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MICROBIAL TREATMENT OF AGRICULTURE WASTES IN THE KINGDOM OF BAHRAIN

BY

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A Thesis submitted in partial fulfillment of the requirements for the degree of

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Supervised by
Prof. Dr. Essam Ghanem

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DECLARATION

I declare that this work is the result of my own investigation and that it has not already been accepted in substance for any degree, nor is it being currently submitted for any other degree.

Signed: _____ date: 3/4/2007

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بسم الله الرحمن الرحيم

IN THE NAME OF ALMIGHTY GOD THE MERCIFUL, THE COMPASSIONATE

"كلوا واشربوا من رزق الله ولا تعثوا في الأرض مفسدين"

صدق الله العظيم

سورة البقرة : 60

DEDICATE

I dedicate this thesis to my beloved husband and son for their understanding and continual support.

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ABSTRACT

A new approach to shorten and improve the composting efficiency of plant residues using allochthonous diverse enzymatically active groups of bacteria and actinomycetes was applied. The approach also included timely inoculations of exponentially growing allochthonous cultures at start, after 3 weeks and 6 weeks respectively. The allochthonous microorganisms used in the present investigation were isolated from post composted materials. Isolation criteria were based on selection of enzymatically diverse groups of bacteria and actinomycetes able to produce cellulolases, pectinases, amylases and proteases and possess positive interactive relationships. Ash content, total organic carbon (TOC), total nitrogen (TN), C/N ratio and extracellular enzymatic activities were used as parameters to measure microbial activity, length of composting period, stability and ripeness. Four bins properly designed to provide better aeration (each 50 liter capacity) were used as experimental containers. Each bin contained 4 kg plant residues. Bins number 2, 3 and 4 were inoculated with enzymatically diverse groups divided on the basis of their enzymatic activities as cellulolytic and pectinolytic microorganisms in bin number 2; amylolytic and proteolytic microorganisms in bin number 3 and all enzymatically active groups in bin number 4. Bin 1 was kept without bioinoculation and left for the effect of its indigenous microflora. Results were recorded numerically and statistically presented. Transformation of recorded numerical data in terms of efficiency of microbial activity proved the assumed approach where in bin number (4) microbial activities reached 99.9% compared to bin number (1) 85.5%, bin number (2) 95.0% and bin number (3) 96.3%.

Moreover, C/N ratio of 10 used as indicator of stability and ripeness was recorded earlier in bin number 4 i.e. within 3-4 weeks while the other bins showed delayed stability and ripeness. Significant results at the $p < 0.05$ level using Tukey index were recorded. All significant variations were in favor of the treatment of bin number 4. The new finding reached in the present investigation was discussed in view of its environmental sustainability importance for agriculture solid waste treatment and recycling.

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ABBREVIATIONS

ASP	Aerated Static Pile
cfu	Colony forming unit
CMC	Carboxymethylcellulose
CYC	Czapek-Yeast-Casamino
DSW	Domestic Solid waste
EPA	Environmental Protection Agency
ISP	International Streptomyces Project
MO	Microorganism
SPANOVA	Split plot Analysis of Variance
SPSS	Statistical Program for Social Sciences
TN	Total nitrogen
TOC	Total organic carbon

NOMENCLATURE

^{o}C	Degrade centigrade
cm	Centimeter
g	Gram
hrs	Hours
kg	Kilogram
l	Liter
M	Molar
ml	Milliliter
µm	Micrometer
%	Percent
$CaCl_2$	Calcium chloride

$CaCO_3$	Calcium carbonate
CH_4	Methane
CO_2	Carbon dioxide
$CoCl_2$	Cobalt chloride
$CuSO_4$	Cupric sulfate
EDTA	Ethylenediaminetetrraacetic acid
$FeSO_4$	Ferric sulfate
H_3Bo_3	Boric acid
I	Iodine
KBr	Potassium bromide
KCl	potassium chloride
K_2HPO_4	Potassium phosphate
KI	Potassium iodide
LiCl	Lithium chloride
$MgSO_4$	Magnesium sulphate
$MnCl_2$	Manganese chloride
N_2	Nitrogen
NaCl	Sodium chloride
Na_2HPO_4	Sodium phosphate
Na_2MoO_4	Sodium nitrate
NH_4Cl	Ammonium chloride
$(NH_4)_2SO_4$	Ammonium sulfate
O2	Oxygen
$SnCl_2$	Stannous chloride
$ZnCl_2$	Zinc chloride
$ZnSO_4$	Zinc sulfate

Part 1

Chapter 1

INTRODUCTION

Solid waste comprises all the waste arising from human and animal activities that are normally solid and that are discarded as useless or unwanted materials (Tchobanoglous et al., 1993). It is a global problem that needs urgently to be addressed and managed properly toward sustainable municipal solid waste management to be environmentally effective, economically affordable and socially acceptable.

Several solid organic residues, mainly urban and agricultural, are continuously produced and increased dramatically each year wherever there is human habitation in many different forms; such as, organic residual sludge from sewage treatment plants, yard waste and tree trimmings, wood crates and pallets, waste from food processing plants, restaurant food waste, and farm animal waste.

Domestic solid wastes generate serious adverse impacts on health and environment since it is considered to be a major source of pollution that is present wherever human growth and activities are present. Therefore, the practices of disposing those wastes in the sea, landfills, or by incineration are all environmentally polluting and have many dangerous consequences.

The organic waste component of landfill is broken down by micro-organisms, where part of it is converted to liquid 'leachate' containing bacteria, rotting matter and many chemical contaminants. Those leachates are hazardous in nature and can present a serious problem if they reach a watercourse or enter the water table. Digesting organic matter in landfills also generates methane. Although methane could be

utilized as a renewable energy resource, it is considered in this specific situation as a harmful greenhouse gas. Therefore, landfill disposal method is a poor solution for very serious situation, and lead to pollution and spread of phytopathogens.

In fact, the word 'waste' is often an inappropriate term for organic matter, which is often put to good use. The economies of most developing countries dictates that materials and resources must be used to their full potential, and this has propagated a culture of reuse, repair and recycling. `Recycling' means `reprocessing', including composting.

Solutions for organic waste recycling are being demanded urgently by the municipalities' affairs by composting as an answer to the increasingly complex and serious problem of municipal refuse disposal and help protect the environment toward sustainable development which is defined as, the development which meets the needs of the present generation without compromising the ability of future generations to meet their own needs and to achieve sustainability, social, environmental and economic needs must be fully integrated (Porteous, 2000).

As a result emphasis is now on aerobic composting, defined as a microbiological process that converts waste into organic manure rich in plant nutrients and humus (Sharma et al., 1999). Even though recycling organic waste has been known since biblical times, there are many aspects that should be improved such as reduction in overall time required for composting, especially during the winter season (Singh, 2002).

Therefore, in the last few decades, compost from different kinds of waste have been widely used in agriculture in many countries (Greenland, 1980; Parr and Wilson, 1980; Simidchieve et al., 1981; Subba Rao, 1984;

Kardinalovskaya, 1986; Lynch, 1987; Steiner et al., 1987) and used as manures and as substrates in the production of vegetables and flowers in the greenhouse (Hoitink and Poole, 1980; Romano, 1984; Verdonck et al., 1985).

Until recently, interest in composting, in the Kingdom of Bahrain, has been limited to the amateur gardener and some number of farms under a guiding program applied by Ministry of Agriculture.

In Western societies, most large-scale composting has been associated with agricultural rather than domestic wastes (Finstein and Morris, 1975) where nutrient conservation and the maintenance of soil structure tend to be emphasized. However, compost is generally noncompetitive with inorganic fertilizer as a source of nitrogen, phosphorous, and potassium (Toth, 1968; Mays et al., 1973).

Materials which have been considered as inoculants essential to the composting process include: animal manures, garden soil, decomposing (partially composted) material, or bacterial cultures. Actually, the principal considerations for a microorganism to be selected as an inoculant to enhance the composting process are the adaptation to the environment and the size of inoculum (Vargas-Garcia et al., 2005).

Therefore, external microorganisms can be added to composting substrates (Golueke et al., 1954; Kosinkiewiez, 1974; Finstein and Morris, 1975; Wani and Shinde, 1978; Solbraa, 1984; Nakasaki and Akiyama, 1988; Magan et al.,, 1989; Kostov et al., 1991; Requena et al., 1996; Rajbanshi et al., 1998; Elorrieta et al., 2002; Vargas-Garcia et al., 2005) as inoculants composed of actively selected microorganisms.

The inoculants usually used to speed up the composting process and enhance the degradation of organic waste materials in a shorter time. But

this idea has been a controversial subject for a long time and, in fact, contradictory results have been described by those different authors.

There are reports which indicate that external supplementation of proper microorganisms as inoculants are necessary for the composting process to occur (Beca et al. 1992; Kakezawa et al. 1992) or by using a compost starter may affect the composting process in two ways: 1) initiating a desired compost process as the rapid breakdown of components not desired in the final product by selected microbes. 2) Supply compounds that stimulate initial microbial population activity (Solbraa, 1984).

Composting of household organic waste can be accelerated if the number of microorganisms is artificially increased by inoculating or seeding material added to the compost only when the population of native microorganisms is too small (Nakasaki and Akiyama, 1988).

Inoculation or seeding has been reported to be effective in composting sawdust (Finstein and Morris 1975; Golueke et al. 1954).

Humification can be accelerated by some microorganisms active in the biodegradation of lignocellulose (Wani and Shinde, 1978) or decrease the C/N ratio (Gaur et al. 1982; Yadav et al. 1982; Matthur et al. 1986; Elorrirta et al., 2002).

Fungal inoculation of horticultural plant wastes mixtures by a cellulolytic fungus (*Trichoderma sp)* led to faster decrease of C/N ratio and higher formation of humic substances (Elorrirta et al., 2002).

The efficiency of inoculation in natural composting process is a subject of controversy among many authors. While Kakezawa et al. (1990,1992) found an advantage in using *Coriolus versicolor* as inoculua, other authors report dubious effects (Finstein and Morris, 1975; Solbraa, 1984) compost starter contained bacteria, fungi and some minerals give zero or

insignificant effects on spruce bark composting (Solbraa, 1984). Horse manure, composting material, bacterial cultures, and soil was used by Golueke as inoculants in his study of composting vegetable trimmings, straw and paper. None of these inocula accelerate the composting process than those indigenous to the refuse (Golueke et al., 1954).

Gray et al. (1971) reported that using the starter will be efficient to the quality of the final product rather than in the acceleration of the composting process.

Decomposing of wheat straw in soil influenced by the effect of five microbial inoculants treatment consisted of the cellulolytic fungi *Trichoderma harzianum*, *Gliocladium roseum*, *Penicillium hordei*, and the bacteria *Clostridium butyricum* and *Enterobacter cloacae* in different mixtures show none markedly improved decomposition of wheat straw in natural soil (Magan et al., 1989). While there are reports indicate that there are decomposition of sterile wheat straw in vitro using combination of the cellulolytic fungi *Trichoderma* spp and the N_2-fixing *Clostridium butyricum* as inoculants (Lynch and Harper, 1985). Others have reported these improvements using species of cellulolytic *Cellulomonas* and N_2-fixing *Azospirillum* (Halsall and Goodchild, 1986; Halsall and Gibson, 1986).

Active cellulose-decomposing isolates such as a *Streptomyces sp.*, a *Bacillus sp.*, and a *Cephalosporium sp.*, used as inoculants for the decomposition of coniferous sawdust and bark with added N and P lead to increase the activity of composting marginally, but increased the yields of tomato compared with soil enriched with the same nutrients (Kostov et al., 1991).

Hatakka studied lignin-modifying enzymes from selected white-rot fungi and found that white-rot fungi played an important role in lignin degradation (Hatakka, 1994). Nakasaki et al., 1994, reported that a thermophilic bacterium, *Bacillus licheniformis*, could effectively decompose protein and prevent the drop of initial pH values during composting; thus, it could stimulate proliferation of other thermophilic bacteria.

The composting system may not have the desired performance due to improper process operations. For instance, Steven Donald Thomas (1995), completed a study on microbial inoculation with mixed cultures of *Bacillus*# 21415 & *Bacillus sp.* #21522, *Trichoderma reesei* QM9414 and *Trichoderma harzianum* FP108 in composting fish wastes. The inocula, performed well overall but were not always significantly better than the controlled compost piles, depending on the season and combination of inocula.

Composting of grass straw and leaf mix (in-vessel) show significant positive effects with seeding material in total organic carbon and organic matter contents (Rajbanshi et al., 1998).

Ohtaki (Ohtaki et al., 1998) revealed that inoculations could increase the microbial population, formulate beneficial microbial communities, improve microbiological quality and generate various desired enzymes; and thus enhance the conversion of organics and reduce odorous gas emissions.

Shin et al. also studied the enhancement of composting efficiency by adding solid and liquid inoculants. However, the inoculation efficiency was usually affected by competition with indigenous microorganisms (Shin et al., 1999).

The Studies of Lei and Gheynst, 2000, indicated that the inoculated microbial populations and indigenous populations would evolve continuously, leading to variations during different composting stages. This could result in difficulties in describing the relevant inoculation mechanisms. It also indicated that inoculation did not significantly raise the rate of temperature increase, but did increase the time the composting high temperature remained.

Beidou Xi et al., 2005, used a method to improve the composting process efficiency by seeding inoculum containing *B. azotofixams*, *B. megaterium*, *B. mucilaginosus*, cellulolytic strains, and White-rot fungi was very efficient in accelerating degradation rate, reducing odorous gas emissions and stabilizing composting products.

Differences among results in the above findings is due to the complexity of the biological events that take place and the many factors on which the process depends, both biological and nutritional as well as physical and chemical, are considered (Vargas-Garcia et al., 2005).

Moreover, differences might be partially due to various substrates employed in each study (Elorrirta et al., 2002) or due to sampling homogeneity problem (Nakasaki and Akiyama, 1988). Thus, the conditions are different in every situation and, therefore, microbial activity is different too.

Composting of plant residues from different sources (litter fall, yard trimmings, etc) have been studied from various aspects. In field-scale composting of leaves and grass mix show stable compost characteristics after 60 days when the windrows were turned once a month (Michel et al., 1996).

1.1 Research problem

In Bahrain large quantities of solid wastes are produced annually in the major areas of the kingdom. Much of the solid wastes are collected and discarded into Askar Municipal Landfill site. Governmental data showed that generation of municipal solid waste which consisted of domestic, building, commercial, agriculture, industrial and carcasses wastes has increased from 384,232.4 tons in 1997 to 659,847 tons in 2003 recording 72% increases in seven years. Moreover, It has been found that the highest constituent, 55% of those wastes, were domestic, (municipalities' affairs report, 1997-2003) (Table 1.1).

Urbanization in Bahrain has been increasing leading to an increase in domestic waste. At present, Bahrain's production of domestic solid waste was 1.2 kg per capita per day in 2003, compared to 0.99 kg per day in 1997 that is considered to be one of the highest waste generations in the world. From these domestic wastes, more than 50% of what goes into Askar landfill is mainly organic waste (Table 1.2) (municipalities' affairs report, 1987-1995) and is often the most hazardous waste.

Table 1.1: Solid waste generation in the Kingdom of Bahrain during 1997-2003.

Type of waste	Solid Waste Generation (Ton / year)						
	1997	1998	1999	2000	2001	2002	2003
Domestic	231,627.8	240,157.4	237,953.6	234,187.0	274,236.9	279,295.3	293,111.1
Construction	20,541.8	17,145.8	22,601.6	36,129.0	30,621.4	31,458.1	61,149.3
Commercial	93,900.9	99,130.0	99,847.6	109,677.6	150,421.4	170,946.3	253,271.4
Agriculture	12,991.5	13,083.9	13,210.2	15,253.5	19,924.6	18,932.2	20,539.5
Industrial	18,152.4	18,368.2	18,519.8	21,957.8	31,876.1	28,568.5	23,843.5
Carcasses	7,018.0	7,024.4	6,928.1	6,730.3	8,347.8	7,620.1	7,932.4
Total	384,232.4	394,909.6	399,060.9	423,935.2	515,428.2	536,820.5	659,847.0

(Source: Municipalities Affairs-General Directorate for Common Municipality Services.)

Table 1.2: Household wastes in the Kingdom of Bahrain

Household Waste Analysis (%)			
Constituent	**1987**	**1993**	**1995**
Organic Materials	53	56.9	59.07
Paper & Cardboard	16	13.8	12.8
Plastics	14	9.6	7.44
Metals	5	3.5	2.05
Glass	7	4	3.39
Textile	2.5	2.6	2.93
Babies napkins	–	4.8	3.99
Household Hazards	–	–	0.19
Garden Waste	–	–	6.73
Miscellaneous	2.5	4.8	1.41

(Source: Municipalities Affairs-General Directorate for Common Municipality Services.)

Moreover, large quantities of horticultural (green) wastes are produced annually. Green waste (yard waste) is a source of organic material which consists of leaves, tree trimmings, weeds, grass, brush, grass clippings and other plant wastes. These wastes are either burned or dumped into the landfill by municipalities neglecting its significance as valuable resource of organic material that can be recycled for a variety of uses.

Beside increase in waste generation annually, there are other causes of the problem such as:

- Lack of adequate environmental legislation.
- Lack of recycling agencies & establishments.
- No specific rules & regulation to segregate waste.
- No clear environmental standard agreed between major industries, municipalities & ministry of agriculture.

1.2 Research objectives

In the present study, a new approach was applied. It depends mainly on selecting enzymatically active bacteria and actinomycetes from raw and ripened compost. The most active microorganisms were used as inocula for composting of leaves in manually turned and aerated bins. The approach assumes that by increasing the numbers and types of active microorganisms, the process should be accelerated.

Chapter 2

COMPOSTING

Composting nowadays is considered as a general treatment method for recycling municipal solid waste via decomposition with a variety of microorganisms. There are two forms of decomposition:

1.3 Aerobic decomposition:

Bacteria and fungi which thrive in high oxygen conditions are responsible for the decomposition. This form of decomposition occurs in open heaps and containers that allow air to enter. With open heaps and more ventilated containers, compost can be formed in a matter of a few months, and even faster if the organic material is turned regularly. In heaps or bins where aerobic decomposition is occurring, there should be no unpleasant odors (web site, 7).

Composting is the degradation (oxidative biotransformation process) of organic solid (non-aqueous) material as a carbon source by rich and varied microbiota naturally present on the residues that involves aerobic mesophilic respiration and passes through a thermophilic stage (Finstein and Morris, 1975; Elorrirta et al., 2002). Microbial biotransformation of organic wastes yields the stabilized end-product compost that provides several benefits to plants when applied to the soil (Elorrirta et al., 2002).

In composting, provided the right conditions are present, the natural process of decay is speeded up.

Composting methods vary according to the process control mechanisms applied, such as aeration, turning and watering. The process usually

needs long periods from 6 to 12 months, to be accomplished (Kakezawa et al., 1990).

There are two primary methods of aerobic composting:

- Active (or hot) composting, which allows the most effective decomposing bacteria to thrive, kills most pathogens and seeds, and rapidly produces usable compost.
- Passive (or cold) composting, which lets nature take its course in a more leisurely manner and leaves many pathogens and seeds dormant in the pile.

Most commercial and industrial composting operations use active composting techniques. This ensures a higher quality product and produces results in the shortest time. The greatest control, and therefore the highest quality, is generally achieved by composting inside an enclosed vessel which is monitored and adjusted continuously for optimal temperature, air flow, moisture, and other parameters.

Home composters use a range of techniques varying from extremely passive composting (throw everything in a pile in a corner and leave it alone for a year or two) to extremely active (monitoring the temperature, turning the pile regularly, and adjusting the ingredients over time) and combinations of both.

1.3.1 Operational factors for optimal composting:

One of the first tasks in developing a successful composting process is to control the operational factors, thereby getting the right combination of ingredients. Two parameters are particularly and essential importance in this regard: moisture content and the carbon to nitrogen (C/N) ratio, some other parameters are also shown as follows:

1.3.1.1 The correct amount of water (moisture content):

Moisture is essential to all living organisms, and most microorganisms, lacking mechanisms for moisture retention (like skin in human body), are particularly sensitive in this regard. The upper limit of moisture varies with different materials, and is a function of their particle sizes and structural characteristics, both of which affect their porosity. For most compost mixtures, 50-60% water content (water-filled porosity) is the recommended upper limit for moisture content (Poincelot, 1972). Because composting is usually a drying process (through evaporation due to microbially generated heat), initial moisture contents are usually recommended in this upper range.

Moreover, the compost should be kept moist at all times but not soggy because microorganisms induced decomposition most rapidly in the thin films found on the surfaces of the organic particles. If the moisture content falls below a critical level of <30%, microbial activity will decrease and the microbes become dormant, slowing the rate of composting. On the other hand, too much moisture >65%, however, is one of the most common factors leading to anaerobic conditions, decomposition rate decreases, odor problems arise and leaching of

nutrients (Golueke, 1991). Thus, mixing-in additional bulking agent is likely to alleviate the excess moisture problem.

The water status of compost is usually evaluated in terms of the moisture content and is expressed on a wet weight basis. Although moisture content is a useful operational parameter, it does not describe the microbial growth environment in any fundamental way. As in other non-aqueous environments, in compost microbial growth actually takes place in aqueous solution (Finstein and Morris, 1975).

1.3.1.2 The correct ratio of carbon to nitrogen:

Microorganisms require a carbon source, macronutrients such as nitrogen, phosphorous and potassium, and certain trace elements for their growth. Carbon serves primarily as an energy source for the microorganisms, and the basic building block making up about 50 percent of the mass of microbial cells. Some of the energy formed is used for microbial metabolism, and the rest is released as heat. Nitrogen is a critical element for microorganisms because it is a component of the proteins, nucleic acids, amino acids, enzymes and co-enzymes necessary for cell growth and functioning.

In general, materials that are green and moist tend to be high in nitrogen, and those that are brown and dry are high in carbon. High nitrogen materials include grass clippings, plant cuttings, and fruit and vegetable scraps. Brown or woody materials such as autumn leaves, wood chips, sawdust and shredded paper are high in carbon. If compost mix is too low in nitrogen, it will not heat up. If the nitrogen proportion is too high, the

compost may become too hot, killing the compost microorganisms, or it may go anaerobic, resulting in a foul-smelling mess (web site, 5).

The optimum C/N ratio for composting has been reported to be 25-40, but the value varies depending on the substrate (Golueke, 1991) or in the range of 25 to 30 parts carbon for each part nitrogen by weight (25:1 to 30:1). The reason behind this ratio is merely explained by (Finstein and Morris, 1975) as follows: at lower ratios, as are often observed in biosolids (sewage sludges), nitrogen will be supplied in excess and it is often lost from the system as ammonia gas, causing undesirable odors. In contrast, higher C/N ratios (more than 40) mean that there is not sufficient nitrogen (limiting factor) for optimal growth of the microbial populations, as a result the compost will remain relatively cool and degradation will proceed at a slow rate.

