THESIS

BIOLOGICAL ASSESSMENT ON SUITABILITY OF COMPOSTS DERIVED FROM BANGKOK CITY SEWAGE SLUDGE FOR AGRICULTURAL PRODUCTION

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THESIS

BIOLOGICAL ASSESSMENT ON SUITABILITY OF COMPOSTS DERIVED FROM BANGKOK CITY SEWAGE SLUDGE FOR AGRICULTURAL PRODUCTION

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A Thesis Submitted in Partial Fulfillment of
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In order to find a sustainable method of disposal of sewage sludge, a study had investigated the suitability of the product for agricultural use. Monthly sampling and analysis of sewage sludge from three wastewater treatment plants in Bangkok Siphraya, Rattanakosin and Chongnonsi were carried out. Plant nutrient contents were high (i.e. total N from 19 to 38 g kg^{-1}) but the organic matter content level was low. Heavy metal levels varied, depending on which plants the sample were taken from and were sometimes higher than the EU or US regulations for sewage sludge use in agriculture. Faecal coliforms were found to be present in the sludge. However, the C/N ratio of sewage sludge was low. Two organic by-products (wood chips and rice husk) with a high level of carbon content needs to be added. Wood chips and rice husk did not exhibit any significant differences concerning decomposition after a period of 63 days. Under experimental conditions, the use of an activator within the sludge mixtures did not improve the decomposition of organic matter contained in the mixtures of sewage sludge and rice husk or sewage sludge and wood chips.

The growth and the number of earthworms (*Eudrilus euginiae*) during vermicomposting were increased. Total organic carbon levels showed a marked decrease during the vermicomposting. The C/N ratio decreased from 16 in the raw mixture to 14 in the end product. The recorded levels of faecal coliforms decreased from 5.71 to 3.96 log Most Probable Number per gram during the process.

Nuanjun Pasda — Student's signature

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Nuanjun Pasda
March 2008

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LIST OF ABBREVIATIONS

km	=	kilometre
BMA	=	Bangkok Metropolitan Administration
m	=	metre
N	=	nitrogen
P	=	phosphorus
K	=	potasium
Ca	=	calcium
Mg	=	magnesium
Fe	=	iron
Zn	=	zinc
Pb	=	lead
Cd	=	cadmium
EPA	=	Environment Protection Agency
cm	=	centimetre
Cr	=	cromium
Hg	=	mercury
MSW	=	municipal solid waste
C	=	carbon
^{o}C	=	degree celcius
CO_2	=	carbon dioxide
ATP	=	adenosine triphosphate
CEC	=	cation exchange capacity
S	=	sulphur
Ni	=	nicle
HNO_3	=	nitric acid
$HClO_4$	=	perchloric acid
AOAC	=	Association of Official Analytical Chemists
APHA	=	American Public Health Association
MPN	=	Most Probable Number

LIST OF ABBREVIATIONS (continued)

DA	=	Discriminant analysis
OC	=	organic carbon
OM	=	organic matter
g	=	gram
w	=	weight
mm	=	millimetre
ml	=	millilitre
M	=	molar
IU	=	international unit
FPase	=	filter paper cellulase
MUB	=	Modifield Universal Buffer
Min	=	minute
$CaCl_2$	=	calcium chloride
NaOH	=	sodium hydroxide
PHP	=	paranitro phenol
CMC	=	carboxyl metyl cellulose
CFU	=	colony forming unit
dw	=	dry weight
ANOVA	=	analysis of variant
DNS	=	dinitrosalicylic acid
INTF	=	iodonitrotetrazolium formazan
dS	=	desi semen
EC	=	electrical conductivity
CaO	=	Calcium oxide
MgO	=	magnesium oxide
EU	=	European Community Commission

BIOLOGICAL ASSESSMENT ON SUITABILITY OF COMPOSTS DERIVED FROM BANGKOK CITY SEWAGE SLUDGE FOR AGRICULTURAL PRODUCTION

INTRODUCTION

Bangkok is located in the low flat plain of the Chao Praya River delta. It covers more than 1 500 km^2 and at present over ten million inhabitants live and work in this area. The population growth is very prominent as the city had only 1.6 million inhabitants in 1958.

The disposal of wastewater is creating huge problems of pollution in the canals and rivers of Bangkok. The Bangkok Metropolitan Administration (BMA) has been working on a resolution to the problems of wastewater disposal. The Bangkok wastewater treatment project started in 1981. The BMA was granted a budget of more than 20,000 million baht for six wastewater treatment plants covering 192 km^2 with a total capacity of 992,000 m^3 day^{-1}. The first three plants to commence operations were located in Siphraya, Rattanakosin and Chongnonsi. These plants mainly treated wastewater from households and buildings. The Siphraya wastewater treatment plant was the first plant to started operating in 1994. The total area of wastewater collection now covers 2.7 km^2 with a capacity of 30 000 m^3 day^{-1}. The plants at Rattanakosin and Chongnonsi collect wastewater from 4.1 km^2 and 28.5 km^2 with a capacity of 40,000 and 200,000 m^3 day^{-1} respectively.

As the catchment area of wastewater collection increases, the quantity of sewage sludge produced, which is the residue generated during the treatment of domestic wastewater, will similarly increase. In 2005, it was estimated that Bangkok was producing in the region of 108 tons of sewage sludge (dry matter) per day^{-1} and it has been predicted that this figure will reach 168 tons per day^{-1} by 2010. It is more than likely that this figure will also increase in the future. It is obvious that the problems associated with the treatment and disposal of sewage sludge have the potential to become extremely serious in the future.

Current disposal strategies for the disposal of sewage sludge, such as incineration, ocean dumping and land filling are all feasible options in many parts of the world. The utilization of sewage sludge in agriculture is quite common. It can be used as an organic amendment or directly as a soil fertilizer due to its content of N, P, K, Ca, Mg and organic matter.

Composting sewage sludge is increasingly being considered by many municipalities throughout the world as a viable disposal option because of the many advantages composting has over other disposal strategies. The vermicomposting process has already been described for the stabilisation of different natural and anthropogenic wastes, such as urban and industrial sewage sludges. Vermicomposting is the digestion of organic material by earthworms which produce, as an end product, excreta known as casts.

OBJECTIVES

1. To study the physical, chemical, and biological properties of sewage sludge collected from three wastewater treatment plants in the Bangkok area, including the analyses of index of faecal contamination and heavy metals contained in the sludge.

2. To study appropriate methods for composting sewage sludge, such as the use of suitable mixing material(s), effective microorganisms, as well as monitoring the decomposition process and the analysis of heavy metals present in the compost.

3. To study changes in biological and chemical properties during the vermicomposting process.

LITERATURE REVIEW

1. The characteristics of sewage sludge

Sewage sludge is the solid material that settles out during various stages of the treatment of wastewater. Large amounts of sludge are produced by municipal wastewater treatment plants. Negulescu (1985) states that sludge can be classified into two different groups based on several criteria: the first is mineral sludge in which the suspended mineral solid content exceeds 50%; the second is organic sludge in which the suspended organic solid content exceeds 50%. The general characteristics of sludge produced from all four processes of wastewater treatment are:

The first process: Sludge produced from primary settling tanks is grey and has an objectionable odour. Paper, plastic bags and vegetable parings are in evidence. The sludge is sticky and does not drain freely.

The second process: Sludge produced in settling tanks following chemical precipitation takes its colour from the chemicals employed. Its odour is less than that from sludge coming from primary settling tanks.

The third process: Sludge produced in secondary settling tanks after a biological filter has been utilised is brown and flocculent. The sludge coming from low-rate biological filters contains many dead worms and its odour becomes quite offensive.

The fouth process: Sludge produced in secondary settling tanks after processing by activated sludge units (excess sludge) is flocculent and air-dries slowly, even when it is spread out in thin layers. If the biological treatment is efficient, the sludge is golden-brown and has an earthy odour.

Sewage sludge exhibits wide variations in physical, chemical and biological properties according to the origin, type, method of previous treatment and period of storage. Sewage sludge contains organic matter, nitrogen (in both organic and mineral forms) and phosphorus; N and P are both major plant nutrients (Sommers, 1997). In addition, sludge also contains other micronutrients such as copper, iron and zinc (Knudtsen and O'Connor, 1987). All of this provides a good indication that treated sludge might be suitable for use in agricultural production. The potential benefits of using sludge to provide plant nutrients have long been recognised. The effectiveness of sludge as a soil-improving agent depends upon the composition of the sludge, the characteristics of the soil on which it is applied and the plant species to be grown (Polprasert, 1996). However, the risk of heavy metal contamination and the presence of faecal coliform in sludge must be considered.

1.1 Nitrogen and phosphorus in sewage sludge

The chemical composition of sewage sludge is quite varied, concentrations of important chemicals range from 0.1% to 17.6% nitrogen, 0.1% to 14.3% phosphorus and 0.02% to 2.6% potassium (Sabey, 1980). Nitrogen and phosphorus are the most abundant major plant nutrients in sludge (Metcalf and Eddy, 1991). However, only a small proportion of these nutrients are in the inorganic form that can be assimilated by plants. The nitrogen in treated sludge occurs in both organic and inorganic forms. Sewage sludge is used as a good source of nitrogen as well as containing a relatively high phosphorus content. Approximately 70% of phosphorus is in the organic form and can be gradually released to plants as it undergoes mineralisation. This gradual release of phosphorus is efficiently utilised by crops. In addition, sludge also contains other nutrients such as micronutrients (Cu, Fe, Zn) and organic matter. The overall composition of sewage sludge is a very good indication of its suitability for use in agricultural production.

1.2 Heavy metals in sewage sludge

Heavy metals are concentrated in the primary sludge and in the biomass of activated sludge. One major constraint on land application of sewage sludge for soil amendment is the potential heavy metal accumulation in crops, which in turn will transfer up the food chain posing a potential health hazard to humans (Sims and Kline, 1991). Zinc, Cu, Pb and Cd are generally considered to be the metals of greatest concern. The presence of Zn, Cu and Pb is important because they can all be phytotoxic. Concern for Cd arises from its possible entry into the food chain; kidney disorders are the first biochemical signs of Cd toxicity in humans. If these metals move too rapidly in a particular soil they can pollute ground water supplies, especially in areas with a high ground water level.

The heavy metals in sludge can be removed in various ways, such as by mixing with agrowaste, specific microorganisms and anaerobically digested sludge. However, the presence of chemical contaminants in the sludge has necessitated the development of rules and guidelines controlling both the quantity and quality of sludge that can be applied (e.g., CEC, 1986; NZ DoH, 1992; U.S. EPA, 1993).

1.3 Pathogens in sewage sludge

Sewage sludge may contain pathogens; this is one of the limitations of sludge use in agriculture. There are three kinds of microorganisms in sewage that are of concern for their effects on human health: bacteria, viruses and parasites.

The U.S. Environment Protection Agency (EPA) also classifies sludge quality based on indicator bacteria. The Class A indicator standard is less than 1,000 feacal coliforms per gram of dry sludge solids and the Class B indicator standard is less than 3 million feacal coliforms per gram of dry sludge solid (Walsh, 1995). Therefore, it is apparent that class B sludge poses greater health risks to humans.

Sludge should be treated to kill the pathogens before using it in agriculture. Conventional methods used for this purpose are pasteurisation, composting and lime treatment. Suss, 1997, mentioned that digested sludge may still contain some pathogens, for example, *Salmonella*, which can survive for long periods outside of their native environment.

Sludge-borne bacteria and viruses from land application systems are not a serious threat to health because these pathogens are poor competitors outside of the host (Pahren *et al.,* 1979; Elliott and Ellis, 1977). The survival of these organisms in the soil depends on a number of environmental factors. Temperature, sunlight, moisture, the availability of organic matter, soil pH, soil particles, the presence of toxic substances and other competitive organisms all have an influence on bacteria and virus survival in soil and sewage sludge (Dunigan and Dick, 1980; Pahren *et al.,*1979; Hurst *et al.,*1978; Elliott and Ellis, 1977).

Poliovirus 1 was isolated by Tierney *et al.* (1977) from soil in the winter; 96 days after the land had been flooded to a depth of 2.5 cm with Poliovirus 1-inoculated sewage waste. These viruses survived only eleven days when applied using the same method during the summer. Van Donsel and Larkin (1977) concluded that the high surface soil temperature caused the death of the virus and *Mycobacterium bovis* BCG. Sunlight is extremely bactericidal (Elliott and Ellis, 1977). Bacteria and viruses are very susceptible to desiccation and, therefore, soil moisture is important to the survival of these organisms. No enteroviruses were detected after land-applied sewage sludge had dried in the field for three months (Hurst *et al.,* 1978).

1.4 Other characteristics

Sewage sludge is generally not well stabilised and has offensive odours. The moisture content in sewage sludge is usually high. Si phraya sludge has a moisture content of around 80% (JICA, 1999).

2. Utilisation of sewage sludge in agriculture

Sewage sludge is a valuable ecological resource, mostly because of its nitrogen, phosphorus and organic matter content (Bayes *et al.,* 1988) and is an effective soil amendment. There are many advantages to be gained by the application of municipal sewage sludge on agricultural land, such as improving the physical properties and increasing the organic matter content of the recipient soil.

Changes in the properties of sludge amended soils vary with the characteristics of the sludge and the soil (Gupta *et al.,* 1977). In Western Europe, 40-50% of the sludge used requires prior knowledge of the interaction between sludge, soil and plants before its use on agricultural lands (Hue *et al*., 1988). The potential benefits of using sewage sludge to provide plant nutrients have long been recognised. However, the potential negative effects (e.g., the introduction of heavy metals such as Cd, Cr, Hg) on the food chain are of concern (Logan and Chaney, 1983). When sludge is applied to the land it provides major plant nutrients such as N, P, K, micro-plant nutrients such as Cu, Fe and Zn and organic matter for improving the soil structure (e.g. better aeration and water holding capacity). The effectiveness of sludge as a soil-improving agent depends very much upon the composition of the sludge, the characteristics of the soil on which it is to be applied and the plant species to be grown. The application of raw sewage sludge is known to cause a decline in plant growth despite the higher nitrogen content of raw sludge. This is believed to be a combination of ammonium toxicity, the action of phytotoxic intermediary decomposition products and dissolved oxygen deficiency in the root zone caused by enhanced microbiological activity. The nitrogen content of raw sludge varies depending on the type of treatment method employed, such as a dry weight of between 0.8 and 5% for sludge produced using the activated sludge unit process (Negulescu, 1985).

Pathogenicity is one of the major concerns when sewage sludge is applied to cropland. In Ontario, Canada, sludge must be stabilised before land application; anaerobic digestion is a popular method for sewage sludge stabilisation. However, waiting periods between sludge spreading and harvesting or grazing are still required: two to six months for grazing, twelve months for commercial sod production, and fifteen months for small fruits (Sludge and Waste Utilisation Committee, 1992). The U.S.EPA (1993) allows anaerobically digested sludge to be spread on farmland, providing that the land is withheld from food production for a period of six to sixteen months. Further treatments are also required to eliminate pathogens in order to exceed the restrictions on sludge application. Irradiation is a recognised method of eliminating pathogens in digested sludge (U.S.EPA, 1993; Ward *et al*., 1984). The potential for pathogen transition exists and this can cause public health problems if land application is done improperly. Pathogens in land applied sludge will usually die rapidly, depending on temperature, moisture and exposure to sunlight. To prevent disease transmission, sludge should not be applied to land during a year when edible crops are grown. Sludge application methods and rates should be designed to produce a product that is based on the soil characteristics, to reduce public health concerns.

3. Definition of compost

Composting is the biological process of converting organic matter, in the presence of suitable amounts of air and moisture, into a humus-like product, in which microbial activity is essential for the decomposition and bio-oxidation of organic materials (Haug, 1993; de Bertoldi *et al.,* 1983). Composting is regarded as the most usual method for recycling the organic fraction of municipal solid waste (MSW), since it provides an agricultural amendment for crop production. Composting seems to be a desirable treatment, having the capacity to reduce the volume and weight of the original material by approximately 50%, resulting in a stable product that can be beneficial to agriculture (He *et al*., 1992).

Composting can be used to eliminate pathogens from sewage sludge. For pathogen destruction, the composting process should be carried out at high temperatures (>55^{o}C) for an extended period (U.S.EPA, 1993). Composted sewage sludge also improves the physical properties of the soil and has been shown to increase seedling growth rates in several tree species. Compost, as a fertiliser, carries plant nutrients in various concentrations and functions (Thacker, 1986 ; Chaney, 1980). Much attention has been paid to micro-nutrient and trace metal concentrations in composts. The overall composting time depends on the biological cycles of the microorganisms involved. Although many different types of organism are required to decompose different materials, the necessary variety is usually naturally present and these organisms will thrive when environmental conditions are satisfactory.

4. The composting process

The time necessary to complete the composting process depends on several conditions, namely the carbon to nitrogen ratio, temperature, oxygen, moisture and pH.

4.1 Carbon and Nitrogen

Carbon is a key requirement for composting (Richard, 1960). It is energy and a source of carbon for the microorganisms that do the composting. Microorganisms break down organic matter and produce CO_2, humus and energy. Nitrogen is critical for microbial population growth, as it is a component of the proteins, nucleic acids, amino acids, enzymes and co-enzymes necessary for cell growth and functioning. If nitrogen is limited, microbial populations will remain small and it will take longer to decompose the available carbon. In contrast, if there is an excess of nitrogen, it is often lost from the system in the form of ammonia gas or other nitrogen compounds. Therefore, the carbon to nitrogen ratio (C/N) of a material is an estimate of the relative amounts of these two elements it contains. The optimum C/N ratio has been reported to be 25, but the value varies depending on the substrate (Golueke, 1991). Differing protoplasmic carbon to nitrogen ratios among microbes lead to differing ratios of degradation. Both bacteria and actinomycetes have a protoplasmic C/N ratio of 5:1, while fungi have a 10:1 ratio. Bacteria need 1-2%

nitrogen to degrade one unit of carbon, actinomycetes need 3-6% and fungi need 3-4% (Miller, 1993).

4.2 Simple substrates

Simple carbon compounds such as soluble sugars and organic acids are easily metabolised and mineralised (de Bertoldi *et al*., 1983) and so are degraded at the fastest rate by the microorganisms. In this process, mesophilic microorganisms are predominant and take only a few days.

4.3 Complex substrates

Lignocellulose is the major constituent of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle (Figuge 1). Lignocellulose, such as wood, is mainly composed of a mixture of cellulose (ca. 40%), hemicellulose (ca. 20±30%), and lignin (ca. 20±30%) (Sjostrom, 1993). Lignin is an integral constituent of cell walls and provides plant strength and resistance to microbial degradation (Argyropoulos and Menachem, 1997).

Fungi and actinomycetes play an important role in the decomposition of cellulose and lignin. They excrete enzymes which hydrolyse cellulose and lignin into monosaccharides or oligosaccharides (Gray *et al.*, 1971). Hemicellulose is also a sugar polymer; it is fairly susceptible to microbial attack when compared to other polymers, such as cellulose and lignin.

