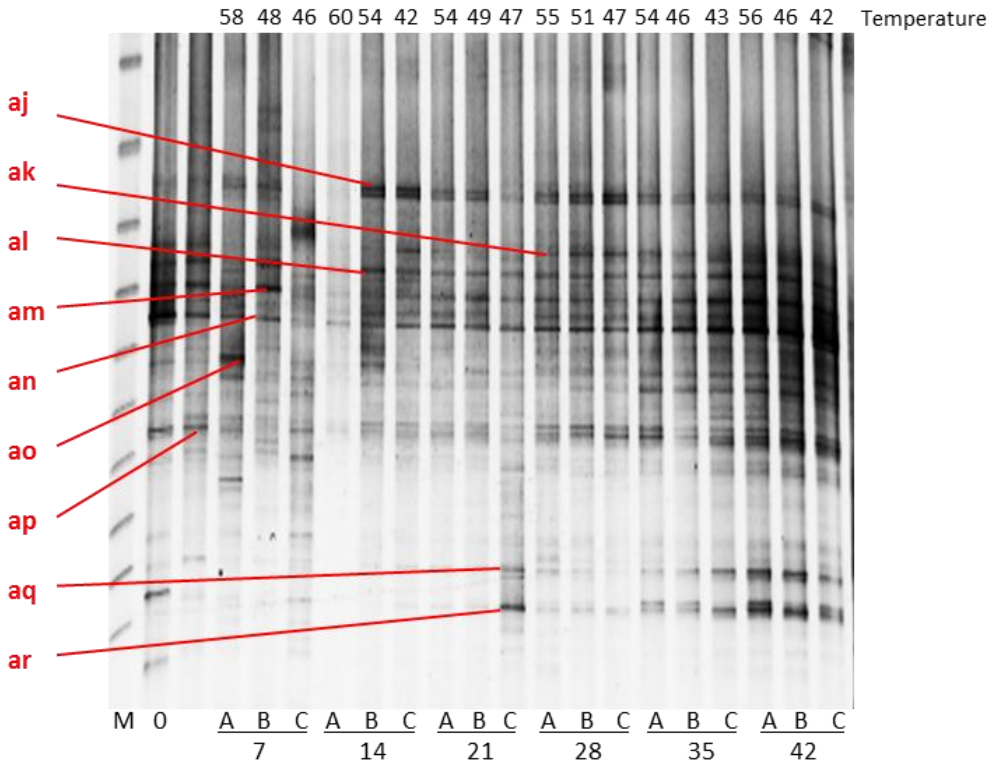


Figure. 2-f. DGGE profile of anaerobic fermentation samples from day 7 to day 42.

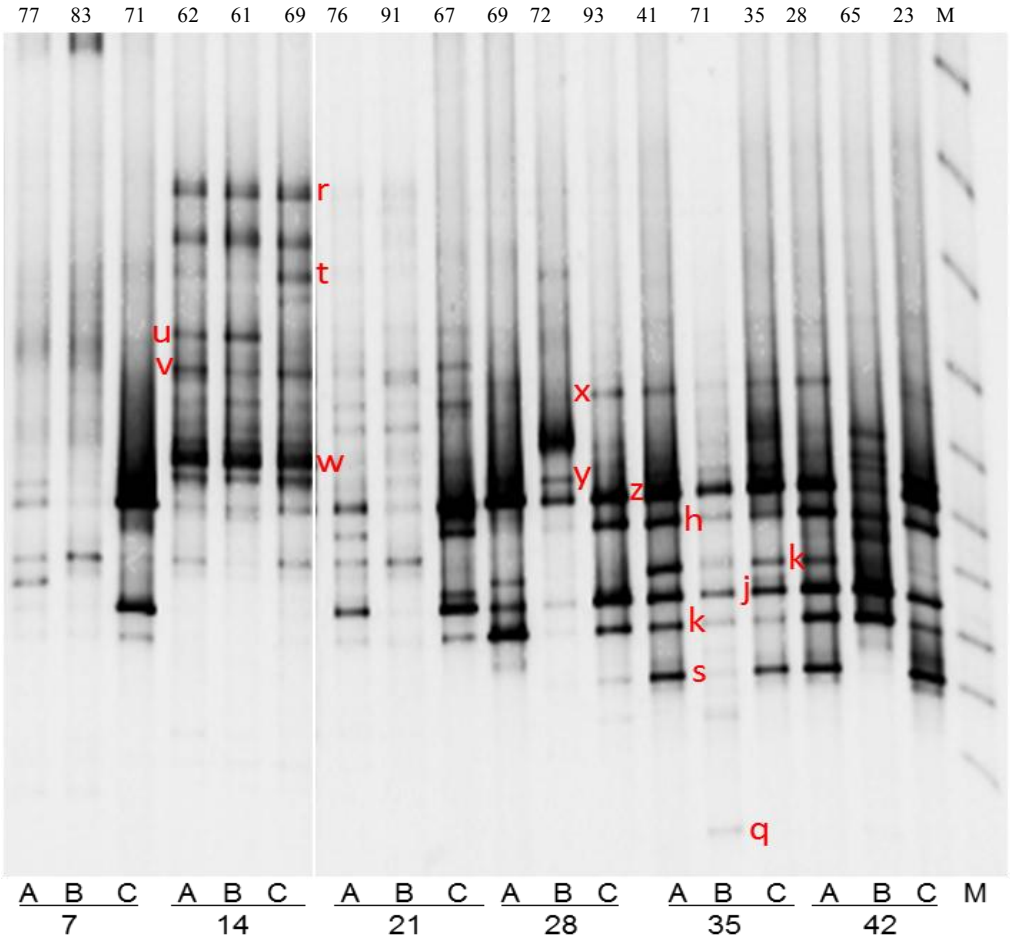


Figure. 2-g. DGGE profile of the samples in the pig manure composting processes from day 7 to day 42. The number expressed below DGGE profiles corresponds to each sample. Each letter indicated the bands sequenced. See Table 2-a for the identification.

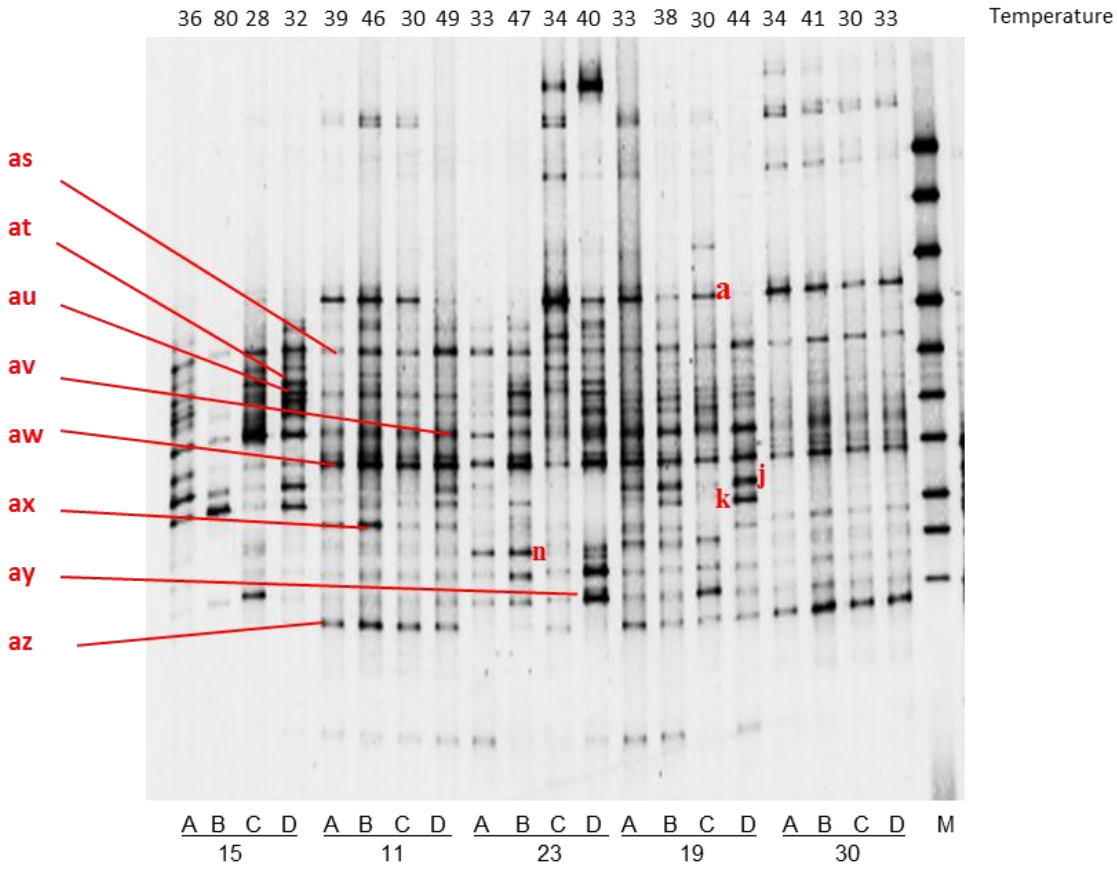


Figure. 2-h. DGGE profile of the sludge from day 11 to day 30. The each red letter indicate the band sequenced. See Table. 2-a for the identification. This is a represent of the bad example for the aerobic ultra high temperature fermentation. It was failed to increase the temperature because of too high moisture.