If woody bulking agents are used for composting, a C/N ratio of 35 to 40 may be used due to the low availability of carbon, where bulking agents play two roles in composting: 1) they provide an extra carbon source to increase the carbon to nitrogen ratio; and 2) they increase the porosity of the composting material which improves air movement (British Columbia, MAF, 1996).

Carbon to nitrogen ratio will be referred to hereafter as the C/N ratio. The C/N ratio can be adjusted to provide optimal amounts of these two crucial elements by mixing together organic materials with suitable contents.

As composting proceeds, the C/N ratio gradually decreases from 30:1 to 10-15:1 for the finished product. This occurs because each time that organic compounds are consumed by microorganisms, two-thirds of the carbon is given off as carbon dioxide. The remaining third is incorporated

along with nitrogen into microbial cells and then later released for further use once those cells die (web site, 5).

1.3.1.3 Sufficient oxygen (aeration):

Since composting is an aerobic process, conditions must promote efficient oxygen transfer and carbon dioxide removal from the medium accomplished by turning and/or aeration of the composted wastes. Oxygen is essential ingredient for successful composting, where it for the metabolism and respiration of aerobic microorganisms. More energy is made available from aerobic respiration than from fermentation. This means that for the same amount of C, higher microbial populations can develop and if higher microbial populations develop, waste decomposition can be accelerated.

Although the atmosphere contains 21% oxygen, aerobic microbes can survive at concentrations as low as 5%. Oxygen concentrations greater than 10% are considered optimal for maintaining aerobic composting.

As microorganisms oxidize various organic molecules present in the waste material for energy, oxygen is used up and carbon dioxide is produced. As biological activity progresses, the oxygen concentration falls and carbon dioxide concentration increases. If the average oxygen concentration in the compost system falls below about 5%, the process will become anaerobic and produce undesirable odors, including the rotten-egg smell of hydrogen sulfide gas.

Some compost systems are able to maintain adequate oxygen passively, through natural diffusion, which is function of the concentration difference between the outside air (21% oxygen) and the oxygen concentration in the interior of the pile. In a passively aerated or windrow

system, diffusion is assisted by natural convection, but that assistance is probably limited to the upper and outer parts of the pile (Miller et al., 1989). Other systems require various methods to maintain aerobic conditions including drilling air holes, inclusion of aeration pipes, forced air flow by blowers and mechanical turning or mixing the compost ingredients. Mixing and turning increase aeration by loosing up, fluff, homogenize and insure even microbial distribution.

It is also a common practice to mix large pieces (bulking agents) that resist decomposition with wastes to create interstitial spaces for the air to flow and diffuse uniformly (Bernal et al., 1996).

1.3.1.4 The type and composition of the organic waste:

Composting process can be accelerated by proper selection of control parameters such as raw material used (Elorrirta et al., 2002) where organic wastes vary considerably in terms of their chemical and physical characteristics. Many organic materials are susceptible to decomposition and degradation during composting. A wide range of microorganisms can easily use readily soluble biodegradable compounds, such as monosaccharides and amino acids. However, polymers such as lignin are considered to some extent, natural barriers that slow down microbial attack.

1.3.1.5 Particle size:

Microbial activity or decomposition generally occurs on or near the surfaces of organic particles, where oxygen diffusion into the aqueous films covering the particle is adequate for aerobic metabolism, and the substrate itself is readily accessible to microorganisms and their

extracellular enzymes. Smaller particle size have more surface area per unit mass or volume than large particles, so if aeration is adequate, small particles will degrade more quickly. Therefore, decomposition rate is proportional to the surface area and inversely proportional to size as shown in (Fig. 1.1).

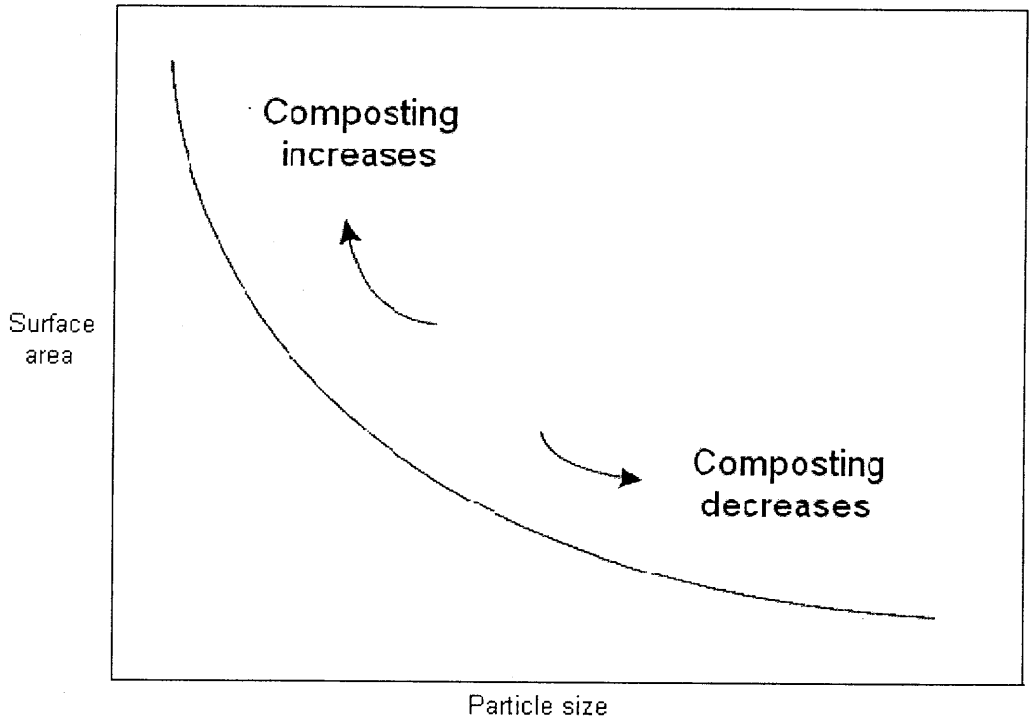


Figure 1.1: The influence of particle size on composting. (Source: Coyne 1999.)

Experiments have shown that the process of grinding compost materials can increase the decomposition rate by a factor of two (Gray and Sherman, 1970). On the other hand, a theoretical calculation by Haug (1993) suggests that for particles larger than 1 mm in thickness and when particles are too small and compact, air circulation through the compost ingredients is inhibited, where oxygen may not diffuse all the way into the center of the particle thus, decreases oxygen available to

microorganisms and ultimately decreases the rate of microbial activity. A common solution is to combine a coarse, relatively insoluble material, called a bulking agent. The mixture of particle sizes improves aeration and can be effective in establishing and maintaining appropriate moisture content for composting. Common bulking agents are tree bark, wood chips, shredded tires, straw and sawdust where the latter is particularly useful for adjusting the moisture content of very wet substrates such as biosolids.

1.3.1.6 The availability of microorganisms:

A successful composting process greatly depends upon the presence of necessary microorganisms and upon the appropriate conditions applied to microbial activity and proliferation (Golueke, 1992). Rapid composting presupposes that a large, active microbial population is present. The microbial population consists of a mixed group of mesophilic and thermophilic bacteria, actinomycetes, and fungi. Inoculating compost with material from a previous compost pile can be beneficial to the composting process because it adds a representative mix of microorganisms in higher numbers (Coyne, 1999).

1.3.1.7 pH level:

Optimum pH is near neutral for most microorganisms and slightly alkaline for actinomycetes important in composting. This translates into a range of about 5.5 to 8.5, but the pH extremes should be avoided. A pH of 6.5 to 7.2 is ideal (Coyne, 1999).

As bacteria and fungi digest organic matter, they release organic acids. In the early stages of composting, these acids often accumulate. The

resulting drop in pH encourages the growth of fungi and the breakdown of lignin and cellulose. Usually the organic acids become further broken down during the composting process. If the system becomes anaerobic, however, acid accumulation can lower the pH to 4.5, severely limiting microbial activity. In such cases, aeration usually is sufficient to return the compost pH to acceptable ranges.

1.3.1.8 Nutrient Balance:

Adequate phosphorus, potassium and trace minerals (calcium, iron, boron, copper, etc.) are essential to microbial metabolism. Normally these nutrients are not limiting because they are present in ample concentration in the compost source materials.

1.3.1.9 Time:

The final consideration is time, which will be a function of all of these other factors and the mechanical manipulation of the compost. Curing is the slow maturation of compost after active composting is accomplished. In (Table 1.3), effects of composting method are shown reflects the different amounts of time it takes to compost different materials.

Table 1.3: Composting times for combinations of methods and materials

Method	materials	Composting Time		Curing Time (Maturation)
		Range	Typical	
Static pile	Leaves	2-3 years	2 years	____
	Well-bedded manure	6 months-2 years	1 year	____
Areated static pile	Sludge/wood chips	3-5 weeks	4 weeks	1-2 months
Windrow, infrequent turning	Leaves	6 months-1 year	9 months	4 months
Passive aerated windrow	Manure	4-8 months	6 months	1-2 months
	Manure bedding	10-12 weeks	____	1-2 months
	Fish waste/peat moss	8-10 weeks	____	1-2 months
Agitated bed	Sludge/yard waste or manure/sawdust	2-4 weeks	3 weeks	1-2 months
Rotating drum	Sludge and/or solid waste	3-8 days	____	2 months

(Source: Carr et al., 1995.)

1.4 Anaerobic decomposition:

The breakdown of the organic material is caused by bacteria and fungi that thrive in low or no-oxygen conditions. It is the type of decomposition that takes place in closed containers. This type of system is more complex and difficult to control and requires complex equipment for larger scale composting (web site, 7). Moreover, anaerobic composting produces undesirable odors and is considered a lengthy process. However, environmental and nutritional conditions are not the only parameters that can affect microbial growth in composting. The presence of other microorganisms can influence the activity of the microorganism tested, either positively or negatively (White et al., 1998). Therefore, in nature, the decomposition of organic waste occurs because of the action of naturally occurring microorganisms such as bacteria and fungi. Small invertebrates, such as earthworms and millipedes, help to complete the process. Composting can convert organic waste into rich, earthy, dark colored compost, or humus, in a matter of a few weeks or months. Under controlled conditions, however, the process can be speeded up and external microorganisms as inoculants can be added to composting substrates to speed up the natural process or to obtain a better final compost product. Effective inoculation has been reported by several researchers (Biey et al., 2000; Baheri and meysami, 2002).

1.5 Composting system types:

Compost systems are categorized on the basis of whether they are batch or continuous processes (Finstein and Morris, 1975). In batch composting, a given quantity of raw waste substrates is allowed to progress through the stages of temperature and microbial succession, finishing with the stabilization stage. In continuous composting, substrates are continually added and the product removed and collected from the reactor.

1.5.1 Batch composting systems:

1.5.1.1 Static Pile Composting:

The simplest batch system is the static pile, where waste materials are merely stacked to produce a pile that is roughly triangle or trapezoidal in shape and not mechanically turned or inverted. A completely static pile may eventually lead to oxygen deficiency in the center of the pile as the microorganisms rapidly decompose the substrates. As a result anaerobic condition leads to decrease in the rate of substrate decomposition to the rate at which oxygen diffuses into the pile and extend the time necessary to completely compost the substrates.

Exhaustion of oxygen in the pile also leads to the production of malodorous end products of anaerobic microbial activity.

1.5.1.2 Windrow Composting:

An engineered improvement of the static pile is the windrow. In this system composting mixture of organic matter or biodegradable waste, like animal manure and crop residues are piled and mixed with a bulking material is placed in elongated piles of compost rows called windrows.

These elongated pile usually constructed to handle the larger volumes of compost substrates generated by municipalities and industries. This configuration facilitates turning, which often expedites composting. Turning the windrow periodically ensures that the outer, cooler, and drier parts of the pile are rotated to the center of the pile where the most rapid microbial activity develops, to improve porosity and oxygen content, mix in or remove moisture. In addition, turning redistributes waste products that accumulate in the central part of the pile.

Commercial windrow turners are large tractor like machines that straddle the windrow as in (photo 1.1). In moving from one end of the windrow to the other, rotating "fingers" beneath the center of the machine turn the windrow, bringing about the mixing action required to ensure rapid and complete composting (website, 1, 5).

1.5.1.3 Aerated Static Pile (ASP) composting:

Aerating compost piles have been developed via a series of perforated pipes is buried in a pile or windrow. A blower mechanism pulls air through the pile and into pipes near the bottom of the compost pile that run parallel to the length of the windrow. Air may also be pumped from the pipes up wards through the pile. This approach obviates the need for turning the pile to achieve aeration. These kinds of windrows are called aerated static piles. A passive ASP system relies on natural air currents, where an active ASP system uses forced aeration to pull or push air through the composting pile, since it allows the compost process to proceed with minimal or no turning, permits piles to be larger. Direction of air flow is sometimes reversed to minimize odors emanating from the pile. Air drawn through the pile when odor levels are high can be filtered

through activated charcoal or finished compost to absorb the odor-causing compounds. Aerated static piles are popular because they provide a higher level of control of the process, minimize the need to turn the pile, and encourage rapid rates of decomposition. For example, complete composting of biosolids can occur within 21 days. Therefore, this system is sandwiched somewhere between windrow and in-vessel technologies with regard to complexity and cost.

Aerated static piles don't need as much space as a windrow operation, but offer rapid biodegradation and process control similar to in-vessel composting, and work well for facilities processing wet materials or a wide variety of feedstock. ASP facilities can be outdoor operations, under roof, or totally enclosed.

Positives of using this composting method include the ability to maintain sufficient oxygen levels for the microbial populations and the removal of excess heat, which can kill desirable microbes and crash the system. However, aerated systems dry out quickly and must be monitored closely to maintain desired moisture levels (website, 6).



Photo 1.1: windrow composting photo. (Source: web site, 1)

1.5.2 Continuous composting systems:

Continuous composting consists of a continuous supply of decomposable substrates kept under strict regulation.

1.5.2.1 In-vessel Composting:

Continuous feed reactors or in-vessel composting is the quickest method of composting, and the most technologically sophisticated. It is an industrial form of composting biodegradable waste that occurs in enclosed reactors like building that has temperature and moisture controlled systems and some consist of metal tanks through which air flow and heat can be controlled. Substrates are continuously fed into the reactor and move through it as decomposition proceeds.

They come in a variety of sizes and have some type of mechanical mixing or aerating system like vertical- or horizontal-flow systems used to aerate and mix the substrates.

In-vessel composting can process larger quantities in a relatively small area more quickly than windrow composting and can accommodate animal products (website, 6).

1.5.2.2 Agitated Bed (Mechanical Windrow) Composting:

Agitated Bed (Mechanical Windrow) composting is the making of long rows of compost by placing the organic material within low concrete walls (troughs) and mixing with mechanized equipment, see (photo 1.2). This process appears simple, but is very inefficient, mechanically intensive, and requires a very large area and building enclosure (website, 2).



Photo 1.2: Agitated Bed (Mechanical Windrow) composting photo.
(Source: web site, 2)

1.5.2.3 Rotating Drum Composting:

The Dano design is one example of the rotating drum composting; consisting of a long cylinder (about 3 m in diameter and 30 m in length) that slowly rotates as substrates are fed into one end. The composting substrates move spirally down the slightly inclined cylinder to emerge from the other end as nearly finished compost. Strict control of the quality and quantity of substrates fed into the reactors and rate of internal movement of the composting wastes allow equilibrium to develop where thermophilic conditions are continuously maintained (photo 1.3).



Photo 1.3: Rotating Drum Composting photo. (Source: web site, 1)

1.5.2.4 Tunnel Composting:

Tunnel composting is an industrial composting technique for biodegradable waste which occurs in side loading tunnels. Air is injected mechanically into the tunnels from the base. Temperature and moisture levels in the compost can be monitored in order to provide optimal composting conditions (website, 6).

The tunnel composting has major processing concept laws. It was originally designed for compacting garbage for trucking. The natural bio-reaction developed heat within the biomass and the decision to use it as a composting system was made.

Its concept is to compress the compost into a dense mass to push it through the system and the need to maintain porosity and aerate the mixture (website, 4).

1.6 Composting benefits:

Composting has many solid waste management benefits such as;

Sanitation:

During composting, pathogenic microorganisms, insect larvae, intestinal parasites and weed seeds are killed rapidly. Nuisances, such as odors, insect breeding, and vermin harborage, can be prevented through the rapid decomposition of the putrescible organic materials (Finstein and Morris, 1975).

Mass and bulk reduction:

Part of the organic carbon is mineralized to carbon dioxide and lost from the material (Finstein and Morris, 1975); therefore, it saves valuable

landfill space and possible contamination of land and water due to landfill 'leachate'.

Environmental:

Compost reduces the amount of waste to be disposed, thus composting contributes to the disposal of wastes and enhances the preservation of the environment (Garcia et al., 1993). Specifically it is an appropriate way to solve problems associated with the disposal of crop residues and garden wastes (Rajbanshi et al., 1998) for example yard waste composting can reduce waste generation by an average of 12% (U.S. EPA, 2000).

Resource recovery:

Most uses for compost are agronomic and produced mainly for reasons of soil husbandry, usually from crop residues and animal excreta, and it is known as artificial manure (Finstein and Morris, 1975) or organic fertilizer. It provides a useful way of returning to the soil mineral plant nutrients in organic form that might otherwise be lost, this stabilized organic waste materials tend to produce a smaller amount of green house gases such as methane CH_4 (Yagi and Minami, 1990). Compost can be used as organic fertilizer on farmland or in the garden by improving the condition of soils and preserve soil fertility (Vargas-Garcia et al., 2005) and as a reservoir of slow releasing nutrients including nitrogen (Sikora and Yakovchenko, 1996). Compost is a good soil conditioner because it is humus-like. Thus, it promotes aggregation and improved aeration in the soil to which it is applied.

1.7 Microorganisms during composting:

1.7.1 Compost environment:

During composting, organic matter transformed by microorganisms into CO_2, biomass, thermal energy (heat) and humus, the relatively stable organic end-product (Fig. 1.2).

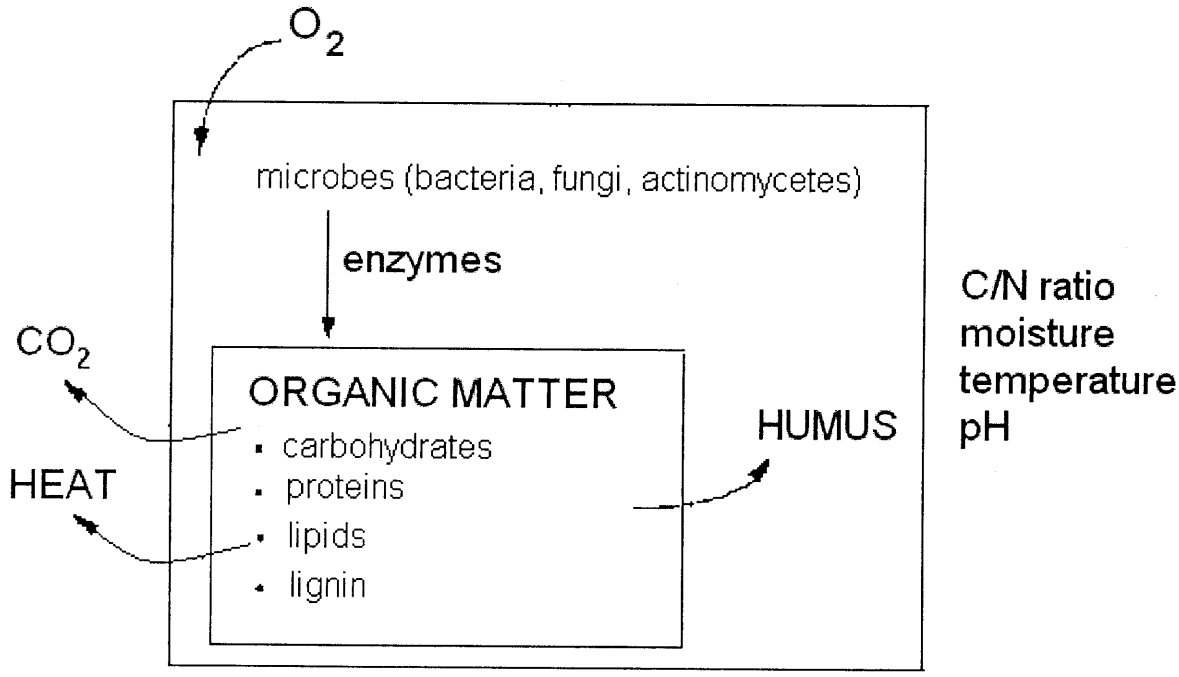


Figure 1.2: The composting process. (Source: Itavaara et al., 1995.)

The organic substrates, bulking agents and amendments used in composting are mostly derived from plant material. The main components of the organic matter are carbohydrates (e.g. cellulose), proteins, lipids and lignin. The capacity of microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for degradation of the substrate. The more complex the substrate, the more extensive and comprehensive is the enzyme system required. Through the synergistic action of microorganisms complex organic

compounds are degraded to smaller molecules which can then be utilized by the microbial cells (Golueke, 1991, 1992).

Under optimal conditions, composting proceeds through three phases: (1) the mesophilic, or moderate-temperature phase, approximately 20° to 40°C, which lasts for a couple of days (2) the thermophilic, or high-temperature phase, above 40°C (Sylvia et al., 1998), which can last from a few days to several months, and finally (3) through a gradual cooling period, the final stabilization or maturation phase which lasts for several months (Fig. 1.3).

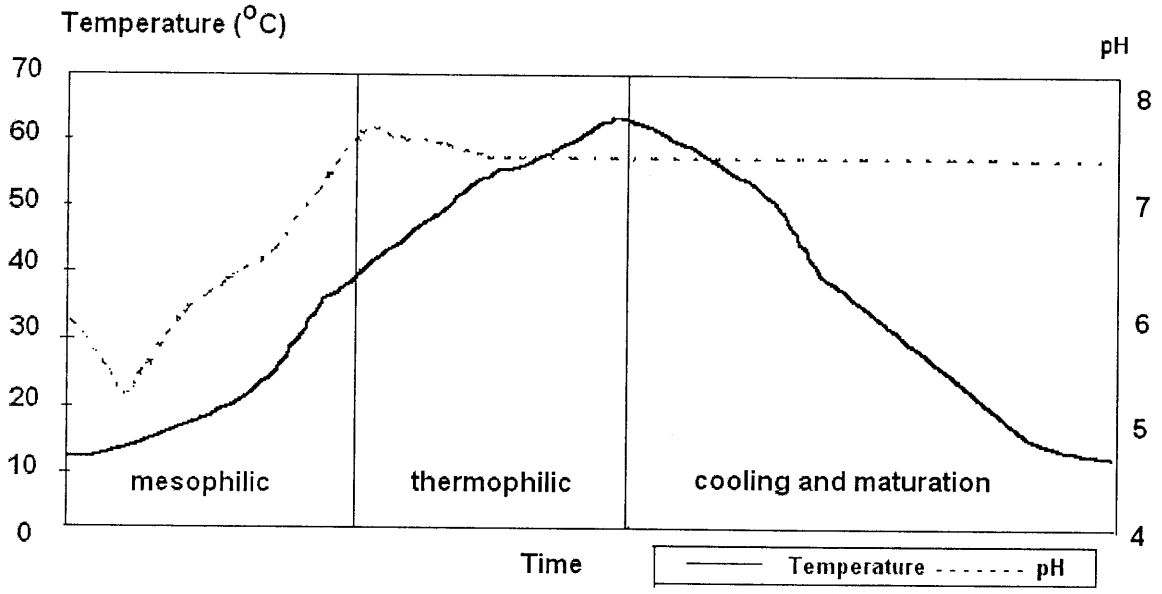


Figure 1.3: Temperature and pH variation during natural composting process. (Source: Golueke 1991.)