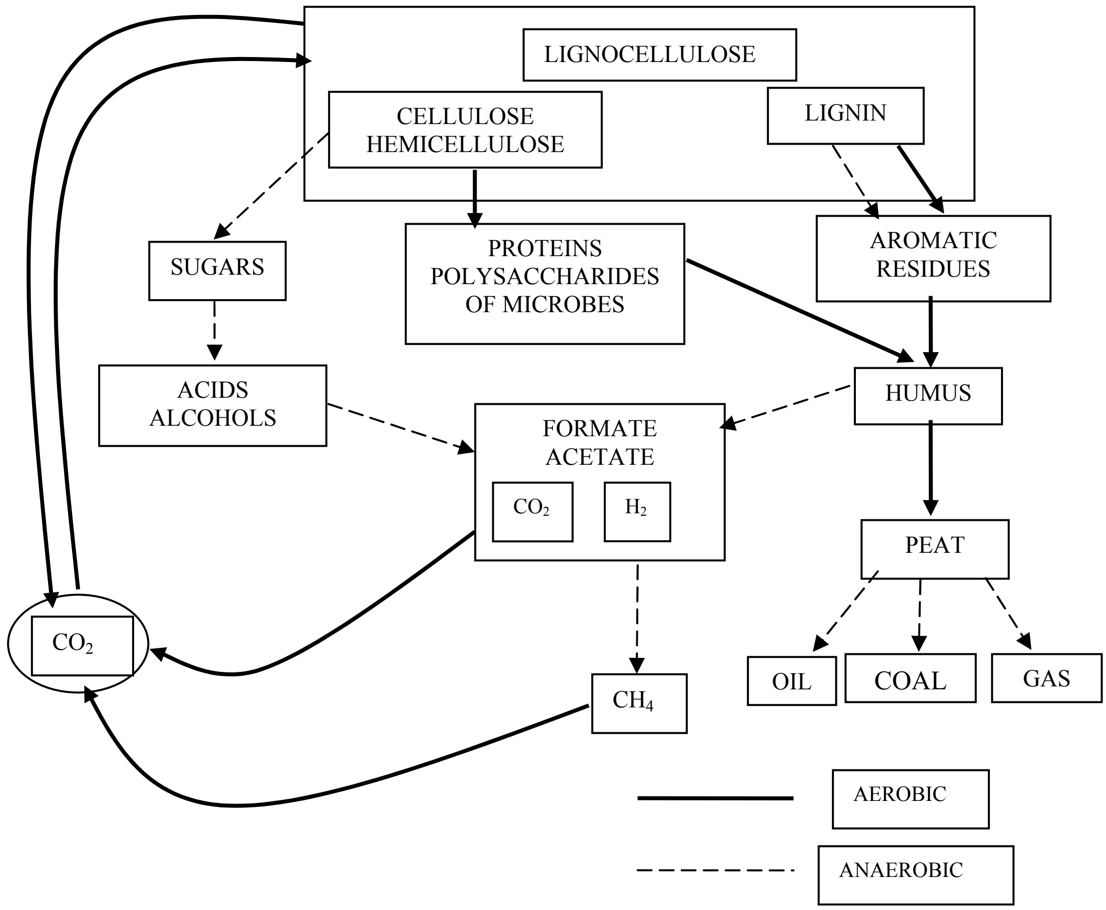
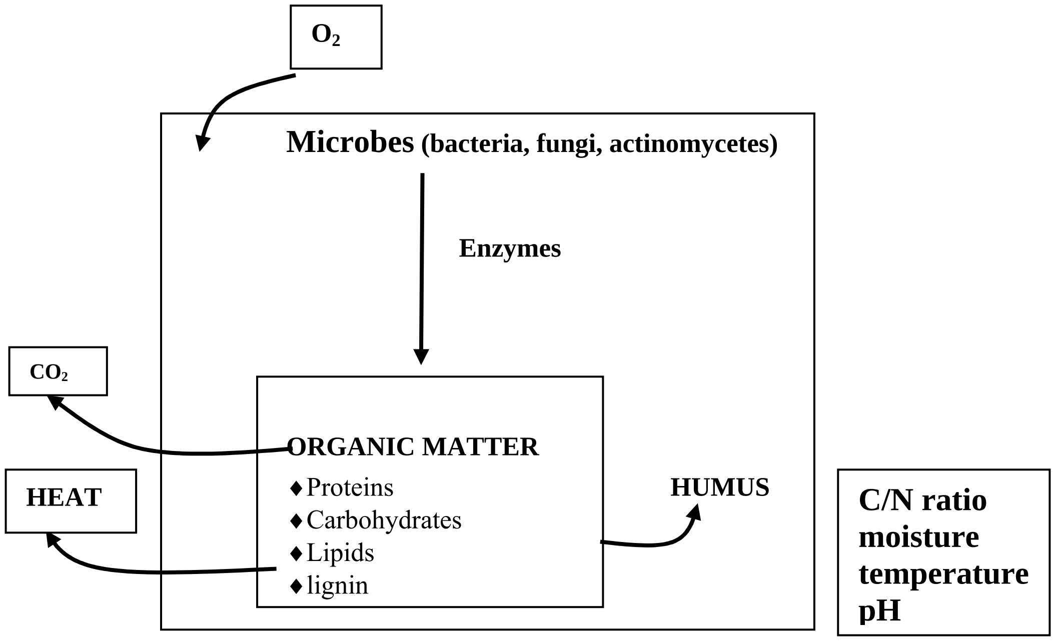


Figure 1 Global carbon cycle.

Source: Brown (1985); Colberg (1988)

4.4 Microorganisms in composting

Many types of microorganisms are required to decompose different materials. During composting, microorganisms transform organic matter into CO_2, biomass, thermoenergy (heat) and a humus-like end-product, (Figure 2).



Figur 2 The composting process.
Source: Itavaara *et al*. (1995).

Different communities of microorganisms predominate during the various composting phases. Bacteria increase rapidly during the first stages of composting. Later, actinomycetes, fungi and protozoa go to work. Microbial population levels are mostly determined by temperature and available food supply (Gray *et al*., 1971). Mesophilic temperature (25-45^{o}C) is developed first in composting followed by thermophilic temperature (50-65^{o}C). After the thermophilic phase, when the temperature of the material falls below 40^{o}C, the mesophilic community returns. A wide range of bacteria have been isolated from different compost environments, including species of *Pseudomonas*, *Klebsiella* and *Bacillus* (Nakasaki *et al*., 1985). Typical bacteria of the thermophilic phase are species of Bacillus, e.g. *B. subtilis*, *B. licheniformis* and *B. circulans.* Strom (1985) reported that as much as 87% of the randomly selected colonies during the thermophilic phase of composting belonged to the genus Bacillus. Many thermophilic species of Thermus have been isolated from compost at temperatures as high as 65°C and even 82°C (Beffa *et al*., 1996). Actinomycetes are bacteria which form multicellular laments, thus they resemble fungi. The genera of thermophilic actinomycetes isolated from compost include *Nocardia*, *Streptomyces*, *Thermoactinomyces* and *Micromonospora* (Waksman *et al*., 1939; Strom, 1985).

Actinomycetes are able to degrade some cellulose and solubilise lignin, tolerate higher temperatures and are important agents of lignocellulose degradation during peak heating, although their ability to degrade cellulose and lignin is not as high as that of fungi (Crawford, 1983; Godden *et al*., 1992). Under adverse conditions, actinomycetes survive as spores (Cross, 1968). Most fungi prefer an acidic environment, but tolerate a wide range of pH, with the exception of Basidiomycotina which do not grow well in environments with a pH above 7.5. Cooney and Emerson (1964) define thermophilic fungi as fungi with a maximum growth temperature of 50°C or higher and a minimum growth temperature of 20°C or higher. Thermotolerant species have a maximum growth temperature of about 50°C and a minimum well below 20°C (Cooney and Emerson, 1964). Crisan (1973) defined thermophilic fungi as fungi with a temperature optimum of 40°C or higher. Of all the species of thermophilic and thermotolerant fungi which are known to have cellulolytic or ligninolytic activity, or which have been found growing in lignocellulose substrate or compost, the predominant mesophilic fungus in the raw material has been identified as *Geotrichum* sp. (von Klopotek, 1962; Nusbaumer and Aragno, 1996) and the thermotolerant fungus *Aspergillus fumigatus* (von Klopotek, 1962).

4.5 Temperature

Temperature is universally a determinant of metabolic activity (Finstein *et al*., 1983). It has been recognised as a key environmental factor for composting conditions. Metabolic activity within a compost pile induces a temperature increase. Activity, represented by heat inside the compost at 50-60^{o}C, will remain intense until the temperature becomes too high for normal living organisms to remain active. Then, as they stop their activity, heat, and thus temperature, will progressively decrease (Figure 3). The insulation of the compost pile affects how heat output influences temperature. There are many reports which state that the optimal temperature is in the range of 55 to 60°C (Finstein *et al*., 1983).

4.6 pH

According to Stuetzenberger *et al*. (1970), in the early stages of composting, the pH decreases to 5.3-5.7. Soluble and easily degradable carbon sources, such as monosaccharides, starch and lipids are utilised by microorganisms in the early stages of composting. The pH decreases because organic acids are formed from these compounds during degradation. In the next stage, microorganisms start to degrade proteins, resulting in the liberation of ammonium and an increase in the pH, approaching neutrality at maturity (Figure 3) (Hellmann *et al*., 1997). Mckinley and Vestal (1985) hypothesised that increased pH could be used as an indirect indicator of high microbial activity.

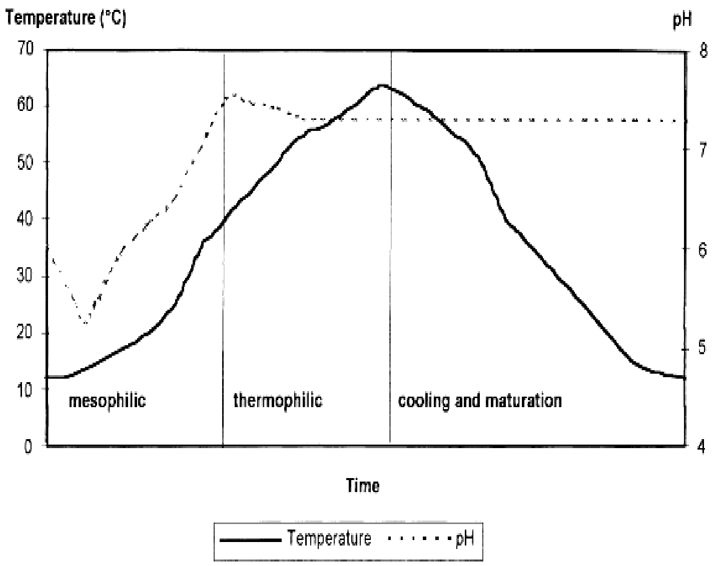


Figure 3 Temperature and pH variation during the natural composting process. Source: Golueke (1991); Tuomela *et al*. (2000)

4.7 Oxygen

Oxygen is an environmental parameter. It is linked to the decomposition process. Oxygen serves as a terminal electron acceptor in microbial respiration (de Bertoldi *et al*, 1983). When there is rapid composting, the oxygen supply can minimise odours because, in anaerobic conditions, the reduced compounds of nitrogen and sulfur, such as amines, sulfamines, mercaptans produce bad smells. The lack of oxygen during composting will result in anaerobic conditions (Brodie *et al*., 2000). The major problem to solve is the aeration rate in the reactor. If the aeration rate is too high, energy transfer in the reactors will increase and the temperature will decrease. If the aeration rate is insufficient the oxygen rate will decrease (Rynk *et al*., 1992). A minimum oxygen concentration of 5% within the pore space of the composting pile is necessary for aerobic conditions (Haug, 1993).

4.8 Moisture

Moisture management requires a balance between two functions: microbial activity and oxygen supply. Microorganisms are able to use organic molecules which dissolve in water and microbes use moisture for their growth. Suler and Finstein (1977) have stated that 50-60% moisture can induce the highest decomposition rate. However, the decomposition rate will be decreased if the moisture content is 70% or more, because too high a moisture content can cause a lack of aeration and the leaching of nutrients (Golueke, 1991). During the composting process, the operating temperature expels water through evaporation. Therefore, water should be added during the composting process, to bring the moisture content level into the optimum range.

5. Compost maturity

Monitoring the composting process and evaluating the maturity is important in terms of product utilisation and is essential for optimal use as a soil amendment and a source of plant nutrients (Mathur *et al*., 1993). Immature composts pose problems of bad smells and fire during storage as well as phytotoxicity and pollution if put to use. Many methods have been proposed to estimate the degree of maturity of compost. The changes of physicochemical and biological properties during sewage sludge composting have been widely studied. According to Morel *et al*. (1984), the maturity of compost may be assessed by the biological stability of the product. The methods may be differentiated into separate areas as follows.

5.1 Respirometry, expressed in terms of carbon dioxide production rate or oxygen consumption rate, provides the most accurate assessment of microbial activity and compost stability, although the measurement of carbon dioxide production does not distinguish between anaerobic and aerobic activity, since carbon dioxide is produced under either process (Lasaridi and Stentiford, 1996). A number of standardised techniques such as BOD5 and COD have been utilised to evaluate the oxygen demand/biodegradability of soluble organics. Respiration measurement methods are now commonplace (Adani *et al*., 2001). Respiration rates were expressed in terms of the relative reduction in oxygen concentration over one hour for compost samples at various stages of decomposition. CO_2 evolution is the most widely acceped microbial respiration test (Chen and Inbar, 1993).

5.2 Analysis of biodegradable constituents: C/N ratio, total organic carbon, polysaccharides. The change in the ratio of the two predominant elements (carbon and nitrogen) within the composting matter has also been considered in terms of stability, for as the readily available organic matter is oxidised and released as carbon dioxide, there is a general reduction in carbon content over time. Inbar *et al*. (1990) reported that the C/N ratio of the solid phase typically begins at 40:1, before dropping quickly at first, then slowing, to finish at about 18:1. Additional thresholds of compost stability include C/N ratios of less than 20 and a C/N final / C/N initial ratio of between 0.6 and 0.75 (Jiménez and Garcia, 1989).

5.3 Study on biochemical parameters (ATP, enzyme activity).

Reports about the evolution of a particular enzyme's activities during composting are very rare. Some attention was paid to cellulase, invertase and alkaline phosphatase (Godden,1983).

5.4 Other methods for estimating the degree of maturity of composts, i.e. the final temperature drops, the degree of self-heating capacity.

6. Sewage sludge compost

Composting sewage sludge is increasingly being considered by many municipalities throughout the world, because it has several advantages over other disposal strategies (Zorpas *et al*., 1999). Firstly, the volume of organic material decreases by about 30-50% during the composting process (Brady and Well, 1996). Secondly, pathogens can be killed due to the heat generated during the thermophilic phase (Wong *et al*., 1995; Furkacker and Haberl, 1995; Finstein *et al*., 1980). Moreover, mature compost also contains natural organic chemicals and beneficial microorganisms that kill or suppress disease-causing microorganisms (EPA, 1998). Lastly, compost can be used as a soil conditioner as many beneficial organic substances are found in sludge (Zorpas *et al*., 1999).

In the sludge composting process, it is necessary to introduce a bulking agent to the sludge because it absorbs moisture, increases porosity and adds a source of carbon to the sludge. Aeration and/or frequent mixing or turning is needed to supply oxygen and remove excess heat. The Environmental Protection Agency (EPA) requires that sludge compost piles reach and maintain a temperature of 55^{o}C for three days. This temperature helps to kill pathogens and most weed seeds. If aerobic conditions are not maintained within the mixture, odours can become annoying. The process is also slowed down, and extended time and space is needed, to make up for inefficiencies in aerating the biomass. In this case, the problems are that a lot of energy is required to aerate the composting material and the end product, the compost, must be disposed of on suitable land making its value as a land fertiliser to farmers less attractive. Therefore, studies into mixing the sludge with a bulking agent have to ensure that the mixture can be aerated adequately for an accelerated aerobic degradation process to occur. The other limitation of land application of sewage sludge compost is the potentially high metal content related to the metal content of the original sludge and the degree of dilution by the bulking agent during composting. Heavy metals can be removed by many methods. Numerous studies have been extensively undertaken on the use of agricultural residues, for the removal of heavy metals through adsorption, ion exchange or chelation. In a laboratory batch, a study using char rice husk for the adsorption of Cu^{2+} and Ni^{2+} has been reported (Yeoh, 1987). A variety of organic material mixtures might be considered suitable for this process.

During composting, increases in pH can cause a loss of N by ammonia volatilisation. This can be minimised by the addition of materials with a high C/N ratio; total enclosure of the system minimises the release of strong odors.

7. Vermicomposting

Earthworms are ubiquitous, relatively large and easy to handle. They can be readily collected and identified. Worms can live for about one year in a worm bin. Since the worm's body is about 90% water, when it dies it will shrivel up and become part of the compost rather quickly. New worms are born and others die all the time. Worms are hermaphrodites, which means they possess both male and female sexual organs in one body. Nonetheless, it still requires two worms to mate. The worms line up in opposite directions near their band, which contains some of the sexual organs. The worms are attached for about fifteen minutes while they exchange sperm cells. Several days later, the eggs come into contact with the sperm cells and form a cocoon, or egg case. The cocoon separates from the worm and fertilisation takes place. On average, two to five baby worms may be found inside the cocoon.

Vermicomposting has already been described for stabilising different natural and anthropogenic wastes, such as urban and industrial sewage sludge (Elvira *et al*., 1996). Vermicomposting is the digestion of organic materials by earthworms which produce excreta known as casts (Chaoui *et al*., 2003). The ability of some earthworms to consume a wide range of organic residues such as sewage sludge, animal wastes, crop residues and industrial refuse has been well reported (Edwards *et al*. 1985; Chan and Griffiths 1988; Hartenstein and Bisesi 1989). The end product, commonly termed vermicompost and obtained as the organic waste passes through the earthworm's gut, is quite different from the parent waste material. There are nearly 6,500 described species of earthworms in the world (Aranda *et al.,* 1999). Only a few are known to be suitable for culturing in organic waste materials.

The best known species with good potential for waste management include: *Eisenia andrei*, *E. fetida*, *Eudrilus euginiae* and *Perionyx excavatus*. These are in the epigeic earthworm species. Most vermicomposting experiments have used epigeic earthworm species, because they possess a higher composting potential (Tripathi and Bhardwaj, 2004). *E. euginiae* is an earthworm species, originally from Africa, which has been bred extensively in the USA, Canada, Europe and Asia for the fish bait market, where it is commonly called the African nightcrawler (Dominguez *et al*., 2001). Its presence in dairy farms was not surprising because it has always been collected locally at sites rich in organic matter (Borges and Moreno, 1994). *E. euginiae* is a large worm that grows extremely rapidly and is suitable as a source of animal feed protein as well as for rapid organic waste conversion (Dominguez *et al*. 2001; Reinecke and Viljoen, 1993). *Eudrilus eugeniae* is already used in vermicomposting (Edwards, 1998). In the vermicomposting process, inoculated earthworms maintain aerobic conditions in the organic waste, ingest solids, convert a portion of the organic material into worm biomass, respiration products and expel the remaining partially stabilised product.

MATERIALS AND METHODS

1. To study the physical, chemical and biological properties of sewage sludge collected from wastewater treatment plants in the Bangkok area, including analysis of faecal coliform *Escherichat coli* and heavy metals contained in the sludges.

1.1 Sample collection and preparation

Dewatered sewage sludge was collected from three central wastewater treatment plants in Bangkok, Thailand (Figure 4). The wastewater treatment processes of Siphraya, Rattanakosin and Chongnonsi plants consist of contact stabilisation, two-stage activated sludge and cyclic activated sludge systems respectively. The samples were collected once a month from November 2001 to October 2002. They were transported to the laboratory and kept at 4°C until biological and physical analysis.

1.2 The chemical and physical analyses

For chemical analysis, sub-samples were dried at room temperature and ground down to pass through a 2 mm sieve. The chemical and physical characteristics were analysed six times a year in the laboratory in order to monitor the changes.

1.2.1 Chemical characteristics such as nutrient content (N, P, K, Ca, Mg, S) organic matter, organic carbon, CEC, pH, some heavy metals.