Table 2-a. Identification of separated bands on the basis of percent to similarity to 16S rDNA and 16S rRNAsequenc

Band	Identification	Accession No.	Similarit (%)	Phylum	Order or Class
a	*Sphingobacteriaceae bacterium Gsoil 524*	EU370954	99	*Bacteroidetes*	*Sphingobacteriales*
b	*Paenibacillus sp. SH-55*	AB162693	94	*Firmicutes*	*Bacillales*
c	*Paenibacillus sp. SBK-15*	AB366356	95	*Firmicutes*	*Bacillales*
d	*Thermoactinomycetaceae bacterium CNR873 PL04*	DQ448805	92	*Firmicutes*	*Bacillales*
e	*Alviniconcha hessleri gill endosymbiont*	AB214932	93	*Proteobacteria*	*Gammaproteobacteria*
f	*Ornithinibacillus sp. XJSL10-7*	GQ903476	90	*Firmicutes*	*Bacillales*
g	*Geobacillus sp. TC-W7*	GQ866911	97	*Firmicutes*	*Bacillales*
h	*Uncultured compost bacterium*	AB437982	100	–	–
i	*Acidimicrobiaceae bacterium IC-180*	AB517669	91	*Actinobacteria*	*Acidimicrobiales*
j	*Planifilum fimeticola*	AB088364	99	*Firmicutes*	*Bacillales*
k	*Planifilum yunnanense*	DQ119659	99	*Firmicutes*	*Bacillales*
l	*Rhodothermus marinus strain yb43*	EU214605	99	*Bacteroidetes*	*Sphingobacteria*
m	*Rhodothermus clarus*	AB252420	100	*Bacteroidetes*	*Sphingobacteria*
n	*Uncultured compost bacterium*	FN667400	98	–	–
o	*Thermaerobacter composti*	AB454087	100	*Firmicutes*	*Clostridiales*
p	*Sphaerobacter thermophilus DSM 20745*	CP001824	100	*Chloroflexi*	*Sphaerobacteria*
q	*Thermus thermophilus*	DQ119659	100	*Deinococcus-Thermus*	*Deinococci*

Continued.

Band	Identification	Accession No.	Similarit (%)	Phylum	Order or Class
r	*Caloramator sp. TH7C1*	GU216701	91	*Firmicutes*	*Clostridiales*
s	*Saccharomonospora viridis*	Z38007	100	*Actinobacteria*	*Actinomycetales*
t	*Clostridium disporicum*	Y18176	98	*Firmicutes*	*Clostridiales*
u	*Uncultured bacterium*	GU320659	99	–	–
v	*Thermoanaerobacter sp. EB3.8*	GU176611	90	*Firmicutes*	*Thermoanaerobacterales*
w	*Tepidanaerobacter sp.T2*	FJ620693	92	*Firmicutes*	*Thermoanaerobacterales*
x	*Paenibacillus polymyxa*	AM268336	92	*Firmicutes*	*Bacillales*
y	*Bacillus sp.R-6770*	FN997655	100	*Firmicutes*	*Bacillales*
z	*Bacillaceae bacterium NS1-3*	AY466703	99	*Firmicutes*	*Bacillales*
aa	*Paenibacillus chitinolyticus*	EF154247	94	*Firmicutes;*	*Bacillales;*
ab	*Ureibacillus thermosphaericus*	AF403021	95	*Firmicutes;*	*Bacillales;*
ac	*Ureibacillus thermosphaericus*	AB300774	98	*Firmicutes;*	*Bacillales;*
ad	*Uncultured compost bacterium*	FN667439	100	*Firmicutes;*	*Bacillales;*
ae	*Bacillus sp. R-7413*	AY422985	100	*Firmicutes;*	*Bacillales;*
af	*Uncultured compost bacterium 5-33*	AB034720	97	*Firmicutes;*	*Bacillales;*
ag	*Geobacillus caldoxylosilyticus*	AY608950	100	*Firmicutes;*	*Bacillales;*
ah	*Geobacillus thermodenitrificans*	AB116106	100	*Firmicutes;*	*Bacillales;*
ai	Geobacillus thermodenitrificans	FN428666	100	*Firmicutes;*	*Bacillales;*

Continued.

Band	Identification	Accession No.	Similarit (%)	Phylum	Order or Class
aj	*Ruminofilibacter xylanolyticum*	DQ141183	99	*Bacteroidetes*	*Bacteroidales*
ak	*Clostridium ultunense strain Esp*	GQ487664	98	*Firmicutes*	*Clostridiales*
al	*Uncultured bacterium clone E82*	FJ205847	98	*Firmicutes*	*Clostridiales*
am	*Uncultured bacterium clone E32*	FJ205814	99	*Firmicutes*	*Clostridiales*
an	*Uncultured bacterium clone 1-1B-28*	F417919	99	*Firmicutes*	*Clostridiales*
ao	*Uncultured bacterium*	AM947522.1	98	*Firmicutes*	*Halanaerobiales.*
ap	*Uncultured bacterium clone 1-2B-19*	JF417939.1	99	*Firmicutes*	*Clostridiales*
aq	*Uncultured bacterium*	AB428540.1	99	–	–
ar	*Uncultured bacterium*	AB274515	99	–	–
as	*Bacillus sp. MHS037*	DQ993294	98	*Firmicutes;*	*Bacillales;*
at	*Bacillus sp. 3LF 16P*	FN666888	100	*Firmicutes;*	*Bacillales;*
au	*Bacillus sp. TAT105*	AB066342	99	*Firmicutes;*	*Bacillales;*
av	*Uncultured compost bacterium*	AB437982	98	–	–
aw	*Uncultured compost bacterium*	FN667033	99	*Actinobacteria.*	-
ax	*Uncultured bacterium clone F1_23X*	AB438017	100	–	–
ay	*Uncultured bacterium clone M58*	EU215310	99	–	–
az	*Uncultured bacterium clone G3DCM-270*	EU037358	98	–	–

Chapter 3

Safe Evaluation and Application of the Aerobic Ultra High Temperature Fermented Compost

Abstract

The toxicity of high temperature compost was evaluated by subchronic toxicity studies and contact toxicity study. No treatment-related sign in subchronic oral toxicity and contact toxicity studies was documented. In subchronic oral toxicity study, male and female ICR mice were given the product of ultra high temperature fermentation which was mixed with drinking water (1 g/ml) for four weeks. All animals survived in the study and showed no treatment-related clinical sign or adverse effects on body weight, food or water consumption, blood biochemistry data or histopathological findings. In contact toxicity study, female ICR mice were kept on the paper clean litter used as control and three types of products of ultra high temperature fermentation for two weeks. No treatment-related changes in body weight, food consumption, water consumption, blood biochemistry data or gross pathology or histopathology was found in any group. Thus, under the experimental conditions used, ultra high temperature compost has no toxicological effect on mice. Then, to assess the feasibility of the product of ultra high temperature fermentation, the usefulness for litters for cattle feedlot and fertilizer for agricultural products were examined. In cattle pens, the product of ultra high temperature fermentation was mixed cornstalks was used as litter. *E. coli* levels in the mixed litter rapidly decreased, suggesting the effectiveness of disinfection. The product of ultra high temperature fermentation (10,000 kg/ha) was sprinkled on arable lands for 19 crops (grass, rice, maize, strawberries, green beans, onions, squash, garlic and so on). The product showed high fertilizer effect on grass, rice and green beans, but not maize, suggesting the usefulness as fertilizer on crops.

Introduction

Composting is a part of the cycling system that move those constituents back to the soil. Composts apply to agricultural soil as nitrogen fertilizer [Kaku *et al.*, 2004]. Composts have microbial decomposition of organic matter under aerobic bacteria where the waste reaches to the manageable, preservable and environmental-safe state that can resolve into soil [Goluke, 1997]. The resulting compost becomes a nourishment source for plants and realizes the circulation of materials. The application of adequate amount of livestock compost does not only nourished crops but also improves the characteristic of the soil physically, chemically and biologically. Same good bacteria from compost produce plant hormones such as auxin and cytokinin that regulate plant growth and bearing fruit. The production of good bacteria in soil, stimulates plant growth and enhances sugar content of fruit. Furthermore, some compost keeps a bacteria group which controls plant disease and acts as a biological pesticide [Nakasaki *et al*., 1998]. Important factor of successful organic farming that is protecting agro-products from continuous cropping hazard by fighting against various bad microbes, worms and insect. Currently, paper or crop residues are widely used for cattle feedlot bedding materials. However, because of increased interest in recycling paper products in recent years, paper is no longer affordable for cattle feedlots. Recently, compost was used as bedding in cattle [Zehnder *et al*., 1997].

In Chapter 1, I confirmed that novel aerobic ultra high temperature fermentation process is good processing method that is high speed at high temperature and easy to use it. It is desirable to use the compost of novel aerobic ultra high temperature fermented for example as manure and bedding for farm animal effectively.

However, I do not understand the safety and the effectiveness as the manure of the fermentation product which which fermented at a ultra high temperature at high speed. In Chapter 2, I found that fermentation compost formed by bacteria more than 17 kinds of dominant bacteria. These bacteria and the substrate which bacteria produce in the middle of fermentation have to confirm whether safe in the soil, plant and animal. In Chapter 3, first, I assessed the safety of the ultra high temperature compost in subchronic toxicity studies and contact toxicity stud it using mice. Furthermore, I was intended to evaluate safety for the use of high temperature compost as a cattle-bedding. Then, the usefulness of ultra high temperature compost and fertilizer were assessed using grass in the farm and rice, maize, strawberries, green beans, onions, squash, garlic and so on in the farmhouses.

Materials and Methods

Experiment 1: Safe evaluation of the aerobic ultra high temperature fermented compost in mice

Materials

Subchronic toxicity:

a. Materials: Aerobic ultra-high temperature fermented compost (Animal resource science center, The University of Tokyo, Kasama, Japan)
b. Treatment water: 200g compost + 2000ml tap water
c. Control water: tap water

Contact toxicity test:

a. Paper clean (SLC, Japan): control
b. Powdered (Intermediate stage)
c. Powdered (End stage)
d. Pellets (End stage (shape deferent)

Animals

Male and female ICR mice were purchased from SLC. Three weeks old male and female mice were used for Subchronic toxicity test, and eight weeks old females were used for contact toxicity test. (Table. 3-a)

	Subchronic toxicity	Contact toxicity
Male	36(n=1/group)	0
Female	36(n=6/group)	40(n=10/group)
Age(week)	3	8-9

Method

Subchronic toxicity experiment: Animals were housed in plastic cage, given MF diet (Oriental Yeast, Tokyo, Japan) and tap water (control group) or treatment water. Observe general symptom might be affect by toxicity and pharmacological including morbidity and mortality. Record the ody weight, daily gain, feed and water consumption. Plasma sample collect. Mice was placed in metabolism cages and fasted for 24h. Then, animals were sacrificed under deep ether anesthesia. Blood samples were collected for biochemical analyses. Plasma was prepared by centrifugation. Measure: Creatine phosphpkinase (CPK), glucose (GLU), urea nitrogen (BUN), creatinine (CREA), total cholesterol (tCHO), glutamic oxaloacetic transaminase (GOT/AST), glutamyltransferase (GGT), glutamic pyruvic transaminase (GPT/AST), high density lpoprotein (HDL), lactate dehydrogenase (LDH), fasting glucose (GLUC), amylase (AMYL), total serum protein (TP), total bilirubin (tBIL), albumin (ALB), alkaline phosphatase (ALP), inorganic phosphorus (iPHS), triglycerides (TG), uric acid (UA), ammonia (NH_3), inorganic phosphorus (iP), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and chloride (Cl) levels were measured using an automatic analyzer (Drychem 400, Fujifilm, Tokyo, Japan). Organs (liver, kidneys, adrenals, brain, heart, thymus, spleen, and ovaries or testes) were removed and fixed in 10% (v/v) phosphate-buffered formalin (pH 7.4) (Wako Pure Chemicals, Osaka, Japan) dehydrated through a graded ethanol series, and embedded in paraffin (Histosec; Merck, Darmstadt, Germany). Serial paraffin-embedded sections 4 μm thick were cut, mounted on glass slides precoated with 3-aminopropyltrimethoxysilane (Sigma Aldrich Chemicals, St. Louis, MO, USA). They were stained with hematoxyline and eosin (HE). Histopathological examination was performed.