The length of the composting phases depends on the nature of the organic matter being composted and the efficiency of the process, which is determined by the degree of aeration and agitation. During the initial stage of decomposition, the mass is at ambient temperature and usually slightly acidic. Soluble and easily degradable carbon sources, such as

monosaccharide, starch and lipids are utilized by microorganisms in the early stage 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.

After the easily degradable carbon sources have been consumed, more resistant compounds such as cellulose, hemicellulose and lignin are degraded and partly transformed into humus (Crawford, 1983; Paatero et al., 1984).

Humus is the end product of the humification process, in which compounds of natural origin are partially transformed into relatively inert humic substances (Tuomela et al., 2000). Humic substances can be considered as a major reservoir of organic carbon in soils and aquatic environments (Aiken et al., 1985).

Organic matters present in compost are usually chemically complex and difficult to fractionate. Aiken et al. 1985 divided humic substances into the following groups: humin (not soluble in water at any pH), humic acids (not soluble in water under acidic conditions) and fulvic acids (soluble in water under all pH conditions).

Composting is a dynamic process carried out by a rapid succession of mixed microbial populations. The main groups of microorganisms involved are bacteria, including actinomycetes, and fungi (Golueke, 1991).

Although the total number of microorganisms does not significantly change during composting, the microbial diversity can vary during the different phases of composting (Atkinson et al., 1996a). The precise nature of succession and the number of microorganisms at each

composting phase is dependent on the substrate and on the preceding microorganisms in the succession (Crawford, 1983).

Different communities of microorganisms predominate during the various composting phases. At the beginning of composting, mesophilic microorganisms predominate, which rapidly break down the soluble, readily degradable compounds. The heat they produce causes the compost temperature to rapidly rise.

As the temperature rises above about 40°C, the mesophilic microorganisms become less competitive and are replaced by others that are thermophilic, or heat-loving. At temperatures of 55°C and above, many microorganisms that are human or plant pathogens are destroyed. Because temperatures over about 65°C kill many forms of microbes and microbial activity decreases dramatically, compost managers use aeration and mixing to keep the temperature below this point.

During the thermophilic phase, high temperatures accelerate the breakdown of proteins, fats and complex carbohydrates like cellulose and hemicellulose, the major structural molecules in plants. As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases; mesophilic bacteria and actinomycetes again dominate (McKinley and Vestal, 1985; Strom, 1985a) for the final phase of "curing" or maturation of the remaining organic matter.

Composting is an aerobic process in general, but anaerobic microenvironments may develop. Atkinson et al. (1996b) estimated that almost 1% of all the bacteria found in municipal solid waste compost were anaerobic. All the anaerobic bacteria found were highly cellulolytic and thus may play a significant role in the degradation of macromolecules. The majority of the mesophilic anaerobic bacteria were

facultative, while under thermophilic conditions more obligate anaerobic bacteria were found (Atkinson et al., 1996b).

1.7.2 Bacteria:

Bacteria are the smallest living organisms and the most numerous in compost; they make up 80 to 90% of the billions of microorganisms typically found in a gram of compost. Because of their small size (ranging from 0.5 to 3.0 μm) bacteria have a very high surface/volume ratio, which allows rapid transfer of soluble substrates into the cell. As a result, bacteria are usually far more dominant and the most nutritionally diverse group of compost organisms, using a broad range of enzymes to chemically break down a variety of organic materials.

Bacteria are single-celled and structured as rod-shaped bacilli, sphere-shaped cocci or spiral-shaped spirilla. Many are motile, meaning that they have the ability to move under their own power. A wide range of bacteria have been isolated from different compost environments, including species of *Pseudomonas*, *Klebsiella* and *Bacillus* (Nakasaki et al., 1985; Strom, 1985a, b).

At the beginning of the composting process (0 - 40°C), mesophilic bacteria predominate. Most of these are forms that can also be found in topsoil.

As the compost heats up above 40°C, thermophilic bacteria take over. The microbial populations during this phase are dominated by members of the genus *Bacillus*, e.g. *B. subtilis*, *B. licheniformis* and *B. circulans* as Strom (1985b) reports that as much as 87% of the randomly selected colonies during the thermophilic phase of composting belong to this genus.

The diversity of bacilli species is fairly high at temperatures from 50-55°C but decreases dramatically at 60°C or above. When conditions become unfavorable, bacilli survive by forming endospores, thick-walled spores that are highly resistant to heat, dryness, radiation and chemical disinfection (Haug, 1993). They are ubiquitous in nature and become active whenever environmental conditions are favorable.

At the highest compost temperatures, Bacteria of the genus *Thermus* have been isolated from compost at temperatures as high as 65°C and even 82°C (Beffa et al., 1996).

Once the compost cools down, mesophilic bacteria again predominate. The numbers and types of mesophilic microbes that recolonize compost as it matures depend on what spores and organisms are present in the compost as well as in the immediate environment. In general, the longer the curing or maturation phase, the more diverse the microbial community it supports.

1.7.3 Actinomycetes:

The characteristic earthy smell of soil is caused by actinomycetes. Actinomycetes are bacteria which form multicellular filaments, thus they resemble fungi. In composting they play an important role in degrading complex organic compounds such as cellulose, chitin, protein and soluble lignin, and they tolerate higher temperatures and pH than fungi. Thus, actinomycetes are important agents of lignocelluloses 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). Their enzymes enable them to chemically break down tough debris such as

woody stems, bark, or newspaper. Under adverse conditions actinomycetes survive as spores (Cross, 1968).

Some species appear during the thermophilic phase as well as the cooling and maturation phase of composting, and can occasionally become so numerous form long, thread like branched filaments that look like gray spider webs stretching through compost. These filaments are most commonly visible toward the end of the composting process in the outer 10 to 15 centimeters of the compost surface.

Thermophilic actinomycetes have been isolated from a wide range of natural substrates, e.g. from desert sand and compost (Cross, 1968). The genera of the thermophilic actinomycetes isolated from compost include *Nocardia*, *Streptomyces*, *Thermoactinomyces* and *Micromonospora* (Waksman et al., 1939b; Strom, 1985a).

1.7.4 Fungi:

Fungi include molds and yeasts, and collectively they are responsible for the decomposition of many complex plant polymers in soil and compost. In compost, fungi are important because they break down tough debris. They spread and grow vigorously by producing many cells and filaments, and they attack organic residues that are too dry, acidic, or low in nitrogen for bacterial decomposition.

Most fungi are classified as saprophytes because they live on dead or dying material and obtain energy by breaking down organic matter in dead plants and animals.

Temperature is one of the most important factors affecting fungal growth; they live in the outer layer of compost when temperatures are high. Other important factors are sources of carbon and nitrogen, and the pH.

Moreover, they are strict aerobes that grow both as unseen filaments and as gray or white fuzzy colonies on the compost surface.

Fungal species are numerous during both mesophilic and thermophilic phases of composting, the majority of fungi are mesophiles which grow between 5°C and 37°C, with an optimum temperature of 25-30°C (Dix and Webster, 1995). However, in the compost environment the elevated temperature means that the small group of thermophilic fungi is an important biodegradation agent. In nature, thermophilic fungi grow in garden compost heaps, bird nests, coal tips, power plant cooling pipes and effluents, in the storage of many agricultural products (like hay, grain etc.) and in piles of wood chips and peat (Sharma, 1989; Dix and Webster, 1995).

Cooney and Emerson (1964) define thermophilic fungi as fungi with a maximum growth temperature of 50°C and a minimum growth temperature of 20°C. Thermotolerant species have a maximum growth temperature of about 50°C and a minimum well below 20°C (Cooney and Emerson, 1964). Crisan (1973), however, defines thermophilic fungi as fungi with a temperature optimum of 40°C.

Part 2

MATERIALS AND METHODS

2.1 MATERIALS

2.1.1 Samples:

Compost samples were obtained from different home made compost stored at room temperature in sterile, clean plastic bags for laboratory investigation.

2.1.2 Media:

2.1.2.1 Isolation medium of mesophilic Streptomycetes:

Streptomycetes were isolated on ISP medium 4, which is composed of (g/l): K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 1.0; NaCl, 1.02; $(NH_4)_2SO_4$, 2.0; $CaCO_3$, 2.0; soluble starch, 10.0 and 1.0 ml solution of trace elements, the medium was solidified by adding 20.0, agar in distilled water (Ryckeboer et al., 2003). The solution of trace elements consisted of 0.1 g $FeSO_4$. $7H_2O$, 0.1 g $MnCl_2.4H_2O$ and 0.1 g $ZnSO_4.7H_2O$ in 100 ml distilled water. The pH of the medium was adjusted to 7.2 (Lacey, 1973).

2.1.2.2 Isolation medium of Thermoactinomyces species:

Thermoactinomyces sp. was isolated on Czapek-Yeast-Casamino (CYC); a highly selective isolation medium. It is composed of (g/l): sucrose, 30.0; $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; Kcl, 0.5; $FeSO_4$, 0.01; yeast extract (Oxoid), 2.0; casamino acids (Difco), 6.0 and agar, 15.0 in distilled water. The medium was adjusted to pH 7.2 autoclaved, cooled before adding membrane – filtered solution of 25 µg/ml novobiocin which inhibits the growth of all associated bacteria capable of growth at 50°C and 50 µg/ml cycloheximide to suppress fungal growth (Cross and Attwell, 1974). The

sample diluents contain gelatin, 0.5 g/l. Suitable dilutions are spread on the surface of the isolation medium and incubated at 55°C for 72 hrs.

2.1.2.3 Isolation medium for cellulolytic activities:

Cellulolytic bacteria and actinomycetes were isolated first on a medium composed of (g/l): $(NH_4)_2SO_4$, 1.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; NaCl, 0.001; agar, 15.0 and carboxymethylcellulose (CMC),10.0 in distilled water (Ryckeboer et al. 2003). Selected colonies were inoculated again onto CMC agar, containing 0.1% carboxymethylcellulose, 1.5% bacto-tryptone, 0.5% bacto-soyton, 0.5% NaCl and 1.5% agar. After growth, the plates were stained with 0.1% congo-red solution (Sigma Chem. Co.). The stained plates were destained with 0.1 M NaCl, colonies which had halo zone were selected as cellulolytic microorganisms.

2.1.2.4 Isolation medium for amylolytic activities:

Amylolytic bacteria and actinomycetes were isolated on a medium consisting of two layers. The lower layer contained minimal Delafield medium prepared with 0.033 M KH_2PO_4-Na_2HPO_4 buffer (pH 6.8) containing (g/l): NH_4Cl, 1.0; $MgSO_4$.7H2O, 0.5; ferric ammonium citrate, 0.05; and $CaCl_2.2H_2O$, 0.005 in distilled water (Delafield et al., 1965) (approx. 15 ml per Petri plate), the upper layer (approx. 5 ml per Petri plate) contained w/v: starch, 1.0 % and agar, 2% in distilled water (Ryckeboer et al., 2003).

Lugol's solution (0.5% I_2 and 1.0% KI in distilled water) was used for selection where all colonies, which produced clearing zones on this medium, were recorded as amylolytic microorganisms.

2.1.2.5 Isolation medium for proteolytic activities:

Proteolytic microorganisms were counted on milk agar, prepared as follows:

Solution A:

Nutrient agar powder (Oxoid Ltd) 23.0g.

Distilled water 700.0 ml.

Solution B:

Skimmed milk powder (Oxoid Ltd) 30.0 g.

Distilled water 300.0 ml.

Each solution was autoclaved separately then mixed together. The proteolytic microorganisms develop bright zones, while the medium remains turbid in case of non producing microorganism (Ryckeboer et al., 2003).

2.1.2.6 Isolation medium for pectinolytic activities:

Pectinolytic organisms cause liquefaction of the pectin gel. It is isolated on a medium consisting of two layers. The lower layer, the (Base agar) composed of (g/l): K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NH_4Cl, 1.0; $CaCl_2.2H_2O$, 3.0; Tris-Hcl buffer (1M, pH8), 100 ml; agar, 15.0 and trace element solution, 1.0 ml in distilled water (Gude, 1973). The upper layer (Pectin gel) contained 2% (w/v) pectin is dissolved in water of 70°C and autoclaved for 10 minutes at 110. Cold solution is poured as a thin layer onto base agar plates. The pectin will form a gel within 5 hours.

The solution of trace elements consisted of (mg/l): $MnCl_2.4H_2O$, 100.0; $CoCl_2$, 20.0; $CuSo_4$, 10.0; $Na_2MoO_4.2H_2O$, 10.0; $ZnCl_2$, 20.0; LiCl, 5.0; $SnCl_2.2H_2O$, 5.0; H_3Bo_3, 10.0; KBr, 20.0; KI, 20.0 and (EDTA) Na-Fe^{3+} salt (trihydrate), 8000.0 in distilled water, sterilized by filtration.

2.1.2.7 Medium for the detection of microbial interaction:

Active microorganisms were inoculated on a medium composed of:

Plant extract was used as organic supplementary material to mimic the actual growth environment of compost microorganisms.

The medium was prepared according to supervisor recommendation as follows:

(1) Plant supplement: 250 g plant material was autoclaved in 2 L distilled water for steam extraction. The autoclaved material was filtered and kept under refrigerator temperature.

(2) Double strength component of Czapek Dox medium containing (g/l): sucrose, 3.0; $NaNO_3$, 3.0; K_2HPO_4, 1.0; $MgSO_4$, 0.5; Kcl, 0.5; $FeSO_4$, 0.01 and agar, 15.0.

(3) Mix 500 ml of (1) + 200 ml of (2) + 300 ml distilled water.

The medium is adjusted to pH 7.2 autoclaved, and poured into petridishes.

2.1.2.8 Media for measuring enzyme activities:

Different plant components including starch, pectin, protein and cellulose were used as substrates for detecting enzyme activities of microbial inocula and to monitor microbial activities during composting stages. Each medium was supplemented with calcium chloride, 0.2 g; and agar, 13 g.

2.1.2.9 Nutrient broth:

Nutrient broth was used for growing the active isolated mesophilic and thermophilic bacteria; it was incubated in shaking incubator at 37°C and 55°C respectively. It contained (g/l): lab-lemco powder, 1.0; yeast extract, 2.0; peptone, 5.0; sodium chloride, 5.0.

2.1.2.10 International Streptomyces Project (ISP) medium 4 broth:

ISP medium 4 broth, composed of (g/l): K_2HPO_4, 1.0; $MgSO_4.7H_2O$, 1.0; NaCl, 1.0; $(NH_4)_2SO_4$, 2.0; $CaCO_3$, 2.0; soluble starch, 10.0 and 1.0 ml solution of trace elements (as indicated above) in distilled water (Ryckeboer et al., 2003).

2.1.2.11 Preservation medium:

After purification, cultures were preserved on rich media slants, i.e. nutrient agar for bacteria, inorganic salt starch 3 medium (ISP-3; Difco, Becton Dickinson) for streptomycetes. Bacterial cultures were lypholized and stored at 2°C.

2.1.3 Reagents and chemicals:

2.1.3.1 Reagents and chemicals for determination of total organic carbon (TOC):

Potassium dichromate solution (1M):

- Potassium dichromate 49.024 g
- Deionized distilled water 600.0 ml

Dilute to 1000 ml with deionized distilled water.

Sulfuric acid, concentrated (95-97%)

Concentrated phosphoric acid

Sodium fluoride

Indicator solution:

Phenanthroline Ferrous Sulfate (ferroin)

Ferrous ammonium sulphate (0.25 M):

- Ferrous ammonium sulphate 98.0 g
- Concentrated sulphuric acid 20.0 ml

Dilute to 1000 ml with deionized distilled water.

2.1.3.2 Reagents and chemicals for determination of total nitrogen (TN):

Digestion mixture:

- Selenium catalyst	0.42 g
- Lithium sulphate	14.0 g
- Hydrogen peroxide (30%)	350.0 ml
- Concentrated sulphuric acid	420.0 ml

Color reagent:

- Sodium salicylate	34.0 g
- Sodium citrate	25.0 g
- Sodium tartrate	25.0 g
- Deionized distilled water	750.0 ml
- Sodium nitroprusside	0.12 g

Components were dissolved in distilled water and the final volume was made up to 1000 ml.

Alkaline hypochlorite solution:

- Sodium hydroxide	30.0 g
- Deionized distilled water	750.0 ml
- Sodium hypochlorite solution	10.0 ml

Components were dissolved and the final volume was made up to 1000 ml with distilled water.

2.1.3.3 Reagents and chemicals for humus production:

- Sodium carbonates	0.1 N
- Potassium permanganate	0.1 N
- Oxalic acid	0.1 N
- Sulfuric acid	30 %

2.1.3.4 Stains for staining slides of microorganisms:

Gram staining used to make slides of bacteria and actinomycetes.

Crystal Violet stain:

- Crystal violet 2.0 g
- Ethanol (95%) 20.0 ml
- Ammonium Oxalate (1%) 80.0 ml

Dissolve the component and let stand for 24 hours and filter.

Gram Iodine:

- Iodine 1.0 g
- Potassium Iodide 3.0 g

Components were dissolved in 300.0 ml distilled water. Store in an amber bottle.

Decolorizer:

- Ethyl Alcohol (95%)

Safranin:

- Safranin 2.5 g
- Ethanol (95%) 10 ml

Add this solution to 100.0 ml distilled water.

2.1.4 Organisms:

Used isolated organisms: A total of 13 bacteria and actinomycetes were selected according to their higher enzymatic activities. They were symbolized as follows:

Bacteria: 3BP37, 13BD37, 1BD37, 2BM37, 1BP55, 12BM55, 1BC55, 3BP55.

Actinomycetes: 5AD37, 1AS37, 8AD37, 5AS37, 1AT55, where A stands for actinomycetes, B stands for bacteria, C stands for CMC isolation media, P stands for pectin isolation media, M stands for milk isolation media, D stands for Delafield isolation media, S stands for *Streptomycetes* isolation media and T stands for *Thermoactinomyces* isolation media.

2.1.5 Equipment and other materials:

2.1.5.1 Autoclave (market forge sterilmatic) model.

2.1.5.2 Growth incubator at 37°C and 55°C (Gallen kamp) model.

2.1.5.3 Growth shaking incubator at 37°C and 55°C (Gallen kamp orbital incubator) model.

2.1.5.4 Vortex mixer (Thermolyne, type 37600 mixer).

2.1.5.5 Micropipette (Finnpipette, F88186 and F29072).

2.1.5.6 Spectrophotometer (Thermo spectronic, Geaesys 20).

2.1.5.7 Digital camera (Casio, EX-Z40).

2.1.5.8 Compound microscope.

2.2 METHODS

2.2.1 Plating method to isolate active microorganisms:

2.2.1.1 Preparation of compost samples:

Compost suspensions were prepared by the addition of 10 g (wet weight) different compost samples to 90 ml of 0.85% (w/v) sterile sodium chloride solution in 250 ml Erlenmeyer flasks (Ryckeboer et al., 2003).

These suspensions were shaken at 100 r.p.m. for 30 min at 25°C. Serial dilutions of these initial suspensions were made in sterile sodium chloride solution. Aliquots (0.1 ml) of 10^{-3}, 10^{-4}, and 10^{-5} were spread with a Drigalski rod in Petri plates containing the required medium. Three plates were used per dilution.

2.2.1.2 Incubation, isolation and purification:

Incubation was at 37°C for mesophilic microorganisms and at 55°C for thermophilic microorganisms. After 24 h, 48 h and 72h of incubation, all thermophilic and mesophilic proteolytic, cellulolytic, pectinolytic and amylolytic bacteria were isolated. Mesophilic streptomycetes were isolated after 72 h of incubation which show aerial mycelium (powdery, wrinkled, or pasty). When these colonies were isolated, their plates had the typical earthy odor associated with actinomycetes (Savage et al., 1973). Occasionally, more than one colony type appeared on the streak plates; in such cases, each type was restreaked until pure cultures of all were obtained.

2.2.1.3 Active microorganisms and inocula preparation:

Isolated mesophilic and thermophilic microorganisms were tested on different substrate media for enzyme activity such as cellulase, pectinase, amylase and protease and the strongest enzyme activity produced by those

bacteria and actinomycetes were detected by measuring the hydrolyzed zones, using appropriate indicators.

The results were expressed as the mean of two ratios calculated by dividing the estimated diameter of hydrolyzed zones by the estimated diameter of the colony growth (cm/cm).

The most active microorganisms were chosen and inoculated in suitable broth media.

2.2.2 Composting experiment:

2.2.2.1 Composting bins:

Composting experiment was carried out in the nursery of University of Bahrain and it was being implemented in four rotating bins with perforated cover as shown in (photo 2.1), approximately 60 cm height, 35 cm width and 36 cm length holding approximately 4 kg of leaf waste material. The headspace between the cover and the top of leaf waste in each bin was about 15 cm. Those bins were kept in plastic tent as shown in (photo 2.2) to provide a warm condition because the study was performed in January (winter season).

Those manual rotating bins have been provided with central aeration system "Aeration Tube" as shown in (photo 2.3) to give enough oxygen needed by the aerobes for better and faster decomposition of the waste. In addition, the center aeration tube with its cross bar assists in the break up and separation of compost as it is turned preventing the materials from turning into a ball and just sliding back and forth when tumbled.

Aerobic conditions were maintained by periodically turning the composting material to allow all parts of the bin to receive oxygen and to prevent the

onset of anaerobic putrefaction which is characterized by objectionable odors and a slow rate of decomposition.

The design of the system ensures appropriate aerobiosis during composting phases.