1.2.2 Physical characteristics such as moisture content.

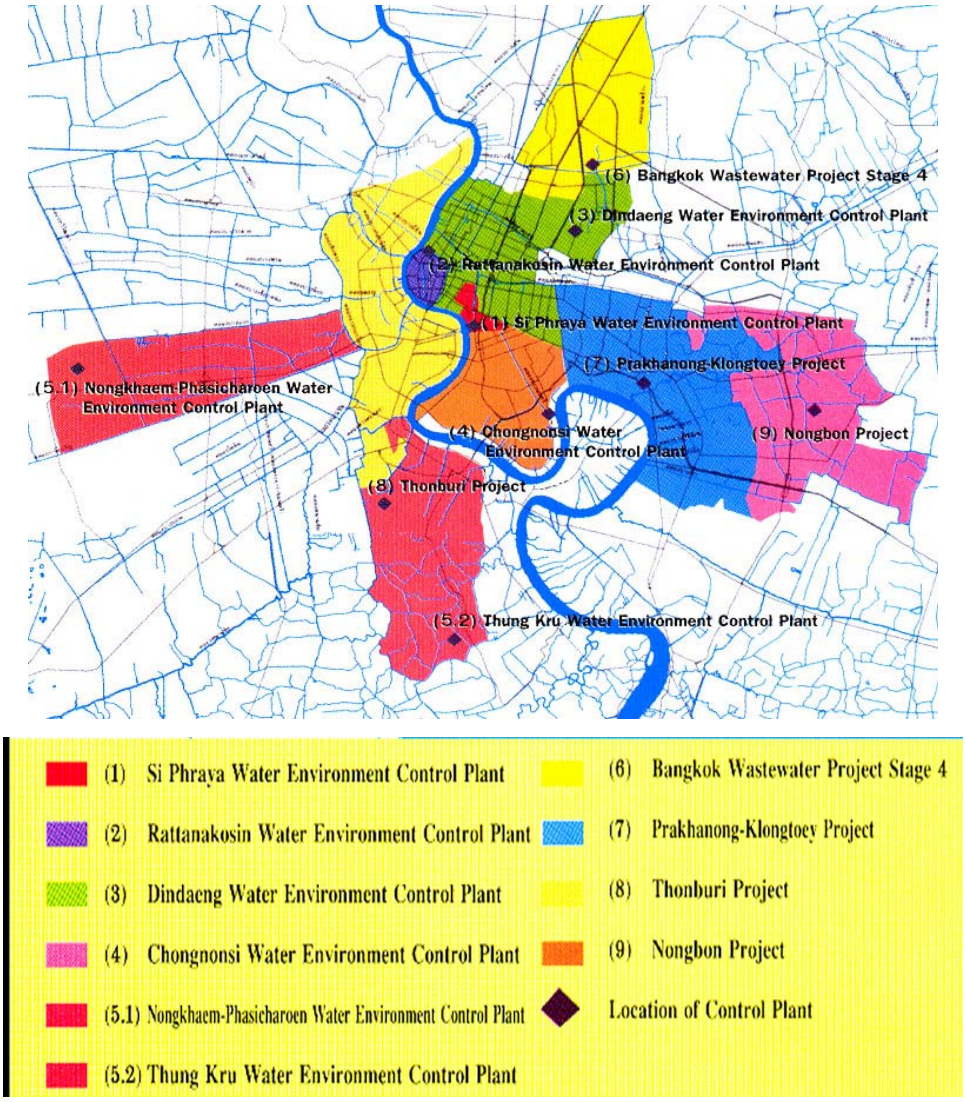


Figure 4 Wastewater Treatment Project of BMA**.**

1.2.3 Rapid determination of pH and electrical conductivity, in the mixture with water at a ratio of 1:10, and organic carbon using the Walkley-Black method (Nelson and Sommers, 1982) was measured from the samples collected every month. Other chemical analyses such as total nitrogen (N), phosphorus (P), potassium (K), calcium (CaO), magnesium (MgO), sulphur (S) and total metals (Fe, Mn, Zn, Cu, Cr, Cd, Pb, Ni, Hg) were analysed on the samples collected every two months. Total N was determined by a micro Kjeldahl procedure (Bremner, 1965). Sludge was digested in HNO_3 and $HClO_4$ for determination of total P by a colorimetric procedure (Murphy and Riley, 1962) and total metals were measured by atomic absorption spectroscopy (AOAC, 1980).

1.2.4 The moisture content of sludge was measured as weight loss in drying at 80°C, to avoid the loss of nitrogen during drying.

1.3 Biological analysis

Subsamples of the sewage sludge stored at 4°C were analysed for biological characteristics every two months using procedures given by APHA (1992). Microbial populations, i.e. total bacteria and fungi, cellulolytic bacteria and fungi, were counted using a serial dilution plate count technique. Faecal coliforms and *Escherichia coli* (*E. coli*) were determined by the Most Probable Number (MPN) method.

1.4 Phytotoxicity

Sewage sludge extracts for phytotoxicity assessment were prepared by shaking 2 g of fresh sewage sludge with 20 ml of distilled water; the suspensions were filtered. Chinese cabbage (*Brassica chinensis*) was used as test plant. Two pieces of No. 2 Whatman filter paper were placed in a sterile disposable Petri dish. The filter paper was wetted with 9 ml of sewage sludge extract and 30 seeds were added. Distilled water served as a control. After four days, the percentage of seed germination in sewage sludge extract was calculated and root elongation was measured (Zucconi *et al.*, 1981).

1.5 Data analysis

Means and standard deviation were used to present the evolution of sewage sludge characteristics, measured on composite samples. Automatic classification and discriminant analysis (DA) were used to colate the data from the different localisation and time sampling studies into homogeneous groups of data in order (i), to identify if the location of the treatment station is a source of variability in the quality of the sewage sludge and (ii), to identify the factors able to differentiate the sources of data on the quality of the sewage sludge.

2. To study appropriate methods for composting sewage sludge, such as the use of suitable mixing material(s), effective microorganisms (LDD1), as well as monitoring the decomposition process and the analysis of heavy metals present in the compost.

2.1 Selection of organic materials for aeration in sewage sludge composting

Dewatered sewage sludge was collected from three wastewater treatment plants in Bangkok: Siphraya, Rattanakosin and Chongnonsi. The samples of sewage sludge were then separately mixed with two types and three rates of organic materials to improve aeration. The materials for mixing were wood chips from sawmill in Bangkok and rice husk from rice mill in Pathumthani province. The characteristics of the raw materials are presented in Table 1. The treatements of sludge composting were prepared as follows.

- Sole Siphraya sewage sludge
- Siphraya sewage sludge + wood chips at the ratio 85:15(w/w)
- Siphraya sewage sludge + wood chips at the ratio 80:20(w/w)
- Siphraya sewage sludge + wood chips at the ratio 75:25(w/w)
- Sole Rattanakosin sewage sludge
- Rattanakosin sewage sludge + wood chips at the ratio 85:15(w/w)
- Rattanakosin sewage sludge + wood chips at the ratio 80:20(w/w)
- Rattanakosin sewage sludge + wood chips at the ratio 75:25(w/w)

Triplicates of each mixture (1,300 g fresh weight per box) were placed in plastic containers (17 x 17 cm height x diameter) with a cover, to allow gas exchange, so as to maintain aerobic conditions in the incubator. The moisture content has been maintained at 60 – 70% during incubation periods at 55°C. A 75 g aliquot was sampled from each treatment on days 1, 7, 14, 28, 49, 56, 63 for chemical and biological analyses. Before each sampling, the box contents were thoroughly mixed in order to homogenise and maintain aeration of the mixture.

Table 1 Chemical characteristics of woodchip and rice husk.

Characteristics	Woodchip	Rice husk
OC (%)	51.28	38.05
N (%)	0.15	0.37
P_2O_5 (%)	0.008	0.092
K_2O (%)	0.018	0.518
pH	4.55	6.08
Soluble sugar (%)	11	5
Hemicellulose (%)	4	19
Cellulose (%)	56	39
Lignin (%)	28	16
Ash (%)	<1	28

2.2 Effect of biological activators on sewage sludge compost production

Dewatered sewage sludge was collected from the Siphraya and Rattanakosin wastewater treatment plants in Bangkok, while the wood chips came from a sawmill in Bangkok, the rice husk was from rice mill in Pathumthani province.

Prior to this experiment, a previous study (experiment 1) had been conducted to select the mixture which produced the fastest rate of decomposition. The wood chips or rice husk at 15, 20 and 25% of fresh weight basis, were mixed with sewage sludge and incubated at 55°C. The highest decrease of OM of wood chips and rice husk has been selected for this experiment to measure the changes of the chemical and the biological properties and the effect of a microbial activator on those properties during sewage sludge incubation. The microbial activator, LDD 1 which is provided from the Department of Land Development used in this experiment contains different types of microorganisms: two bacteria (*Bacillus* sp), two actinomycetes (*Streptomyces* sp), and four fungi (*Scopulariopsis* sp., *Helicomyces* sp., *Chaetomium* sp. and *Trichoderma* sp.). The application rate recommended by the Land Development Department for this activator is 100 grams (g) for one ton of fresh organic material. Eight organic matter mixtures had been prepared in a laboratory. Sewage sludge was mixed with wood chips at a ratio of 80:20 (weight/weight; w/w) on a fresh weight basis, with and without a microbial activator: The other treatments were sludge mixed with rice husk at a ratio of 80:20 (w/w) on a fresh weight basis, with and without a microbial activator: Triplicates of each mixture (1,300 g fresh weight per box) were set in plastic containers (17 x 17 cm height x diameter) with a cover allowing gas exchanges in order to maintain aerobic conditions. Each activator at 0.13 g rate (13 ml of a suspension dilution in water) was added to the corresponding mixture activator treatments. The moisture content was maintained at 60 – 70 % during incubation periods at 55°C in an incubator. Sub-samples from each treatment were collected on days 1, 7, 14, 28, 49, 56, 63 for chemical and biological analyses. Before each sampling, the materials in the box were thoroughly mixed in order to homogenise and maintain the aeration of the mixtures.

2.3 Chemical analysis

For chemical analysis, the samples collected during incubation periods were dried and ground to pass through a 2 mm sieve. Organic matter and nitrogen content levels were measured in all samples. The organic matter content was estimated by the calculation: weight of dry sample – weight of ash remaining after burning at 550°C for 1 hour. Percentage of carbon was calculated from the following equation.

$$\%C = \frac{100 - \%ash}{1.8}$$

Total nitrogen was determined by using the Kjeldahl method (Bremner, 1965). The sludge was digested in HNO_3 and $HClO_4$ for determination of total P by a colorimetric procedure (Murphy and Riley, 1962), total metals were measured by atomic absorption spectroscopy (AOAC, 1980). The pH (1:10, compost: water) was measured.

2.4 Biological analysis

2.4.1 Microbial and enzymatic analysis

At days 1, 7, 14, 28, 49, 56, 63, the carbon dioxide (CO_2) content was measured directly in the plastic containers (17 cm x 17 cm height x diameter) containing samples. The method was developed from soil respiration measurement (Naganawa *et al.,* 1989). Plastic boxes were closed with the cover and sealed with plastic tape for twenty-five to thirty minutes (min), the concentration of CO_2 in the headspace of the boxes was measured by pumping air (1 litre per minute) via a hole made in the cover to an infrared gas analysis system (ZFP5, Fuji Electronics Co. Ltd, Japan) connected to a digital multimeter, for output collection.

At the same intervals, enzymatic activity was determined in the fresh samples. Cellulase activity was determined by incubating an assay mixture containing 0.5 ml of organic mixture extract (1:10/mixture:water; weight/volume) and filter paper (Whatman No.1; 1x6 centimetres) in 0.5 ml of 0.02 Molar (M) phosphate buffer (pH 6.0) for 1 hr at 50°C. The amount of reducing sugar formed was estimated using the dinitrosalicylic acid method (DNS) (Miller, 1959). One international unit (IU) of cellulase activity using filter paper as a substrate (FPase) was defined as reducing sugar from substrate produced per minute using glucose as a standard. Xylanase activity was estimated in the organic mixture extract using 1% xylan in an acetate buffer solution (pH 5.5) as a substrate. The measurement of reducing sugar released during incubation at 65°C for 10 min was accomplished dinitrosalicylic acid testing using xylose as a standard. Phosphatase activity was determined by adding 0.2 ml of toluene, 4 ml of Modified Universal Buffer (MUB, pH 6.5) and 1 ml of *p*-nitrophenylphosphate solution to 0.5 g of mixture, incubated at 37°C for 30 min (Tabatabai and Bremner, 1969). After incubation, 1 ml of 0.5 M calcium chloride ($CaCl_2$) and 4 ml of 0.5 M sodium hydroxide (NaOH) was added, the suspension was then filtered and the amount of *p*-nitrophenol (PNP) released was measured using a spectrophotometer at 410 nanometres. A control was prepared in the same way as the samples, except that the substrate (*p*-nitrophenylphosphate) was added after incubation and immediately before the addition of $CaCl_2$ and NaOH.

The thermophilic cellulolytic bacteria and fungi populations were counted using the dilution agar-plate method (Lorch *et al.*, 1995) with carboxyl methyl cellulose (CMC) as the cellulose substrate, without any other source of carbon. The fresh homogenised mixture sample at 10 g weight was transferred into a 250 ml flask containing 90 ml of sterile distilled water to obtain a dilution of 10^{-1}. The flask was then shaken for fifteen minutes. One millilitre of this suspension was poured into a test tube containing 9 ml of sterile distilled water (10^{-2}) and was mixed using a vortex mixing device (Torika mixer MA-1) and subsequently serially diluted up to the appropriate dilution depending upon the samples. One millilitre of the last three dilutions was poured into sterile Petri dishes (3 replications). The CMC medium was then added to each plate. Incubation was carried out at 55^0C in the incubator. Dilutions presenting 30-300 colonies were counted. The results of microbial population study were expressed in a log basis of CFU (Colony Forming Unit) per gram of dry weight (log CFU g^{-1} dw).

2.5 Statistical analysis

As the recorded data was repeated through measuring the same items over a long period, the effect of the different types of bulking agent (woodchips vs rice husk) and the effect of activator use (with vs without), as well as the interaction between the two types of treatments, were analysed using the two-way ANOVA method to allow for random effects.

3. To study the methods of vermicomposting sewage sludge

3.1 Preparation of the materials

Siphraya sewage sludge was mixed with wood chips at a ratio of 80:20(w/w) on a fresh weight basis, with and without a microbial activator. The sewage slude mixtures were put into the plastic boxes (17.3 cm x23.0 cm x 8.5 cm width x length x height). The mixures were composted under the optimum conditions which the moisture content of the mixture was maintained at 75-80% throughout the vermicomposting phase and the containers were maintained in darkness at room temperature. The clitellated earthworms (*Eudrilus euginiae*) were placed in the mixture material at a ratio 30:1(w/w). The controls were not inoculated with earthworms. Each treatment was replicated four times, shown as follows:

Treatment 1: sewage sludge + wood chips + activator (LDD 1) (SWA)
Treatment 2: sewage sludge + wood chips + activator (LDD 1)+ earthworm (SWAE)
Treatment 3: sewage sludge + wood chips (SW)
Treatment 4: sewage sludge + wood chips + earthworm (SWE)

On day twenty, the earthworms were removed and their quantity and weight were measured. The samples were collected at the begining and at the end of vermicompostion (twenty days) to analyze the chemical and biological properties.

3.2 Chemical analysis

Total organic carbon (TOC) and total nitrogen (N) were determined by dichromate oxidation (M.A.P.A., 1986) and Kjeldahl (Jackson, 1958) methods, respectively. The C/N ratios were calculated from TOC and N analysis.

3.3 Biological analysis

Cellulase activity was determined by incubating an assay mixture containing 0.5 ml of sample extract (1:10/sample:water; weight/volume) and filter paper (Whatman No.1; 1x6 centimetres) in 0.5 ml of 0.02 Molar (M) phosphate buffer pH 6.0 for 1 hour at 50^{o}C. The reducing sugar formed was estimated using the dinitrosalicylic acid (DNS) method. Xylanase activity in the sample extract was estimated using 1% xylan in acetate buffer solution pH 5.5 as a substrate. The measurement of reducing sugar released during incubation at 65^{o}C for 10 min was estimated by DNS using xylose as a standard. To determine dehydrogenase activity, 0.4% 2-p-iodophenyl-3 p-nitropheny-5-tetrazolium chloride (INT) was used as a substrate. Iodonitrotetrazolium formazan (INTF) produced in the reduction of INT was measured with a spectrophotometer at 490nm.

The total numbers of actinomycetes and bacteria were counted using the standard dilution plate count method. While faecal coliform was determined by using the Most Probable Number (MPN) method.

3.4 Statistical analysis

As the recorded data was repeated through measuring the same items over a long period, the effect of the earthworms and the effect of activator use (with vs without), as well as the interaction between the two types of treatments, were analysed using the two-way ANOVA method to allow for random effects.

RISULTS AND DISCUSSION

1. To study the physical, chemical, and biological properties of sewage sludge collected from three wastewater treatment plants in the Bangkok area, including the analysis of index of faecal contamination and heavy metals contained in the sludges.

1.1 Physical characteristic

The moisture content of the three sludge samples was measured after drying the material at 80°C up to constant weight. The moisture ranged from 73 to 86% after the sludge had been processed through the belt press. The moisture content did not vary during the sampling period. As sewage sludge has a high moisture content, a large percentage of the moisture in the sludge should be evacuated.

1.2 Chemical characteristics

1.2.1 pH, electrical conductivity (EC) and total organic carbon (TOC)

Monthly data of pH and EC of sewage sludge from the three wastewater treatment plants from November 2001 to October 2002 are presented in Figure 5. The pH ranged from 6.1 to 7.9. Chongnonsi sludge had a pH higher than sludge from the other two plants, especially in April and May 2002; additionally, EC was low in this sludge. EC was not very high in all of the sludge samples, except in Rattanakosin where it was 3.5 and 3.0 dS m^{-1} in September and October respectively. It is close to the critical level of 4 dS m^{-1} (Richards, 1960). Organic matter is one component of sludge and a source of organic carbon when sludge is applied to soil. It is useful for improving the soil structure. In addition, soil microorganisms use organic matter as an energy source. The organic C content was variable (110-240 g kg^{-1} in three sludges) during the sampling period (Figure 6). The data shows that Siphraya sludge had the highest organic C content. It might be the population in the catchment area of Siphraya plant had the highest and the part of catchment area was slum. Rattanakosin and Chongnonsi sludge were not much different. Considering the samples taken from the three waste water treatment plants, the sludge had a similar evolution of C content with the highest observed in March and April.