Contact toxicity experiment: Animals were fed in plastic cage with paper litter (control) or ultra high temperature compost, given MF diet (Oriental Yeast) and tap water, and observed general toxicity and pharmacological effects, morbidity and mortality during the test. Body weight, daily gain, feed and water consumption was recorded. During test, estrus stage in each female was estimated by taking a vaginal swab. In brief, moistened sterile cotton swab was gently and slightly inserted into the vagina. The fluid from the cotton swab was then smeared onto a relatively small area of a prenumbered slide. Slides were preserved using a cytofixative. One day before collection of samples for the clinical biochemistry and pathology evaluation, each animal was placed in metabolism cages and fasted for 24h.

Experiment 2: the feasibility of the cattle bedding test

Mixed ultra high temperature compost (2.5 m^3) and sawdust (2.5 m^3) used it as bedding. As a control group, I used only sawdust (5 m^3) as bedding. Seven Japanese black (BW: approximately 500 kg of) was fed with the cattle barn of the area of 5.4 m× 10.7 m. Repeated the mixed ward and control ward three times in turn. The sample was taken on the day10th and 20th from examination, at five spots of the bedding. The sample examined the number of coliform bacteria and the bacterial community.

Experiment 3: the feasibility of organic fertilizer test

Fertilize the compost on grass

To examine quantity of appropriate fertilization of the compost, the product of was sprinkled on grass each 0, 1000 and 2000 kg/ha. The kind of the grass used Italian ryegrass. To examine the compost effect as the fertilizer with chemical fertilizer and

general compost, three types of fertilizer was sprinkled to grass by for each 1000 kg/ha. To achieve a fertilizer effect, surrounded to lime in addition to all type fertilizers. The effect was judged based on weight of the grass after a crop.

Fertilize the compost on rice and culinary plants

The sompost (10,000 kg/ha) was sprinkled on arable lands for 19 crops (rice , maize , strawberries , green beans , onions , squash , garlic, cabbage, eggplant, oats, broad beans, potato, peanut, gourd, watermelon, dahlia and zucchini). I performed questionary survey about the effect.

Results and Discussion

Experiment 1: Safe evaluation of the aerobic ultra high temperature fermented compost in mice

Subchronic toxicity: The ICR mice orally treated by the compost solution for 29weeks. The general symptoms and body weight changes were observed for 29 week. No deaths and no toxicological changes associated with the dosage of the compost were observed during the tested periods. And no abnormal findings in the gross examination were determined. No toxicities related with the dosage of the compost were observed in general symptom, body weight gains (Fig.3-a) food intake (Fig. 3-b), water intake (Fig. 3-c), blood chemistry. And in the pathological studies (necropsy and histopathological observation), toxicological changes caused by compost were not observed.

Contact toxicity experiment: Female ICR mice were fed in the plastic cage bedded with paper clean as a control group and three shapes of compost for two weeks. The general symptoms and body weight changes were observed for 2 week (Fig.3-d). No deaths and no toxicological changes associated were observed during the tested periods. And no abnormal findings in the gross examination were determined. No toxicities related with the dosage of the compost were observed in general symptom, body weight gains, food intake and water intake.

From these results, the oral administrations of the aerobic ultra high temperature fermented compost at least did not show subchronic toxicities in mice, and substances are safe.

Experiment 2: the feasibility of the cattle bedding test

The coliform bacteria, was not confirmed from the compost and but it was detected by sawdust 2.8×10^4 cfu/g and cattle manure (5.1×10^6 cfu/g). The number of coliform bacteria of the mixed at the day 10 significantly less than control ($p<0.05$), and even at the day 20 there was no deference was recognized (6.5×10^5 cfu/g) with the control group (3.2×10^5 cfu/g). From these results, it was revealed that compost could use for the bedding material instead of.

The structure of the bacterial community was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE). The band pattern of the mixed ward was significantly differed that of sawdust ward. Three bands appeared more clearly than sawdust ward. From the results in Chapter 2, expected that it is each *Acidimicrobiaceae bacterium, Planifilum fimeticola, Planifilum yunnanense*. It is thought that these bacteria prevent an increase of *E. coli*.

Experiment 3: the feasibility of organic fertilizer test

The product of aerobic ultra high temperature fermentation (10,000 kg/ha) was sprinkled on arable lands for 19 crops (grass, rice, maize, strawberries, green beans, onions, squash, garlic and so on). The amounts of grass production were higher than the general compost and were equal to chemical fertilizer. The product showed high fertilizer effect on rice. Because initial growth was very better with the kidney beans than a year using the chemical fertilizer, I reduced the number of times and quantity of the additional fertilizer, but the harvest increased 30%. The growth situation was thought to be approximately equal to chemical fertilizer with the onions, but not maize only in 19 kinds.

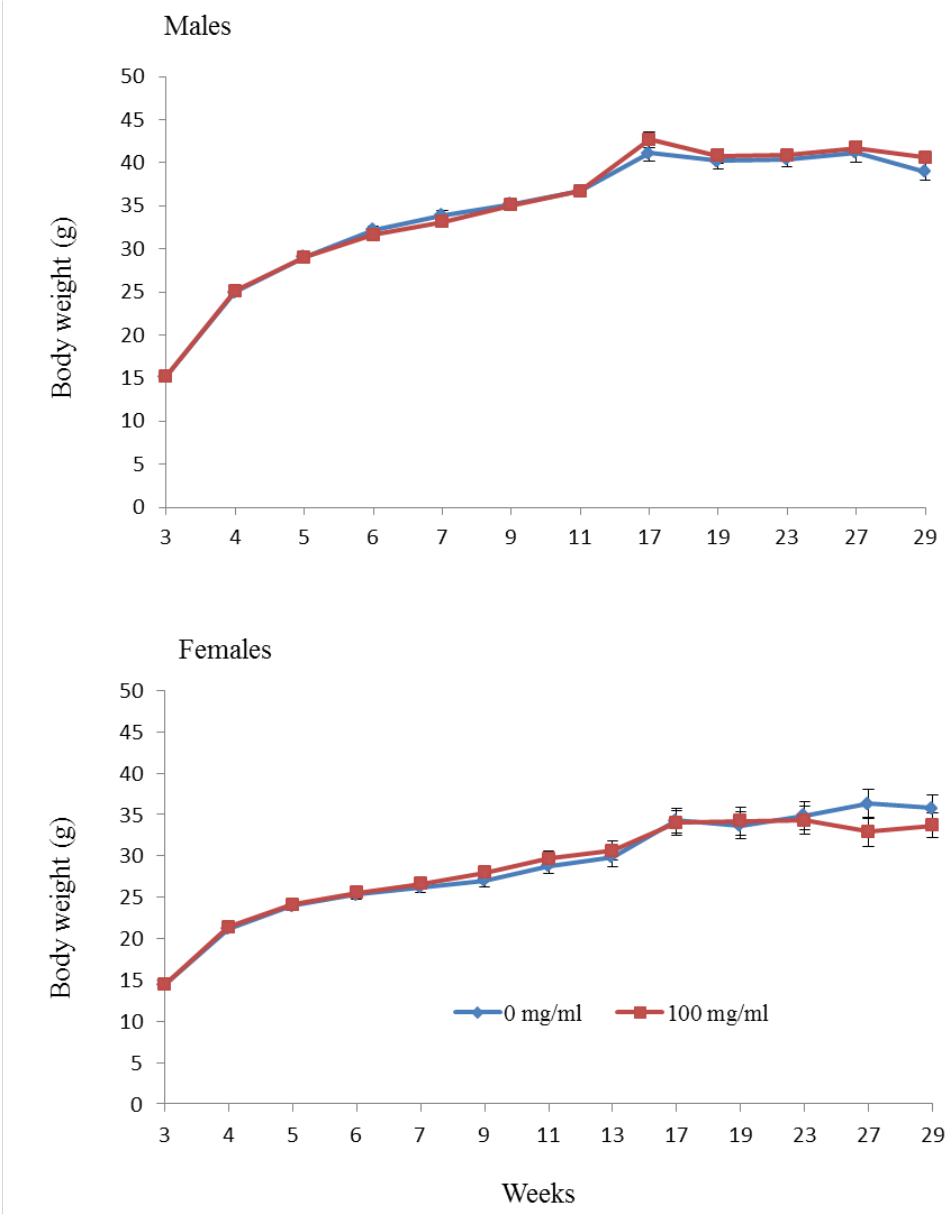


Figure. 3-a. Mean body weights transition after oral treatment by the compost. There no significant changes both of male and female.

