Studies on the Microbial Ecology of Open Windrow Composting

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I certify that this thesis is the true and accurate version of the thesis approved by the examiners.

Signed [redacted]

Director [redacted]

Date 15/10/98

ABSTRACT

Due to the pressure of recent legislative changes (eg: the EU Landfill Directive and the imposition of a Landfill Tax), composting as a waste disposal technique is now being viewed as the preferred alternative to the landfilling of organic waste. However, while composting has been practised in one form or another for 2500 years, the underlying principles behind the microbial ecology of composting, is poorly understood. In order to obtain an insight into the ecology and hence, the critical nature of the composting process, a number of low cost open-windrows containing urban botanical wastes were established. These windrows were subjected to microbial and physico-chemical analysis over the initial period of exothermically active composting (25 days). This study demonstrated that, whilst average temperatures within open windrows can reach in excess of 65°C, the sustainability and range of these temperatures depended upon the windrow bulk density. Windrows with bulk densities of 600kgm^{-3} had a larger insulation factor and thus, were able to sustain high temperatures for longer periods. However, these windrows were more susceptible to the development of areas of low temperature (cold spots) at depths below 20cm. Windrows with bulk densities of 400kgm^{-3} had smaller insulation factors and therefore, lost heat at a faster rate than windrows with higher bulk densities. This loss of heat was observed to be the case with the windrow surface layers, but they exhibited fewer cool spots at lower levels. This study found that the average microbial population of windrow material was 2.29×10^{13} CFU kg^{-1} and that each microbial cell could generate between 6.33 and 8.56×10^{-13} Mjkg^{-1}. This resulted in the generation of between 1.13 and 1.70 Mjkg^{-1} °C^{-1} of heat energy. Contrary to the published literature, this study observed that temperatures above 65°C did not result in the significant loss of ammonia from the windrow. However, high levels of ammonia did suppress the formation of nitrate within the windrows. Experiments investigating microbial population kinetics within the windrows indicated that observed changes were proportional to temperature up to 60°C, when a reduction in population numbers was observed between 60°C and 65°C. However, between 60°C and 70°C population levels increased once again. It was also noted that at the start of the composting process, 13 different microbial species or genera could be identified. However, after 17 days of exothermic composting, this had been reduced to 2 genera, including a novel large bacterial species belonging to the genus *Bacillus*. This study also showed that samples of windrows exposed to temperatures above 55°C for 48 hours did not eliminate mesophilic or psycrotrophic microbial populations as previously assumed by other workers, but only suppressed their metabolism during the high temperature period.

ACKNOWLEDGEMENTS

Before embarking on any journey, especially one of discovery, there is only one thing that can be relied upon for certain (and then only tentatively), the starting point. There will be many distractions, blind alleys, dead ends and wrong turns along the way before reaching the final destination. Before starting this journey, I was fortunate to find a first class support team who kept me on the 'straight and narrow', ensuring I did not become lost the minute I came to a crossroad. The core of my support crew was undoubtably that of my family, and in particular my wife Dawn who, for over a period of five years supported, encouraged and read every thing I wrote (twice) without injury or favour, giving only suggestions on 'improvements'. Another vital component of my support team was my director of studies, Dr. Phillip Collier, who also read everything twice, and never ran out of suggestions for further 'improvements'. I thank him for his patient guidance and encouragements along the way. My thanks goes out to Dr. Kevan Gartland and his team of dedicated composters for helping to make waste derived composting more acceptable to the horticultural industry. I also acknowledge The Composting Association for ensuring I was cognizant on the latest legislation. To Rachel Brooks I am particularly indebted for spending her evenings proofreading flawed manuscripts when she must have had better things to do. I am also grateful to the continuing support of the councillors and officers of the former City of Dundee District Council Cleansing Department, now the Environmental and Consumer Protection Department of The City of Dundee Council, and in particular, Alistair Lamont, for making the journey possible in the first place.

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INTRODUCTION

Composting.

Composting, for the purposes of this thesis, is the aerobic biodegradation of organic material (commonly a waste material), with a high temperature (thermophilic) stage (Unaogu *et al* 1994; Stenbro-Olsen *et al* 1995a; Stentiford *et al* 1985). Composting is not new. It has been practised by farmers as part of their agricultural practices, for centuries. In Japan and China, composting is reputed to go back at least 2500 years and in Europe, the use of composted waste material to sustain soil fertility, can be dated from medieval times. Despite this, the twentieth century has seen a steady decline in the practice, with composting becoming principally a domestic gardening pursuit. Over the last five years however, changes to environmental legislation, socioeconomic and political factors as well as advances in composting technology, have seen composting re-emerge not as an agricultural process, but as a major feature of the waste disposal industry.