Photo Credit: Author

Photo 2.1: Experimental composting bins



Photo Credit: Author

Photo 2.2: Bins kept in plastic tent

2.2.2.2 Composting substrate preparation:

Dry leaves and small tree branches were used as substrates for composting. They were collected from University of Bahrain campus. These waste materials were chopped and cut into small pieces approximately 2.5 cm by shredder as shown in (photo 2.4). As Gray et al. (1971) recommend a particle size of 1.3 to 7.6 cm (0.5 to 2 inches). Thus, shredded materials or smaller particle size can be degraded more quickly than larger particles of the same material and insure proper distribution of inocula.

Plant wastes (dried leaves) were enriched by adding an average of 2 per cent (dry weight) nitrogen fertilizer as urea, and 0.2 per cent phosphorus as superphosphate because when composting high carbon materials such as dry leaves, additional nitrogen required to reduce the C/N ratio to the optimal range.

The moisture content was adjusted to 600 g kg^{-1} wet weight (Rajbanshi et al., 1998). This level helps to ensure good aeration for oxidative process and provides sufficient moisture for heterotrophic microbes in the decomposition process (Sylvia et al., 1998). Furthermore, water was added when necessary to maintain optimum moisture content.

Temperature observations of the bins were made daily with mercury thermometer at a mid-depth of the center of the bin. Aerobic condition makes possible the accumulation of sufficient heat from bacterial metabolism to raise the temperature of the biomass to the thermophilic range.

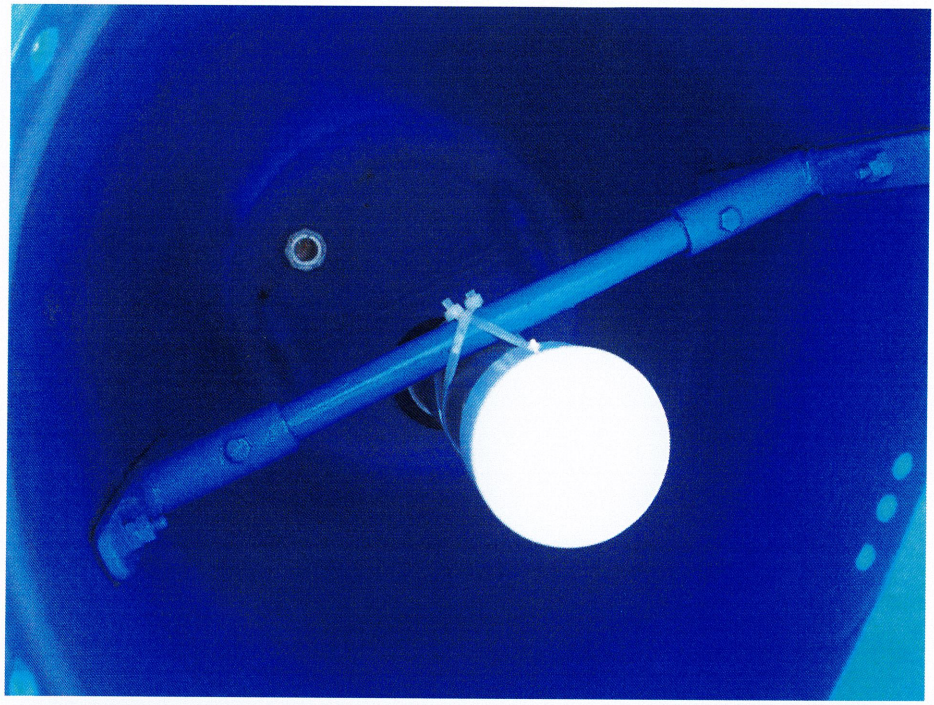


Photo Credit: Author

Photo 2.3: Aeration Tube in composting bin



Photo Credit: Author

Photo 2.4: Shredding machine

2.2.2.3 Inoculation:

Each bin was inoculated with active microbial suspension mixtures (approximately10^4 to 10^5 *cfu* ml^{-1}) for each isolate, 50 ml active culture / kg substrate, and referred as inoculated treatments.

Inoculation was made at start, after 3 weeks and after 6 weeks according to the following scheme:

Bin 1: non-inoculated treatment (control).

Bin 2: 5AD37, 1AT55, 3BP37, 1BP55, 1AS37, 13BD37, 12BM55.

Bin 3: 8AD37, 1BD37, 1BC55, 5AS37, 1AT55, 2BM37, 3BP55.

Bin 4: 5AD37, 1AT55, 3BP37, 1BP55, 1AS37, 13BD37, 12BM55, 8AD37, 1BD37, 1BC55, 5AS37, 2BM37, 3BP55.

2.2.3 Enzyme activity:

Active microorganisms were tested for their extra cellular enzymes production by measuring the diameter of the hydrolyzed zones.

2.2.4 Compost Chemical analysis:

Analysis of the compost material was made from the raw material to the finished product at days 0, 14, 28, 42, 56, 70, 84, 98, 112, and 126.

Determinations included ash%, moisture content%, total organic carbon (TOC) %, total nitrogen (TN) %, and C/N ratio.

Humus was analyzed at the end of the composting process by using Stevenson method (1994).

Grab samples were taken from several different parts of the bin and thoroughly mixed. 100 g portion were removed, two portions for carbon and nitrogen analyses and the third one for ash determinations.

Percentage moisture was determined on the 100 g sample to be used in ash determinations by observing the weight loss during a 24-hour period of drying at 104°C. The samples were then ignited in a muffle furnace at 500°C and percentage ash calculated (Golueke et al., 1954).

Total nitrogen (TN) and total organic carbon (TOC) of the compost material were estimated by using Keeney and Nelson method (1982) and Walkey and Black's Rapid Titration method (1934), respectively.

2.2.5 Statistical analysis:

The data was subjected to the analysis of variance using General Linear Model (SPANOVA) "SPSS 13.0" software. Whenever significant results ($p<0.05$) among the parameters were obtained, analysis was then followed by multiple comparison analysis (Tukey) to locate the differences.

Part 3

RESULTS

3.1 Isolation of total active microorganisms:

The present study focused on isolating bacteria and actinomycetes that produce different enzymes able to degrade plant materials, and make use of those active microorganisms as inocula to enhance bio-composting.

Bacteria and actinomycetes were isolated from different post composted materials. All isolates were selected based on their enzymatic activities and optimum growth temperatures to isolate mesophilic and thermophilic and/or thermotolerant bacteria from different selective media such as CMC, pectin, milk, Delafield, *Streptomycetes* and *Thermoactinomyces* isolation media.

Tables 3.1, 3.2, 3.3, 3.4 show the total numbers, types of isolates and their related growth temperatures of bacteria and actinomycetes that produce cellulolytic, pectinolytic, amylolytic and proteolytic activities respectively.

A total number of 209 isolates were obtained during the isolation process. They were divided as: 5 cellulolytic bacteria grown at 37°C; 7 cellulolytic bacteria grown at 55°C; 18 cellulolytic actinomycetes grown at 37°C; 35 pectinolytic bacteria grown at 37°C; 22 pectinolytic bacteria grown at 55°C; 37 pectinolytic actinomycetes grown at 37°C;. In addition to that, 6 amylolytic bacteria grown at 37°C; 14 amylolytic bacteria grown at 55°C; 31 amylolytic actinomycetes grown at 37°C; 5 proteolytic bacteria grown at 37°C; 10 proteolytic bacteria grown at 55°C; 18 proteolytic actinomycetes grown at 37°C and one cellulolytic and proteolytic actinomycete grown at 55°C.

Table 3.1: Total numbers, isolate types and related growth temperatures of **cellulolytic** bacteria and actinomycetes.

Serial #	Isolate code	Isolate type	Optimal incubation period (h)	Growth Temperature (C^0)	Other enzymatic activities
1	1BC37	Bacteria	24	37	Pectinolytic
2	2BC37	Bacteria	24	37	Pectinolytic
3	3BC37	Bacteria	24	37	Pectinolytic
4	3BP37	Bacteria	48	37	Pectinolytic Proteolytic
5	6BP37	Bacteria	24	37	Pectinolytic Amylolytic
6	1BC55	Bacteria	72	55	Pectinolytic Amylolytic Proteolytic
7	1BP55	Bacteria	24	55	Pectinolytic Amylolytic
8	3BP55	Bacteria	24	55	Pectinolytic Amylolytic Proteolytic
9	7BM55	Bacteria	24	55	Pectinolytic Proteolytic
10	10BM55	Bacteria	24	55	Pectinolytic Amylolytic Proteolytic
11	11BM55	Bacteria	24	55	Pectinolytic Amylolytic Proteolytic
12	12BM55	Bacteria	24	55	Pectinolytic Amylolytic Proteolytic
13	1AC37	Actinomycetes	96	37	Pectinolytic Amylolytic
14	4AC37	Actinomycetes	72	37	Pectinolytic Amylolytic Proteolytic
15	6AC37	Actinomycetes	96	37	Pectinolytic Amylolytic
16	1AP37	Actinomycetes	48	37	Pectinolytic Proteolytic
17	2AP37	Actinomycetes	48	37	Pectinolytic
18	4AP37	Actinomycetes	72	37	Pectinolytic

Table 3.1: (continued)

19	6AP37	Actinomycetes	48-72	37	Pectinolytic Proteolytic
20	1AD37	Actinomycetes	72	37	Pectinolytic Amylolytic Proteolytic
21	2AD37	Actinomycetes	72	37	Pectinolytic Amylolytic Proteolytic
22	3AD37	Actinomycetes	48	37	Pectinolytic Amylolytic Proteolytic
23	5AD37	Actinomycetes	72	37	Pectinolytic Amylolytic Proteolytic
24	8AD37	Actinomycetes	72	37	Pectinolytic Amylolytic
25	9AD37	Actinomycetes	72	37	Pectinolytic Proteolytic
26	2AS37	Actinomycetes	72	37	Pectinolytic Amylolytic Proteolytic
27	7AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
28	10AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
29	12AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
30	13AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
31	1AT55	Actinomycetes	24-48	55	Proteolytic

Table 3.2: Total number, types of isolates and related growth temperatures of **Pectinolytic** bacteria and actinomycetes.

Serial #	Isolate code	Isolate type	Optimal incubation period (h)	Growth Temperature (C^0)	Other enzymatic activities
1	1BC37	Bacteria	24	37	Cellulolytic
2	2BC37	Bacteria	24	37	Cellulolytic
3	3BC37	Bacteria	24	37	Cellulolytic
4	4BC37	Bacteria	24	37	ND
5	5BC37	Bacteria	24	37	ND
6	6BC37	Bacteria	24	37	ND
7	7BC37	Bacteria	24	37	Amylolytic
8	2BP37	Bacteria	24	37	Proteolytic
9	3BP37	Bacteria	48	37	Cellulolytic Proteolytic
10	4BP37	Bacteria	24	37	Amylolytic
11	5BP37	Bacteria	48	37	ND
12	6BP37	Bacteria	24	37	Cellulolytic Amylolytic Proteolytic
13	12BD37	Bacteria	24-48	37	ND
14	13BD37	Bacteria	72	37	ND
15	24BD37	Bacteria	24-48	37	ND
16	26BD37	Bacteria	48	37	ND
17	30BD37	Bacteria	24	37	ND
18	31BD37	Bacteria	24	37	ND
19	32BD37	Bacteria	24	37	ND
20	1BM37	Bacteria	24	37	ND
21	2BM37	Bacteria	24	37	Proteolytic
22	3BM37	Bacteria	24	37	ND
23	4BM37	Bacteria	24	37	ND
24	5BM37	Bacteria	24	37	ND
25	7BM37	Bacteria	24	37	Proteolytic
26	9BM37	Bacteria	24	37	ND
27	2BS37	Bacteria	48	37	ND
28	4BS37	Bacteria	48	37	ND
29	7BS37	Bacteria	24-48	37	ND
30	9BS37	Bacteria	24	37	ND
31	11BS37	Bacteria	24-48	37	ND

Table 3.2: (continued)

32	12BS37	Bacteria	24	37	ND
33	14BS37	Bacteria	24	37	ND
34	15BS37	Bacteria	48	37	ND
35	17BS37	Bacteria	72	37	ND
36	1BC55	Bacteria	72	55	Cellulolytic Amylolytic Proteolytic
37	1BP55	Bacteria	24	55	Cellulolytic Amylolytic
38	2BP55	Bacteria	48	55	Amylolytic
39	3BP55	Bacteria	24	55	Cellulolytic Amylolytic Proteolytic
40	1BD55	Bacteria	72	55	Amylolytic
41	2BD55	Bacteria	72	55	Amylolytic
42	4BD55	Bacteria	72	55	Amylolytic
43	5BD55	Bacteria	72	55	Amylolytic
44	1BM55	Bacteria	24	55	Proteolytic
45	2BM55	Bacteria	24	55	ND
46	3BM55	Bacteria	24	55	Proteolytic
47	6BM55	Bacteria	24	55	ND
48	7BM55	Bacteria	24	55	Cellulolytic Proteolytic
49	8BM55	Bacteria	48	55	Amylolytic Proteolytic
50	10BM55	Bacteria	24	55	Cellulolytic Amylolytic Proteolytic
51	11BM55	Bacteria	24	55	Cellulolytic Amylolytic Proteolytic
52	12BM55	Bacteria	24	55	Cellulolytic Amylolytic Proteolytic
53	5BS55	Bacteria	24	55	Amylolytic
54	8BS55	Bacteria	48	55	Amylolytic
55	1BT55	Bacteria	24	55	ND
56	2BT55	Bacteria	48	55	ND
57	3BT55	Bacteria	48	55	ND

Table 3.2: (continued)

58	1AC37	Actinomycetes	96	37	Cellulolytic Amylolytic
59	2AC37	Actinomycetes	72	37	Amylolytic
60	3AC37	Actinomycetes	72	37	Amylolytic Proteolytic
61	4AC37	Actinomycetes	72	37	Cellulolytic Amylolytic Proteolytic
62	5AC37	Actinomycetes	96	37	Amylolytic Proteolytic
63	6AC37	Actinomycetes	96	37	Cellulolytic Amylolytic
64	1AP37	Actinomycetes	48	37	Cellulolytic Proteolytic
65	2AP37	Actinomycetes	48	37	Cellulolytic
66	3AP37	Actinomycetes	72	37	Proteolytic
67	4AP37	Actinomycetes	72	37	Cellulolytic
68	5AP37	Actinomycetes	48-72	37	Amylolytic Proteolytic
69	6AP37	Actinomycetes	48-72	37	Cellulolytic Proteolytic
70	1AD37	Actinomycetes	72	37	Cellulolytic Amylolytic Proteolytic
71	2AD37	Actinomycetes	72	37	Cellulolytic Amylolytic Proteolytic
72	3AD37	Actinomycetes	48	37	Cellulolytic Amylolytic Proteolytic
73	4AD37	Actinomycetes	48	37	Amylolytic
74	5AD37	Actinomycetes	72	37	Cellulolytic Amylolytic Proteolytic
75	6AD37	Actinomycetes	72	37	Amylolytic
76	7AD37	Actinomycetes	72	37	Amylolytic Proteolytic
77	8AD37	Actinomycetes	72	37	Cellulolytic Amylolytic
78	9AD37	Actinomycetes	72	37	Cellulolytic Proteolytic

Table 3.2: (continued)

79	1AS37	Actinomycetes	72	37	Amylolytic
80	2AS37	Actinomycetes	72	37	Cellulolytic Amylolytic Proteolytic
81	3AS37	Actinomycetes	48	37	Amylolytic
82	4AS37	Actinomycetes	72	37	Amylolytic Proteolytic
83	5AS37	Actinomycetes	72	37	Amylolytic Proteolytic
84	6AS37	Actinomycetes	72	37	Amylolytic Proteolytic
85	7AS37	Actinomycetes	72	37	Cellulolytic Amylolytic
86	8AS37	Actinomycetes	72	37	Amylolytic
87	9AS37	Actinomycetes	72	37	Amylolytic
88	10AS37	Actinomycetes	72	37	Cellulolytic Amylolytic
89	11AS37	Actinomycetes	72	37	Amylolytic
90	12AS37	Actinomycetes	72	37	Cellulolytic Amylolytic
91	13AS37	Actinomycetes	72	37	Cellulolytic Amylolytic
92	14AS37	Actinomycetes	72	37	Amylolytic Proteolytic
93	15AS37	Actinomycetes	48-72	37	Amylolytic
94	16AS37	Actinomycetes	72	37	Amylolytic

ND: not detected

Table 3.3: Total number, types of isolates and related growth temperatures of **Amylolytic** bacteria and actinomycetes.

Serial #	Isolate code	Isolate type	Optimal incubation period (h)	Growth Temperature (C^o)	Other enzymatic activities
1	7BC37	Bacteria	24	37	Pectinolytic
2	4BP37	Bacteria	24	37	Pectinolytic
3	6BP37	Bacteria	24	37	Cellulolytic Pectinolytic
4	1BD37	Bacteria	72	37	ND
5	15BS37	Bacteria	48	37	Pectinolytic
6	17BS37	Bacteria	72	37	Pectinolytic
7	1BC55	Bacteria	72	55	Cellulolytic Pectinolytic Proteolytic
8	1BP55	Bacteria	24	55	Cellulolytic Pectinolytic
9	2BP55	Bacteria	48	55	Pectinolytic
10	3BP55	Bacteria	24	55	Cellulolytic Pectinolytic Proteolytic
11	1BD55	Bacteria	72	55	Pectinolytic
12	2BD55	Bacteria	72	55	Pectinolytic
13	4BD55	Bacteria	72	55	Pectinolytic
14	5BD55	Bacteria	72	55	Pectinolytic
15	8BM55	Bacteria	48	55	Pectinolytic Proteolytic
16	10BM55	Bacteria	24	55	Cellulolytic Pectinolytic Proteolytic
17	11BM55	Bacteria	24	55	Cellulolytic Pectinolytic Proteolytic
18	12BM55	Bacteria	24	55	Cellulolytic Pectinolytic Proteolytic
19	5BS55	Bacteria	24	55	Pectinolytic
20	8BS55	Bacteria	48	55	Pectinolytic
21	1AC37	Actinomycetes	96	37	Cellulolytic Pectinolytic

Table 3.3: (continued)

22	2AC37	Actinomycetes	72	37	Pectinolytic
23	3AC37	Actinomycetes	72	37	Pectinolytic Proteolytic
24	4AC37	Actinomycetes	72	37	Cellulolytic Pectinolytic Proteolytic
25	5AC37	Actinomycetes	96	37	Pectinolytic Proteolytic
26	6AC37	Actinomycetes	96	37	Cellulolytic Pectinolytic
27	5AP37	Actinomycetes	48-72	37	Pectinolytic Proteolytic
28	1AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Proteolytic
29	2AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Proteolytic
30	3AD37	Actinomycetes	48	37	Cellulolytic Pectinolytic Proteolytic
31	4AD37	Actinomycetes	48	37	Pectinolytic
32	5AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Proteolytic
33	6AD37	Actinomycetes	72	37	Pectinolytic
34	7AD37	Actinomycetes	72	37	Pectinolytic Proteolytic
35	8AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic
36	1AS37	Actinomycetes	72	37	Pectinolytic
37	2AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic Proteolytic
38	3AS37	Actinomycetes	48	37	Pectinolytic
39	4AS37	Actinomycetes	72	37	Pectinolytic Proteolytic
40	5AS37	Actinomycetes	72	37	Pectinolytic Proteolytic
41	6AS37	Actinomycetes	72	37	Pectinolytic Proteolytic
42	7AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic

Table 3.3: (continued)

43	8AS37	Actinomycetes	72	37	Pectinolytic
44	9AS37	Actinomycetes	72	37	Pectinolytic
45	10AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic
46	11AS37	Actinomycetes	72	37	Pectinolytic
47	12AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic
48	13AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic
49	14AS37	Actinomycetes	72	37	Pectinolytic Proteolytic
50	15AS37	Actinomycetes	48-72	37	Pectinolytic
51	16AS37	Actinomycetes	72	37	Pectinolytic

ND: not detected

Table 3.4: Total number, types of isolates and related growth temperatures of **Proteolytic** bacteria and actinomycetes.

Serial #	Isolate code	Isolate type	Optimal incubation period (h)	Growth Temperature (C^o)	Other enzymatic activities
1	2BP37	Bacteria	24	37	Pectinolytic
2	3BP37	Bacteria	48	37	Cellulolytic Pectinolytic
3	2BM37	Bacteria	24	37	Pectinolytic
4	7BM37	Bacteria	24	37	Pectinolytic
5	8BM37	Bacteria	24	37	ND
6	1BC55	Bacteria	72	55	Cellulolytic Amylolytic Pectinolytic
7	3BP55	Bacteria	24	55	Cellulolytic Amylolytic Pectinolytic
8	1BM55	Bacteria	24	55	Pectinolytic
9	3BM55	Bacteria	24	55	Pectinolytic
10	5BM55	Bacteria	72	55	ND
11	7BM55	Bacteria	24	55	Cellulolytic Pectinolytic
12	8BM55	Bacteria	48	55	Amylolytic Pectinolytic
13	10BM55	Bacteria	24	55	Cellulolytic Amylolytic Pectinolytic
14	11BM55	Bacteria	24	55	Cellulolytic Amylolytic Pectinolytic
15	12BM55	Bacteria	24	55	Cellulolytic Amylolytic Pectinolytic
16	3AC37	Actinomycetes	72	37	Pectinolytic Amylolytic
17	4AC37	Actinomycetes	72	37	Cellulolytic Pectinolytic Amylolytic
18	5AC37	Actinomycetes	96	37	Amylolytic Pectinolytic
19	1AP37	Actinomycetes	48	37	Cellulolytic Pectinolytic

Table 3.4: (continued)

20	3AP37	Actinomycetes	72	37	Pectinolytic
21	5AP37	Actinomycetes	48-72	37	Amylolytic Pectinolytic
22	6AP37	Actinomycetes	48-72	37	Cellulolytic Pectinolytic
23	1AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Amylolytic
24	2AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Amylolytic
25	3AD37	Actinomycetes	48	37	Cellulolytic Pectinolytic Amylolytic
26	5AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic Amylolytic
27	7AD37	Actinomycetes	72	37	Pectinolytic Amylolytic
28	9AD37	Actinomycetes	72	37	Cellulolytic Pectinolytic
29	2AS37	Actinomycetes	72	37	Cellulolytic Pectinolytic Amylolytic
30	4AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
31	5AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
32	6AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
33	14AS37	Actinomycetes	72	37	Pectinolytic Amylolytic
34	1AT55	Actinomycetes	24-48	55	Cellulolytic

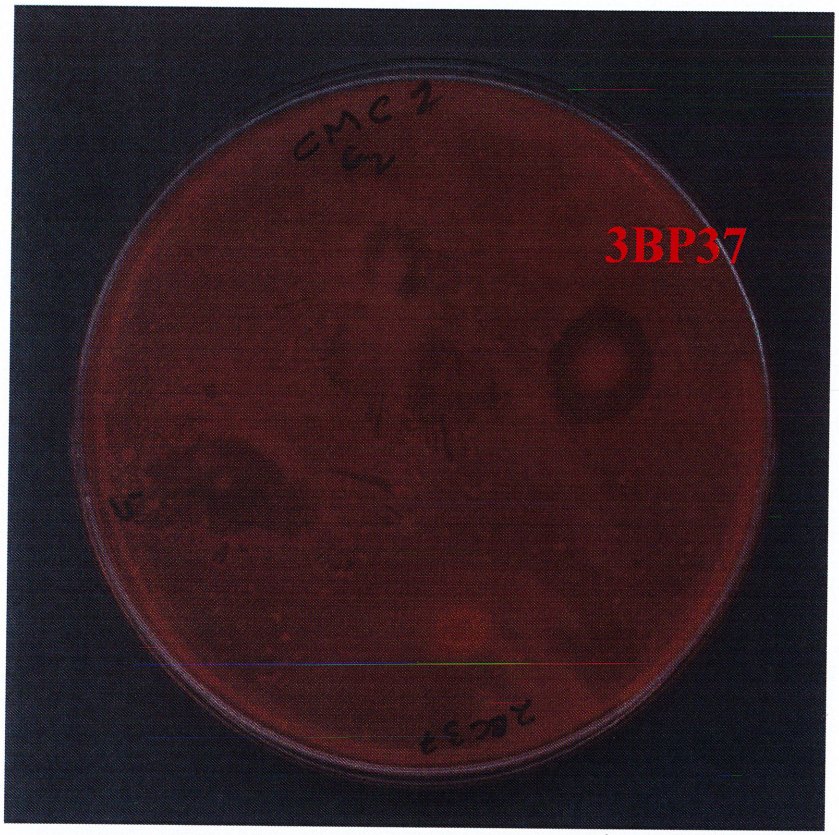
ND: not detected

3.2 Selection of the most enzymatically active bacteria and actinomycetes:

The most active bacteria and actinomycetes selected on the basis of higher enzymatic activities are 24 bacteria and 17 actinomycetes.
Selection also took into consideration activities at 37°C and 55°C.
The following results show the selected enzymatically active groups.