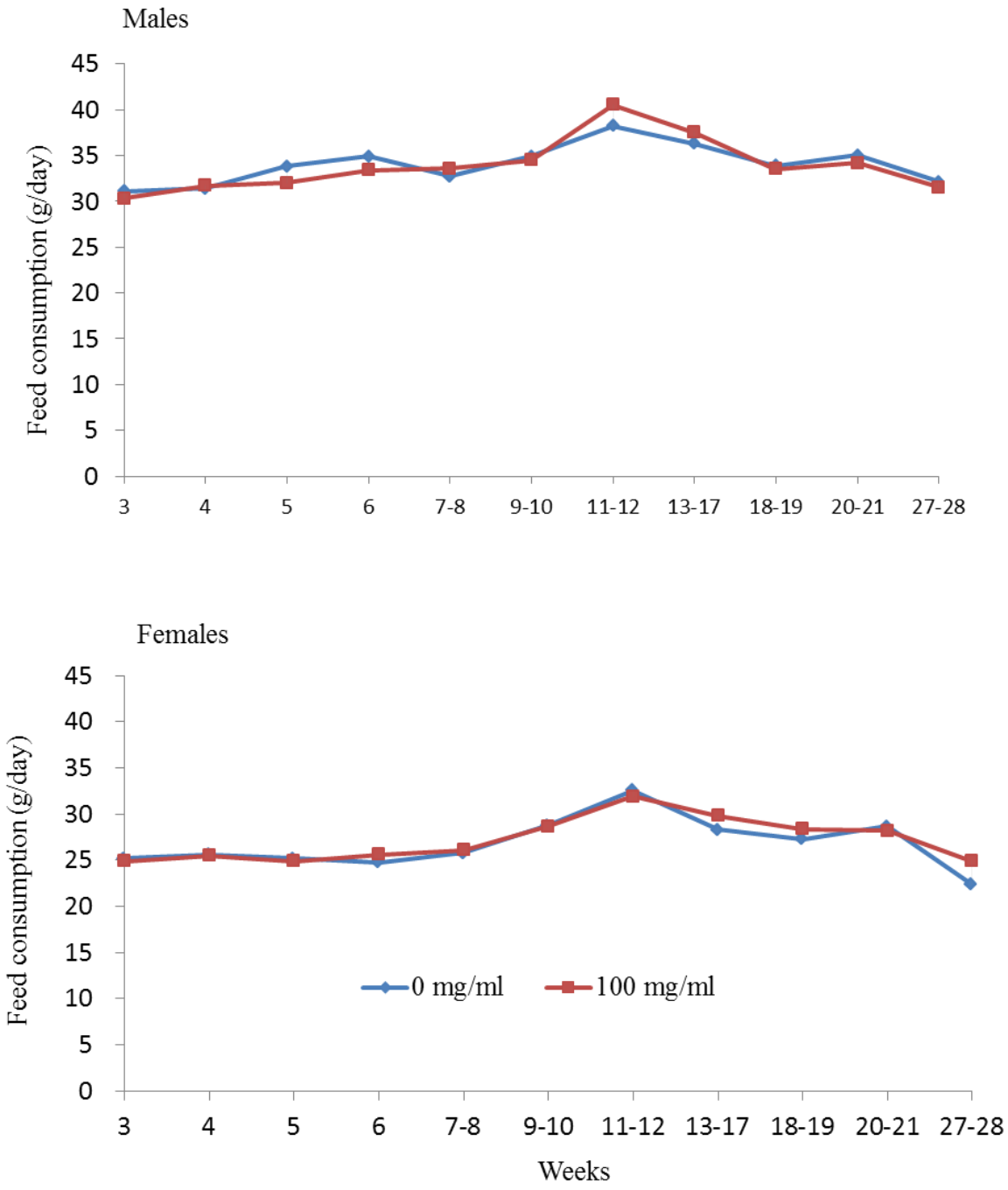


Figure. 3-b. Average transition of the feed consumption of the male and female mice treated by compost liquid. There is no significant changes both of male and female.

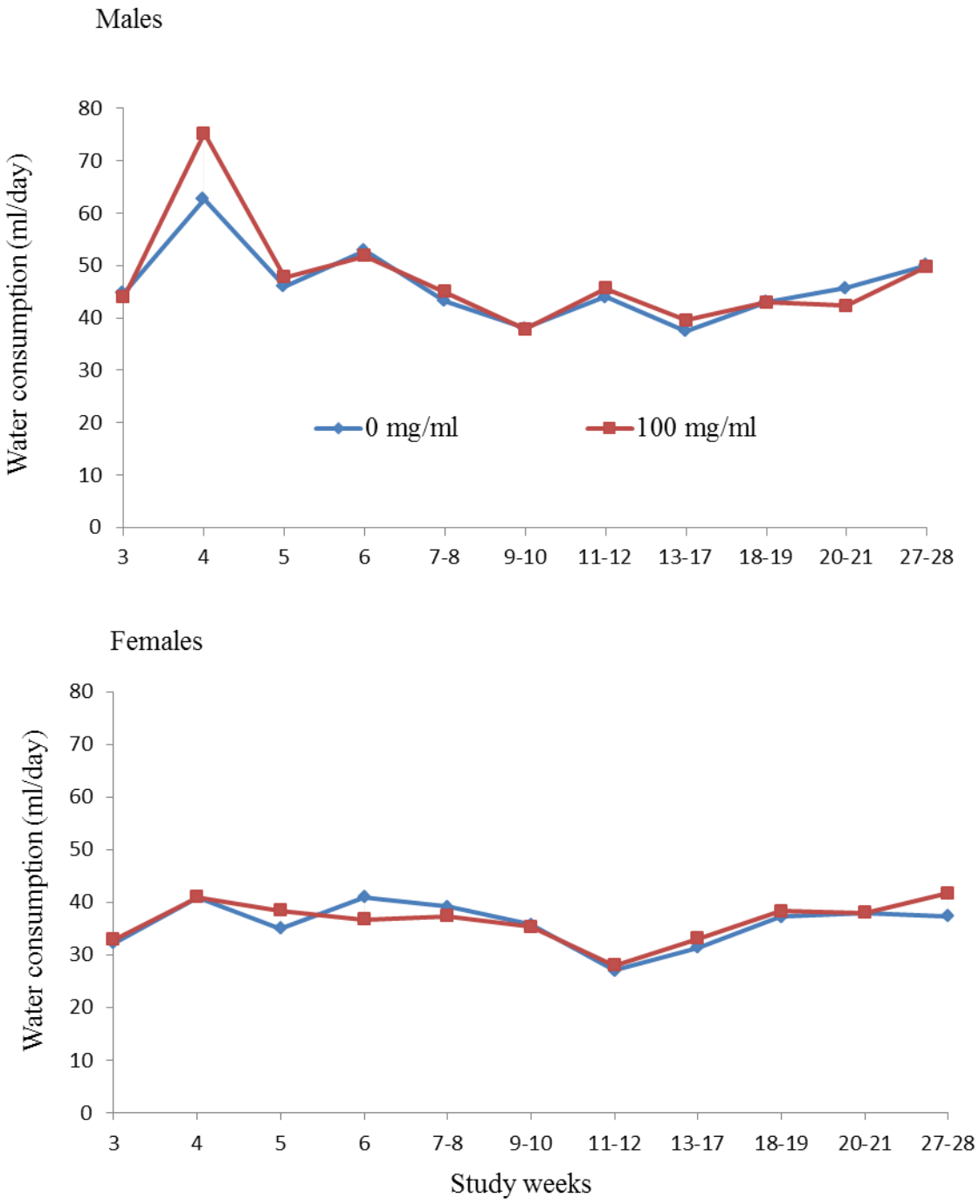


Figure. 3-c. Average transition of the water consumption after oral treatment by the compost liquid. There is no significant changes both of male and female.

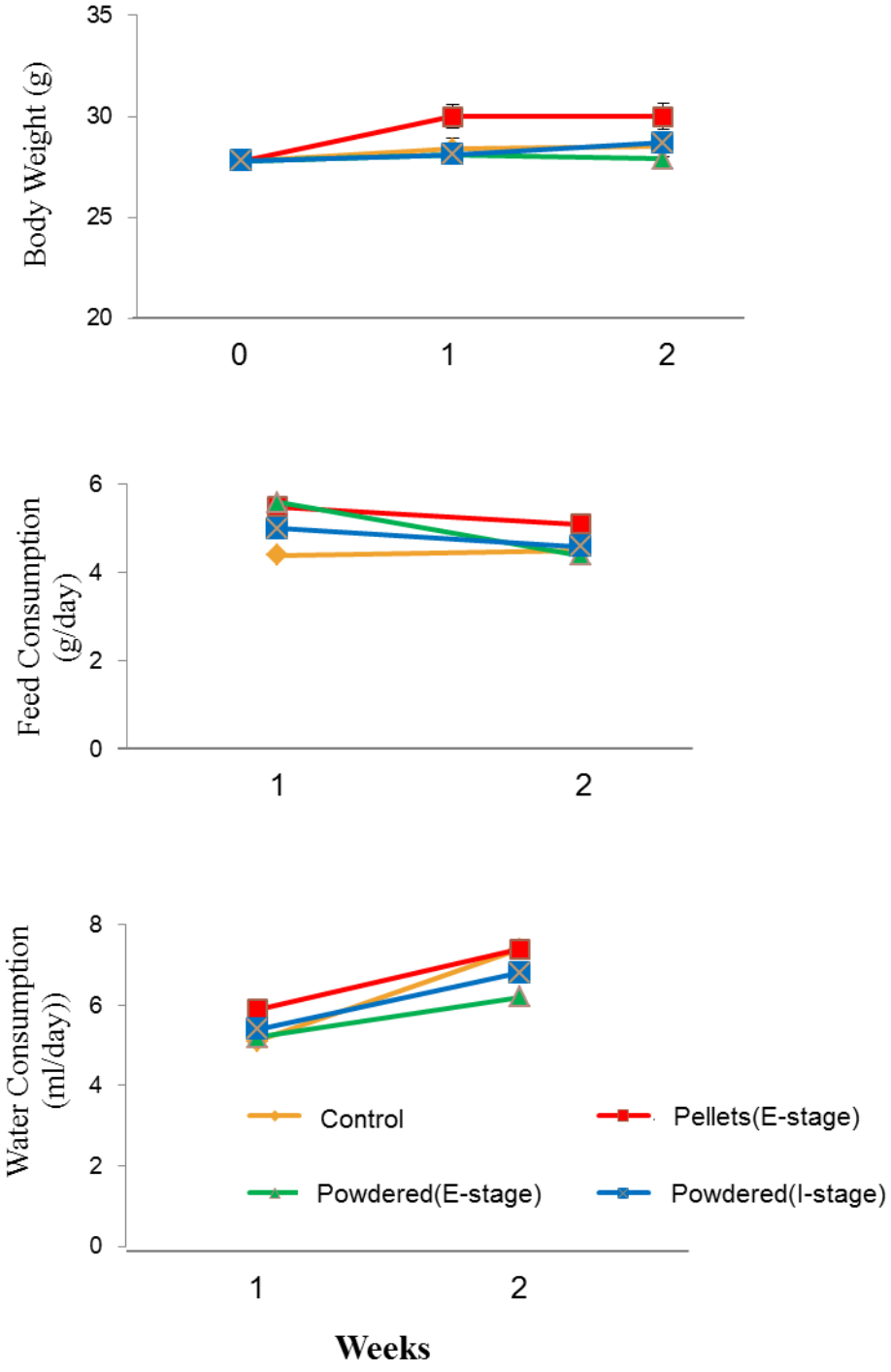


Figure. 3-d. Mean body weights, feed consumption and water consumption for female ICR mice. (Contact toxicity study)