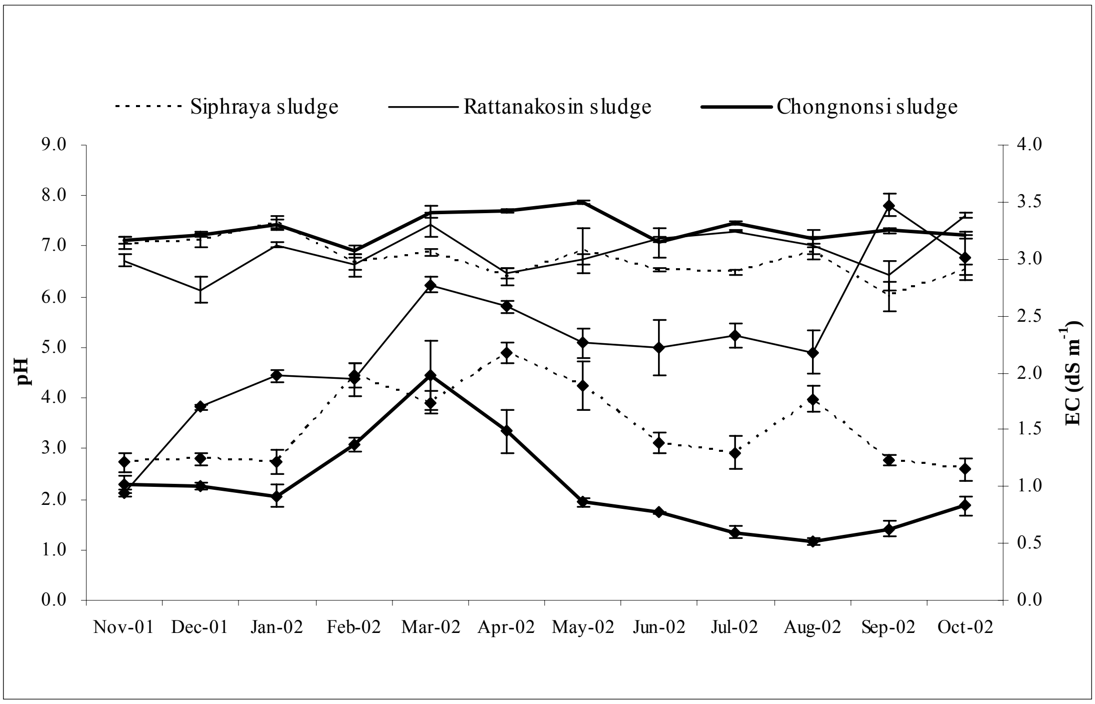


Figure 5 Annual evolution of pH (◊) and EC (♦) in the three sewage sludges from Bangkok wastewater treatment plants. (Mean of 5 replications).

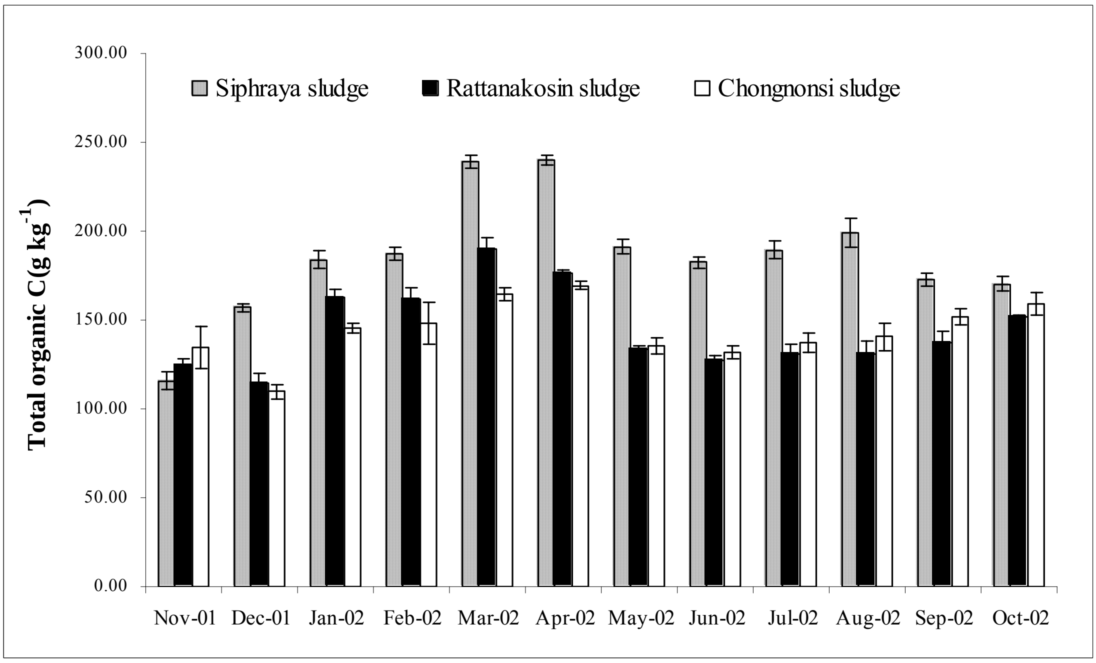


Figure 6 Evolution of total organic carbon (TOC) content in the three sewage sludges from Bangkok wastewater treatment plants. (Mean of 5 replications, bars indicate standard deviation).

1.2.2 Plant nutrients

Major plant nutrients are present in sewage sludge, especially N and P. According to Sommers (1977), sewage sludge is very rich in N and has good potential for use as compost. In this study, the nutrient content varied according to sludge type and sampling time. Total N content ranged from 19.7 to 38.7 g kg^{-1} in the three types of sludge (Figure 7). Total N content was the highest in Siphraya sludge according to the hightest in the influent of Siphraya plant. Consequently, the C/N ratio is low (around 6), indicating that the decomposition rate will also be low. This should be assessed as this study measured the total N produced only, giving no indication of the actual availability of N to the microorganisms. Total P, K and S contents were not much changed during the sampling periods, except that Rattanakosin sludge showed a large amount of sulphur in the month of August. Total P ranged from 9.4 to 14.0 g kg^{-1}, the maximum being in Chongnonsi sludge. K and S contents were the highest in Rattanakosin sludge for nearly all sampling periods, except K in June, which was higher in Chongnonsi sludge. Total K in Chongnonsi sludge is higher than in Siphraya sludge. Siphraya sludge had more total S than Chongnonsi sludge. The maximum K and S were 6.0 and 25.4 g kg^{-1} respectively in August

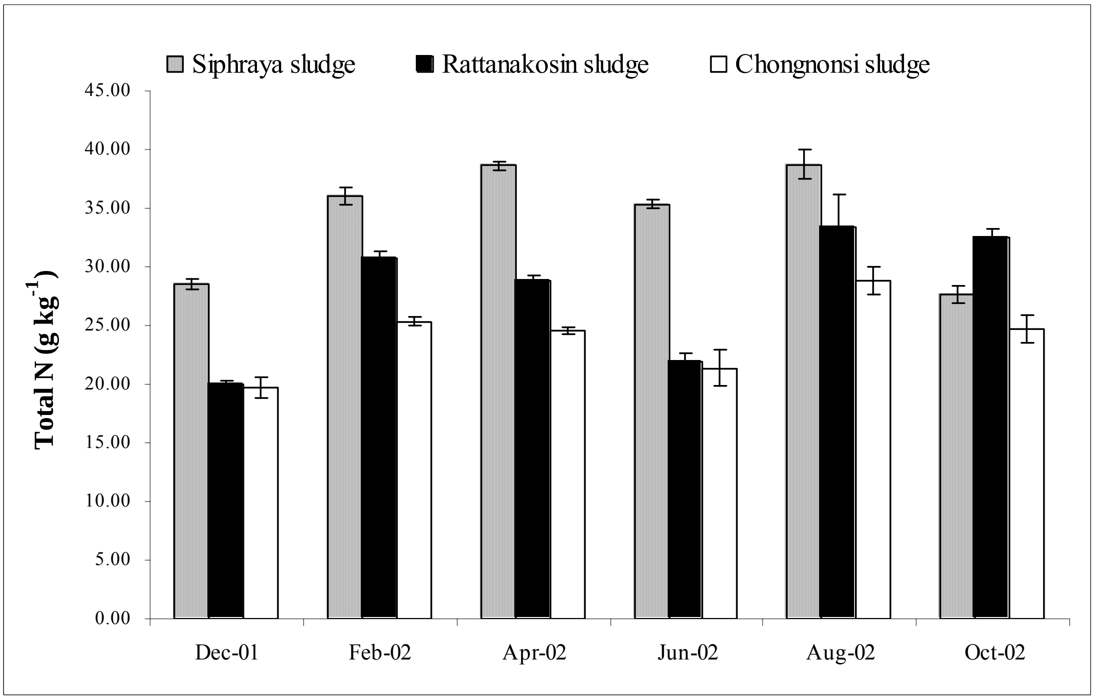


Figure 7 Annual evolution of total N content in the three sewage sludge from Bangkok wastewater treatment plants. (Mean of 5 replications, bars indicate standard deviation).

Chongnonsi sludge had total CaO content higher than the others. It increased from December to June. The highest contents were 33.8 g kg^{-1}, 32.4 g kg^{-1} and 42.8 g kg^{-1} in Siphraya, Rattanakosin and Chongnonsi sludge respectively. Total MgO content (4.9 to 9.2, 6.0 to 8.9 and 5.3 to 10.5 g kg^{-1} in Siphraya, Rattanakosin and Chongnonsi sludge respectively) was not much different amongst the three, except in February and June where Chongnonsi sludge was the highest. Table 2 shows the data of nutrient contents for the three sludge samples compared to other organic by-products. Total N, P and S content in the sludge was higher than in the organic waste samples. Total K content in the organic wastes was higher than in the sludge. Considering the plant nutrient contents, sludge could be used for agricultural purposes, even if some complement nutrients should be supplied to the crops for balanced nutrition. However, the data presented in Table 2 is for the total content of the elements and part of them might not be available directly to crops.

Table 2 The comparison of nutrient contents in the three sewage sludges and some agricultural waste (g kg^{-1}).

Materials	Minimum/ maximun	Total N	Total P	Total K	CaO	Mg	S
Siphraya sludge	Minimum	27.6 (0.7)	9.7 (0.24)	3.0 (0.3)	15.0 (0.4)	4.9 (0.1)	9.0 (0.9)
	Maximum	38.7 (1.3)	11.9 (0.3)	5.0 (0.5)	33.8 (1.1)	9.2 (0.5)	14.0 (1.5)
Rattanakosin sludge	Minimum	20.0 (0.3)	9.4 (0.5)	3.3 (0.3)	12.3 (1.6)	6.0 (0.1)	8.3 (1.0)
	Maximum	33.4 (0.3)	12.5 (0.1)	6.0 (0.4)	32.4 (3.1)	8.9 (0.2)	25.4 (1.9)
Chongnonsi sludge	Minimum	19.7 (0.9)	10.1 (0.2)	3.7 (0.5)	21.7 (0.6)	5.3 (0.2)	6.5 (0.6)
	Maximum	25.4 (0.4)	14.0 (0.4)	5.1 (0.3)	42.8 (2.1)	10.5 (0.2)	11.6 (0.8)
**Corn stoack		12.9	1.1	6.6	4.6	2.8	2.7
**Wood chip		3.0	0.4	3.0	4.5	3.5	0.8
**Rice husk		3.5	0.4	12.6	17.6	1.3	0.3
**Rice straw		10.5	1.9	9.0	25.9	7.8	0.6

Values in parentheses are standard deviation of 5 replications

Source:** Limtong and Leaungvutiviroj (1997)

1.2.3 Heavy metals

The major concern of sludge for land application is heavy metal contamination. High concentrations of heavy metals may be toxic to plants, animals and human beings (Barker, 1997; Chaney, 1980). However, the United States Environmental Protection Agency (USEPA) and European Community Commission (EU) have established regulations for the quality of sludge. Table 3 shows the comparison of total heavy metal concentrations for the three sludge samples and USEPA, EU regulation limits of sludge for land application. As a result, total heavy metal concentrations in the sludge are lower than both regulation limits, except for copper concentrations in Rattanakosin sludge (highest every month) and cadmium in Rattanakosin which was higher than the limitation in every month as well as cadmium in Rattanakosin and Chongnonsi sludge in October.

Table 3 Comparison of heavy metal contents in the three sewage sludges and USEPA (ceiling concentration limits for land application), EU (ceiling concentration limit for agricultural use) (mg kg-1).

Heavy metals	Siphraya sludge		Rattanakosin sludge		Chongnonnsi sludge		USEPA	EU
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum		
Fe	18,740	35,920	18,157	46,800	19,432	38,820	-	-
	(196)	(1,282)	(275)	(10,073)	(406)	(1,066)		
Mn	1,488	3,020	436	2,696	1,778	3,520	-	-
	(94)	(74)	(13)	(83)	(137)	(147)		
Cd	N.D.	9.0	N.D.	176	N.D.	184	85	40
		(2.8)		(2)		(8)		
Cr	45	151	110	347	363	588	3,000	-
	(8)	(62)	(15)	(89)	(27)	(92)		
Cu	1,040	1,700	6,100	16,620	420	700	4,300	1,750
	(135)	(0)	(129))	(116)	(40)	(89)		
Pb	2.3	120	2.5	486	2.5	195	840	1,200
	(0.8)	(12)	(0.6)	(22)	(0.7)	(6)		
Hg	0.45	0.94	0.15	0.37	0.17	0.44	57	25
	(0.10)	(0.60)	(0.04)	(0.04)	(0.05)	(0.07)		
Ni	31	88	166	398	136	263	420	400
	(14)	(11)	(10)	(10)	(10)	(14)		
Zn	137	1,380	124	2,260	28	3,604	7,500	4,000
	(19)	(74)	(7)	(49)	(0.4)	(114)		

Values in parentheses are standard deviation of 5 replications

The total heavy metal concentration changed according to the source of the sludge and the sampling time. Chongnonsi sludge had the highest average values of Cr, Mn and Zn contents. The measured chromium in Chongnonsi sludge was quite different from the other sludges in that it reached its maximum level in August, but in Siphraya and Rattanakosin sludge the maximum level of Cr was in June. The highest Mn concentration was found in April in Siphraya and Chongnonsi sludge; October for Rattanakosin sludge. Total Zn concentration was the highest in Chongnonsi sludge except in the month of April for Rattanakosin sludge. Rattanakosin sludge has a higher concentration of Zn than Siphraya sludge. The range of concentration was very wide, from 28 mg kg^{-1} in April to 3 604 mg kg^{-1} in October. The three sludge samples had only small amount of Zn in April and August. The minimum and maximum heavy metal concentrations are shown in Table 3.

The total concentrations of four heavy metals namely Cu, Fe, Ni and Pb were much more obvious in Rattanakosin sludge. Figure 8 shows the changes of Cu in the three sludges during the sampling period. The Cu content in Rattanakosin sludge was far higher than the other two sludges and was higher than USEPA and EU regulation limits at every sampling time. This might indicate the presence of some small industrial effluent within the zone and commercial small industrial zone where the silverware and copperware plants are located.

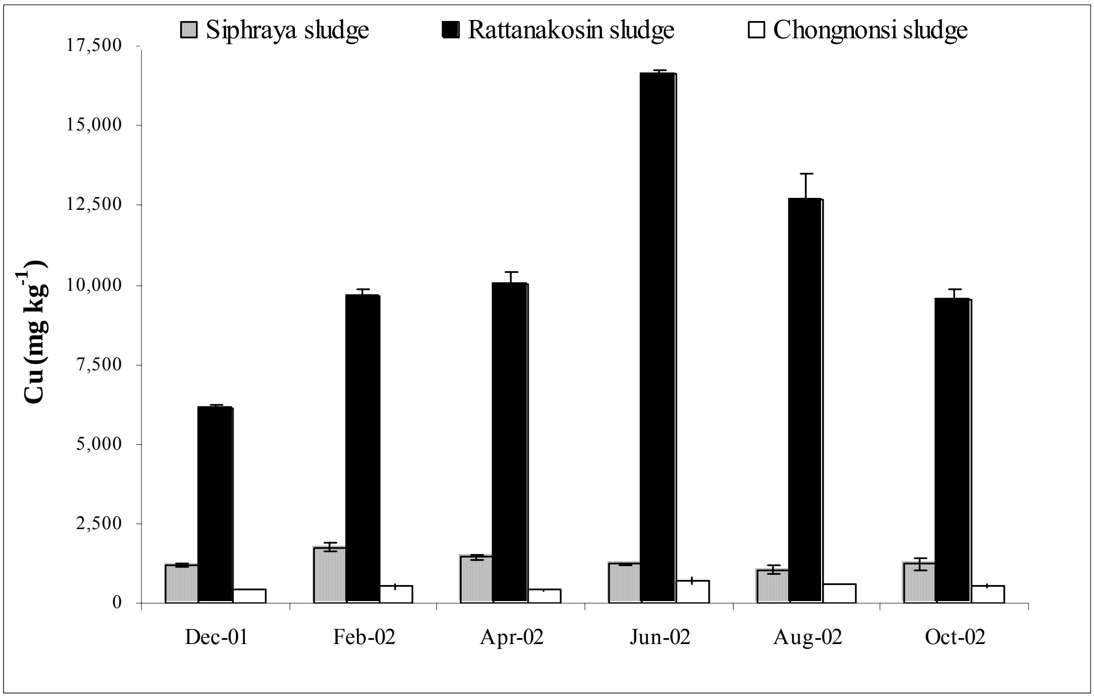


Figure 8 The amount of total Cu in the three sewage sludges taken from Bangkok wastewater treatment plants (Mean of 5 replications, bars indicate standard deviation).

Some important variations also were found in total Cd and Hg. Cadmium concentration levels in the three sludge samples were low from December to June (0.8 to 23.2 mg kg^{-1}). In August, Cd was still low in Siphraya sludge, but not in Rattanakosin and Chongnonsi where the Cd amount was higher than USEPA and EU regulations. The highest content of total Hg (within the range of 0.15 to 0.94 g kg^{-1}) was found in Siphraya sludge, but this range of concentration was still lower than USEPA and EU regulations.

1.3 Biological characteristics

The populations of total bacteria and fungi in all sludges ranged from 10^5 to 10^{10} and from 10^2 to 10^5 cells per gram of dry solid respectively. In the same samples, cellulolytic bacteria and fungi ranged from 10^4 to10^{10} and from 10^2 to10^5 cells per gram of dry solid respectively (data not presented). The microbial population may be increased in soil when sewage sludge is used for land application as organic carbon and nutrients are supplied to soil microorganisms (Lima *et al*., 1996). However, the presence of pathogenic microorganisms is one of the main limiting factors for land application of sludge. The types of pathogens may vary according to the season, possibly as a result of the seasonality associated with certain enteric infections (Toranzos and Macros, 2000). The presence of pathogenic microorganisms in sewage sludge indicates that an intrinsic public health risk exists following such land application. Faecal coliforms and *Escherichia coli* (*E. coli*) are enteric bacteria possibly present in wastewater and sewage sludge. The number of faecal coliforms and *E. coli* in sewage sludge from the Chongnonsi plant was very high. The Most Probable Number (MPN) was 10^7-10^{13} per gram of dry solid. Faecal coliforms in the sludge Siphraya and Rattanakosin were from 10^4 to 10^6 and from 10^3 to 10^5 per gram of dry solid respectively (Table 4). A USEPA regulation, under Title 40 - Code of Federal Regulations Part 503 (40 CFR 503) classifies sludge based on indicator bacteria. The class A standard is less than 1000 MPN faecal coliforms (including *E. coli*) per gram of dry sludge and class B standard is less than 3 million MPN coliforms per gram of dry sludge. Faecal coliform numbers in sewage sludge from Chongnonsi were higher than both of these two classes. For the same sampling period, Siphraya and Rattanakosin sludge pathogen contents were higher than Class A. Thus, the sludge from the three BMA sewage treatment plants studied should be stabilised to reduce the number of pathogens before land application.

Table 4 Population of some index of faecal contamination in the three sewage sludges (MPN g^{-1} dry weight).