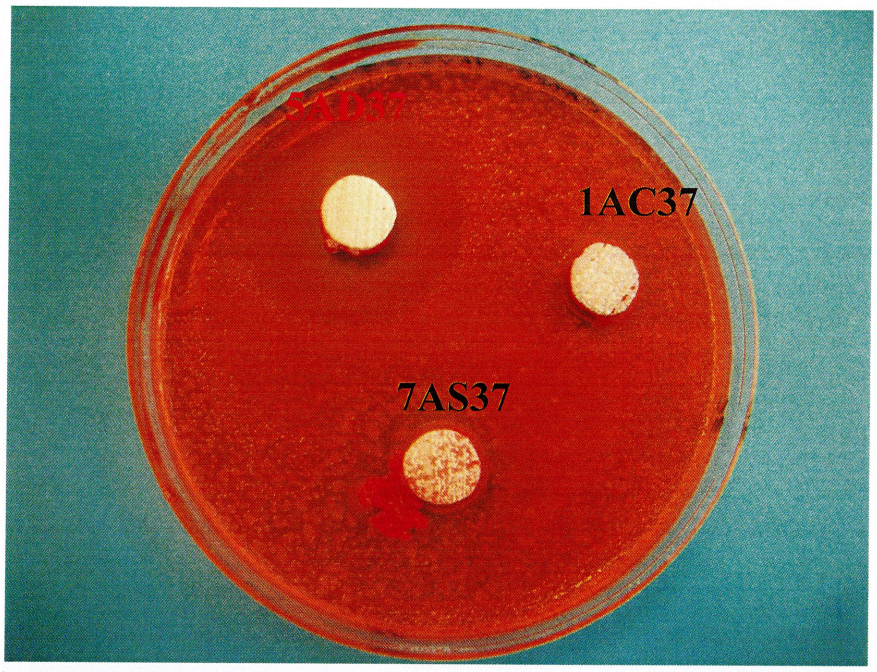
3.2.1 Selection of the most active cellulolytic bacteria and actinomycetes:

Bacteria and actinomycetes that showed higher cellulolytic activities on CMC medium are shown in (photo 3.1 and photo 3.2) respectively.
Selection was based quantitatively on the width of the activity zones which means that more width more active.
The most active selected bacteria and actinomycetes are listed below as follows:
Cellulolytic bacteria active at 37°C: 3BP37, 1BC37, 6BP37 and 2BC37.
Cellulolytic bacteria active at 55°C: 1BP55.
Cellulolytic actinomycetes active at 37°C: 5AD37, 7AS37 and 1AC37.
Cellulolytic actinomycetes active at 55°C: 1AT55.

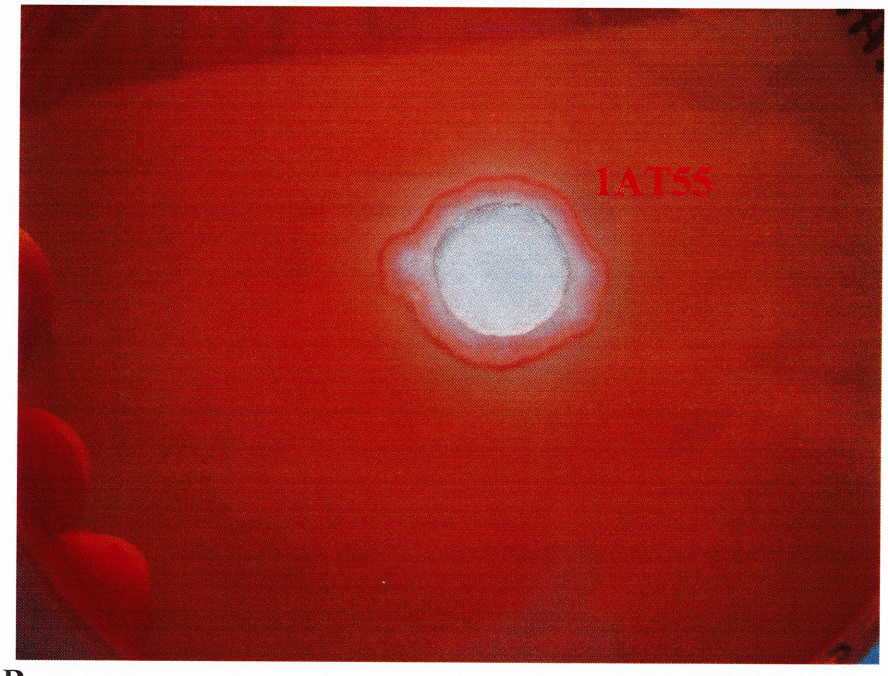


Photos Credit: Author

Photo 3.1: Cellulolytic active bacteria 3BP37 isolated at 37°C on CMC medium.



A.



B.

Photos Credit: Author

Photo 3.2: Different cellulolytic active actinomycetes isolated at 37°C & 55°C on CMC medium.
A: Cellulolytic actinomycete isolated at 37°C: 5AD37.
B: Cellulolytic actinomycete isolated at 55°C: 1AT55.

3.2.2 Selection of the most active pectinolytic bacteria and actinomycetes:

Bacteria and actinomycetes that showed higher pectinolytic activities on pectin medium are shown in (photo 3.3 and photo 3.4) respectively.

Selection was based quantitatively on the width of the activity zones i.e. more width more active.

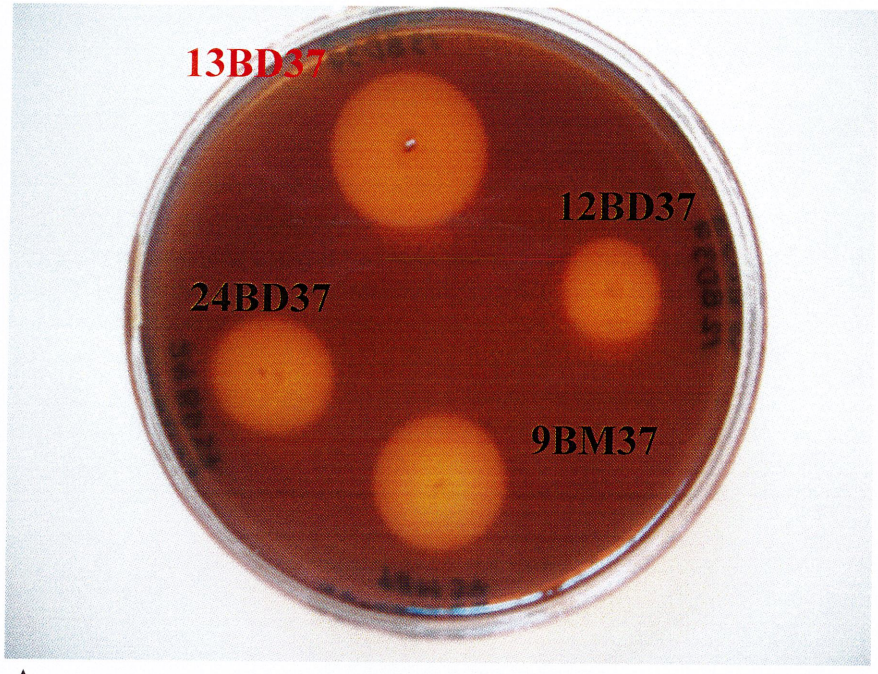
The most active selected bacteria and actinomycetes are listed below as follows:

Pectinolytic bacteria active at 37°C: 13BD37, 9BM37, 11BS37, 6BP37 and 5BC37.

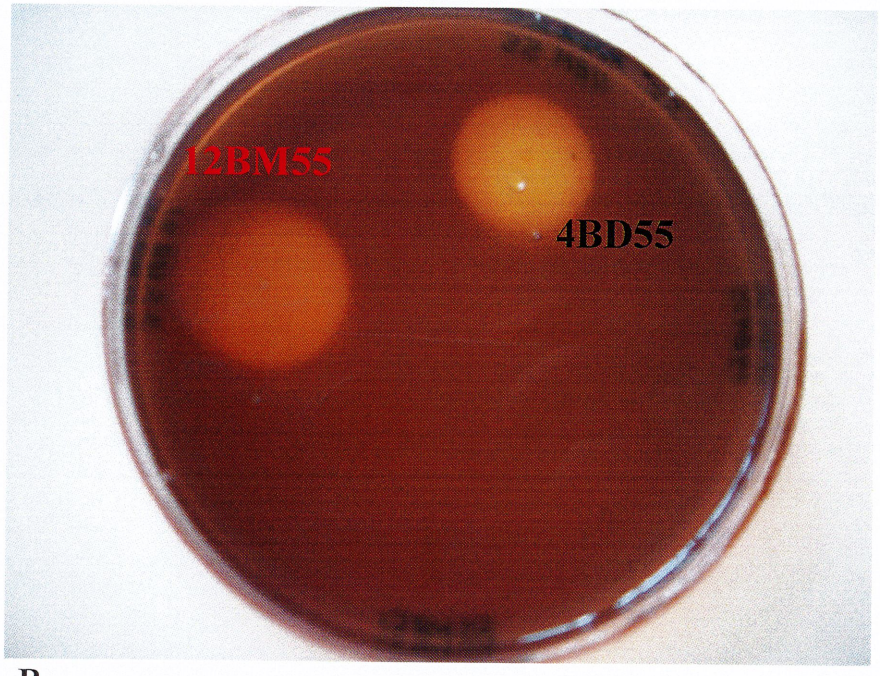
Pectinolytic bacteria active at 55°C: 1BC55, 12BM55, 4BD55, 8BS55 and 1BT55.

Pectinolytic actinomycetes active at 37°C: 1AS37, 1AC37, 1AD37 and 5AP37.

Pectinolytic actinomycetes active at 55°C: not detected.



A.



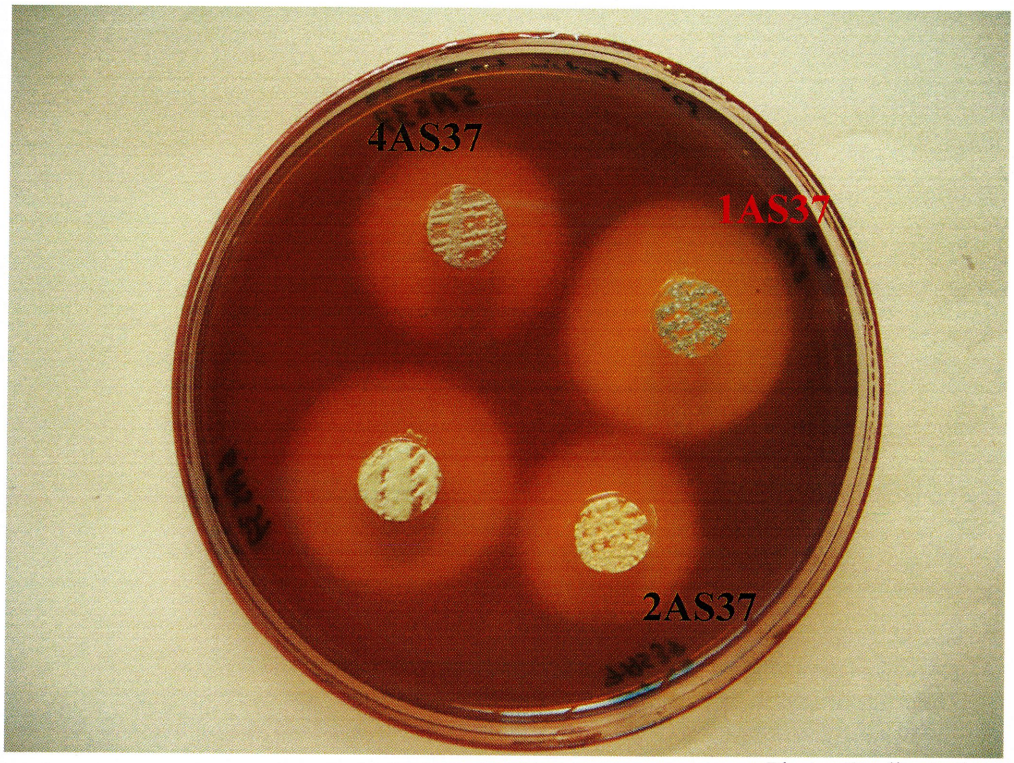
B.

Photos Credit: Author

Photo 3.3: Different pectinolytic active bacteria isolated at 37°C & 55°C on pectin medium.

A: Pectinolytic bacteria isolated at 37°C: 13BD37.

B: Pectinolytic bacteria isolated at 55°C: 12BM55.



Photos Credit: Author

Photo 3.4: Different pectinolytic active actinomycetes isolated at 37°C on pectin medium.
Pectinolytic actinomycete isolated at 37°C: 1AS37.

3.2.3 Selection of the most active amylolytic bacteria and actinomycetes:

Bacteria and actinomycetes that showed higher amylolytic activities on Delafield medium are shown in (photo 3.5 and photo 3.6) respectively. Selection was based quantitatively on the width of the activity zones i.e. more width more active.

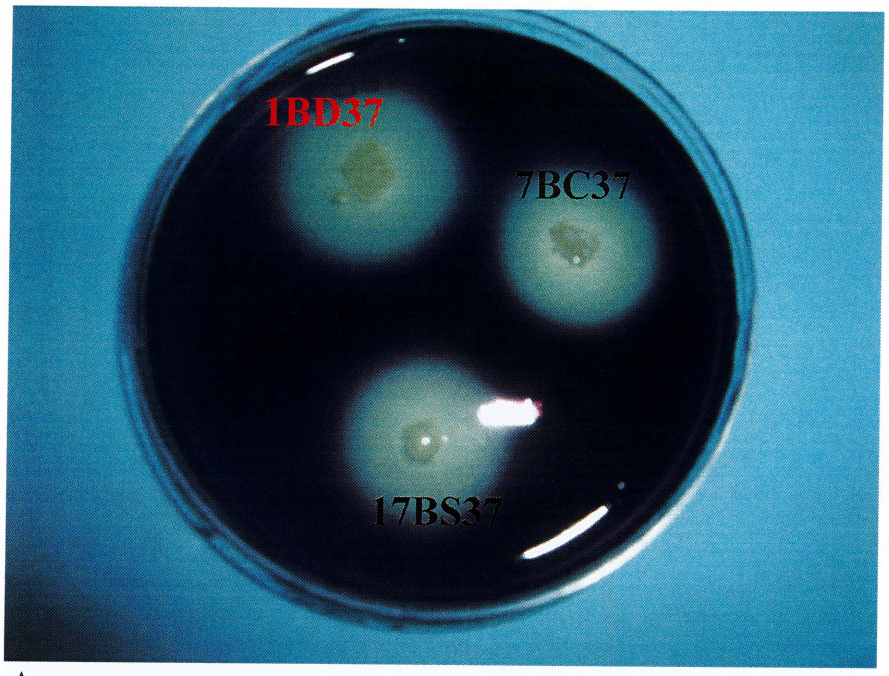
The most active selected bacteria and actinomycetes are listed below as follows:

Amylolytic bacteria active at 37°C: 1BD37, 17BS37, 7BC37 and 4BP37.

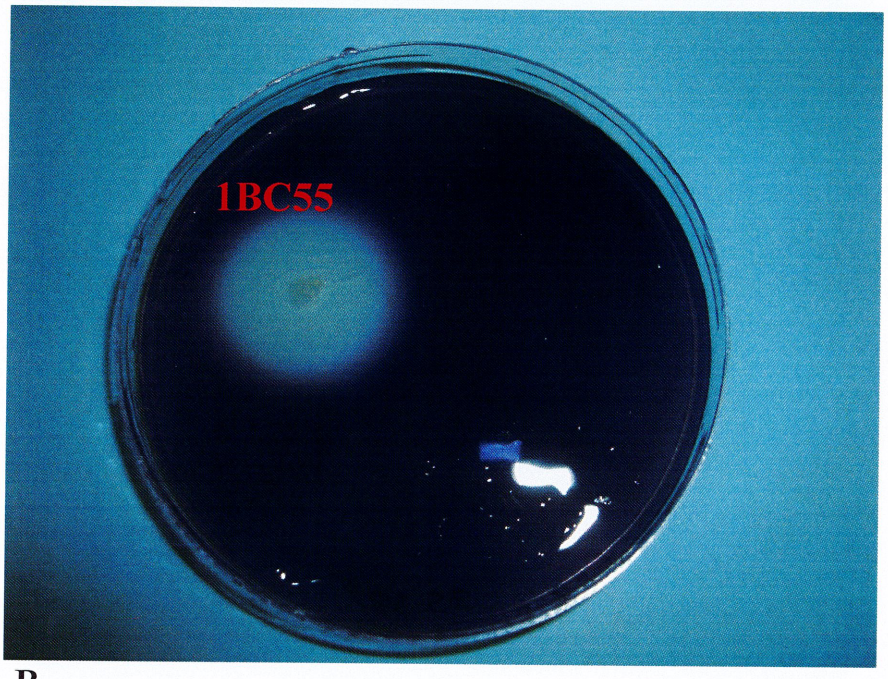
Amylolytic bacteria active at 55°C: 1BC55, 8BS55 and 2BD55.

Amylolytic actinomycetes active at 37°C: 8AD37, 1AS37, 1AC37 and 5AP37.

Amylolytic actinomycetes active at 55°C: not detected.



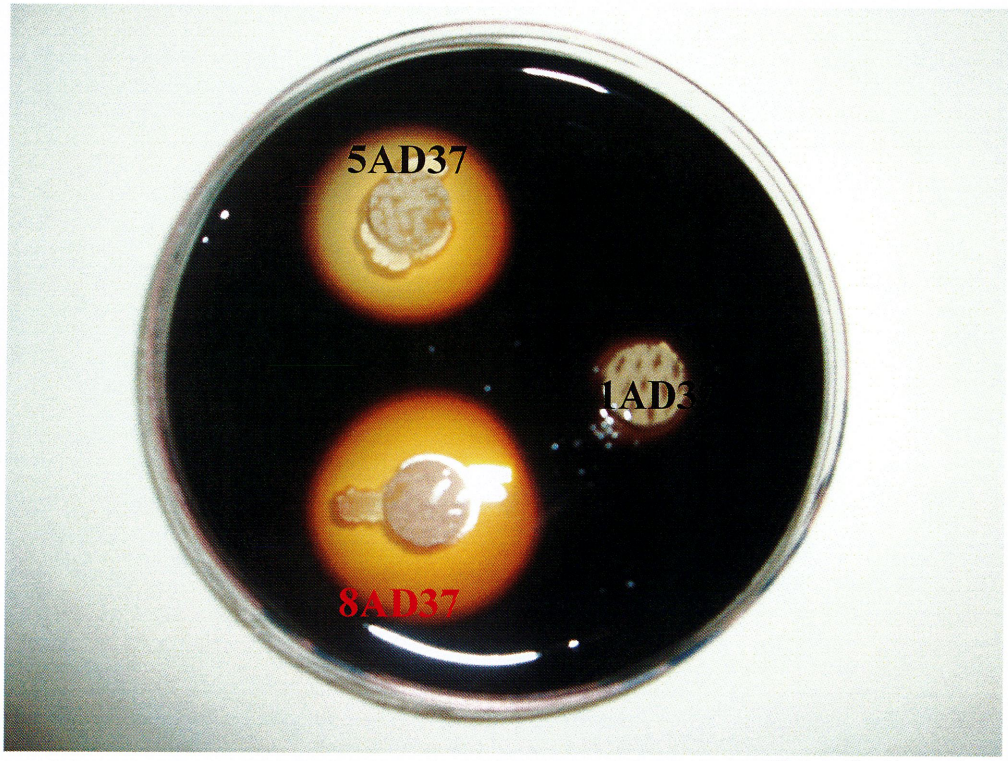
A.



B.

Photos Credit: Author

Photo 3.5: Different amylolytic active bacteria isolated at 37°C & 55°C on Delafield medium.
A: Amylolytic bacteria isolated at 37°C: 1BD37.
B: Amylolytic bacteria isolated at 55°C: 1BC55.



Photos Credit: Author

Photo 3.6: Different amylolytic active actinomycetes isolated at 37°C on Delafield medium.
Amylolytic actinomycete isolated at 37°C: 8AD37.

3.2.4 Selection of the most active proteolytic bacteria and actinomycetes:

Bacteria and actinomycetes that showed higher proteolytic activities on milk medium are shown in (photo 3.7 and photo 3.8) respectively.

Selection was based quantitatively on the width of the activity zones i.e. more width more active.