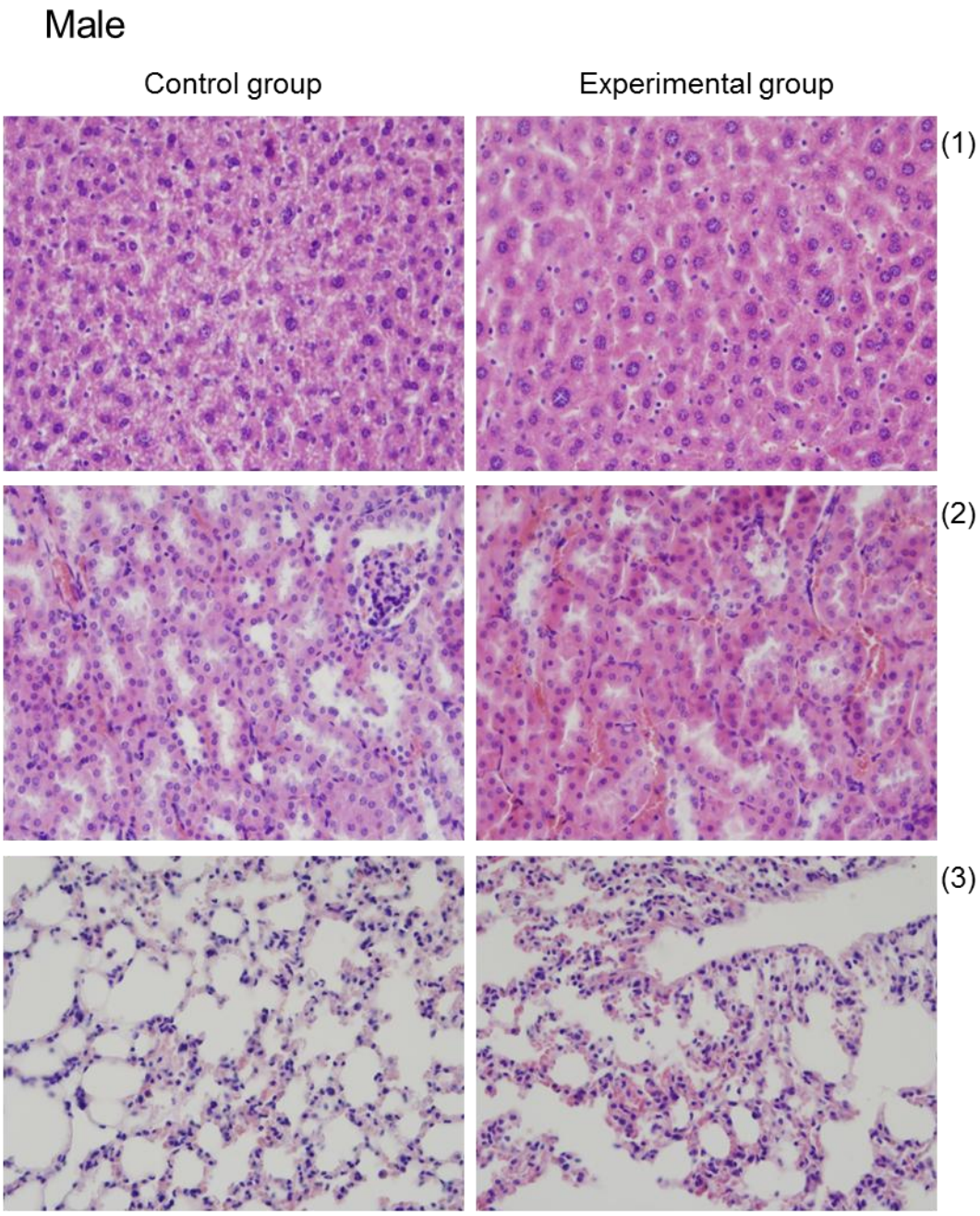


Figure. 3-d-1. Safe evaluation of the aerobic ultra high temperature fermented compost in mice (subchronic toxicity). (1) Liver; (2) kidney; (3) lung.

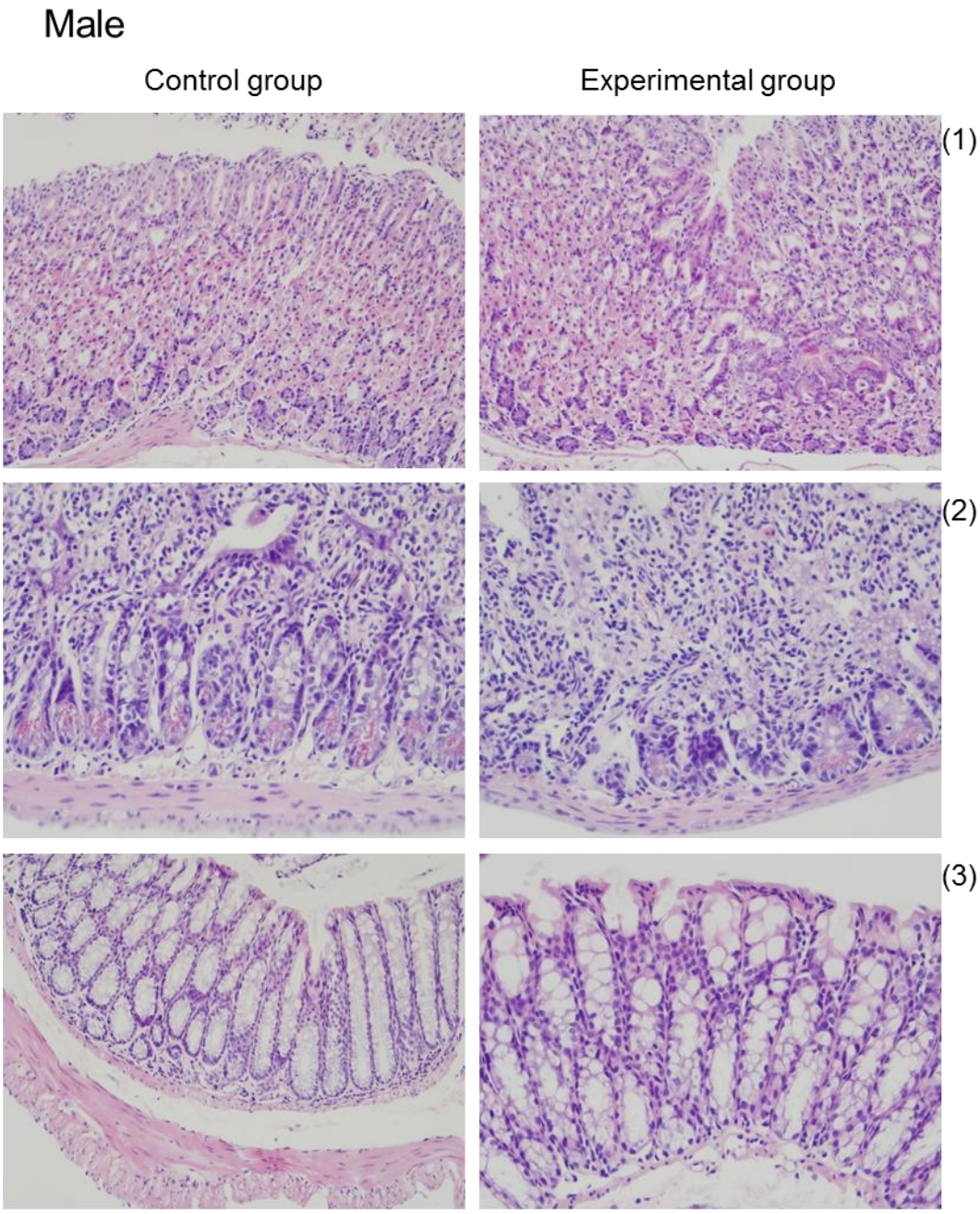


Figure. 3-d-2. Safe evaluation of the aerobic ultra high temperature fermented compost in mice (subchronic toxicity). (1) Stomachr; (2) small intestine; (3) large intestine.