Date	index of faecal contamination	Siphraya sludge	Rattanakosin sludge	Chongnonsi sludge
December 2001	**Faecal coliforms**	2.28×10^{5}	8.72×10^{5}	1.54×10^{12}
	E. coli	1.56×10^{5}	8.72×10^{5}	1.81×10^{12}
February2002	**Faecal coliforms**	6.96×10^{4}	1.51×10^{4}	1.14×10^{7}
	E. coli	5.88×10^{4}	3.24×10^{3}	1.09×10^{7}
April 2002	**Faecal coliforms**	7.02×10^{4}	1.5×10^{4}	1.6×10^{12}
	E. coli	4.76×10^{4}	3.14×10^{3}	9.37×10^{10}
June 2002	**Faecal coliforms**	2.86×10^{5}	1.51×10^{4}	4.74×10^{12}
	E. coli	1.82×10^{5}	3.14×10^{3}	4.74×10^{12}
August 2002	**Faecal coliforms**	2.4×10^{6}	5.03×10^{5}	4.4×10^{11}
	E. coli	2.3×10^{6}	4.97×10^{5}	4.4×10^{11}
October 2002	**Faecal coliforms**	3.88×10^{5}	3.22×10^{5}	2.36×10^{13}
	E. coli	4.29×10^{5}	3.04×10^{5}	2.58×10^{13}

1.4 Phytotoxicity

The percentage of Chinese cabbage seed germination in three sludge extracts was compared with a control (distilled water). The seed germination show no significant difference. According to Wang and Keturi (1990), measuring the seed germination rate of Chinese cabbage was regarded as a less sensitive method than root elongation when used as a bioassay for the evaluation of phytotoxicity. For root elongation, Chongnonsi sludge showed a highly significant difference from the water control, while Siphraya and Rattanakosin sludge extracts were not significantly different from the control. However, the average root elongation was 4.7, 5.0, 4.0 and 2.9 mm in the control, Siphraya, Rattanakosin and Chongnonsi sludge respectively. This indicates that Chongnonsi sludge should not be supplied to crops as fresh product, as it will reduce root growth and, as a consequence, the nutrient absorption of the crop will be reduced.

1.5 Discriminant analysis

Automatic classification confirms that the three stations (Siphraya, Rattanakosin and Chongnonsi) are different (more than 80% of the data of the same station are directed in the same group). In order to look for homogeneity within the sludge characterisation data, they have been compared using discriminant analysis (Figure 9). Three groups of data have been found, corresponding to the three plants, so that it was not possible to treat them as a whole. Rattanakosin was differentiated by EC values higher than the others. For Siphraya and Chongnonsi, pH and organic carbon levels explain the differences. It has not been possible to correlate the sewage sludge composition data with rainfall data. Even if part of the rainwater received on the zone is diluting the wastewater treated by the plants, the rainfall has not had a significant impact on the composition of the sewage sludge.

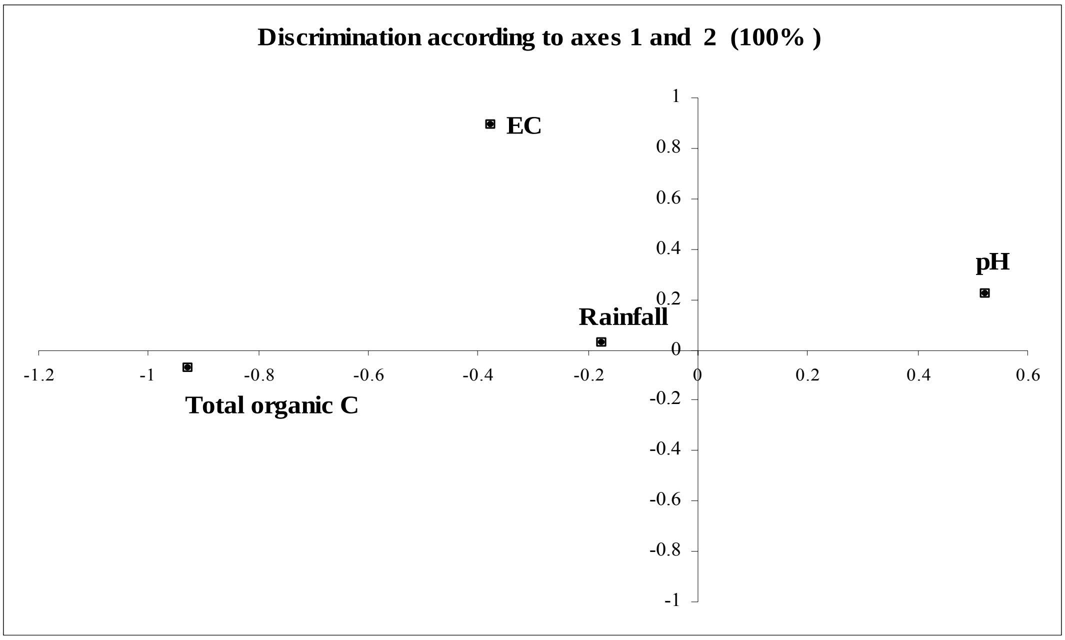


Figure 9 Heterogeneity of the sewage sludge characteristics data (EC, pH, Organic Carbon) from Bangkok wastewater treatment plants.

2. To study appropriate methods for composting sewage sludge, such as the use of suitable mixing material(s), effective microorganisms, as well as monitoring the decomposition process and the analysis of heavy metals present in the compost.

2.1 Suitable mixing materials

Dewatered sewage sludge from Siphraya and Rattanakosin treatment plants was mixed with organic materials, wood chips and rice husk. The ratios for each sludge and organic material mix were 85:15, 80:20, and 75:25 (w/w).

- Sole Siphraya sewage sludge (SS)
- Siphraya sewage sludge + wood chips at ratio 85:15 (w/w) (SSW1)
- Siphraya sewage sludge + wood chips at ratio 80:20 (w/w) (SSW2)
- Siphraya sewage sludge + wood chips at ratio 75:25 (w/w) (SSW3)
- Siphraya sewage sludge + rice husk at ratio 85:15 (w/w) (SSR1)
- Siphraya sewage sludge + rice husk at ratio 80:20 (w/w) (SSR2)
- Siphraya sewage sludge + rice husk at ratio 75:25 (w/w) (SSR3)
- Sole Rattanakosin sewage sludge (RS)
- Rattanakosin sewage sludge + wood chips at ratio 85:15 (w/w) (RSW1)
- Rattanakosin sewage sludge + wood chips at ratio 80:20 (w/w) (RSW2)
- Rattanakosin sewage sludge + wood chips at ratio 75:25 (w/w) (RSW3)
- Rattanakosin sewage sludge + rice husk at ratio 85:15 (w/w) (RSR1)
- Rattanakosin sewage sludge + rice husk at ratio 80:20 (w/w) (RSR2)

- Rattanakosin sewage sludge + rice husk at ratio 75:25 (w/w) (RSR3)

Triplicates of each mixture were collected during the incubation periods every week from 0 to 42 days. At the day 42, the mixture materials had a fine texture dark color. It often characterizes mature composts. Organic carbon and nitrogen content were measured in all samples.

The organic carbon content level decreased as the duration of incubation increased (Table 5, 6). The percentage of organic matter content decrease during Siphraya sewage sludge incubation was highest in pure sludge. The decrease of organic carbon in the mixing material at the ratio 80:20 of sludge and materials was the higher than at the other ratios.

In this experiment, the total nitrogen percentage decreased during the first seven days; thereafter, the N content increased in all the treatments (Table 7, 8). The increase in nitrogen content during the incubation process is in agreement with other studies. When organic matter is decomposing, carbon dioxide is released and, as N is expressed in percent of the dry weight, the N percentage is increased, as long as there is no N loss. Studies indicate that the nitrogen content shows a decrease during composting, mostly through ammonia volatilisation. Ammonia volatilisation during sludge composting might be caused by anaerobic conditions, alkalinity and a low C/N ratio.

This experiment was a preliminary test for the selection a suitable mixing ratio for the two materials, wood chips and rice husk. The decrease of organic carbon (OC) during 42 days of incubation was high in the 20% and 25% wood chip mixtures. The decrease of OC content was the highest in the 20% mixture with rice husk. Consequently, a level of 20% of wood chips and rice husk has been selected from this experiment to study the chemical and biological properties of the mixtures and the effect of a microbial activator on those properties during sewage sludge incubation.

Table 5 The changes of total carbon during Siphraya sewage sludge composting (%).

Treatments	Composting period (days)							%Decreasing
	0	**7**	**14**	**21**	**28**	**35**	**42**	
SS	31.99	30.16	29.10	29.35	28.22	27.91	27.11	15.25
SSW1	43.93	42.84	42.66	42.37	40.35	40.73	40.57	7.66
SSW2	45.74	45.00	45.14	42.27	43.48	41.26	41.88	8.44
SSW3	47.58	45.48	45.97	45.20	44.05	45.20	45.26	4.88
SSR1	39.30	38.72	36.59	35.42	35.30	35.24	34.23	12.90
SSR2	39.20	38.72	39.39	38.57	36.33	37.79	33.40	14.81
SSR3	39.67	39.34	37.99	34.99	34.93	38.99	35.74	9.92

Table 6 The changes of total carbon during Rattanakosin sewage sludge composting (%).

Treatments	Composting period (days)							%Decreasing
	0	**7**	**14**	**21**	**28**	**35**	**42**	
RS	24.61	22.23	20.08	20.95	20.51	19.60	20.12	18.25
RSW1	38.07	37.44	38.10	36.01	36.04	37.15	34.49	9.41
RSW2	40.69	38.73	41.74	36.78	41.87	40.17	36.23	10.97
RSW3	41.49	39.54	40.97	38.51	39.39	39.94	39.31	5.26
RSR1	32.65	32.28	32.73	31.24	30.65	32.53	30.77	5.75
RSR2	38.60	36.25	35.32	35.44	33.12	33.44	32.71	15.26
RSR3	37.23	35.48	36.04	32.49	32.49	37.14	32.85	11.76

Table 7 The changes of total nitrogen during Siphraya sewage sludge composting (%).

Treatments	Composting period (days)						
	0	**7**	**14**	**21**	**28**	**35**	**42**
SS	4.09	3.58	3.57	3.56	3.58	3.53	3.55
SSW1	2.31	2.1	2.09	2.03	2.36	2.41	2.07
SSW2	1.93	1.78	1.61	1.82	1.83	1.89	1.99
SSW3	1.63	1.5	1.44	1.52	1.52	1.71	1.78
SSR1	2.09	2.06	1.90	1.99	1.95	1.92	1.85
SSR2	2.03	1.71	2.04	2.09	2.08	2.05	2.05
SSR3	1.72	1.73	1.79	1.73	1.79	1.75	1.79

Table 8 The changes of total nitrogen during Rattanakosin sewage sludge composting(%).

Treatments	Composting period (days)						
	0	**7**	**14**	**21**	**28**	**35**	**42**
RS	3.04	2.74	2.67	2.76	2.98	2.87	2.85
RSW1	1.64	1.78	1.61	1.68	1.56	1.48	1.45
RSW2	1.51	1.48	1.51	1.54	1.63	1.56	1.58
RSW3	1.37	1.30	1.32	1.37	1.38	1.30	1.39
RSR1	1.75	1.66	1.60	1.63	1.55	1.55	1.58
RSR2	1.51	1.53	1.28	1.41	1.65	1.63	1.56
RSR3	1.41	1.25	1.17	1.19	1.49	1.50	1.41

2.2 The effective microorganisms

The microbial activator used in this experiment contains two bacteria (*Bacillus* sp), two actinomycetes (*Streptomyces* sp), and four fungi (*Scopulariopsis* sp., *Helicomyces* sp., *Chaetomium* sp. and *Trichoderma* sp.). Four organic matter mixtures were prepared in a laboratory. Sewage sludge was mixed with wood chips at a ratio of 80:20 (w/w) on a fresh weight basis, with and without a microbial activator: SWA (Sewage sludge + Wood chips + Activator), SW (Sewage sludge + Wood chips). The other treatments were sludge mixed with rice husk at a ratio of 80:20 (w/w) on a fresh weight basis, with and without a microbial activator: SRA (Sewage sludge + Rice husk + Activator), SR (Sewage sludge + Rice husk). Triplicates of each mixture were collected during the incubation periods from 0 to 63 days. At the day 63, the mixture materials had a fine texture dark color. It often characterizes mature composts. Organic carbon and nitrogen content were measured in all samples

2.2.1 Siphraya sewage sludge composting

2.2.1.1 Changes in pH

At the beginning of incubation, pH values varied between 6.6 and 6.7 in SW and SWA, 7.1 to 7.3 in SR and SRA (Table 9). The pH of woodchip mixtures was lower than the pH of those containing rice husk, due to the low pH of wood chips (pH= 4.6), but without the influence of an activator. From seven to fourteen days, the pH of all treatments rose to 7.8 in woodchip mixtures and and 7.7 in rice husk mixtures. The increase of value may be due to the release of ammonium and the nitrification at this stage of the incubation process as observed by some authors. After fourteen days of incubation, the pH had decreased slightly to 6.2-6.4 and on to the end of incubation (56 to 63 days). This pH evolution was not significantly different from one mixture to another, with or without an activator. In fact, these mixtures were within the pH range of municipal composts, from 5 to 8.

Table 9 pH change during Siphraya sludge incubation.

Treatments	Composting period (days)						
	0	**7**	**14**	**28**	**49**	**56**	**63**
SW	6.57	6.68	7.84	6.71	6.91	6.43	6.37
	(0.94)	(0.86)	(0.42)	(0.33)	(0.19)	(0.28)	(0.15)
SWA	6.68	6.65	7.67	6.77	7.11	6.25	6.27
	(0.77)	(0.22)	(0.56)	(0.30)	(0.23)	(0.18)	(0.16)
SR	7.13	6.99	7.39	6.91	6.96	6.11	6.25
	(0.73)	(0.72)	(0.36)	(0.36)	(0.51)	(0.12)	(0.03)
SRA	7.31	7.22	7.73	7.00	7.17	6.26	6.23
	(0.51)	(0.39)	(0.14)	(0.19)	(0.10)	(0.19)	(0.05)

2.2.1.2 Changes in organic matter, total nitrogen and the C/N ratio.

The agricultural by-products added for the purposes of experimentation noticeably increased the organic matter content of the sewage sludge from less than 40% to 70%; higher with wood chips than with rice husk. The organic matter content decreased with time (Table 10). The evolution of organic matter content during sewage sludge incubation was not significantly different between processes with activators and processes without activators. Theoretically, the organic matter content is significantly different according to the type of bulking agent added to the sewage sludge. Carbon is used as a source of energy for microbial activity, while nitrogen is a component of proteins, nucleic acids, amino acids, enzymes and co-enzymes.

In this experiment, the total nitrogen percentage decreased during the first seven days then later increased in all of the treatments. The measured increase in nitrogen content during the incubation process is in agreement with other studies. When organic matter is decomposing, carbon dioxide is released and, as N is expressed as a percentage of the dry weight, the N percentage increases as long as there is no loss. Studies indicate that the nitrogen content decreases during the composting process, mostly through ammonia volatilisation. Ammonia volatilisation during sludge composting might be caused by anaerobic conditions, alkalinity and a low C/N ratio. At the end of incubation, the total nitrogen content was not significantly different according to the bulking agent used or the use of an activator.

Table 10 Organic matter content (OM) and total nitrogen content (N) evolution during Siphraya sludge incubation.

Treatments		**Composting period (days)**						
		0	**7**	**14**	**28**	**49**	**56**	**63**
SW	OM	70.74	66.82	68.90	70.96	69.86	66.20	62.01
	N	1.72	1.63	1.83	1.91	1.75	2.03	1.91
SWA	OM	71.28	71.14	69.61	68.45	67.19	68.42	62.17
	N	1.78	1.64	1.72	1.79	1.84	1.88	2.00
SR	OM	66.87	60.71	66.24	59.78	58.55	59.99	60.25
	N	1.99	1.52	1.68	1.68	1.96	2.02	1.99
SRA	OM	67.10	65.25	63.52	61.09	58.97	60.19	60.84
	N	2.07	1.62	1.81	1.83	2.19	2.05	2.19

2.2.1.3 Changes in heavy metals.

Table 11 shows the comparison of total heavy metal concentrations for the Siphraya sludge mixtures and USEPA, EU regulation limits of sludge for land application. As a result, total heavy metal concentrations in Siphraya sewage sludge with mixture materials were lower than both regulation limits.

Table 11 Heavy metal contents in Siphraya sludge incubation(mg kg^{-1}).

Heavy metals	0 DY				63 DAYS				USEPA	EU
	SW	SWA	SR	SRA	SW	SWA	SR	SRA		
Fe	18.27	14.18	11.78	17.37	18.51	22.16	22.38	22.64	-	-
Mn	0.22	0.14	0.21	N.D.	0.12	0.12	0.28	0.23	-	-
Al	22.89	21.93	14.91	28.84	26.12	28.56	28.68	30.02	-	-
Cu	882.25	617.25	640.00	3,666	1,574	1,090	1,604	1,072	4,300	1,750
Zn	868.88	649.19	612.41	773.06	1,175	1,320	1,347	1,401	7,500	4,000
Pb	69.25	58.75	47.50	78.25	91.25	96.75	100.00	100.25	840	1,200
Ni	150.50	166.75	109.00	299.50	66.50	70.75	245.00	158.25	420	400
Cr	315.15	342.38	233.48	409.20	148.23	116.88	463.38	308.28	3,000	-
Cd	0.83	0.65	0.58	0.48	0.81	1.11	1.13	1.29	85	40

2.2.1.4 CO_2 respiration rate

The aerobic composting process produces CO_2 resulting from microbial decomposition of organic matter. Respirometry is a suitable method of measuring compost maturity. The CO_2 emission levels showed a significant peak during the initial seven days, as shown in Figure 10. During this period, the CO_2 came from the decomposition of easily available organic matter in the sewage sludge. Thereafter, the CO_2 emission level decreased in all treatments. The treatment using an activator showed similar rates of CO_2 to the one without an activator up until day 28; the rate of CO_2 emission for SS + wood chips and an activator was significantly higher than for other treatments, whilst at the same time the treatment with rice husk and an activator was significantly lower. During the three last dates at the end of the incubation period, sewage sludge with wood chips had respiration levels lower than with rice husk, showing more stability than rice husk mixtures. CO_2 emission in the mature compost was significantly lower than in raw mixtures. However, different types of compost contained various organic inputs and therefore the rates of microbial activity were different.

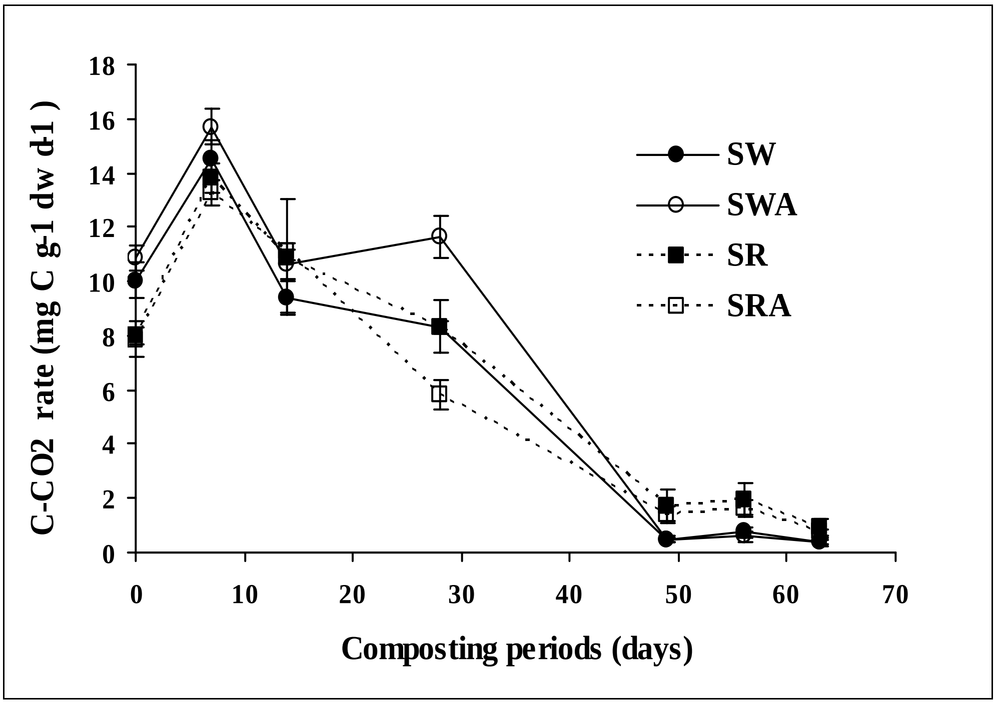


Figure 10 The CO_2 emission rate during thermophilic incubation of Siphraya sludge mixed with organic products and activator.