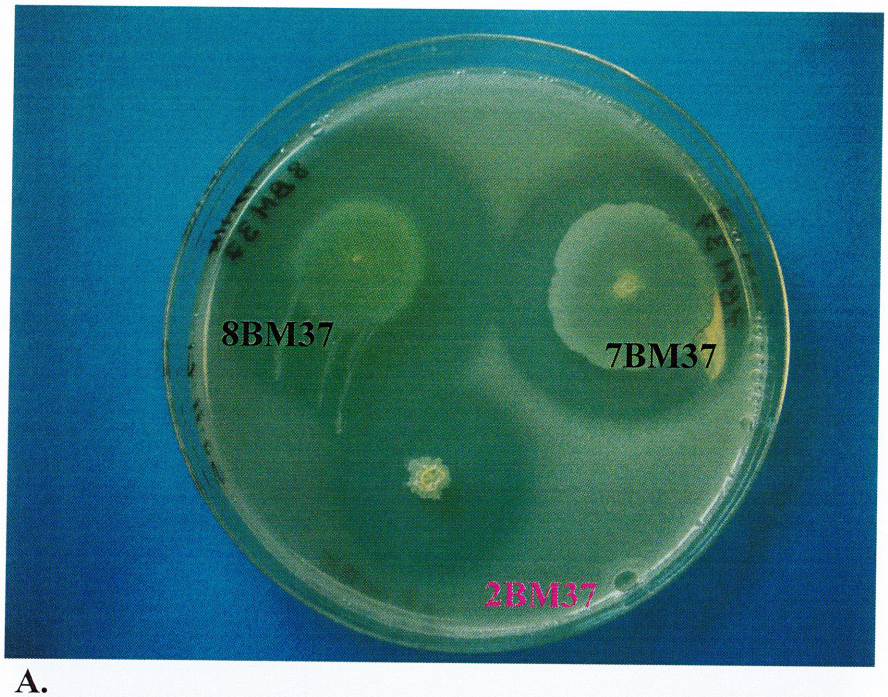
The most active selected bacteria and actinomycetes are listed below as follows:

Proteolytic bacteria active at 37°C: 2BM37, 8BM37, 2BP37 and 3BP37.

Proteolytic bacteria active at 55°C: 3BP55, 1BC55 and 5BM55.

Proteolytic actinomycetes active at 37°C: 9AD37, 3AD37, 5AS37, 3AC37, 2AD37, 4AC37, 6AP37, 5AP37, 5AC37 and 2AS37.

Proteolytic actinomycetes active at 55°C: 1AT55.



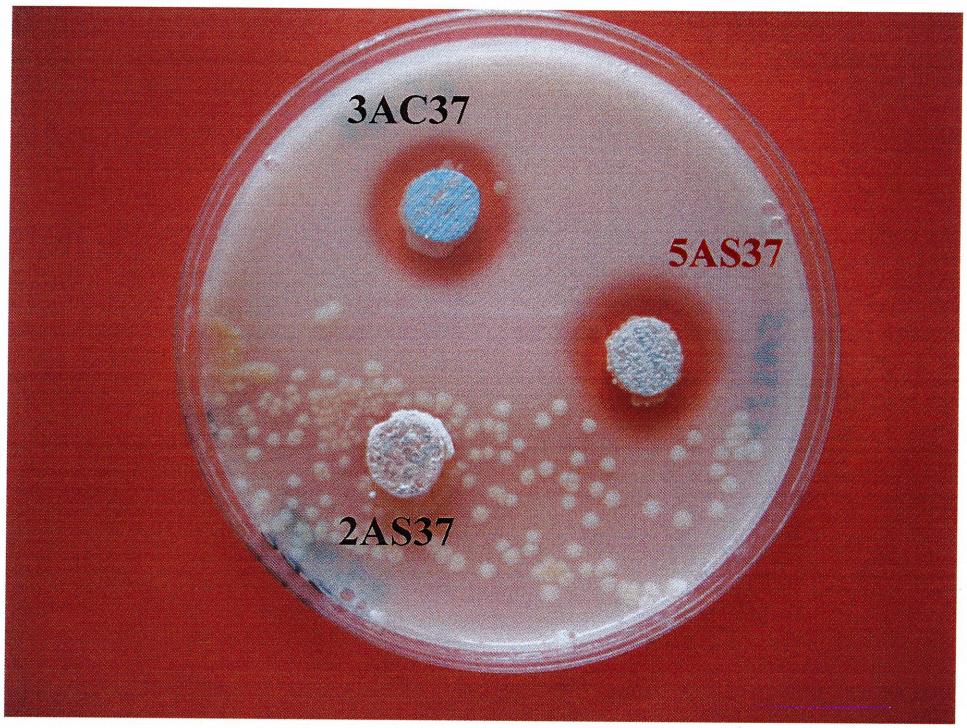
A.



B.

Photos Credit: Author

Photo 3.7: Different proteolytic active bacteria isolated at 37°C & 55°C on milk medium.
A: Proteolytic bacteria isolated at 37°C: 2BM37.
B: Proteolytic bacteria isolated at 55°C: 3BP55.



Photos Credit: Author

Photo 3.8: Different proteolytic active actinomycetes isolated at 37°C on milk medium.
Proteolytic actinomycete isolated at 37°C: 5AS37.

3.3 Assessment of bacteria/bacteria/actinomycetes interaction:

The aim of this experiment is to answer the following question:

What will happen to the selected bacteria and actinomycetes when they are mixed together and inoculated in one bin?

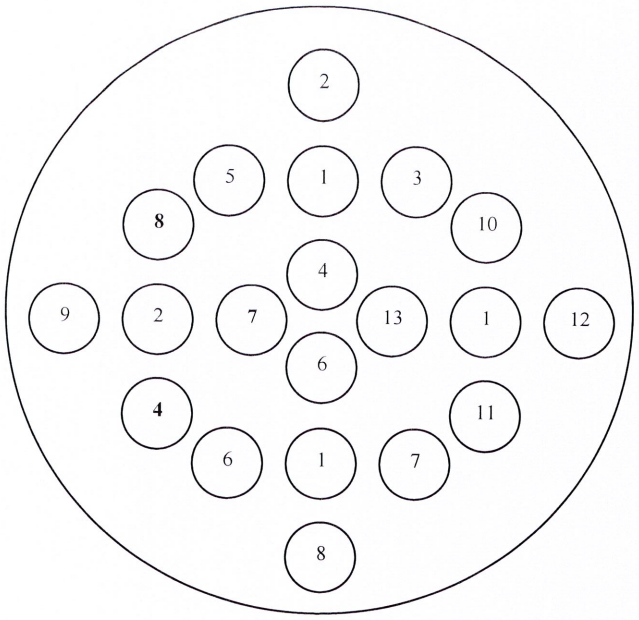
An interaction experiment that shows the interaction between selected bacteria and actinomycetes was designed. Plant extract was used as a substrate for growth to moderately mimic the nutrient composition of raw plant waste. All selected bacteria and actinomycetes (41) were inoculated on the surface of interaction medium using a pre-designed scheme. The scheme insures testing all species in different combinations.

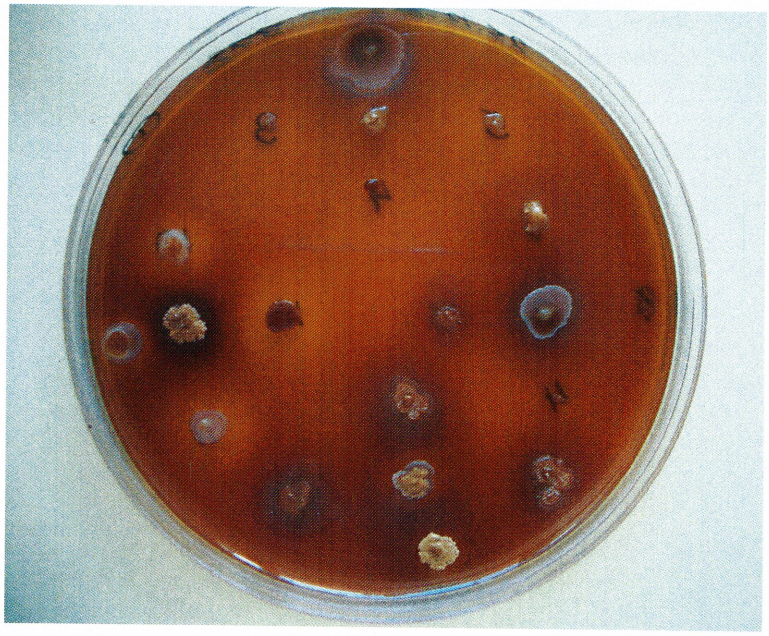
3.3.1 Assessment of bacteria/bacteria interaction for the most active bacterial species grown at 37°C:

All selected active bacterial species that grow on 37°C and used in this study was inoculated on the surface of interaction medium according to a pre-designed interaction scheme. Bacterial individuals were inoculated in a way that allows surface growth to interact. Synergistic and antagonistic activities were recorded.

Results showed positive relations between all active bacteria. None of the tested species showed antagonistic effect against each others. Some grow under certain restriction such as 1BC37 (4), 13BD37 (7) and 2BM37 (8). They produced clear zones on the substrate that indicate high assimilation.

Interaction scheme and interaction photographs are shown in (photo 3.9).





17BS37(1), 11BS37(2), 7BC37(3), **1BC37(4)**, 5BC37(5), 1BD37(6), **13BD37(7),** **2BM37(8),** 9BM37(9), 4BP37(10), 2BP37(11), 3BP37(12), 6BP37(13).

Photo Credit: Author

Photo 3.9: Bacteria/bacteria interaction for the most active bacterial species grown at 37°C.

3.3.2 Assessment of bacteria/bacteria interaction for the most active bacterial species grown at 55°C:

All selected active bacterial species that grow on 55°C and used in this study were inoculated on the surface of interaction medium as mentioned before. Synergistic and antagonistic activities were recorded. Results showed positive relations between all active bacteria. None of the tested species showed antagonistic effect against each others.

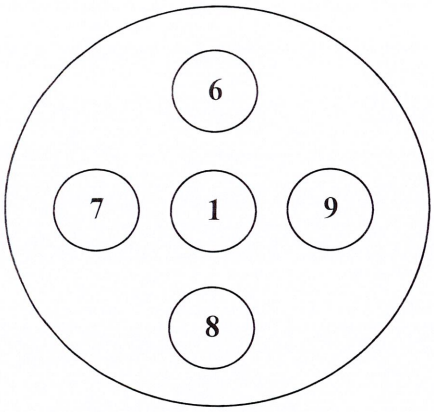
3.3.3 Assessment of actinomycete/ actinomycete interaction for the most active actinomycetes species grown at 37°C:

All selected active actinomycete species that grow on 37°C and used in this study were inoculated on the surface of interaction medium according to a pre-designed interaction scheme. Actinomycete individuals were inoculated in a way that allows surface growth to interact. Synergistic and antagonistic activities were recorded.

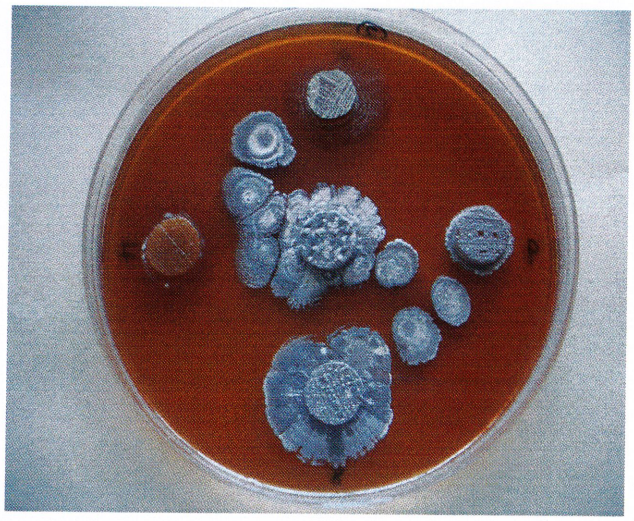
Results showed positive relations between all active actinomycete. None of the tested species showed antagonistic effect against each others.

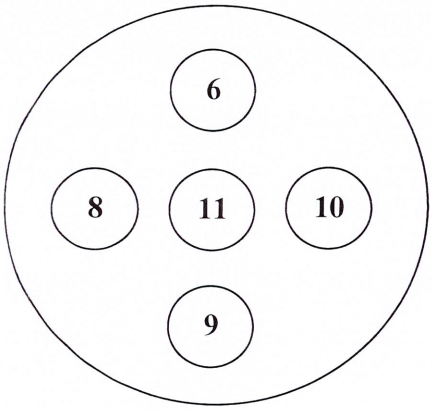
However, some species showed overgrowth but not antagonistic activity as shown between colonies 1&8, 1&9, 11&8, 11&9.

Interaction scheme and interaction photographs are shown in (photo 3.10).



1AS37 (1), **8AD37 (6)**, 9AD37 (7), **5AD37 (8)**, 1AD37 (9)





8AD37 **(6)**, **5AD37 (8)**, 1AD37 (9), 5AP37 (10), **5AS37 (11)**

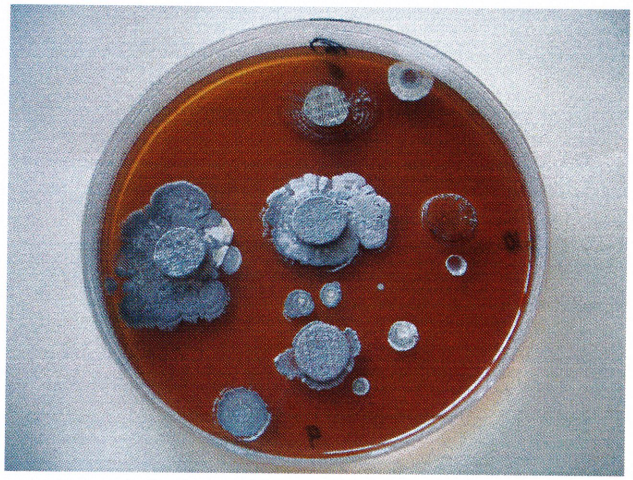


Photo Credit: Author

Photo 3.10: Actinomycete/actinomycete interaction for the most active actinomycete species grown at 37°C.

3.4 Composting:

Composting experiment was carried out in 4 bins and through 126 days. The four bins were prepared as follows:

Bin 1: Raw waste from plant materials (4 kg) kept without inoculation all the time. The normal microflora of plant material was left to carry on their activities without any interference. Moisture was kept within the normal range all the time (Table 3.5).

Bin 2: Raw waste from plant materials (4 kg) plus additional bacterial and actinomycetes inocula. Cellulolytic bacteria 3BP37, *Bacillus* 1BP55, pectinolytic bacteria 13BD37, *Bacillus* 12BM55, Cellulolytic actinomycetes *Streptomyces* 5AD37, cellulolytic *Thermoactinomyces* 1AT55 and pectinolytic actinomycetes *Streptomyces* 1AS37 were inoculated into this bin to enhance degradation of cellulose and pectin. Moisture was kept within the normal range all the time (Table 3.5).

Bin 3: Raw waste from plant materials (4 kg) plus amylolytic bacteria 1BD37, 1BC55, proteolytic bacteria *Bacillus* 2BM37, 3BP55, amylolytic actinomycetes *Streptomyces* 8AD37, proteolytic actinomycetes *Streptomyces* 5AS37 and proteolytic *Thermoactinomyces* 1AT55. Those groups were added to enhance amylolytic and proteolytic activities in bin 3. Moisture was kept within the normal range all the time (Table 3.5).

Bin 4: Raw waste from plant materials (4 kg) plus all 13 enzymatically active bacteria and actinomycetes mentioned in previous sections. Addition of extra inocula is hoped to enhance the composting process. Moisture was kept within the normal range all the time (Table 3.5).

Mixed individual samples from each bin were analyzed on 2-weeks interval during the 126 days composting experiment. The analyzed parameters were ash, moisture, total organic carbon (TOC) and total nitrogen (TN).

Data from total organic carbon and total nitrogen were used to produce the C/N ratio index.

Analysis data were shown in (Table 3.5) presented according to incubation periods viz: Start (0 day), 14, 28, 42, 56, 70, 84, 98, 112, 126 days respectively.

Table 3.5: Two weeks interval measurements of some parameters determining the progress of composting process during 126 days. The ratio C/N was used to indicate microbial activity and compost ripeness.

Treatments	Time (days)	Chemical analysis					Treatments	Time (days)	Chemical analysis				
		Ash *	Moisture †	TOC *	Nitrogen *	C/N			Ash *	Moisture †	TOC *	Nitrogen *	C/N
Bin 1[a]	0	28.3	71.3	40.0	1.4	29.1	Bin 3[c]	0	28.2	73.9	41.0	1.5	31.0
	14	30.5	73.4	29.0	1.6	17.9		14	29.2	74.3	26.5	1.6	16.5
	28	32.2	75.3	21.8	1.5	15.4		28	31.4	74.3	21.4	1.6	13.6
	42	73.3	71.5	17.9	2.1	8.8		42	71.0	79.1	16.2	2.4	6.9
	56	70.6	78.2	15.3	2.8	5.7		56	71.2	79.5	15.6	3.1	5.2
	70	70.9	78.6	28.0	6.4	4.4		70	71.4	77.1	25.7	8.4	3.0
	84	68.9	77.0	30.2	16.7	1.8		84	69.8	77.1	29.0	12.2	2.4
	98	68.8	71.3	26.5	15.9	1.7		98	72.9	72.3	25.8	14.4	1.8
	112	67.5	72.8	27.3	14.6	1.9		112	67.6	74.3	22.3	13.1	1.7
	126	66.9	72.6	25.2	13.5	1.9		126	68.5	72.1	25.7	15.7	1.6
Bin 2[b]	0	26.0	74.6	32.5	1.3	25.6	Bin 4[d]	0	23.2	71.0	35.8	1.4	27.3
	14	26.8	74.1	22.6	2.1	11.6		14	24.0	71.7	22.5	2.2	10.4
	28	29.0	74.4	19.5	1.7	11.3		28	24.4	71.6	19.2	1.9	10.0
	42	70.8	78.5	15.8	2.6	6.3		42	69.4	75.9	15.2	2.9	5.3
	56	70.9	78.5	15.1	3.2	4.7		56	69.3	75.4	15.1	3.4	4.5
	70	70.5	78.4	27.0	10.4	2.6		70	71.3	78.0	26.2	7.9	3.3
	84	70.0	76.5	30.0	13.3	2.3		84	70.3	72.9	29.0	17.8	1.6
	98	72.2	70.7	23.4	15.6	1.5		98	72.1	71.2	23.1	19.8	1.2
	112	70.7	69.7	25.9	16.0	1.6		112	68.7	67.6	24.4	14.1	1.7
	126	70.9	73.3	24.5	12.3	2.0		126	70.8	69.0	26.8	19.7	1.4

All values presented are mean of three replicates.
All values are given in percentage except C/N ratio.
*Values expressed as per cent dry weight.
† Values expressed as per cent wet weight.
[a] uninoculated (control).
[b] inoculated by (Cellulolytic and Pectinolytic-decomposing MO).
[c] inoculated by (Amylolytic and Proteolytic-decomposing MO).
[d] inoculated by (Cellulolytic, Pectinolytic, Amylolytic and Proteolytic-decomposing MO).

3.4.1 Changes of ash during 126 days of incubation in the 4 bins under investigation:

Ash content was used as an indicator of complete oxidation of organic compounds present in the raw material as well as an indicator of microbial activities since this process is mainly microbial.

As shown in (Table 3.5) the initial ash content (%) in each bin differ from the other three bins (28.3, 26.0, 28.2, 23.2 % in bins 1, 2, 3 and 4 respectively). This could be attributed to low oxidation rate of organic compounds by indigenous microflora prior to the onset of the composting experiment. All bins showed increase in % ash before day 42 (73.3, 70.8, 71.0, and 69.4 in bins 1, 2, 3 and 4 respectively). More incubation did not affect the ash content which showed a relatively fixed trend (Table 3.5 and Fig. 3.1).

Statistical analysis indicated significant changes at the 0.05 level between bins and during different incubation periods. The significant changes were in favor of bin number 4 over the other 3 bins. However, bin 2 showed also significant changes at the 0.05 level compared to bin 1 and bin 3 (Table 3.6).

Efficiency of microbial oxidation of organic compounds (mineralization) was calculated in terms of ash content of the samples and correlated to incubation periods (Table 3.7 and Fig. 3.2).

Results showed that bins 2 and 4 contained active microbes that enhance faster mineralization of TOC viz: 63.3 and 66.6 % respectively. Interestingly the four bins reached maximum mineralization rates during relatively the same incubation period but ripeness of the compost was mostly presented in bin 4.

Figure 3.1: Percent of ash in the four bins measured on 2 weeks interval over a period of 126 days

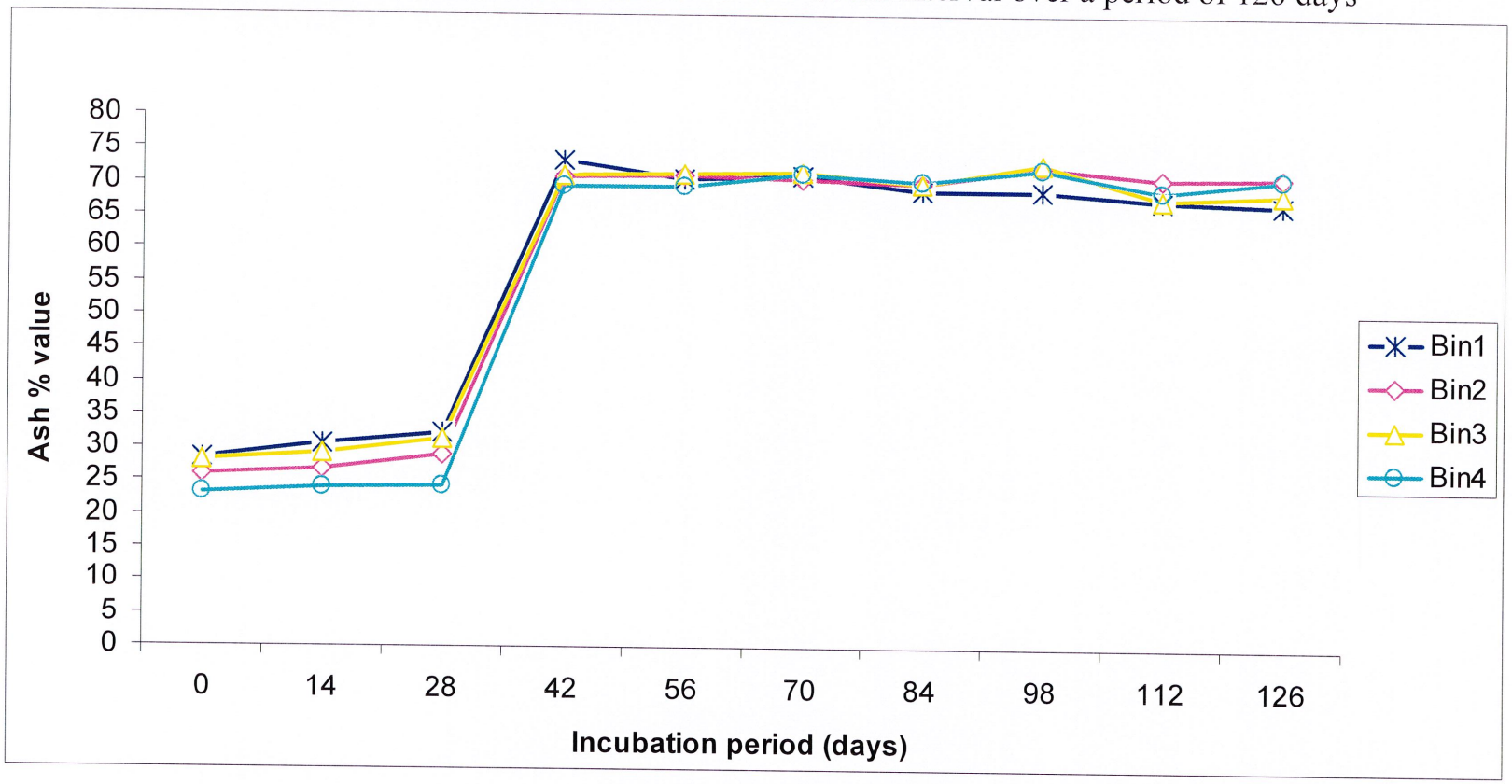


Table 3.6: multiple comparisons for the four treatments in ash content (Tukey).