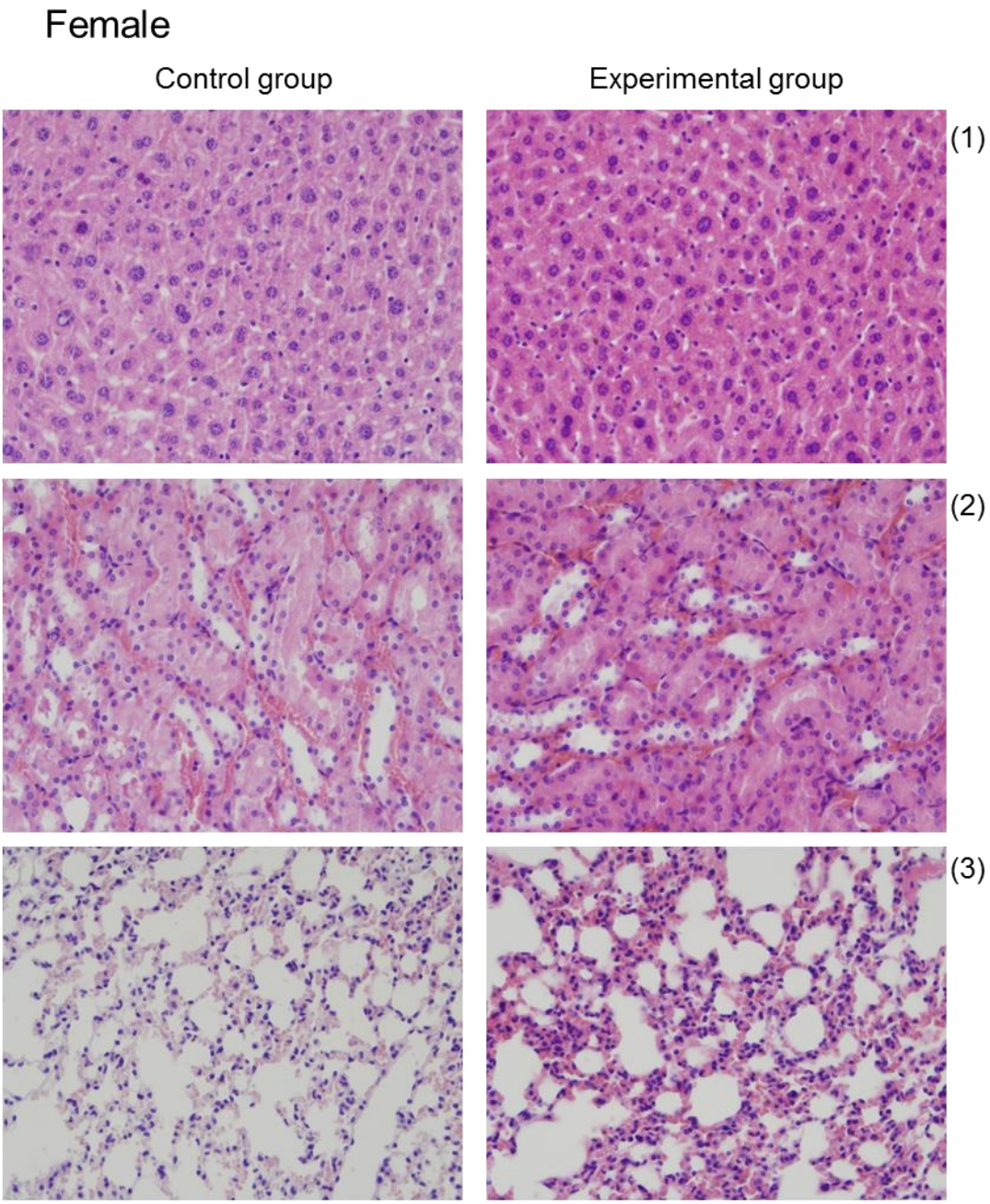


Figure. 3-d-3. Safe evaluation of the aerobic ultra high temperature fermented compost in mice (subchronic toxicity). (1) Liver; (2) kidney; (3) lung.

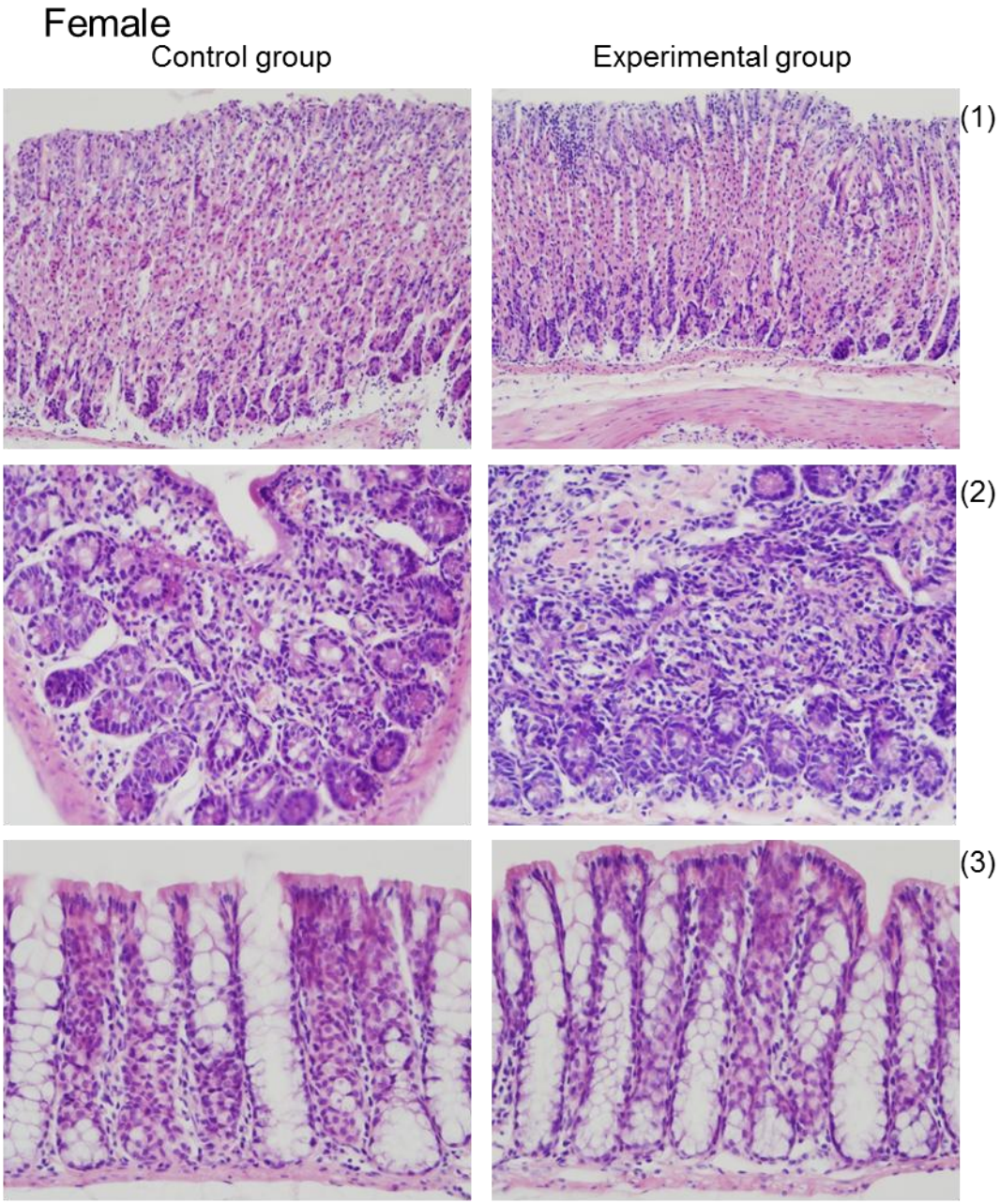


Figure. 3-d-4. Safe evaluation of the aerobic ultra high temperature fermented compost in mice (subchronic toxicity). (1) Stomachr; (2) small intestine; (3) large intestine.

Table. 3. biochemical examination of serum in mice for 29 weeks

	Female		Male	
	Control	dose group	Control	dose group
TP-PⅢ g/dl	4.7±0.4	5.1±0.4	4.8±0.6	5.2±0
ALB-P g/dl	2.6±0.2	2.8±0.2	2.5±0.3	2.7±0.1
TBIL-PⅢ mg/dl	0.5±0.2	0.5±0.1	0.5±0.1	0.5±0.1
GOT/AST-PⅢ U/l	101±94	45±10	67±18	64±30
GPT/ALT-PⅢ U/l	5.3±0.6	8±5.3	7.7±2.9	9±2
ALP-PⅢ U/l	207±75	183±48	119±37	164±39
GGT-PⅢ U/l	3.3±0.6	4±2	3.7±0.6	2±1.7
LDH-PⅢ U/l	177±48	182±73	247±75	307±67
LAP-P U/l	44±6	44±4	47±8	49±1
CPK-PⅢ U/l	35±2	30±6	34±6	35±6
AMYL-PⅢ U/l	3747±946	2971±493	4130±390	4019±253
NH3-PⅡ ug/dl	573±311	400±58	300±44	327±61
TCHO-PⅢ mg/dl	94±20	112±7	166±11	176±48
HDL-C-PⅢ mg/dl	70±21	87±9	134±12	146±25
TG-PⅢ mg/dl	128±34	92±9	73±17	97±12
UA-PⅢ mg/dl	5±0.7	2.9±0.8	3.1±1.6	3±0.3*
BUN-PⅢ mg/dl	30.3±15.9	22.9±5.1	27±4.3	31.4±3.6
CRE-PⅢ mg/dl	0.2±0.2	0.1±0.1	0.1±0.1	0.1±0
GLU-PⅢ mg/dl	256±73	224±29	200±37	225±29
Ca-PⅢ mg/dl	8.4±0.5	8.5±0.1	7.6±1	7.7±0.3
IP-PS mg/dl	11.8±1.5	12.6±1	10.2±0.8	12.1±0.8
Mg-PⅢ mg/dl	2.8±0.5	2.7±0.1	2±0.4	2.6±0.4
Na mEq/l	150±2	147±2	146±8	151±3
K mEq/l	10.1±3	12.2±1.6	7.7±1.2	10.2±2.2
Cl mEq/l	115±5	114±3	109±7	118±3

Values are means for groups of 6 mice.

* Mean significantly different from control ($p<0.05$).

The survival rates were statistically analysis by Fisher ’s exact test .

General Discussion

The amount of solid wastes produced is increasing along with economic development. After the 1940s, the intensive trend appeared in the livestock industry in Japan, and the total discharge amount of excrement was kept more than 94 million ton per year [Chino, 2000]. With the development of large-size livestock farming, the large amount of animal dung becomes a serious problem of environment pollution. The aerobic composting is an effective approach to treat animal dung and transform it to resources. Composting is an environmentally less burden technology because of its recycling capability of organic wastes discharged from industrial and municipal plants and livestock farming. However, unfortunately, a number of problems have appeared in many of these plants for example immature compost and malodorous. Application of compost in agricultural practice could potentially cause contamination of foodstuffs with pathogenic bacteria such as *E. coli* O157 [Cieslak *et al*., 1993]. The decline of the fermentation temperature produces hygienic unsafely. Recently, the purpose of the composting of domestic animal feces is kept the safety for the crops, and deaden *E. coli* other pathogen and the seeds of weeds by the craze for fermentation, and to secure hygienic safety. Thus, in the present study, I carried out the effective treatment method on the feces and urine using the aerobic ultra high temperature fermentation bacteria in The Animal Resource Science Center, Graduate School of Agricultural and Life Sciences, The University of Tokyo. This fermentation system is superior in fermentation temperature, more than 110 ℃, organic resolution speed and reduction of volume. Saiki [1978] and Zinder [1986] reported that the inside temperature reached up to 75-80 ℃ in conventional aerpbic composting systems. In comparison with such conventional systems, novel aerobic ultra high temperature fermentation system maintained the inside temperature of the compost more than 110 ℃ (ultra high temperature) during more than

one month. This aerobic ultra high temperature fermentation system significantly hastened the fermentation speed. In conventional aerobic fermentation system, fermentation period is more than three months, but only four- to six-week-fermentation period is need in novel aerobic ultra high temperature fermentation system. Moreover, the volume of the organic wastes reduces less than 1/10 in novel aerobic ultra high temperature fermentation system.