2.2.1.5 Enzyme activity

The cellulase activity in all treatments revealed two distinctly different phases (Figure 11). Within the first phase, cellulase activity was weak at the beginning of the incubation period. After this initial lag phase, activity increased rapidly to the maximum concentration obtained at 28 days. Thereafter, cellulase activity slightly decreased and then remained almost constant for all treatments. In the early stage, the soluble substances, including soluble sugar, and organic acids present in the sewage sludge were decomposed. The high concentration of enzymes in the later stage should be an indication of the decomposition of complex substances, such as cellulose.

Xylanase activity within the organic matter mixtures was low at the beginning of the incubation period (Figure 12). The activity of xylanase increased rapidly just after the beginning of incubation and did not decrease after that. The activities of both enzymes within the four treatments were not much different. The trends of enzyme activity matched the CO_2 evolution during the first 28 days of incubation, as the enzymes' activities were closely related to microbial activity. However, after 28 days the level of CO_2 was significantly lower, but enzyme activity remained at the same level; this might be due to the fact that these were extracellular enzymes.

Phosphatase is an enzyme which uses hydrolysis in the process of dephosphorylation of an organic phosphorus compound into an inorganic form available to plants. Phosphatase activity was high at the beginning of mixture incubation, reaching maximum activity at day 28 (Figure 13). This result agrees with the measurement of enzyme activity during manure composting, even though this paper indicates that the activity is maintained at the same level throughout the period of composting. In this experiment, a decrease in phosphatase activity was observed in the later stage (after 28 days), similar to alkaline phosphatase activity in fly ash compost where activity decreased as composting time increased.

After fourteen days of incubation, enzyme activity in the woodchip mixture was higher than with rice husk, either with or without an activator. This may indicate that the composition of the bulking agent could affect some enzyme activity. Even where the P content in wood chips was low, there was a higher level of enzymes and organic matter than those in rice husk. The C/N ratio calculated for wood chips and for rice husk were 342 and 103 respectively. Both materials contained cellulose and hemicellulose at almost the same levels, but the mineral (ash) content was higher in rice husk (high level of silica) when compared to wood residues.

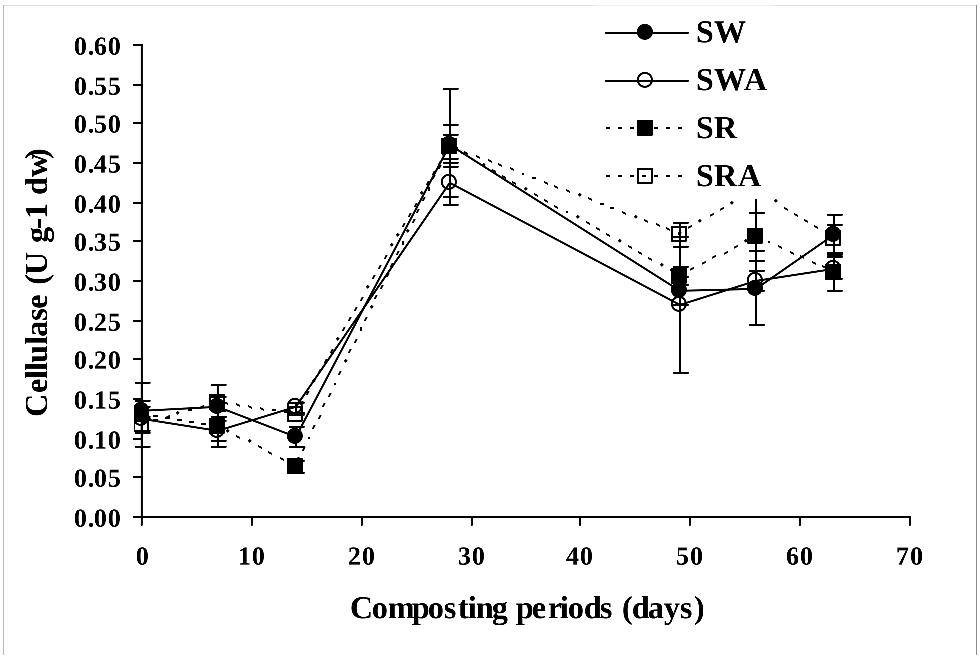


Figure 11 Evaluation of cellulase activity during Siphraya sludge incubation period.

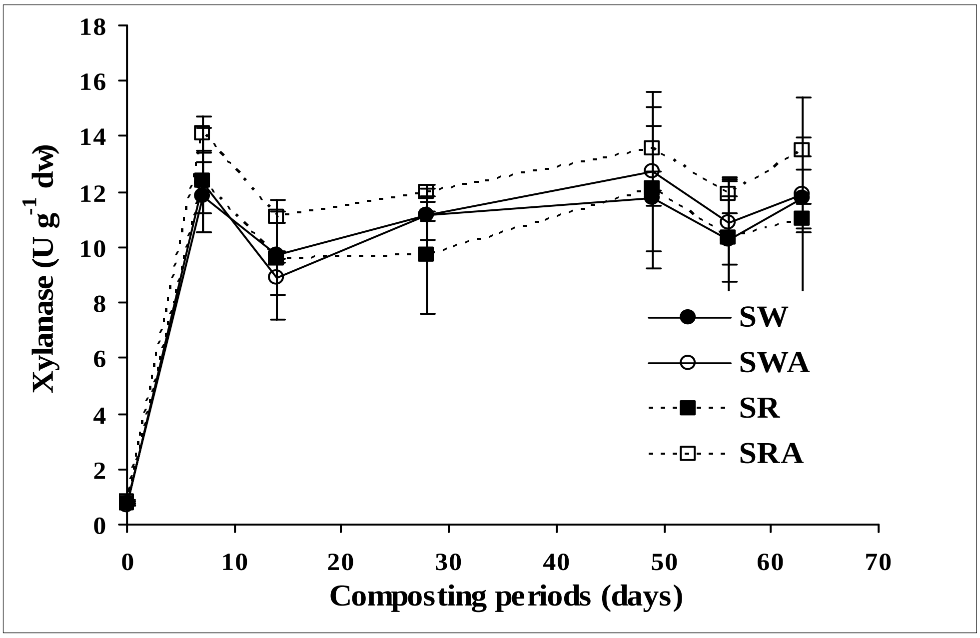


Figure 12 Evaluation of xylanase activity during Siphraya sludge incubation period.

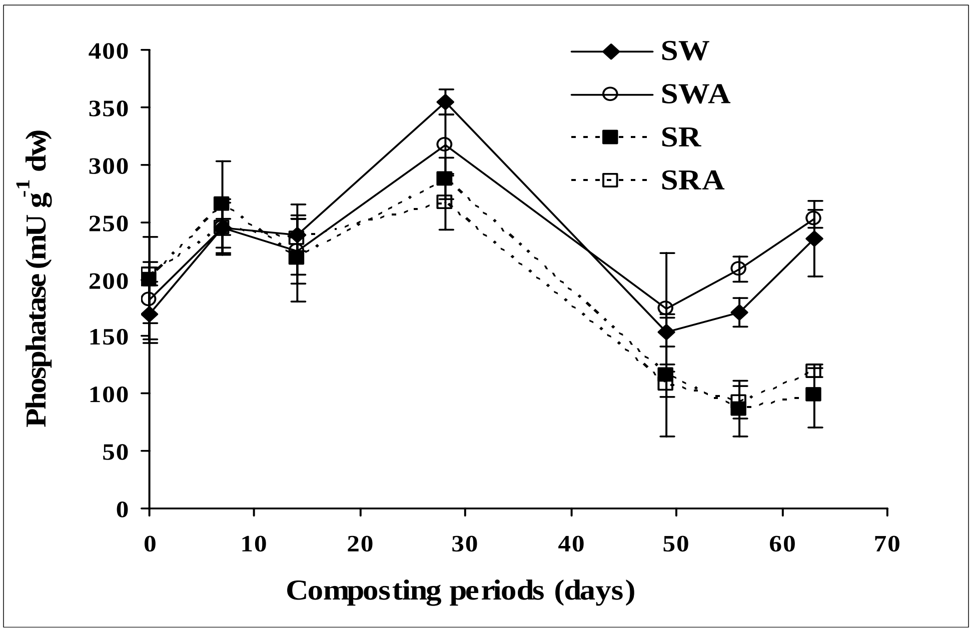


Figure 13 Evaluation of phosphatase activity during Siphraya sludge incubation period.

2.2.1.6 Microbial population

The microbial populations contained in the sewage sludge mixtures showed a higher level of bacteria in comparison to fungi at the beginning of incubation in all treatments (Table 12). During the first fourteen days, cellulolytic bacteria levels fluctuated in all treatments. The treatment with an activator had a higher bacteria population than treatments without an activator, indicating that the strains released by the activator proliferated in the mixtures. After day fourteen, bacteria levels were seen to decrease in both woodchip mixtures, but not in the rice husk mixtures. The bacteria population level was low at the end of incubation. During this experiment, the thermophilic cellulolytic fungi population was lower than the bacteria population. This might be due to the fact that bacteria can have a rapid transfer of soluble substrates (Haug, 1993). Another hypothesis could be that the temperature of 55°C was unfavourable to the growth of thermophilic fungi, which have an optimum temperature of 40 to 50°C. Fungi population changes were similar to bacteria evolution, fluctuating during the first fourteen days and then slightly decreasing until the end of the experiment with very heterogeneous counting results when compared to bacteria counts. Moreover, no correlation has been observed between enzyme activity and thermophilic microorganism levels during composting.

Table 12 Thermophilic bacteria cellulolytic (TBC) and fungi (TFC) evolution during Siphraya sludge incubation.

Treatments	Composting period (days)						
	0	7	14	28	49	56	63
TBC_SW	7.66 (0.46)	7.29 (0.68)	7.18 (0.58)	6.63 (0.97)	6.79 (0.81)	6.72 (0.90)	6.50 (0.92)
TBC_SWA	8.28 (0.44)	8.42 (0.60)	7.32 (0.51)	6.87 (0.54)	6.34 (0.49)	7.54 (0.45)	5.52 (0.28)
TBC_SR	7.48 (0.94)	8.91 (0.62)	6.45 (0.31)	7.32 (0.92)	7.92 (0.95)	8.02 (0.97)	6.91 (0.96)
TBC_SRA	8.85 (0.52)	8.86 (0.30)	8.50 (0.64)	7.38 (0.98)	8.21 (0.21)	8.28 (0.20)	7.70 (0.46)
TFC_SW	3.18 (0.14)	3.97 (0.19)	4.03 (0.21)	3.44 (0.30)	3.35 (0.01)	2.36 (0.05)	1.88 (0.11)
TFC_SWA	4.73 (0.30)	3.93 (0.14)	4.92 (0.07)	3.58 (0.30)	3.25 (0.06)	2.48 (0.02)	1.56 (0.07)
TFC_SR	5.33 (0.62)	5.36 (0.54)	4.80 (0.32)	3.61 (0.33)	3.27 (0.66)	2.71 (0.19)	1.89 (0.06)
TFC_SRA	5.22 (0.42)	4.78 (0.49)	4.78 (0.09)	4.06 (0.40)	3.36 (0.54)	3.04 (0.04)	2.70 (0.37)

2.2.2 Rattanakosin sewage sludge composting

2.2.2.1 Changes in pH

Increases in the pH of Rattanakosin sludge mixtures were observed in the period between seven and fourteen days of incubation (Table 13). The increase of value may be due to nitrification and the release of ammonium. After fourteen days of incubation, the pH slightly decreased in all mixtures. This pH evolution was not significantly different from one mixture to another, with or without an activator.

Table 13 pH change during Rattanakosin sludge incubation.

Treatment	Composting period (days)						
	0	**7**	**14**	**28**	**49**	**56**	**63**
RW	5.51	6.93	7.11	6.66	7.00	6.23	6.23
RWA	5.62	6.95	6.74	6.47	6.76	6.22	6.22
RR	5.48	7.40	7.31	6.53	6.77	6.11	6.14
RRA	5.46	6.82	7.25	6.49	6.66	6.19	6.15

2.2.2.2 Changes in organic matter, total nitrogen

The level of organic matter content decreased as the incubation period increased (Table 14). The evolution of the organic matter content during sewage sludge incubation tended to increase in the sludge mix containing an activator. The organic matter content was significantly different according to the bulking agent added to the sewage sludge. Woodchip mixtures had higher organic matter content levels than rice husk mixtures.

In this experiment, the total nitrogen content varied between 1.2 to 1.9%. The percentage of nitrogen evolution did not differ in the mixtures. However, at the end of incubation the nitrogen content was more than 1%, noticeably higher than compost made from other agricultural products.

Table 14 Organic matter content (OM) and total nitrogen content (N) evolution during Rattanakosin sludge incubation(%).

Treatment		Composting period (days)						
		0	7	14	28	49	56	63
SW	OM	54.70	54.17	56.17	54.31	52.88	54.12	49.10
	N	1.41	1.48	1.48	1.41	1.43	1.28	1.46
SWA	OM	53.62	51.62	52.01	52.77	55.77	50.84	47.41
	N	1.34	1.46	1.38	1.48	1.94	1.25	1.51
SR	OM	56.55	54.84	50.72	50.53	50.15	47.23	45.81
	N	1.58	1.62	1.76	1.76	1.77	1.48	1.62
SRA	OM	55.14	54.60	50.15	50.70	45.36	46.65	44.57
	N	1.50	1.56	1.40	1.59	1.89	1.62	1.50

2.2.2.3 Changes in heavy metals.

Table 15 shows the comparison of total heavy metal concentrations for Rattanakosin sludge mixtures and USEPA, EU regulation limits of sludge for land application. As a result, total heavy metal concentrations in Rattanakosin sewage sludge with mixture materials were lower than both regulation limits, except for copper concentrations according to the the high Cu content in Rattanakosin sludge (highest every month).

Table 15 Heavy metal contents in Rattanakosin sludge incubation(mg kg^{-1}).

Heavy metals	0 DY				63 DAYS				USEPA	EU
	RW	RWA	RR	RRA	RW	RWA	RR	RRA		
Fe	17.18	18.76	17.33	15.35	25.87	25.34	26.26	29.29	-	-
Mn	0.30	0.44	0.60	0.40	1.64	1.46	1.67	1.77	-	-
Al	28.36	38.09	29.89	29.99	46.24	44.86	46.07	52.98	-	-
Cu	11,424	8,249	11,874	3,859	11,274	11,424	12,274	13,449	4,300	1,750
Zn	1,088	910.88	1,142	700.09	1,683	1,672	1,730	1,960	7,500	4,000
Pb	125.00	104.00	133.50	73.25	190.25	190.50	199.00	227.50	840	1,200
Ni	395.00	191.50	300.25	185.25	290.00	322.25	448.25	460.50	420	400
Cr	487.03	203.23	392.43	338.53	262.35	278.30	451.83	403.43	3,000	-
Cd	0.39	0.30	0.40	0.41	0.56	0.59	0.56	0.61	85	40

2.2.2.4 Changes in CO_2 emission rates

CO_2 production was caused by the mineralisation of the compost's organic matter (Bernal *et al*., 1998). During the first seven days of incubation, levels of CO_2 increased immediately as a result of the decomposition of easily available organic matter in the sludge. However, the lower level of CO_2 emission in the Rattanakosin sludge mixture, in comparison to the Siphraya sludge mixture, was due to the higher levels of organic matter content in Siphraya sludge. After ten days of incubation, the measured decrease of CO_2 emissions mainly resulted from the decomposition of cellulose and lignin in the organic material. The treatment using an activator showed a higher a rate of CO_2 emission than the ones without an activator, especially in the rice husk mixture (Figure 14).

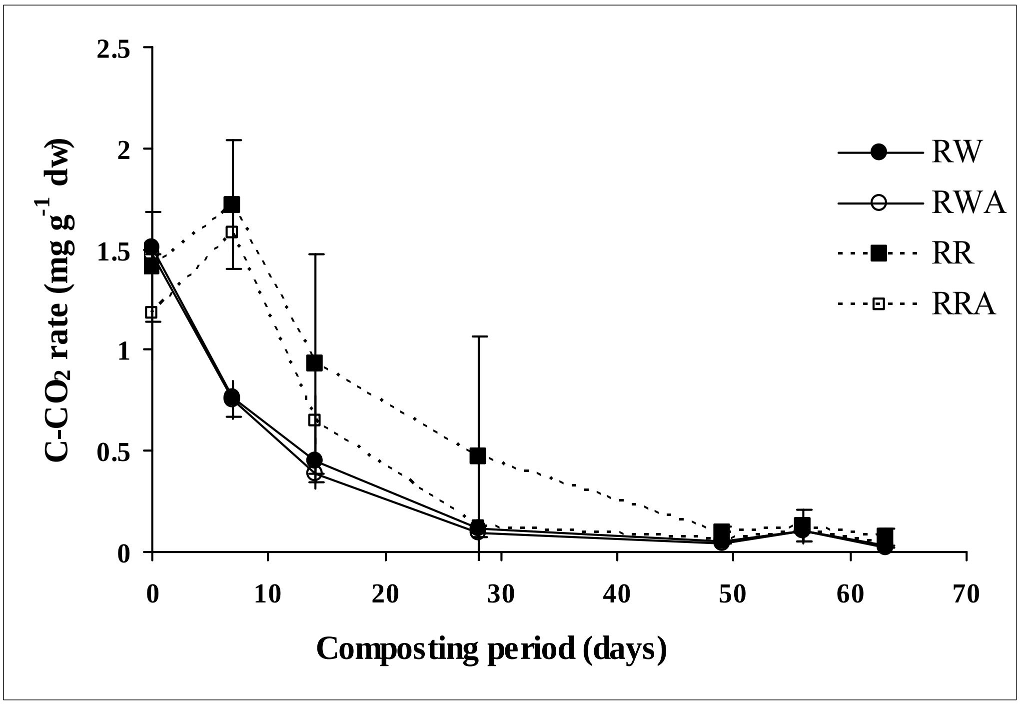


Figure 14 The CO_2 emission rate during thermophillic incubation of Rattanakosin sludge mixed with organic products and activator.

2.2.2.5 Enzyme activity

The cellulase system consists of three general classes of enzymes: cellobiohydrolases, endoglucanases and fl-glucosidase, which together act synergistically to convert cellulose to glucose. Cellulase activity in all treatments showed two distinctly different phases (Figure 15) similar to trends observed in Siphraya sludge composting. Within the first phase, cellulase activity was weak at the beginning of incubation until, after ten days, it increased rapidly to the maximum concentration obtained at 28 days. Following that, cellulase activity slightly decreased and thereafter remained almost constant for all treatments. The cellulase activity during sewage sludge composting when using an activator was higher than without using one, because the microbial activators were the effective cellulolytic microorganisms.

The trends of xylanase activity were shown to correspond to cellulase activity (Figure 16). However, xylanase activity was higher than cellulose activity. According to previous research (Sarkar and Prabhu, 1982), the decomposition rate of hemicellulose was higher than that of cellulose.

Phosphatase activity in all treatments decreased markedly with an increase in the composting time (Figure 17). The rice husk mixture appeared to have a more adverse effect on phosphatase activity than the woodchip mixture. Also, the treatments using activators demonstrated higher phosphatase activity, which was mainly due to the activity of the thermophilic microorganisms in the activator.