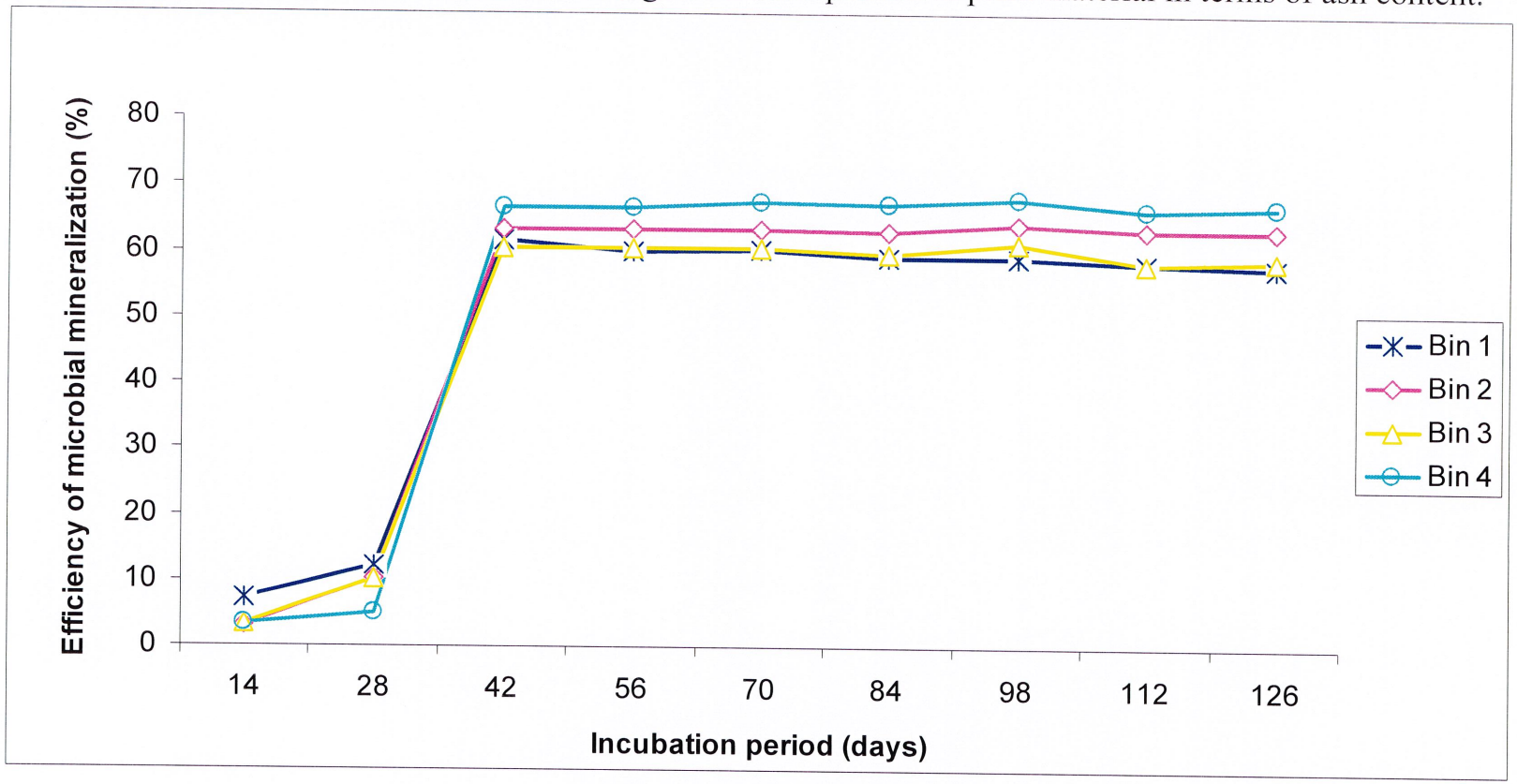
(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Bin 1	Bin 2	-.0180	.41546	1.000
	Bin 3	-.3637	.41546	.817
	Bin 4	1.4080	.41546	.038*
Bin 2	Bin 1	.0180	.41546	1.000
	Bin 3	-.3457	.41546	.838
	Bin 4	1.4260	.41546	.036*
Bin 3	Bin 1	.3637	.41546	.817
	Bin 2	.3457	.41546	.838
	Bin 4	1.7717	.41546	.012*
Bin 4	Bin 1	-1.4080	.41546	.038*
	Bin 2	-1.4260	.41546	.036*
	Bin 3	-1.7717	.41546	.012*

Based on observed means.
*The mean difference is significant at the 0.05 level.

Table 3.7: Efficiency of mineralization of organic carbon present in the plant material during 126 days of incubation calculated in terms of ash content of each bin.

Incubation periods (days)	Efficiency of microbial oxidation (%)			
	Bin 1	Bin 2	Bin 3	Bin 4
14	7.2	3.0	3.4	3.4
28	12.1	10.3	10.2	4.9
42	61.4	63.3	60.3	66.6
56	60.0	63.3	60.4	66.5
70	60.1	63.1	60.5	67.3
84	58.9	62.9	59.6	67.0
98	58.9	63.9	61.3	67.8
112	58.1	63.2	58.3	66.2
126	57.7	63.3	58.8	66.8

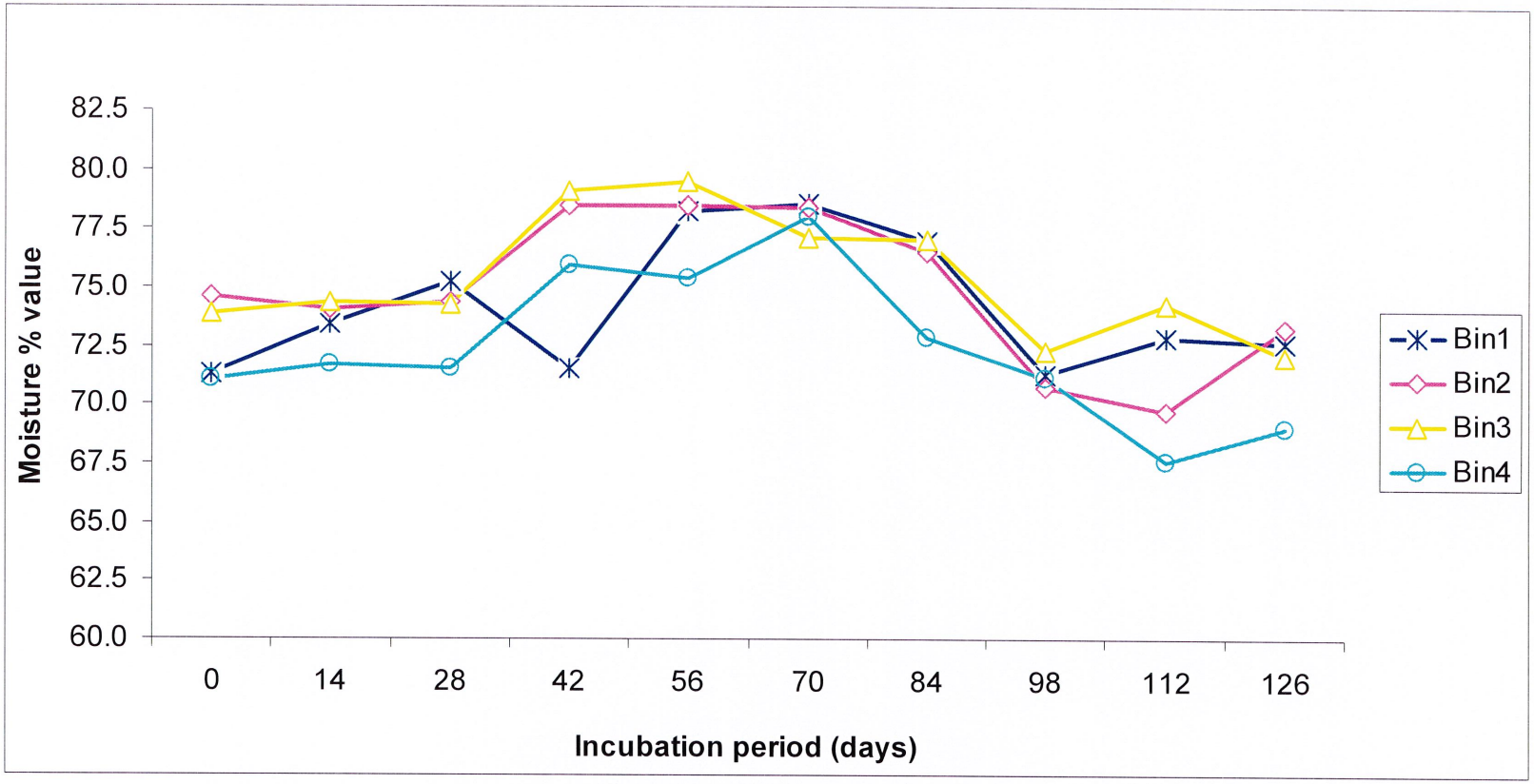
Figure 3.2: Efficiency of mineralization of organic carbon present in plant material in terms of ash content.



3.4.2 Moisture content of the four bins during 126 days incubation period:

Measurements of the moisture content were shown in (Table 3.5 and Fig. 3.3). Results showed some changes in moisture measurements between bins and during different sampling periods. This could be attributed to variations in microbial activities in each bin as well as heating effect of biological reactions. However, moisture was not used as ripeness indicator in the present study but it was used to correct the moisture content which was recorded to self increase by time due to active growth and respiration of microorganisms, as well as low incubation temperatures during the study period. This increase limits the addition of more water to the bins during subsequent inoculations.

Figure 3.3: Changes in moisture content in the four bins during the composting experiment.



3.4.3 Total organic carbon changes of the four bins during 126 days measured on 2-weeks interval:

Total organic carbon showed obvious decrease in all bins during the composting period (Table 3.5 and Fig. 3.4). Decrease in TOC was achieved after 56 days of incubation in all bins where TOC % were (15.3, 15.1, 15.6 and 15.1 in the four bins respectively). Data showed that bin 4 recorded earlier decreases in TOC within 42 days (15.2 %) compared to other bins 1, 2 and 3 respectively (17.9 %, 15.8 % and 16.2 %). This might reflect that enrichment of bin 4 with various enzymatically active microbes increase the composting rate.

Statistical analysis of TOC between bins and between incubation periods approved significant decrease in bin 4 at 0.05 levels (Table 3.8).

Calculating the efficiency of microbial oxidation of TOC from the residual TOC in each bin revealed very interesting observations (Table 3.9 and Fig. 3.5). Microbialy treated bins 2, 3 and 4 proved to be more efficient in oxidizing the residual TOC than that of the untreated control within 56 days of incubation. This means that allochthonous microflora (Bioinoculants) increase the composting rate and shorten the time required for ripeness. Hence, enzymatically active groups supplemented to bin 4 showed efficient oxidation of 99.94 % of the residual carbon compared to the normal microflora of the untreated control which showed 85.5 %.

Interestingly the efficiency of the microflora present in the four bins was reduced dramatically after 56 days.

Total carbon content increased at high rate after 56 days. This is confirmed by measuring the total humus content in each bin. Results showed that: humus formed due to microbial activities during the whole

study period reached 0.12%; 0.197%; 0.197% and 0.17% in bins 1, 2, 3 & 4 respectively.

Figure 3.4: Percent change in TOC as indicator of mineralization measured on 2-weeks interval over a period of 126 days.

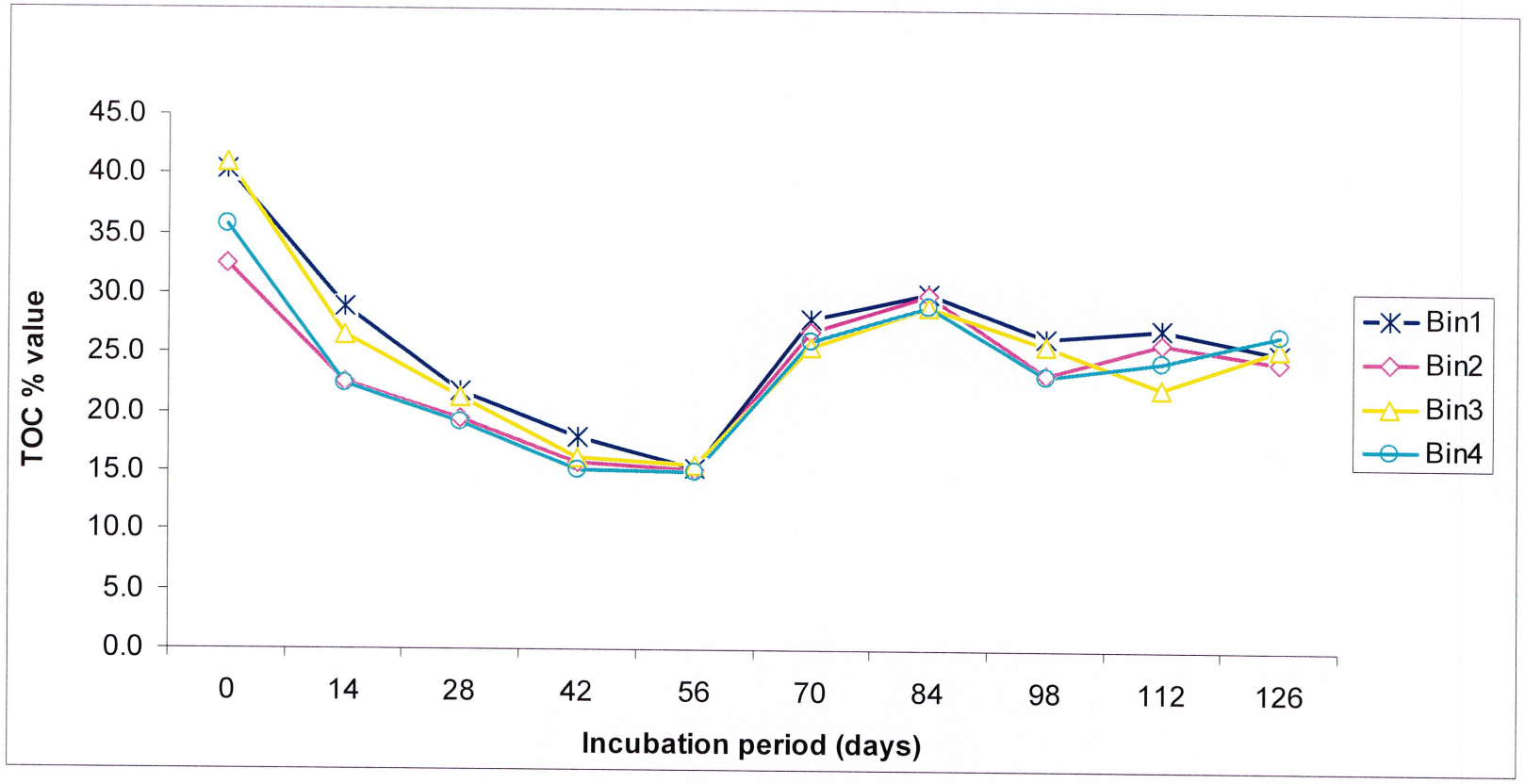


Table 3.8: multiple comparisons for the four treatments in TOC content (Tukey).

(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.
Bin 1	Bin 2	2.5260	.59349	.012*
	Bin 3	1.2410	.59349	.234
	Bin 4	2.4330	.59349	.015*
Bin 2	Bin 1	-2.5260	.59349	.012*
	Bin 3	-1.2850	.59349	.212
	Bin 4	-.0930	.59349	.999
Bin 3	Bin 1	-1.2410	.59349	.234
	Bin 2	1.2850	.59349	.212
	Bin 4	1.1920	.59349	.261
Bin 4	Bin 1	-2.4330	.59349	.015*
	Bin 2	.0930	.59349	.999
	Bin 3	-1.1920	.59349	.261

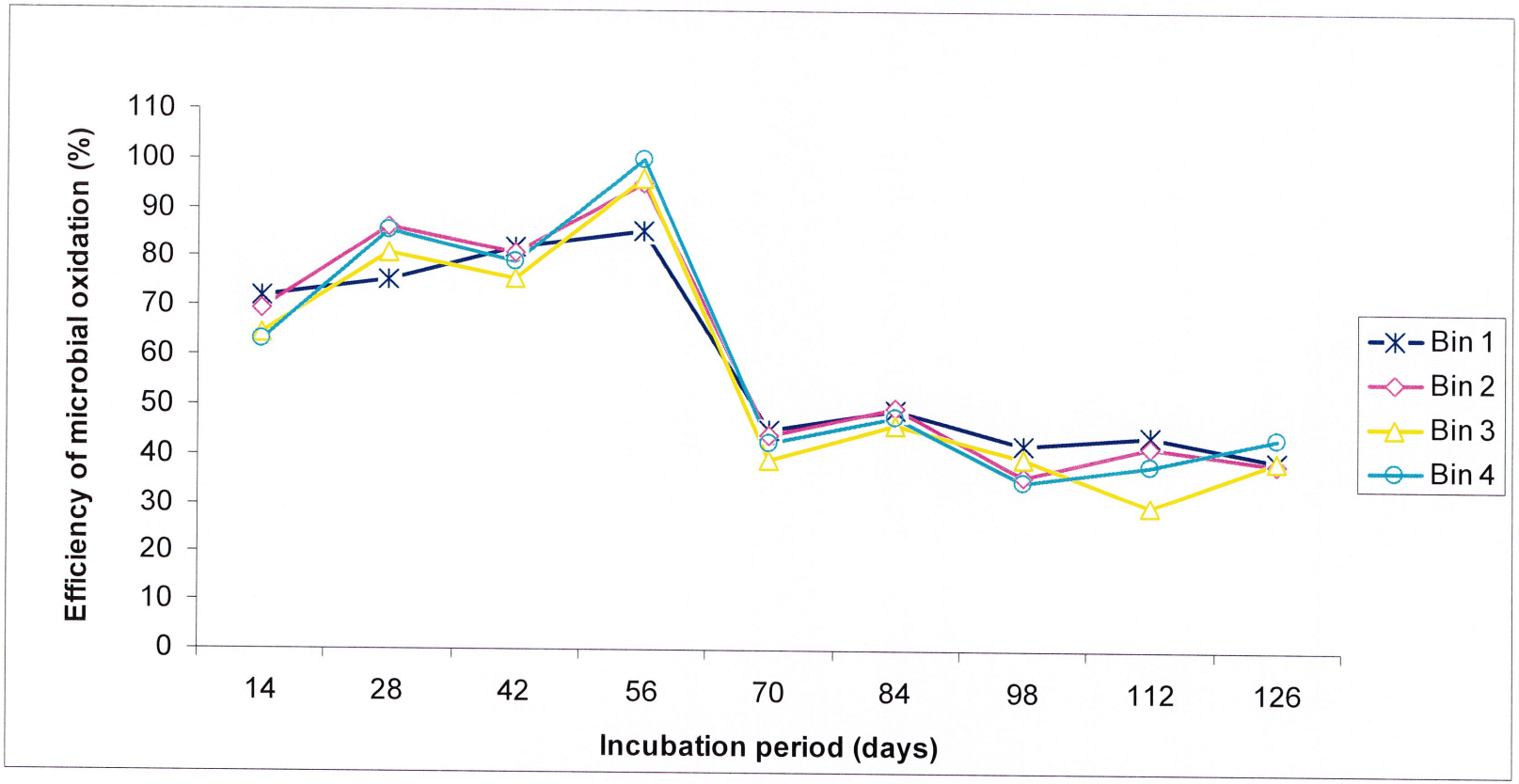
Based on observed means.

*The mean difference is significant at the 0.05 level.

Table 3.9: Efficiency of microbial oxidation of TOC % in the plant material during 126 days of incubation calculated in terms of residual TOC in each bin.

Incubation periods (days)	Efficiency of microbial oxidation (%)			
	Bin 1	Bin 2	Bin 3	Bin 4
14	71.8	69.5	64.6	63
28	75.2	86.2	80.8	85.3
42	82.1	81.0	75.7	79.2
56	85.5	95.0	96.3	99.94
70	45.0	44.1	39.3	42.4
84	49.3	49.7	46.2	47.9
98	42.3	35.8	39.5	34.6
112	43.9	41.7	30.0	38.1
126	39.3	38.4	39.3	43.7

Figure 3.5: Efficiency of microbial oxidation of TOC.



3.4.4 Changes of total nitrogen and the resulting C/N ratio in the four bins during 126 days measured on 2-weeks interval:

Total nitrogen in the other hand, was measured accordingly during the 126 days of incubation on 2-weeks interval. Results showed increase in total nitrogen with increasing incubation days (Table 3.5 and Fig. 3.6).

The aim of measuring total nitrogen is not only to record the % increase or decrease but also to use those data in calculating the C/N ratio of the system which is a practical monitoring index of ripeness confirmed at C/N level of 10.

Understanding the practical importance of C/N ratio of 10, the present results showed that the ripeness of the compost started after day 28 and end on day 42 in the uninoculated control bin 1. As well in bin 2 the ripeness of the compost started at earlier times, day 14 and continued to day 28. Bin 3 is more or less similar to bin 1 in the ripeness period. Interestingly C/N ratio of 10.4 was recorded on day 14 in bin 4 indicating shorter ripeness time (Fig. 3.7). It could be indicated that the ripeness period in bin 4 started shortly after the beginning of the experiment and reached maximum ripeness on day 28 where C/N recorded 10.

Figure 3.6: Percent change in nitrogen content in the four bins measured on 2-weeks interval during 126 days.