In Chapter 1, to examine the most suitable condition of the aerobic ultra high temperature fermentation, I firstly examined spatial characteristics of aerobic ultra high temperature fermentation process, and physicochemical change in the fermentation process. In Chapter 2, changes in the structure of bacterial flora communities during aerobic ultra high temperature fermentation process was assessed by PCR-DGGE techniques. In Chapter 3, I assessed the safety of the ultra high temperature compost. Then, the usefulness of ultra high temperature compost as a cattle-bedding and fertilizer for farm were assessed.

In Chapter 1, I examined physical and chemical characteristics in aerobic ultra high temperature fermentation process. The temperature and the moisture rate became heterogeneous spatially in the fermentation process because of influence of the aeration pipe. The highest temperature (117 ℃) was recorded about 100 cm from the surface of the compost. The temperature at 10 cm above the floor and at 10 cm below the face of compost was more than 70 ℃, suggesting that the waste pile is a good thermal insulator and little heat can escape from the inside of the waste pile. I examined the influence of fermentation temperature and decomposition of organic matter in aeration, to determine the optimal conditions for aerobic ultra high temperature fermentation. The fermentation temperature was affected the aeration rate. To maintain high temperature

(more than 110 °C, maximum 117 °C) appropriate aeration rate is essential. The aerobic condition was not kept in low aeration rate. The composts near the air-pipe were dried in high aeration rate. As for the evaporation of the water and organic matter resolution, to regulate appropriate aeration rates was key point to keep the fermentation. I found that fermentation temperature and the fermentation situation change by adjustment of the air slightly. I found that fermentation process of zero aeration rates, the temperature was below 60 °C during the experiment, most of the water does not fly, almost stopped fermentation, I considered to anaerobic microbial process.

In Chapter 2, I investigated the structure of bacterial flora communities in aerobic high temperature fermentation processes and evaluated the bacterial succession during the composting processes. The structure of the bacterial community was analyzed by PCR-DGGE technique and genomic sequencing. Based on phylogenetic analysis, the dominant bacteria belonged to 17 kind, six phyla (*Actinobacteria*, *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Chloroflexi* and *Deinococcus-Thermus*) were identified. These dominant bacteria may play crucial role to keep high temperature during the composting processes. Especially, six bacteria (*Acidimicrobiaceae bacterium*, *Planifilum fimeticola*, *Planifilum yunnanense*, *Thermaerobacter composti* and *Thermus thermophiles*) are considered to be important for high temperature fermentation. Among six dominant bacteria, I make hypothesis that *Thermus thermophilus* is key bacterium and is connected with the ultra high temperature fermentation process.

However, in the bacterial community were detected the new bacteria by the ultra high temperature. Adversely, the DGGE band was not found from some samples of fermentation temperature more than 100 °C. Bacteria may be present throughout the composting process as active or dormant cells, or as spores. Only their numbers and

level of activity change during the composting process [Gentleman *et al*., 2004]. The band did not appear in DGGE on sample of the fermentation at low aeration, but can found that fermentation advanced. Therefore, it is thought that the bacteria work on the resolution again when fermentation temperature falls. I thought that *Thermus thermophilus* is connected with the ultra high temperature decreases by the fermentation process. Because, when *Thermus thermophilus* band appears in DGGE gel only, fermentation process can maintain the high temperature. I found that the fermentation not to provide air, sequences are grouped to either class *Bacteroidetes* of class *Firmicutes* and that most sequences belong to the members of class *Clostridia*. In fact, the bacteria grouped in class *Clostridia* are often found in cow rumen fluid [Whitford *et al*., 1998] and cow manure [Ozutsumi *et al*., 2005]. It was proved that is anaerobic process. I conclude it when process can not contributed to establish ecosystem to easily use oxygen for from the early days to the last time of the composting by only mixing the materials every 7 days. Aerobic ultra high temperature fermentation of pig manure, the best fermentation temperature reached 105 ℃ and maintained for 40 days more than 80 ℃. It showed that aerobic ultra high temperature fermentation with pig feces was also possible. The sequences are grouped to either class *Bacillales* and *Actinomycetales*. That most sequences belong to the members of class *Bacillales*. It thought that four members are of inoculum origin. The fermentation of sludge, all samples was mesophilic (28-49 ℃) state. The sequences are grouped to either class *Bacillales* and *Actinomycetales*. That most sequences belong to the members of class *Bacillales*. It could not detect any high temperature bacteria such as members of class *Thermus*. The DGGE pattern of sample of the sludge fermentation process was significantly different from those of the mix manure and pig manure process. But, *Planifilum fimeticola* and

Planifilum yunnanense appear by fermentation of this type well. As for the two bacteria, thought that it may be saved to ultra high temperature fermentation bacteria such as *Thermus thermophilus.*

In Chapter 3, I assessed the safety of the end-product of compost. The toxicity of high temperature compost was evaluated by subchronic toxicity studies and contact toxicity study. In subchronic oral toxicity study and contact toxicity study, No treatment-related changes in body weight, food consumption, water consumption, blood biochemistry data or gross pathology or histopathology was found in any group. Thus, under the experimental conditions used, ultra high temperature compost has no toxicological effect on mice. Then, to assess the feasibility of the product of ultra high temperature fermentation, the usefulness for litters for cattle feedlot and fertilizer for agricultural products were examined. In cattle pens, the product of ultra high temperature fermentation was mixed cornstalks was used as litter. *E. coli* levels in the mixed litter rapidly decreased, suggesting the effectiveness of disinfection. The product of ultra high temperature fermentation (10,000 kg/ha) was sprinkled on arable lands for 19 crops (grass, rice, maize, strawberries, green beans, onions, squash, garlic and so on). The product showed high fertilizer effect on grass, rice and green beans, but not maize, suggesting the usefulness as fertilizer on crops.

Based on the description above, the aerobic ultra high temperature fermentation method is very advanced system. This method has been demonstrated not only reduce animal waste to 10% and malodorous but also kill disease-causing germs like *E. coli*, other pathogen and the seeds of weed. The product of this method is a good organic fertilizer to increase the agricultural yield and is effective in the treatment of the land.

References

Agnew J.M, Leonard J.J, Feddes J, Feng Y. Amodified air pycnometer for compost air volume and density determination. 2003; Canadian biosystems engineering. 2003; 45: 627-635.

Beeffa T, Blanc M, Lyou PF, Vogt G, Marchiani M, Fischer JL, Aragno M. Isolation of thermua strains from hot composts. Appl. Environ Microbiol. 1996; 62(5): 1723-1727.

Bernal M, Alburquerque J, Moral R. Composting of animal manures and chemical criteria for compost maturity assessment. A review. Bioresource Technology. 2009; 100: 5444–5453

Bernal S, Mazuelos E, Garcia M, Aller A, Martinez M, Gutierrez M. Evaluation of CHROMagar Candida medium for the isolation and presumptive identification of species of Candida of clinical importance. Diagnostic Microbiology and Infectious Disease.1996; 24: 201-204.

Bonitoa G, Omoanghe S. Isikhuemhenb, Vilgalysa R. Identification of fungi associated with municipal compost using DNA-based techniques. Bioresource Technology. 2010; 101(3): 1021-1027.

Brouwer R, Langford L.H, Bateman LI, Turner R.k. A meta-analysis of wetland contingent valuation studies. Regional Environmental Change. 1999; 1(1): 47-57

Burton C, Turner C. Manure management: treatment strategies for sustainable

agriculture. 2nd Edition. Silsoe Research Institute, Wrest Park, Silsoe, Bedford, UK. 2nd Edition. 2003.

Carlyle R.E., Norman A.G. Ther mogenesis in the decomposition of plant material. Parr Ⅱ. Factors in volved. 1941; 41: 699-724.

Chapman P, Siddons C, Manning J, Cheetham C. An outbreak of infection due to verocytotoxin-producing *Escherichia coli* O157 in four families: the influence of laboratory methods on the outcome of the investigation. Epidemiol Infect; 1997; 119: 113-119

Chen Y, Inbar Y. Chemical and spectroscopic analyses of organic matter transformations during composting in relation to compost maturity. In: H.A.J. Hoitink and H.M. Keener (eds.) Science and Engineering of Composting: Design, Environmental, Microbiology and Utilization Aspects. Renaissance Publications, Worthington, OH, U.S.A.1993; 551-600.

Chino M. The present conditions and problem of the biological waste recycling. Livestock Industry's Environmental Information. 2000; 9: 2-8

Cieslak, P. R., Barrett, T. J., Griffin, P. M., Gensheimer, K. F., Beckett, G.,Buffington, J. and Smith, M. G.: Escherichia coli O157:H7 infection from a manured garden. The Lancet, 1993; 342, 367

Coia J. Clincal, microbiological and epidemiological aspects of *Escherichia coli* O157 infection. FEMS Immunology and Medical Microbiology. 1998; 20: 1-9

Das K, Keener H.M. Process control based on dynamic propemes in composting: Moisture and compaction considerations. European Commission Int. Conf. "The science of composting", May 30-June 2, 1995; Bologna, Italy.

Dees PM, Ghiorse WC. Microbial diversity in hot synthetic compost as revealed by PCR-amplified rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiology Ecology. 2001; 35: 207–216.

Deportes I, Benoit-Guyod J, Zmirou D. Hazard to man and the environment posed by the use of urban waste compost: a review. The Science of the Total Environment. 1995; 172: 197-222

de Bertoldi M, Vallini G, Pera A. The biology of composting: A review. Waste Manage. 1983; 1: 157–176.

Finelli L, Crayne E, Pilot K, Spitalny K. Enhanced detection of sporadic *Escherichia coli* O157:H7 infections-New Jersey 1994. Morbidity and Mortality Weekly Report. 1995; 44: 417-418

Finstein M, Morris M. Microbiology of municipal solid waste composting. Adv. Appl. Microbiol. 1975; 19: 113–151.