Composting, the early years.

For centuries, composting was a simple matter of throwing vegetable and animal waste into pits or leaving it in surface piles to decompose naturally. The process was not controlled to any large extent (other than perhaps covering with soil or occasionally turning) and was left (mostly undisturbed) for up to a year, before being returned to the fields. Those early composting techniques were none the less effective in recycling plant nutrients and are still practised today, essentially unchanged, in domestic gardens. The first recorded major change in composting is accredited to the Chinese around 2500 years ago. China, with a high and expanding population, developed a method of combined composting not only as a key process in the maintenance of soil fertility (essential for food production), but also as a waste disposal technique, by including human faecal material mixed with animal manures and vegetable waste in the compost heaps. The Chinese at that time, appeared to have had a degree of understanding of the composting process, as witnessed by the development of the partly aerated

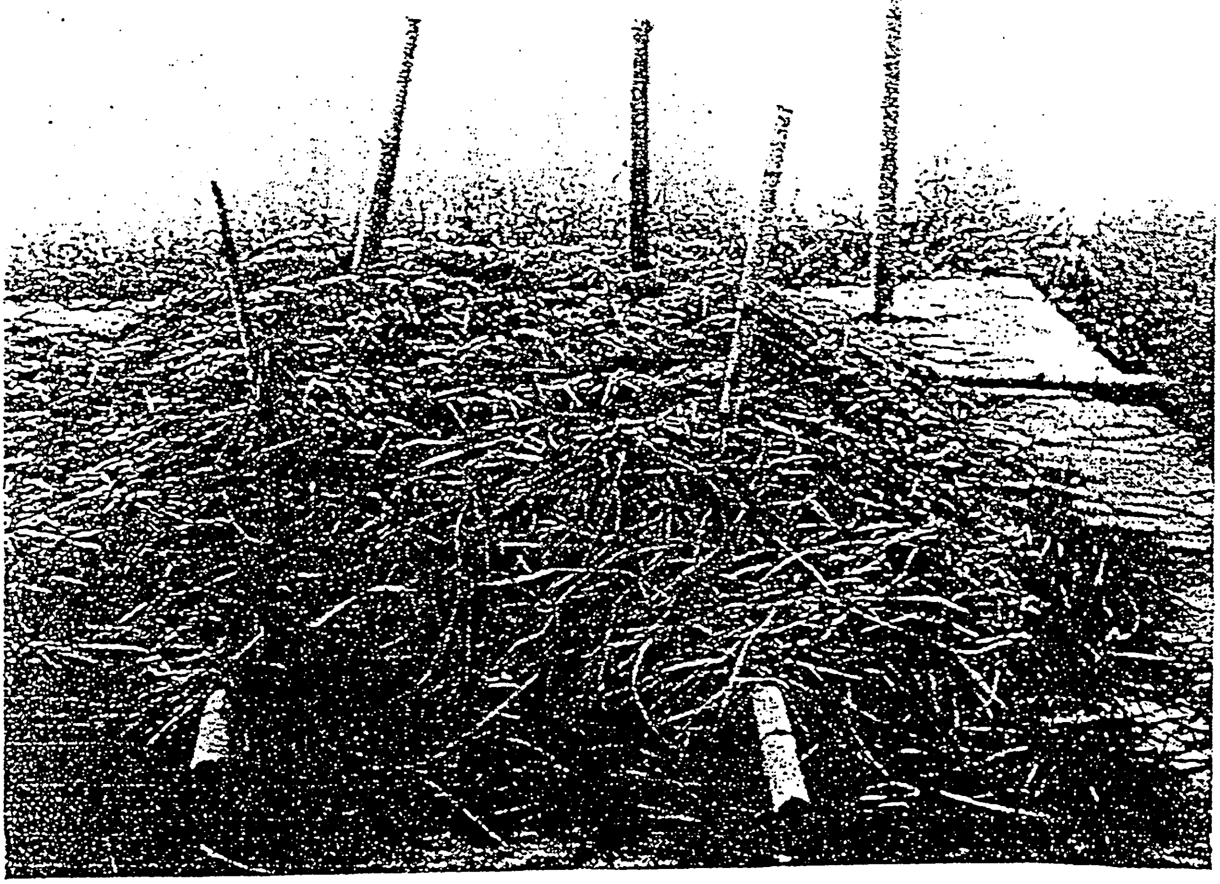
static pile (aeration facilitated with bamboo poles; (Fig.1)). Such systems are still to be found today in many developing countries (Polprasert 1996).