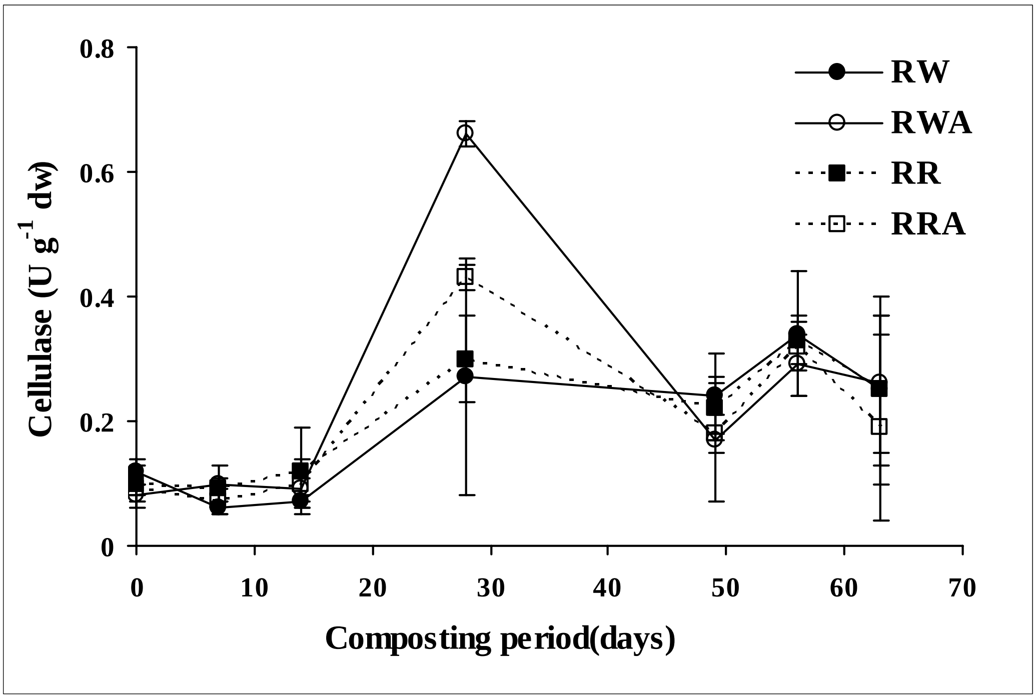


Figure 15 Evaluation of cellulase activity during Rattanakosin sludge incubation period.

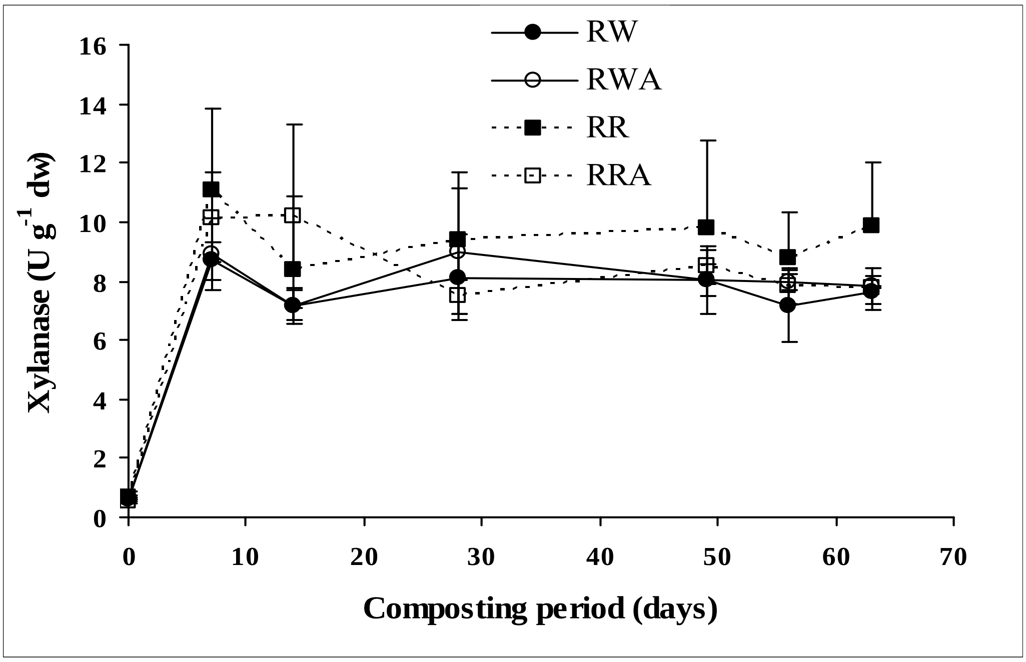


Figure 16 Evaluation of xylanase activity during Rattanakosin sludge incubation period.

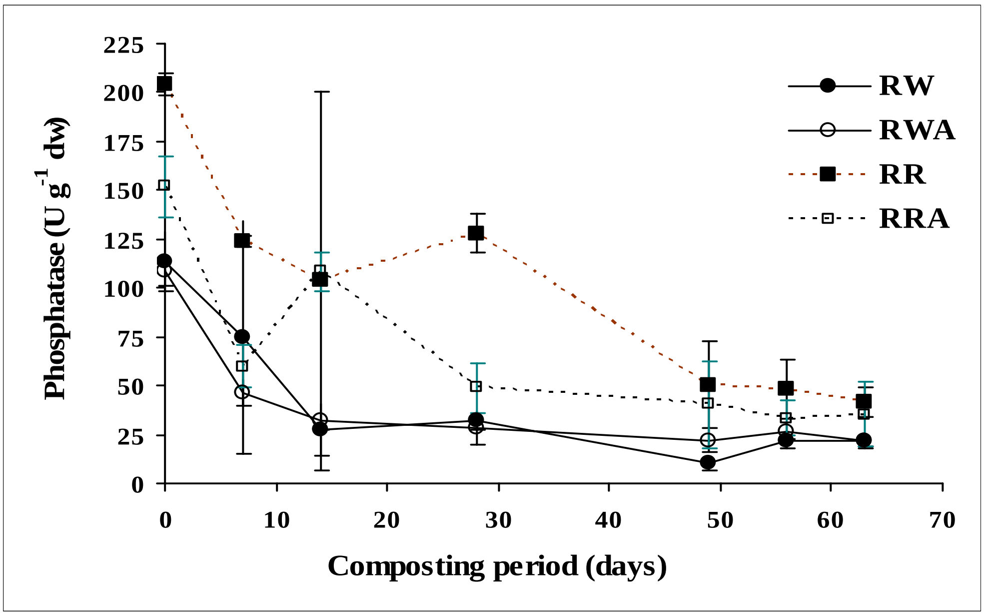


Figure 17 Evaluation of phosphatase activity during Rattanakosin sludge incubation period.

2.2.2.6 Microbial population

Microbial populations contained in the Rattanakosin sewage sludge mixtures showed a higher level of bacteria compared to fungi at the beginning of incubation in all treatments (Table 16), indicating that the easily decomposable organic matter was used primarily by bacteria and then followed by the growth of fungi. During the first fourteen days, levels of cellulolytic bacteria tended to increase in all treatments. The treatment with an activator had a higher bacteria population than without an activator, indicating that the strains released by the activator were living in the mixtures. After the fourteenth day, a decrease was observed in all mixtures.

Table 16 Thermophilic bacteria cellulolytic (TBC) and fungi (TFC) evolution of Rattanakosin sludge incubation.

Treatments	**Composting period (days)**						
	0	**7**	**14**	**28**	**49**	**56**	**63**
TBC_RW	6.25 (0.05)	7.75 (1.20)	5.71 (0.24)	6.11 (0.79)	5.88 (0.80)	5.66 (0.13)	4.84 (0.12)
TBC_RWA	6.69 (0.62)	8.17 (1.17)	7.24 (1.36)	5.39 (0.51)	5.23 (0.24)	5.34 (0.28)	4.19 (0.85)
TBC_RR	7.79 (1.59)	6.50 (0.42)	7.07 (1.60)	6.51 (0.68)	6.15 (1.57)	6.69 (1.49)	6.06 (1.67)
TBC_RRA	7.46 (0.43)	6.50 (0.31)	7.00 (0.17)	3.89 (0.44)	5.97 (0.52)	5.32 (0.12)	5.04 (0.08)
TFC_RW	3.63 (0.17)	3.64 (0.10)	3.69 (0.10)	1.98 (0.73)	1.20 (0.01)	0.83 (0.43)	0.57 (0.98ป
TFC_RWA	3.70 (0.06)	4.03 (0.47)	3.40 (0.15)	2.03 (0.79)	0.84 (0.01)	1.84 (0.62)	0.90 (0.56)
TFC_RR	5.18 (0.53)	5.07 (1.13)	5.07 (0.26)	4.04 (0.24)	3.03 (0.34)	1.93 (0.73)	1.60 (0.46)
TFC_RRA	4.86 (0.56)	5.33 (0.42)	5.06 (0.58)	4.09 (0.11)	2.05 ((0.80)	0.77 (0.33)	1.28 (0.11)

3. To study the methods for vermicomposting of sewage sludge

3.1 Growth of earthworms

The changes in worm biomass and total number of worms for all mixtures over the observation period are shown in Table 17. The total biomass of earthworms showed a significant increase from day one to day twenty. The highest increase occurred in treatment four. The increase was 52.1% and 66.0% in the inoculated earthworm mixture, with and without PD1 respectively. However, their growth rates in the corresponding twenty day period were 1.26 and 1.28, respectively.

Dominguez *et al*. (2001) reported that the total biomass production per mature worm at 25°C was 1.38. However, the maximum weight gain of *E. eugeniae* was 280 mg per week. This paper showed evidence of weight gain at 420 and 426 mg per week. Thus, these results showed that both sludge mixtures, with and without PD1, seem to be suitable growth mediums for earthworms. These mixtures probably provide earthworms with organic matter which is easily metabolised, and nutrients in the sludge that favour the growth of worms. However, the total number of earthworms in the mixtures was not much changed in the period between one and twenty days.

Table 17 Growth of earthworm (*Eudrilus euginiae*).

Treatments	**0 day**		**20 days**	
	Total number of earthworms	**Total weight of earthworms(g)**	**Total number of earthworms**	**Total weight of earthworms(g)**
SWA	0	0	0	0
SWAE	10.25	8.88	10.75	13.54
SW	0	0	0	0
SWE	11.25	8.880	11.50	14.75

3.2 Changes in chemical properties

The total organic carbon (TOC) content decreased in all treatments, indicating mineralisation of the organic matter (Table18). The decrease in the TOC content of treatments two and four were 7.27% and 10.16%, respectively. However, the results from the control sample (without worms) indicated a much lesser reduction in the TOC. Total N content ranged from 1.31 – 1.71%. The C/N ratio is one of the indicative parameters of the decomposition process (Chen and Inbar, 1993). It usually decreases during the decomposition process, as a result of carbon respiration. The C/N ratio decreased from 16.32 to 14.73 and 14.37 to 14.14 in treatments two and four, respectively. The C/N ratio remained unchanged in the treatment without worms.

Table 18 Changes in total organic carbon content (TOC), total nitrogen content (N) and C/N ratio.

Treatment	0 day			20 days		
	TOC (%)	**N (%)**	**C/N**	**TOC (%)**	**N (%)**	**C/N**
SWA	22.01	1.35	16.32	21.47	1.31	16.46
SWAE	22.01	1.35	16.32	20.41	1.38	14.73
SW	24.49	1.71	14.37	23.98	1.67	14.40
SWE	24.49	1.71	14.37	22.00	1.55	14.14

3.3 Changes in biological properties

3.3.1 Enzymatic activity

Changes in enzyme activity throughout the vermicomposting process are reported in Figures 18, 19 and 20. The high initial activity of these enzymes is reflected in the high microbial activity (Benitez *et al.,* 1999). The presence of a high content of degradable organic compounds (available substrate) in the sewage sludge might have stimulated enzyme synthesis; as the amount of available substrate decreased, the level of enzyme activity decreased accordingly (Ceccanti and Garcia, 1994). Reported levels of cellulase and xylanase activity, which are extracellular enzymes, showed a decrease during vermicomposting in the treatment with inoculated earthworms. However, the addition of an activator had no measured effect on these particular enzyme's activities.

Dehydrogenase activity in soils and other biological systems has been used as a measure of overall microbial activity (Garcia *et al.*, 1997). These results showed a decrease of dehydrogenase activity. According to Benitez *et al.* (1999), after two weeks, dehydrogenase activity decreased sharply. The stabilisation of dehydrogenase activity would indicate that most of the easily available organic matter was decomposed during the early stages of the vermicomposting process.

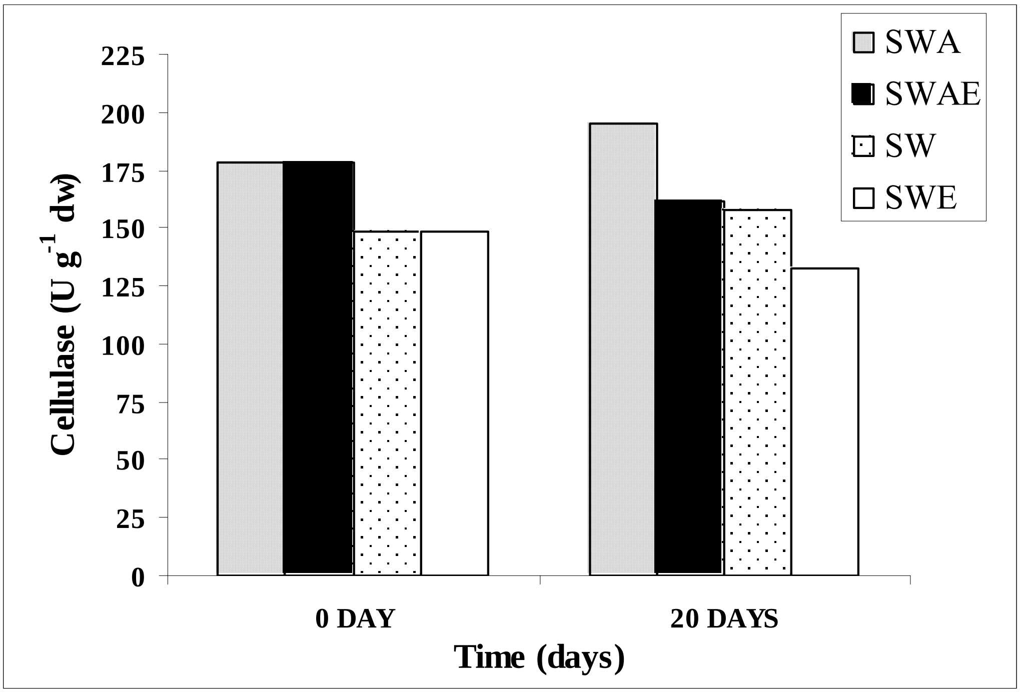


Figure 18 Changes in cellulase activities during vermicomposting.

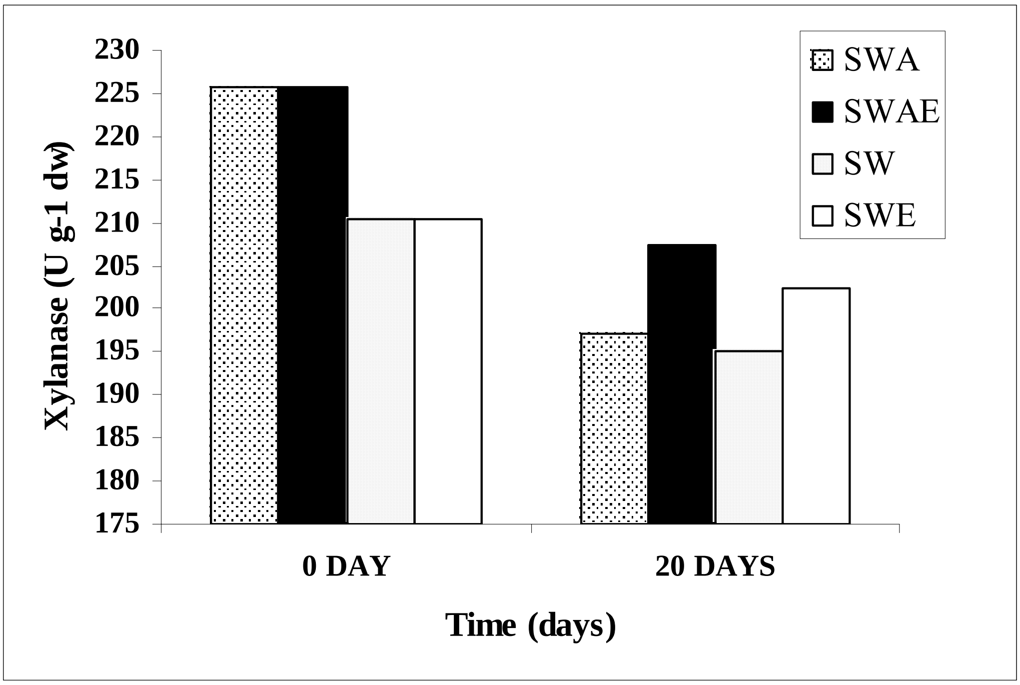


Figure 19 Changes in xylanase activities during vermicomposting.

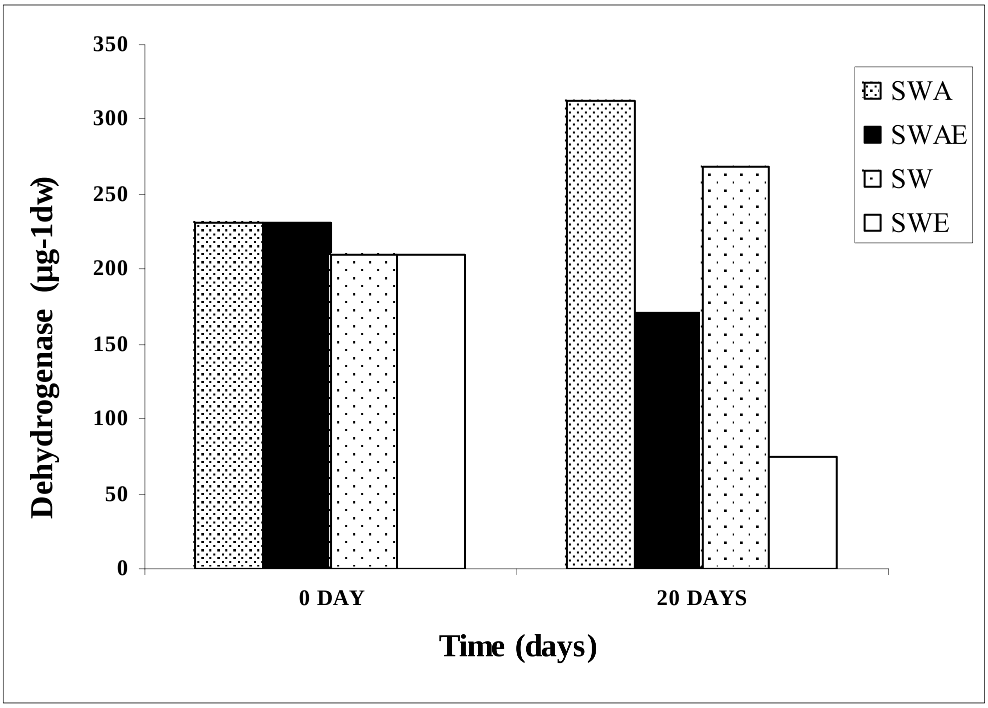


Figure 20 Changes in dehydrogenase activities during vermicomposting.