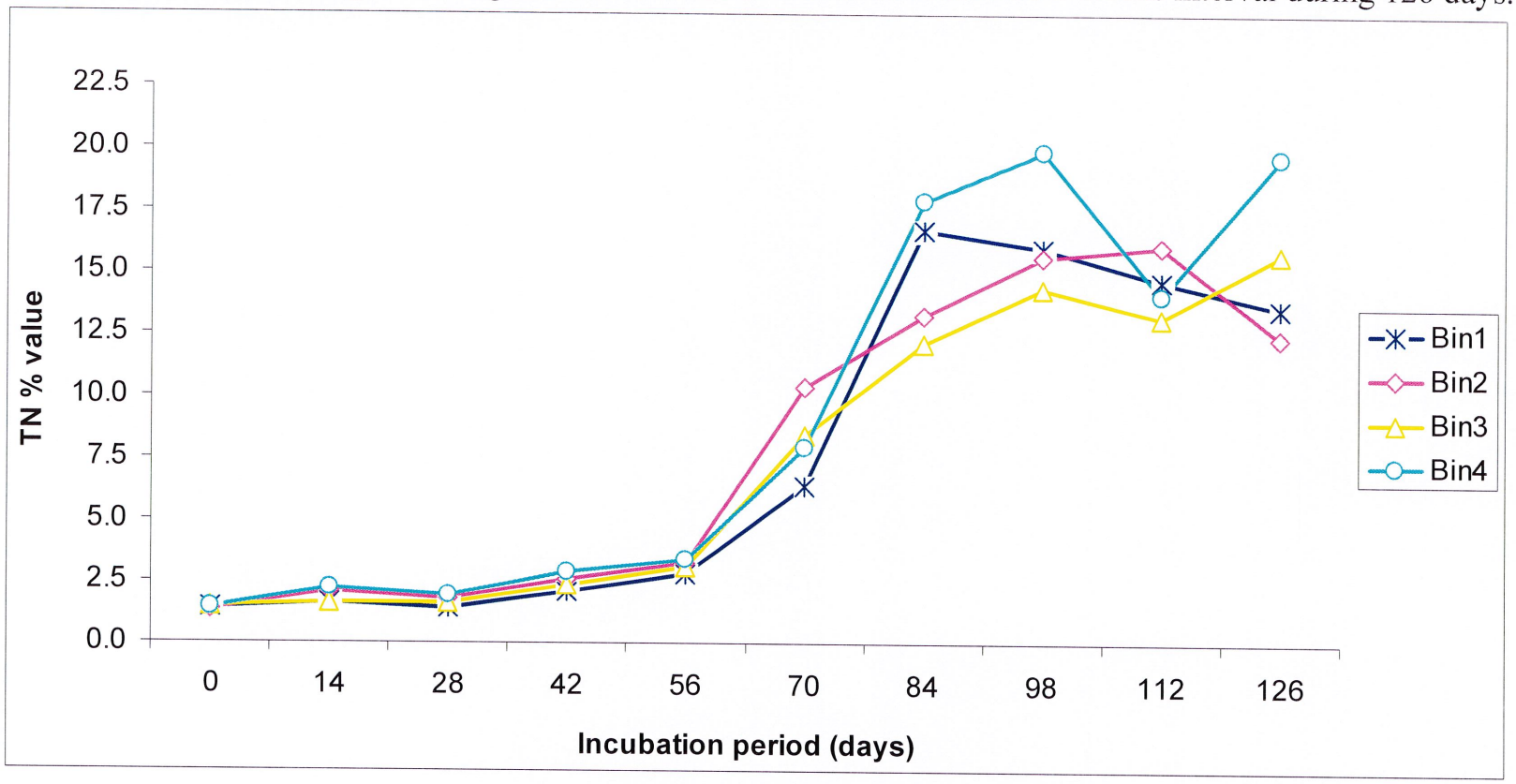
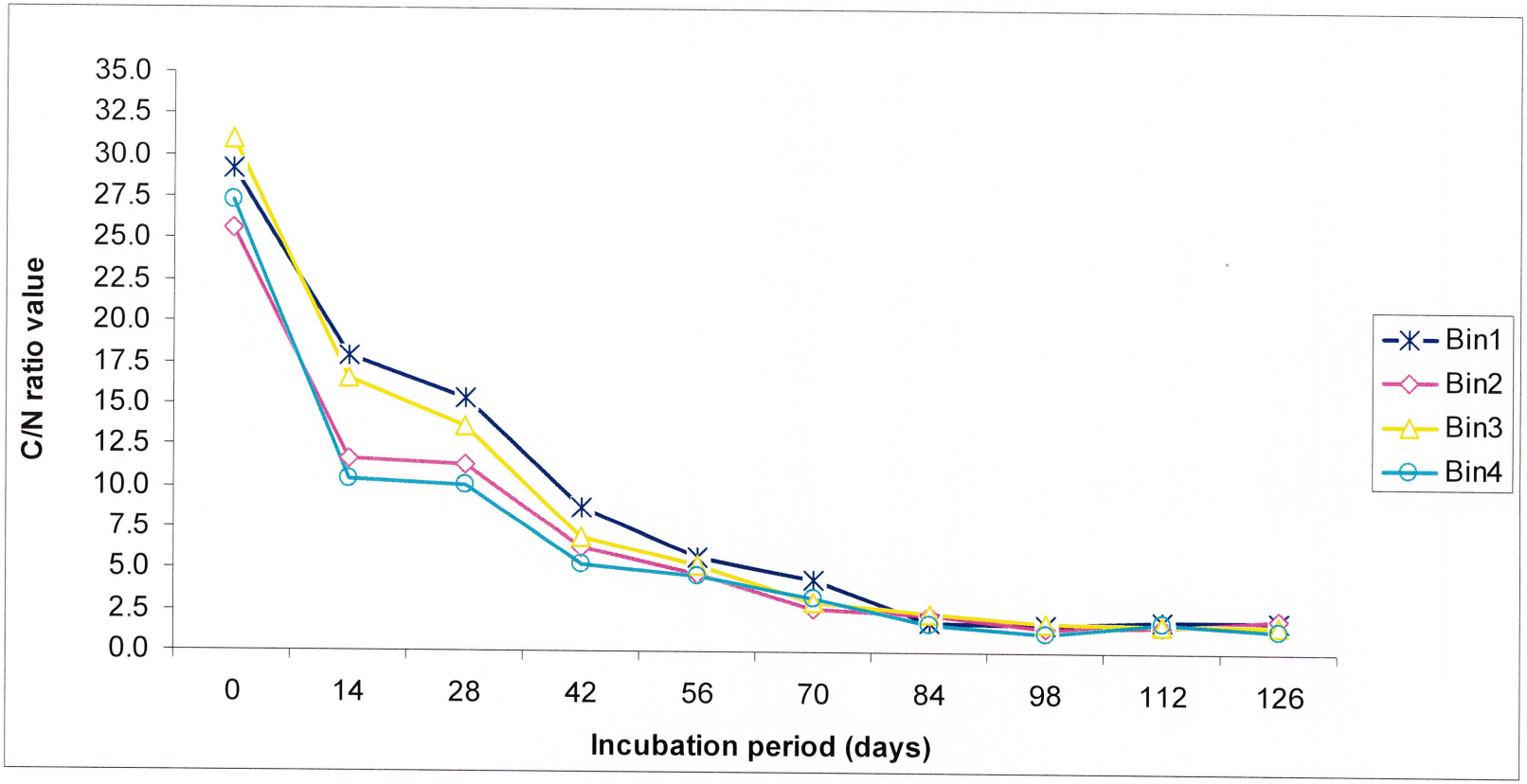


Figure 3.7: Changes in C/N ratio in the four bins during the composting period (126 days) recorded on 2-week interval.



3.4.5 Extracellular enzyme activities measured during 126 days of incubation in the 4 bins under investigation:

Measurements of the extracellular enzyme activities (clearing zone diameter) in the 4 bins were shown in (Table 3.10). Results showed that cellulolytic enzyme activity was present in all the bins at 20 days of composting incubation, and then it was not detected at later incubation periods due to the degradation activities. In comparison, proteolytic activity appeared in Bin 4 at 81 and 95 days after the degradation of plant cellulose. However, pectinolytic and amylolytic enzyme activities were present in all of the bins through all the composting period.

Table 3.10: Extracellular enzyme activities measured during 126 days of composting process in the four bins under investigation.

Treatments	Time (days)	Enzyme activity (diameter of clearing zones in cm)			
		Cellulolytic	Pectinolytic	Amylolytic	Proteolytic
Bin 1[a]	20	1.9	2.4	1.5	ND
	67	2.0	2.9	2.1	1.7
	81	ND	2.5	2.0	ND
	95	ND	2.6	2.0	ND
Bin 2[b]	20	2.0	2.5	1.6	ND
	67	ND	2.7	1.8	ND
	81	ND	2.8	1.8	ND
	95	ND	2.7	2.0	ND
Bin 3[c]	20	2.0	2.3	1.6	ND
	67	ND	2.8	1.6	ND
	81	ND	2.4	1.5	ND
	95	ND	2.6	1.6	ND
Bin 4[d]	20	1.9	2.4	1.5	ND
	67	ND	2.7	1.9	ND
	81	ND	2.4	1.6	2.4
	95	ND	2.7	1.7	3.0

All values presented are mean of three replicates.
ND (not detected).
[a] uninoculated (control).
[b] inoculated by (Cellulolytic and Pectinolytic-decomposing MO).
[c] inoculated by (Amylolytic and Proteolytic-decomposing MO).
[d] inoculated by (Cellulolytic, Pectinolytic, Amylolytic and Proteolytic-decomposing MO).

Part 4

DISCUSSION

The present study introduces a new approach to composting agriculture wastes that took into account physiological and ecological factors such as biodiversity physiological youth of inoculants, enzymatic activities, population interactions, substrate enrichment with nutrients, aeration, temperature and moisture. The proposed approach targeted the importance of environmental sustainable development focusing on recycling agriculture wastes and developing new techniques towards improving the composting processes.

Alternative composting approaches utilized either the biotransformation abilities of indigenous microorganisms (time consuming treatment) or seeding post composted materials or addition of external microorganisms to the preexisting indigenous microorganisms to arbitrary shorten the composting time (Vargas-Garcia et al., 2005; Elorrirta et al., 2002; Rajbanshi et al., 1998; Requena et al., 1996; Kostov et al., 1991; Magan et al., 1989 and others). Such approach has been considered a controversial subject for a long time where contradictory results have been described.

Therefore, enzymatically active bacteria and actinomycetes were enriched, selected and isolated from mature composted local agriculture waste. Cellulolytic, pectinolytic, amylolytic and proteolytic bacteria and actinomycetes were prepared as mixed potential inoculants to small scale composting experiments.

Cellulose which is a major component of plant materials specially leaves and soft wood, where it represents about 45-55 % of the plant dry weight (Wood and Kellogy, 1988a). Based on this fact, cellulolytic bacteria and

actinomycetes were targeted as main components of the external inoculants.

On the other hand pectin is expected to be another important component of the composted material under study, where it is the main component of the middle lamellae (Wood and Kellogy, 1988b). As a result pectinolytic bacteria and actinomycetes were also considered.

Moreover, amylolytic and proteolytic bacteria and actinomycetes were also used in the present study to enhance the degradation of soluble components released from plant cells after being attacked by cellulolytic and pectinolytic enzymes. Keeping the above consideration in mind, selection of enzymatically active bacteria and actinomycetes were made from post composted materials. Selection strategy focused on enzymatically active diverse groups of bacteria and actinomycetes. Metabolic diversity of selected microorganisms necessitates variations in the degradative pathway; an advantage targeted for the first time in the present study. This explains why diverse isolates of bacteria and actinomycetes were selected. For example many bacteria such as *Bacillus* 1BC37, 2BC37 and 3BC37 species were isolated on the basis of high extracellular cellulolytic activity only. Others such as *Bacillus* 3BP37 was selected on the basis of extracellular cellulolytic activity which also possess the capability of proteolytic activity. Therefore, many other isolates used in the present study were selected on the basis of producing more than one extracellular enzyme.

Many factors were considered when applying the current sustainable approach that took into consideration one important target which is to shorten the composting time and improve product quality. This includes:

1. Ability to degrade plant extracts as shown in the results section 3.3.
2. Positive interactions between members of the potential inoculants.
3. Timely inoculation of exponentially growing cultures at start, after 3 weeks and after 6 weeks.
4. Regular mixing of the components to maintain good aeration.
5. Adding water, on demand, to keep the required moisture effective.
6. Eliminating the role of cellulolytic fungi which are in fact opportunistic pathogens and aeroallergens transmitted during composting process such as *Aspergillus fumigatus* as described by (Austwick, 1974; Clark et al., 1984 and Strom, 1985b).

Assessment of microbial activity were monitored using increase in ash content, decrease in carbon content, changes in nitrogen and in the C/N ratio.

Ash content increased due to microbial oxidation of organic compounds during 42 days of composting process. Two bins (2 & 4) showed increasing microbial activity (63.3 and 66.6 % respectively) over the other two bins (1 & 3) 61.4 and 60.3 % respectively. Bin 2 contained cellulolytic and pectinolytic members, Bin 4 contained all enzymatically active bacteria and actinomycetes.

Since cellulose and pectin constituted high proportion of organic compounds present in the substrate under study, it would be accepted that microbial activities in bin 2 is higher than bin 1 and bin 3. Significant changes in ash content and hence microbial activities at $p < 0.05$ level were recorded in bin 4, which was previously inoculated with all enzymatically active bacteria and actinomycetes.

It was clearly apparent that increase in ash content is proportional to the extent of decomposition. Loss in carbon confirms the above argument where it showed obvious decrease during the composting period. Hence, the correlation between the decreasing of organic carbon and increase in ash content reflected high microbial activity since both results were due to microbial action. This was confirmed in part when the efficiency of microbial activity reaches 66.6 % in case of ash production and 99.94 % in the other part in case of TOC aerobic oxidation during 56 days.

The decrease in TOC during composting is used as an index of stabilization of compost which was achieved between weeks 4 and 8. Charest and coworkers indicated that stabilization of the compost needs at least 12 weeks to start stabilization. The same author showed that celluloses and hemicelluloses were mainly biodegraded during the first 8 weeks (Charest et al., 2004). Interestingly, the output of the present study recorded that cellulolytic bacterial activities ceased within 4 weeks. A result that strongly showed that cellulolytic microbes start the composting process. In other words, composting of plant materials needs cellulolytic microorganisms to act on the plant skeletal structure -cellulose- during the first phase of composting. This activity combined with pectinolytic microbial activities releases the internal soluble cellular components including starchs, proteins as major organic materials, fats and lipids as minor organic materials.

Amylolytic microorganisms started their activity during the first few days of composting supporting the above argument. Moreover, proteolytic activities were recorded later and showed fluctuations of activity. These fluctuations were correlated to the availability of the substrate -protein-

and increase of nitrogen content which is a major component of enzyme structure, refer to (table 3.10).

Nitrogen has often been recognized as a limiting factor for microbial growth and activity during decomposition of plant materials (Recous et al., 1995). However, experiments of plant residues showed different results, ranging from positive to negative effects on the decomposition rate (Fog, 1988). Resource quality, microclimatic conditions and decomposers efficiency are major factors regulating composition and activity of decomposer communities and hence the process of decomposition and nutrient release (Neely et al., 1991 and Agren et al., 2001). Thus the effect of added nitrogen on decomposition may depend on the plant material as degradation is influenced by nutrient content and anatomical structure of the material. Parameters such as N source and time scale of the decomposition process also influence the effect of added nitrogen.

In the present study, it has been shown that the ratio of N immobilized to C mineralized increased with time and suggested that there was a high microbial demand on N during the time of release of soluble and easily degradable plant materials, while the nitrogen demand was lower when the recalcitrant carbon compounds were decomposed in the initial phase. The current finding is not as the same as that of (Recous et al., 1995) who found that N demand was higher at the early onset of the composting process. Since nitrogen content was monitored extensively during the present study, it was clear that nitrogen increased gradually due to increased activities of the diverse microbial groups present as bioinoculants, or in other words more synthesis of enzymes.

During composting the C/N ratio decreased to around 10 in all the four bins under investigation which indicated the biological stability of the compost. The ratio C/N of 10 is used as a monitor of stability and ripeness by many authors (Bernal et al., 1996; Bernal et al., 1998; Pare et al., 1998; Mathur et al., 1993).

In Bin number 4, C/N ratio reached 10 within four weeks indicating that the idea of using diverse physiological groups of microorganisms shorten the period required for the ripeness and stability of the compost under the conditions of the present study. Other bins showed stability but within longer period (6 weeks).

In the present study, assessment of the thermophilic phase was neglected due to the small scale experiment (4kg) and the small composter volume 50l which were considered barriers for correct monitoring of the thermophilic phase. Many authors with special reference to Rajbanshi et al., 1998 and Rajbanshi and Inubushi, 1998 recommended neglecting the role of temperature for small scale composters.

Composting experiments have been performed intensively during the last decades. Primarily the studies have focused on rural and urban wastes, often with the aim of reducing volume and avoiding nutrient losses (Witter and Lopez-Real, 1988; Martins and Dewes, 1992; Sánchez-Monedero et al., 2001 and Dresbøll and Thorup-Kristensen, 2004). More recently, the focus has also been on composting of plant residues to produce growing media (Jensen et al., 2001; Prasad and Maher, 2001 and Garcia-Gomez et al., 2002). Major topics in composting research have been process control and characterization of maturity and stability criteria.

The present research has focused on process control aspect in view of bioinoculation strategy; diversity and avoidance of opportunistic pathogens were discussed in the light of the results recorded due to enzymatically active diverse groups of bacteria and actinomycetes.

This new technical finding quietly satisfies the concept of environmental sustainable development in the sense that, recycling plant residues into usable products will minimize environmental pollution, gas emission, nutrient loss and biological and chemical air pollution. In addition to that, it has a share in safeguarding the quality of underground water resources.

CONCLUSION

Agriculture wastes are overlooked in the kingdom of Bahrain. The annual agriculture wastes showed remarkable increase reached 20,539.5 tons in year 2003.

Composting of agriculture wastes becomes a time consuming task. Therefore, in the present study, a new approach to reduce the time of composting was successfully presented. It depends mainly on enriching the agriculture waste with metabolically diverse groups of bacteria and actinomycetes as avoiding opportunistic fungal pathogens.

Results of the study worth large scale application and it could be adopted by the Municipalities and included among their plans towards recycling of solid wastes to satisfy sustainably developed environmental policies.

RECOMMENDATION

- Plan to minimize waste generation by stating goals or legislative mandates to drastically reduce the volume of waste being sent to landfills.
- Reuse and recycle waste materials as much as possible.
- Raise population awareness and provide retail home composting bins for the residents.
- Municipality should collect agriculture waste at source divided into organic and inorganic matter.
- Interaction between the Bahrain University and community achieved by establishing a compost research and application production unit.
- Plan to have a developed large scale composting plant in Bahrain.
- Reduce the decomposition time which would enable us to potentially convert the waste into value added products in a short time and the derived compost used as substrates for cultivation.
- Bahrain University adopts the preparation of the starter culture.
- Distribution (selling) the cultures at low cost to encourage farmers, interested directorates and others to perform in situ composting to achieve the target of sustainable development.

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الملخص العربي:

تتنوع المشكلات البيئية من حيث الأسباب والنتائج، بيد ان مشكلة المخلفات الصلبة أصبحت قضية عالمية حيث تتولد عن هذه المخلفات تأثيرات خطيرة على البيئة وصحة المجتمع. ومن هنا تزايدت الاهتمامات العلمية والدراسات لحل هذه المشكلات عن طريق تدوير هذه المخلفات وبالتالي تحجيم خطرها على البيئة والمجتمع.

يتناول هذا البحث دراسة لتدوير وتحسين سرعة انتاج السماد العضوي (الكومبوست) من المخلفات العضوية وتحديداً مخلفات النباتات ليستفاد منها في الزراعة والبستنة في مملكة البحرين، وقد تم تفعيل هذا التحسين عن طريق تقصير الفترة الزمنية لنضج السماد العضوي (الكومبوست). وقد اتبع البحث عزل الكائنات الدقيقة (بكتيريا و أكتينوميسيتات) من مصادر تسميد نباتية سابقة النضج، وتم استكثار هذه الكائنات في المعمل لاعداد حقنات نشطة انزيمياً ومن ثم اضافتها للمخلفات النباتية محل الدراسة. وقد كان المعيار الاساسي لاختيار هذه الكائنات الدقيقة الدخيلة ذات التفاعل الايجابي مع بعضها البعض على أساس قدرتها الفائقة على افراز انزيمات قادرة على شطر السليلوز و البكتين و النشا والبروتين.

تم قياس تقدم عملية التحلل ونضج الكومبوست عن طريق تقدير النسبة المئوية لمحتوى الرماد، الكربون العضوي الكلي، النيتروجين الكلي، نسبة الكربون الى النيتروجين و فعالية الانزيمات.

أجريت هذه التجارب العلمية بمشتل جامعة البحرين في أربع حاويات سعة (50 ليتر) احتوت كل واحدة منهن على 4 كجم من المخلفات النباتية.

حاوية رقم 2 و3 و4 طعمت بعدد من الكائنات الدقيقة ذات الانزيمات النشطة على النحو التالي، حاوية رقم 2 طعمت بكائنات دقيقة ذات افرازات انزيمية نشطة قادرة على شطر السليلوز و البكتين فقط، حاوية رقم 3 طعمت بكائنات دقيقة ذات افرازات انزيمية نشطة قادرة على شطر النشا والبروتين فقط، حاوية رقم 4 طعمت بكائنات دقيقة ذات افرازات انزيمية نشطة قادرة على شطر السليلوز و البكتين و النشا والبروتين، بينما تركت حاوية رقم 1 من غير أي تطعيم وتحت تأثير الكائنات الدقيقة الأصلية.

أثبتت نتائج هذه الدراسة أن 99.9 % من النشاط الميكروبي ظهرت في حاوية رقم 4 مقارنة بالحاويات الثلاثة الأخرى كما ان نسبة الكربون الى النيتروجين ذات القيمة 10 والتي استخدمت كمؤشر لنضج السماد العضوي قد تم تسجيلها خلال 3-4 أسابيع في الحاوية رقم 4، مما يؤكد سرعة نضج الكمبوست مقارنة عنه في الحاويات الأخرى.

خضعت نتائج الدراسة للتحليل الاحصائي باستخدام (SPANOVA) و جميع هذه المتغيرات الدالة احصائياً سجلت لصالح حاوية رقم 4.

النتائج الجديدة لهذا البحث تم نقاشها من خلال أهميتها في مجال البيئة والتنمية المستدامة ومعالجة وتدوير المخلفات الصلبة خاصة المخلفات النباتية بغرض الاستفادة من هذه المخلفات في مجال الزراعة، وتحسين التربة.