Gajalakshmi S, Abbasi S. Solid waste management by composting: state of the art. Critical Reviews in Environmental Science and Technology. 2008; 38: 311-400.

Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, Ellis B, Gautier L, Ge Y, Gentry J, Hornik K, Hothorn T, Huber W, Iacus S, Irizarry R, Leisch F, Li C, Maechler M, Rossini AJ, Sawitzki G, Smith C, Smyth G, Tierney L, Yang JY, Zhang J. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004; 5(10): R80.

Golueke C. Biological reclamation of solid wastes. Rodale Press. Emmaus. 1976;

Golueke C. Effect on life style from governmental and free enterprise cooperation. Water Air and Soil Pollution. 1975; 4: 303-309

Hall J E. Nutrient recycling: the European experience. Asian-Aus. J. Anim. Sci. 1999; 12: 667-674.

Harada, Y. A quality standard of the domestic animal feces and urine compost and the judgment method and the problems that were left: organic processing, circulation, use system compost center as an axis. General agriculture study library. 1985; 7: 141-163

Honta K. House of livestock manure composting process. Animal husbandry. 1999; 53: 193-198

Harada, Y. Quality of compost produced from animal wastes. JARQ. 26, 238 -246 (1993)

Itoh Y, Sugita-Konishi Y, Kasuga F, Iwaki M, Hara-Kudo Y, Saito N, Noguchi Y, Konuma H, Kumagai S. Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. Applied and Environmental Microbiology. 1998; 64; 1532-1535

Jakobsen S. Aerobic decomposition of organic wastes 2. Value of compost as a fertilizer. Resources, Conservation and Recycling.1995; 13: 57-71.

Keener H, Elwell D, Monnin M. Procedures and equations for sizing of structures and windrows for composting animal mortalities. Applied Engineering in Agriculture. 2000; 16(6): 681-692.

Kourkia P, Mylonakis T, Flanigan. Enterohemorrhagic *E.coli* O157:H7 an emerging pathogen. Family Physician. 1997; 56: 853-856

Larney F, Hao X. A review of composting as a management alternative for beef cattle feedlot manure in southern Alberta. Bioresource technology. 2007; 98: 3221-3227.

Little C, Roberts D, Youngs E, Louvois J. Microbiological quality of retail imported unprepared whole lettuces: a PHLS food working group study. Journal of Food Protection. 1999; 62: 325-328

Ludwick A.E, Johnston A.M. Organic nutrients. Better Crops. 2002; 86(2): 8-9

Matsuzaki T. An application technology to farm products of the domestic animal feces and urine. The study of the stock raising. 1976; 30: 205-210

Miller FC. Composting of municipal solid waste and its components. In Microbiology of solid waste. Edited by Palmisano, A.C., and Barlaz, N.A. CRC Press, Boca Raton, Fla. 1996; 115–154.

Miller FC, MacGregor ST, Psarianos KM. The Rutgers strategy for compositng: process design and control. Acta Horticulturae. 1992: 75-85

Nakasaki. New operation for production. disease-suppressive compost from grass clippings, Appl. Emviron. Microbiol. 1998; 64(10): 4015-4020

Norman A, Richards L, Carlyle R. Microbial thermogenesis in the decomposition of plant materials. Part1. An adiabatic fermentation apparatus. J. Bacteriol. 1941; 41(6): 689-697

Oenema O. Governmental policies and measures regulating nitrogen and phosphorus from animal manure in European agriculture. Journal of Animal Science. 2004; 82: 196-206

Oshima T, Imahori K. Desription of Thermus Thermophilus comb. Now., a

nonsporulating thermophilic bacterium form Japanese thermal spa. Int J syst bacterial. 1974; 24: 102-112

Ozutsumi Y, Tajima K, Takenaka A, Itabashi H. The effect of protozoa on the composition of rumen bacteria in cattle using 16S rRNA gene clone libraries. Bioscience, biotechnology, and biochemistry. 2005; 69(3):499-506

Parr J. Chemical and biological considerations for land application of agricultural and municipal wastes, organic materials as fertilizer. Soils bulletin. 1975; 27: 227-252

Pedro MS, Haruta S, Hazaka M, Shimada R, Yoshida C, Hiura K, Ishii M, Igarashi Y. Denaturing gradient gel electrophoresis analyses of microbial community from field-scale composter. J. Biosci. Bioeng. 2001; 91: 159-165.

Peter MD, William CG. Microbial diversity in hot synthetic compost as revealed by PCR-ampli¢ed rRNA sequences from cultivated isolates and extracted DNA. FEMS Microbiology Ecology. 2001; 35: 207-216

Romantschuk M, Sarand I, Petanen T, Peltola R, Jonsson-Vihanne M, Koivula T, Yrjala K, Haahtela K. Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. Environ Poll. 2000; 107: 179-185

Saiki T, Beppu T, Arima K. Changes in microbial flora, including thermophiles, during composting of animal manure. Biochemistry of Thermophily. S.M.Friedman, Academic

press. New York, NY. 1978; 103-105.

Schloss PD, Handelsman J. Biotechnological prospects from metagenomics. Curr. Opin. Biotechnol. 2003; 14: 303-310.

Sciancalepore V, Colangelo M, Sorlini C, Ranalli G. Composting of effluent from a new two-phase centrifuge olive mill. Microbial characterization of the compost.1996: Tosicol Environ. Chen. 1996; 55(1-4): 145-148

Sefano G.de, Piacquadio, Sciancalepore R. Compostaggio della sansa di olive dei nuovi impanti a blue fasi. Rifiuti solidi. 1994

Shimura M, Tabuchi T. Relations of the nitrogen density of processing method and river of the stock raising feces and urine. Journal of water and waste. 1997; 39(5): 21-26

Soares H, Cardenas B, Weir D, Switzenbaum M. Evaluating pathogen regrowth in biosolids compost. Biocycle. 1995; 36: 70-74

Stentiford E.I. Composting control: principles and practice. In: de Bertoldi M, Sequi P. Lemmes B, Papi T. [eds] the sience of composting. Blackie academic and professional, London: 1996:49-59

Taiganides E. Composting of feedlot wastes. In: Animal wastes (Ed. E. P. Taiganides). Applied Science Publishers Ltd., Essex, England. 1977: 241-252.

Umeda I, Ono H, Watanabe K, Taguchi K, Oshio S, Nakanishi T, Ito M, Takahara Y, Yasukata K, Asai H, Wakazono S, Mizuno T. Effect of insulation effect and the oils and fats addition of the compost fermenter. Bulletin of the Gifu Prefectural Livestock Research Institute. 1999; 25: 25-32.

Watabe C, Nunofuji M, Naito S, Tani Y, Fujita K. the ups and downs of Escherichia coli and Property of separation bacteria in a composting process of the cow dung. Bulletin of the Shiga Prefectural Agricultural Research Center, Livestock Research and Improvement Institute. 1998; 5: 27-30

Whitford MF, Forster RJ, Beard CE, Gong J, Teather RM. Phylogenetic analysis of rumen bacteria by comparative sequence analysis of cloned 16S rRNA genes. Anaerobe. 1998 Jun; 4(3): 153-63.

Zender CS, Mangalam HJ. Scaling properties of common statistical operators for gridded datasets, Int. J. High Perform. Comput. 2007; 21(4): 485–498

Zinder S. Thermophilic waste treatment systems. Thermophiles: General, Molecular, and Applied Microbiology. T.D. Brock, Wiley, New York. 1986; 257-277

Zucconi F, de Bertoldi M. Compost specifications for the production and characterization of compost from municipal solid wastes. Compost: Production Quality and Use, Elsevier Applied Science, London .1987; 30-50.

謝　辞

まもなく３年の留学生活は終わります．東京大学に留学できるように機会を賜り，本研究の遂行ならびに本論文の執筆にあたり，熱心なご指導，ご鞭撻を賜り，多大なご助言くださり，大変お世話になりました指導教官の東京大学大学院農学生命科学研究科眞鍋昇教授に心から厚く御礼申し上げます．研究計画から学位論文をまとめるまでの全プロセスにおいて，始終一貫暖かいご指導，激励を賜りました本研究室の李俊佑先生に心から深謝申し上げます．特に，博士論文・学会発表の作成にあたっては，お忙しいところ，貴重な時間と精力を割いて私の原稿を一字一句直していただきました．先生から教えていただいた学問に対する熱心さと真面目さを持って，今後の研究に取り組んでいかなければならないと思っています．さらに，研究者としての鋭い研究力，考察力，真理を探求する姿勢の重要性を教示いただき，日常生活に関しても大変お世話になりました．附属牧場で研究を行うにあたって多大なる御指導，ご協力を頂きました元牧場長吉川泰弘先生，塩田邦朗先生，技術職員，事務職員の皆様に深謝いたします．特に堆肥の研究にあたり毎週のサンプルの採取にご協力いただき，研究や生活の面でも大変お世話になりました高橋友継さんと入江猛さんに心より感謝致します．

堆肥の研究・実験の遂行上，ご協力いただきました株式会社山有の山村正一社長，共和化工株式会社の吉村俊治社長に感謝いたします．特に共和化工株式会社環境微生物研究所の大島泰郎先生にはご専門の立場から研究の進め方など多岐に渡り貴重なご意見を賜り心より厚く御礼申し上げます．森屋利幸博士，吉井貴宏博士には本研究の軸となりました微生物菌叢解析方法などの実験手技だけでなく，実験に関する悩みにも快く応じて下さり，大変お世話になりました．山火理紗さんにはお忙しいところサンプルの測定にご協力いただきました。ありがとうございました．

実験操作などを教えてくださった金花子先輩，Kannika WONGPANIT先輩，後藤康文先輩，程園先輩，中川奨麻先輩，小野山一郎氏及び高橋創氏に大変感謝しております．私とともに学生生活を供にした Kabir.A.K.M氏，高紅梅氏，東福望氏に感謝いたします．さらに，日本に滞在中，様々な形で便宜を図ってくださった全ての方々に心から感謝の意を表します．

最後に，３年間の留学生生活に対して，惜しみない援助と激励および心の支えを与えてくれました両親と妹及び親友たちに深く感謝申し上げます．