3.3.2 Microbial population

Total numbers of actinomycetes, bacteria and fungi tended to decrease in the initial period up to the twentieth day of vermicomposting. However, the measured decrease of microbial activity in the treatments without worms was higher than the treatments with worms. Sewage sludge usually contains some pathogens. Faecal coliforms were used as indicators of sludge contamination. Faecal coliform levels in the initial vermicomposting were 5.71 and 4.58 log MPN gram^{-1} in treatments with an activator and without an activator respectively. These population levels subsequently decreased to 4.20, 3.70, 3.92 and 2.00 in treatments one, two, three and four respectively at day twenty of the experiment. The results showed a significant reduction in the presence of faecal coliforms using vermicomposting, because the amount of reduction of pathogens in the treatments with earthworms was higher than those without earthworms.

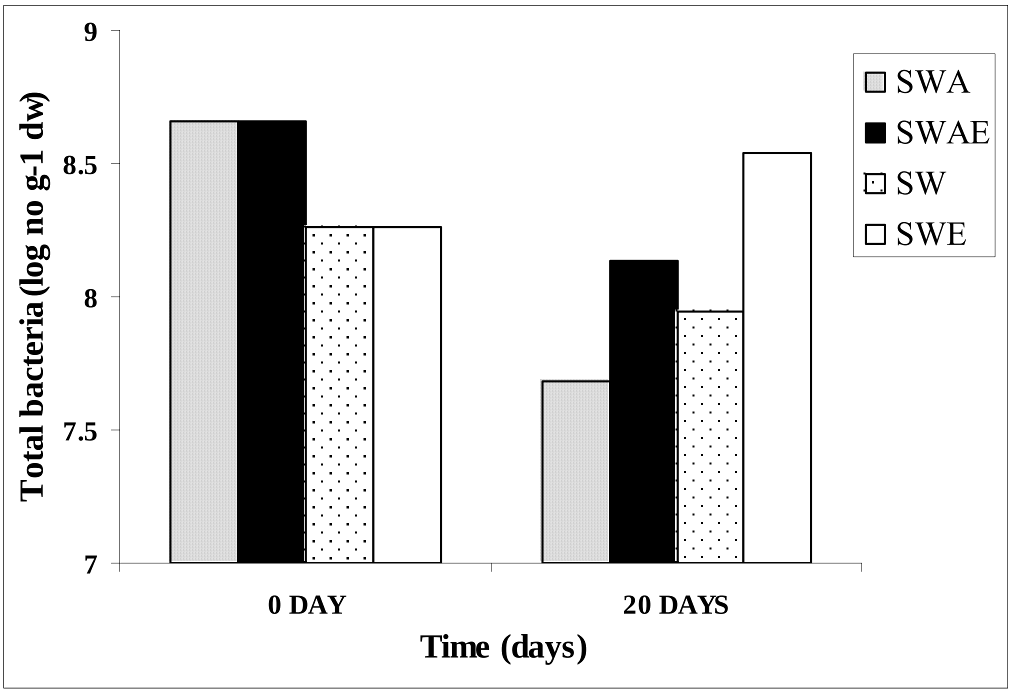


Figure 21 Changes in bacteria population during vermicomposting.

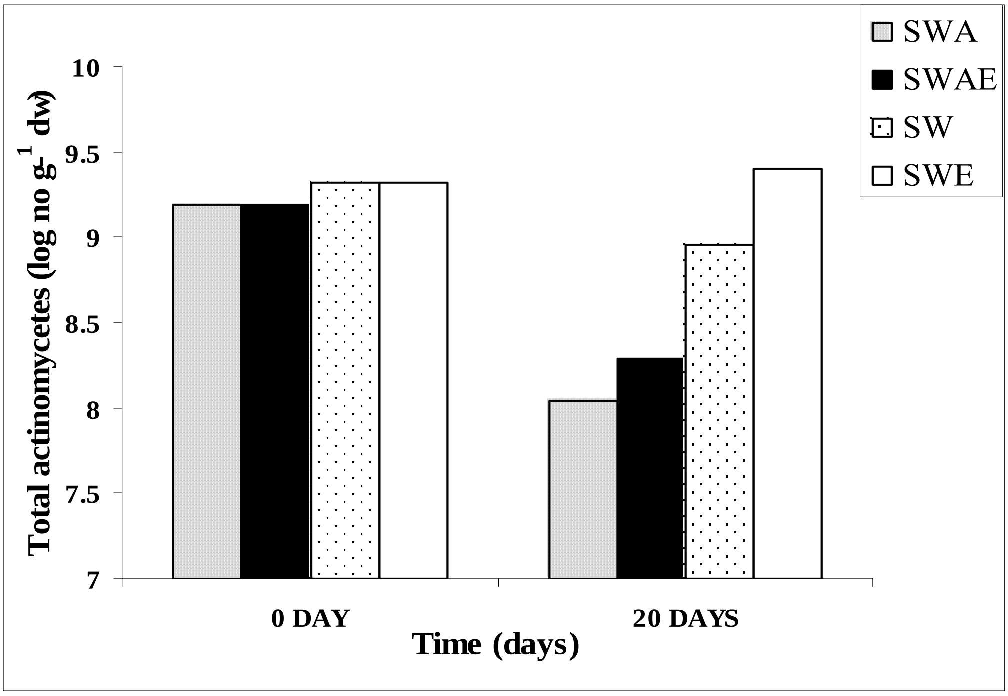


Figure 22 Changes in actinomycetes population during vermicomposting.

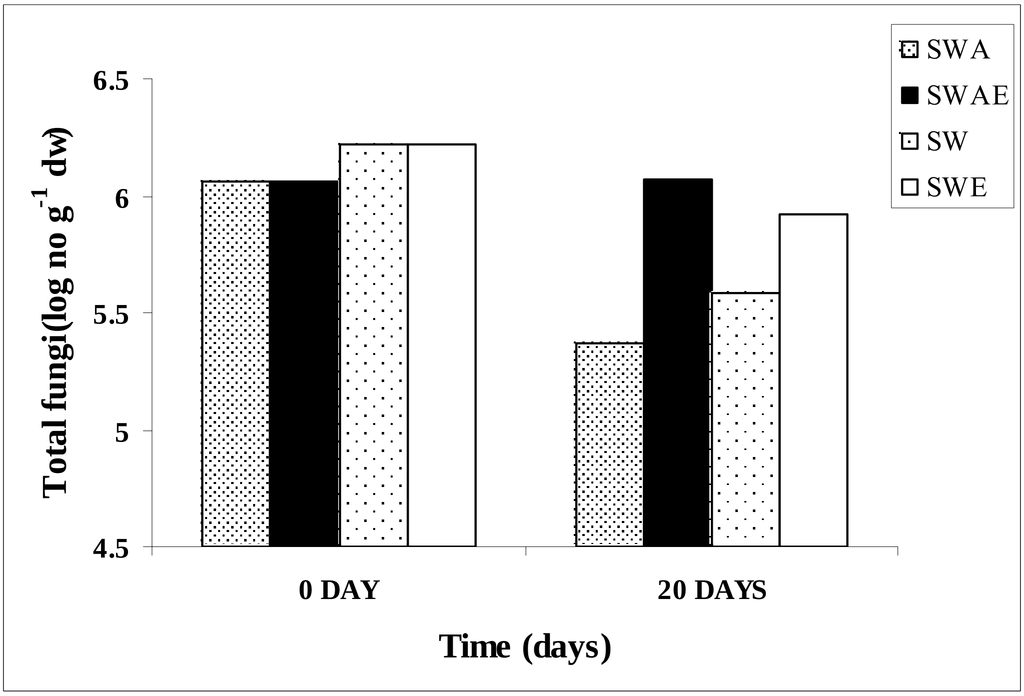


Figure 23 Changes in fungi population during vermicomposting.

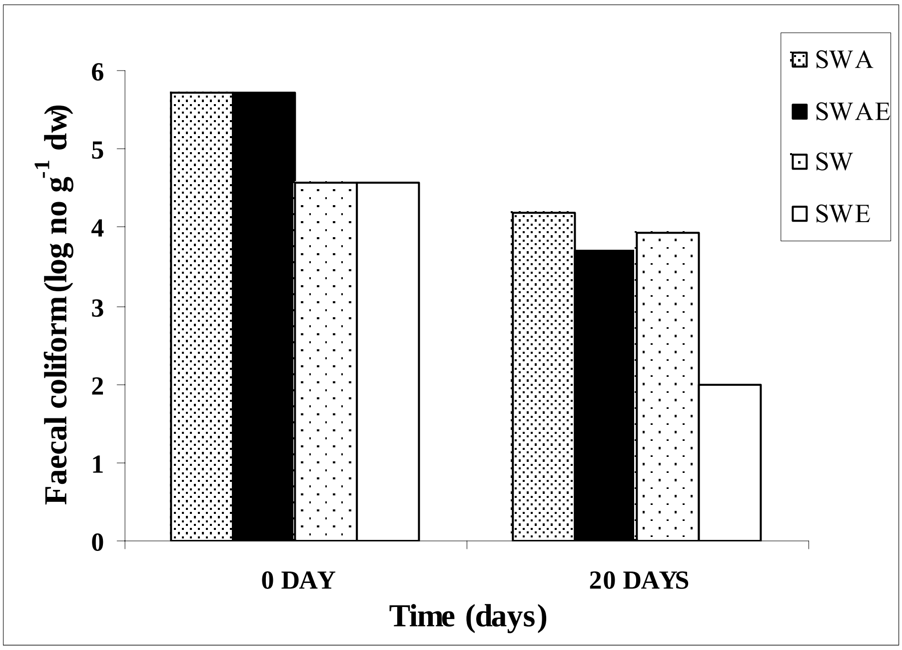


Figure 24 Changes in faecal coliform population during vermicomposting.

CONCLUSION

Using information obtained through determining characteristics on a monthly basis (pH, electrical conductivity and total organic carbon), statistical analysis showed that the sewage sludge samples taken from the three wastewater treatment plants in Bangkok (Siphraya, Rattanakosin and Chongnonsi) were notably different. This is certainly representative of the variability at source of wastewater collection for these treatment plants, particularly concerning the presence of heavy metals (i.e. for all sampling periods, the concentration of copper in Rattanakosin sludge was higher than the USEPA and EU concentration limits of sludge for agricultural application) and faecal contamination, particularly so in the case of Chongnonsi. Analysis of the rainfall cycle and measurements cannot explain the changes to the sludge characteristics. As a consequence, these particular types of sewage sludge cannot be considered as being a beneficially homogeneous component as far as agricultural disposal is concerned. The results of monthly analyses showed trends of evolution of the characteristics for each site. Total N content ranged from 19.7 to 38.7 g kg^{-1} in the three types of sludge. Total N content was the highest in Siphraya sludge according to the hightest in the influent of Siphraya plant. The organic matter content was low (15 to 40%); in accordance with regulations, these kinds of sewage sludge cannot be considered as organic amendments. The total concentrations of four heavy metals namely Cu, Fe, Ni and Pb were much more obvious in Rattanakosin sludge. The Cu content in Rattanakosin sludge was far higher than the other two sludges and was higher than USEPA and EU regulation limits at every sampling time. This might indicate the presence of some small industrial effluent within the zone and commercial small industrial zone where the silverware and copperware plants a located.

In order to improve the value of the sludge from these locations and to use it for agricultural purposes without harmful problems, the sludge must be heated to kill the faecal coliforms (Siphraya and Rattanakosin sludge had faecal coliforms and *E. coli* contents exceeding the USEPA class A standard. Chongnonsi sludge had levels of those pathogens exceeding class B). Lowering the level of faecal contamination can be achieved through composting. However, the average C/N ratio (organic C / total N)was near 6, which is too low for good decomposition. Mixtures with high organic carbon content materials are needed to improve the C/N ratio in order to allow for good decomposition of the organic matter and a reduction of faecal contamination. The impact of the percentage of sewage sludge within the compost mixtures should be measured to assess the level of phytotoxicity when Chongnonsi sludge is used. Composting sewage sludge with organic by-products could be a good way to improve the quality of the final product in order for it to be utilised for agricultural purposes. This experiment showed that mixing wood chips or rice husk with sewage sludge increased the organic matter content of the mixtures (from less than 40 to 70%).

Regarding the choice of agricultural by-product to use as an organic bulking agent, after 63 days of incubation, this experiment showed little difference in organic matter evolution and a higher rate of decomposition with woodchip mixtures. After 63 days of thermophilic conditions, the level of carbon was still high. Incorporating such a by-product might be a good way of improving the organic status of soils. There is a reduced risk of nitrogen starvation in the soil when incorporating such a stable carbon product compared to easily decomposable organic waste.

Even though the microbial activator(LDD 1) used in this experiment gave good results in accelerating the decomposition of agricultural by-products at farm level, when mixtures to be composted contained sewage sludge and rice husk or wood chips, the rate of decomposition did not increase when using this composting activator. The two by-products used in this study contain only small amounts of easily available carbon and the activator used in this experiment had no significant effect. This might be due to the high silica content of rice husk or to the tannins/phenols present in wood chips, reducing the availability of carbon for microorganisms. Further study should be undertaken to improve the decomposition of sewage sludge with organic waste using selected strains of microorganisms and earthworms.

The evolution of biological and chemical parameters during the vermicomposting process showed an increase of biomass. An undesirable waste is converted into a valuable product. Moreover, according to the changes in TOC and the C/N ratio, the decomposition process occurred during vermicomposting and the end result was a lower C/N ratio. Enzyme activity provided a clear indication of dynamic organic matter degradation. In addition, the results indicated that earthworms actually inhibit the growth of faecal coliform in domestic sewage sludge.

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APPENDICES

Appendix A

Publications

Publications

Pasda, N., P. Limtong, R. Oliver, D. Montange and S. Panichsakpatana. 2005. Influence of Bulking agents and Microbial Activator on Thermophilic Aerobic Transformation of Sewage sludge. **Environmental Technology.** 26:1127-1135.

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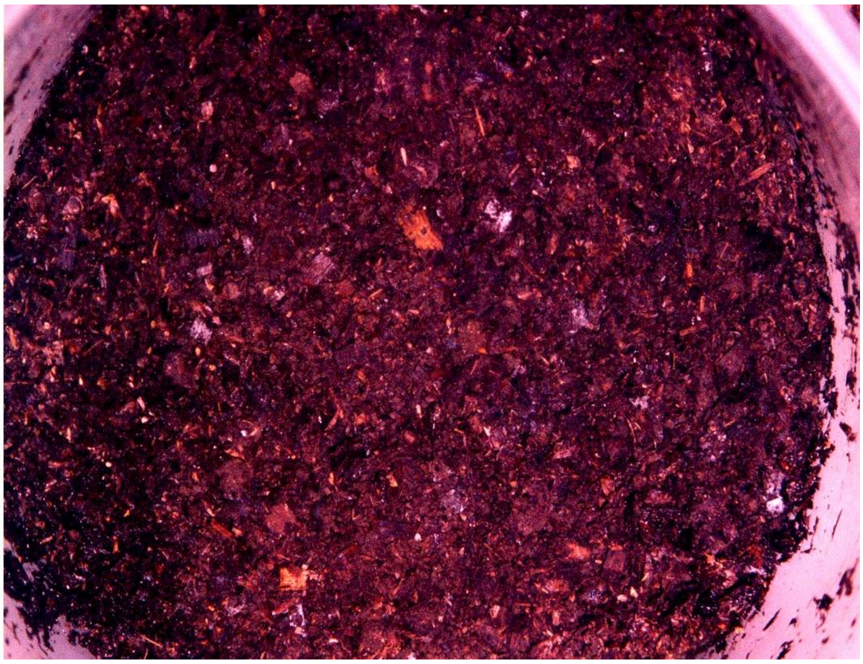
Appendix B

Figure

Appendix Fingure B1 Sewage sludge collection.

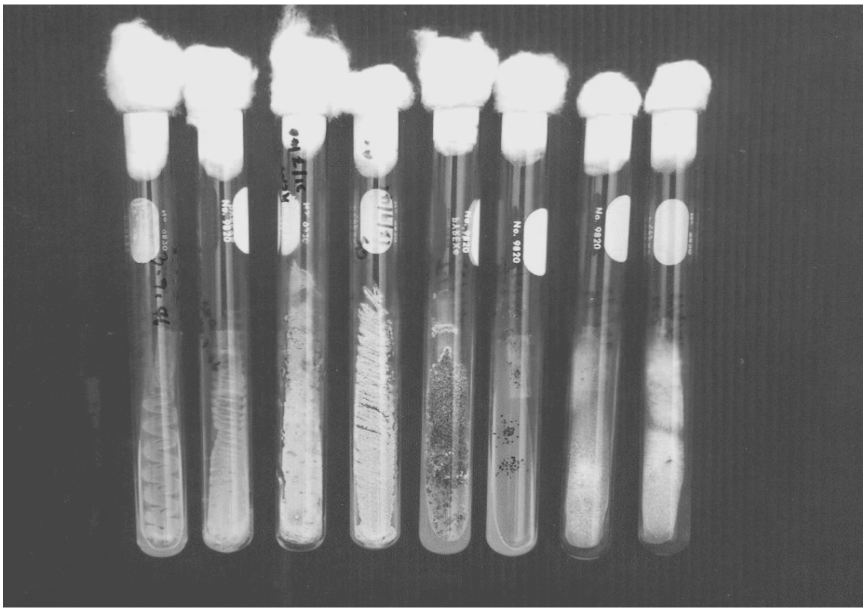
Appendix Fingure B2 Materials for compoting.

Appendix Fingure B3 Mixture of sewage sludge and rice husk.



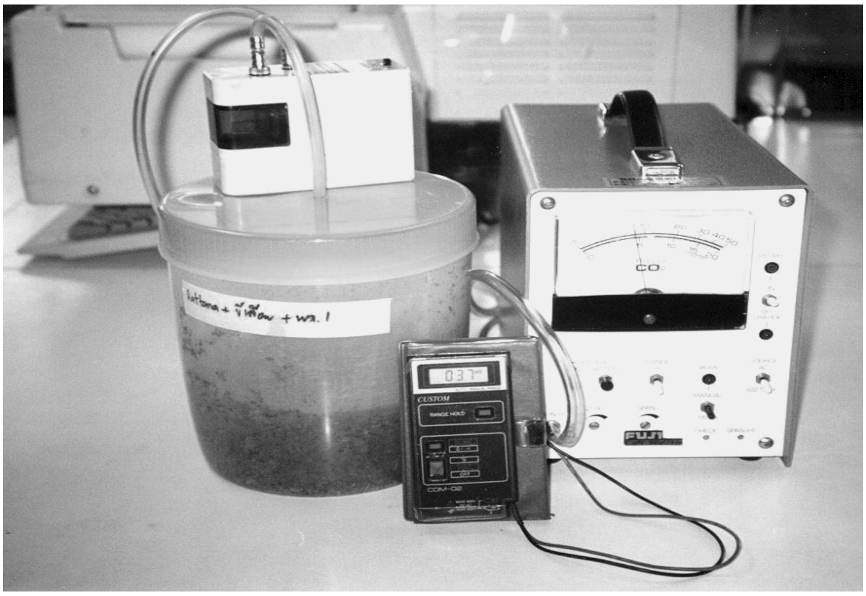
Appendix Fingure B4 Mixture of sewage sludge and woodchips.

Appendix Fingure B5 Microbial activator (LDD1).



Appendix Fingure B6 Microorganisms in LDD1.

Appendix Fingure B7 Composting materials in plastic containers.



Appendix Fingure B8 Carbondioxide measurement.

Appendix Fingure B9 Mixture of sewage sludge and rice husk at the day 0 (left) and 63 days (right).

Appendix Fingure B10 Mixture of sewage sludge and woodchips at the day 0 (left) and 63 days (right)

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