Seventeenth century Europe saw composting becoming a vital part of the arms industry when during the English Civil War (1642-49), it was used in the production of saltpetre (potassium nitrate, KNO_3) for the manufacture of gunpowder. Large areas of the Royal Forests were set aside to provide turf, firs and ferns for the building of compost heaps from which the nitrate was leached out, by steeping the composted material in water (Catton 1983). However, as the gunpowder industry found alternative ways of producing their product, composting as a large scale industry went into decline, relegated as before, to principally an agricultural pursuit.

The Decline of Composting in Europe.

Traditionally towns and cities obtained their food supplies from the surpluses produced by farms outside the towns, while rural populations fed themselves. But as a result of the decline in infant mortality and increases in life expectancy (Gray 1985), large increases in European populations and in particular those of the cities and towns were seen (Brown, *et al.* 1981). With increases in populations, that which was once prime agricultural land, was now needed to house this expansion. The net result of this, was a gradual urbanisation of the countryside. In addition, the steady and sustained population drift away from rural areas brought on by the Industrial Revolution drawing people away from agriculture into factories, resulted in enormous pressures on the remaining farms to produce ever increasing quantities of food, from decreasing amounts of agricultural land (Brown, *et al* 1981; Gray 1985; Sarre 1991). These pressures, and an improved understanding of plant nutrition, paved the way for the large scale use of concentrated plant nutrients (e.g. Guano). The availability of alternative sources of essential plant nutrients was further improved during the early part of the twentieth century by the ability to manufacture large quantities of 'inorganic fertilisers' (i.e. superphosphates (calcium phosphate treated with sulphuric acid)) and ammonium nitrate, (Haber process).

Fig. 1. Chinese aerated static pile (circa 2500BP): Common mix was: 40% crop stalks, 30% agricultural waste or garbage and 30% animal excreta plus 'night soil' (Polprasert 1996).



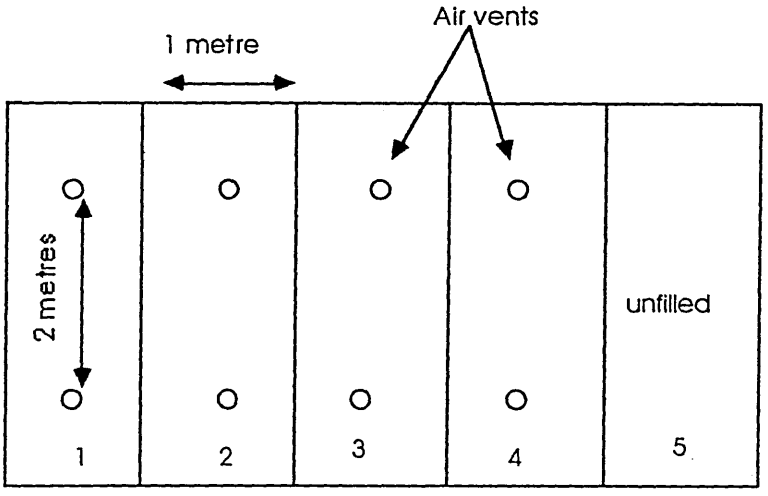
The manufacture of fertilisers, lead to fundamental changes in crop production. Intensive 'chemical' farming had arrived (Brown, *et al* 1981). In 1900, 91% of all forms of nitrogen applied to farm crops in the UK, was from an organic source (compost, animal manures etc.). By 1913, organic sources of nitrogen had dropped to 40%. The use of the so called 'artificial' fertilisers steadily increased to the extent that by 1980, 75% of nitrogen, 70% of potassium and 59% of phosphorous, were supplied to farms in a manufactured form (Sarre 1991). While these figures relate to farming in the developed world, similar trends are seen in nearly all developing countries as a result of the introduction of 'Western' farming practices since 1945. Agricultural waste, the traditional source material for plant nutrients, was now only regarded as a serious waste problem, to be 'managed' by landfilling or burning.

Composting, a quiet renaissance.

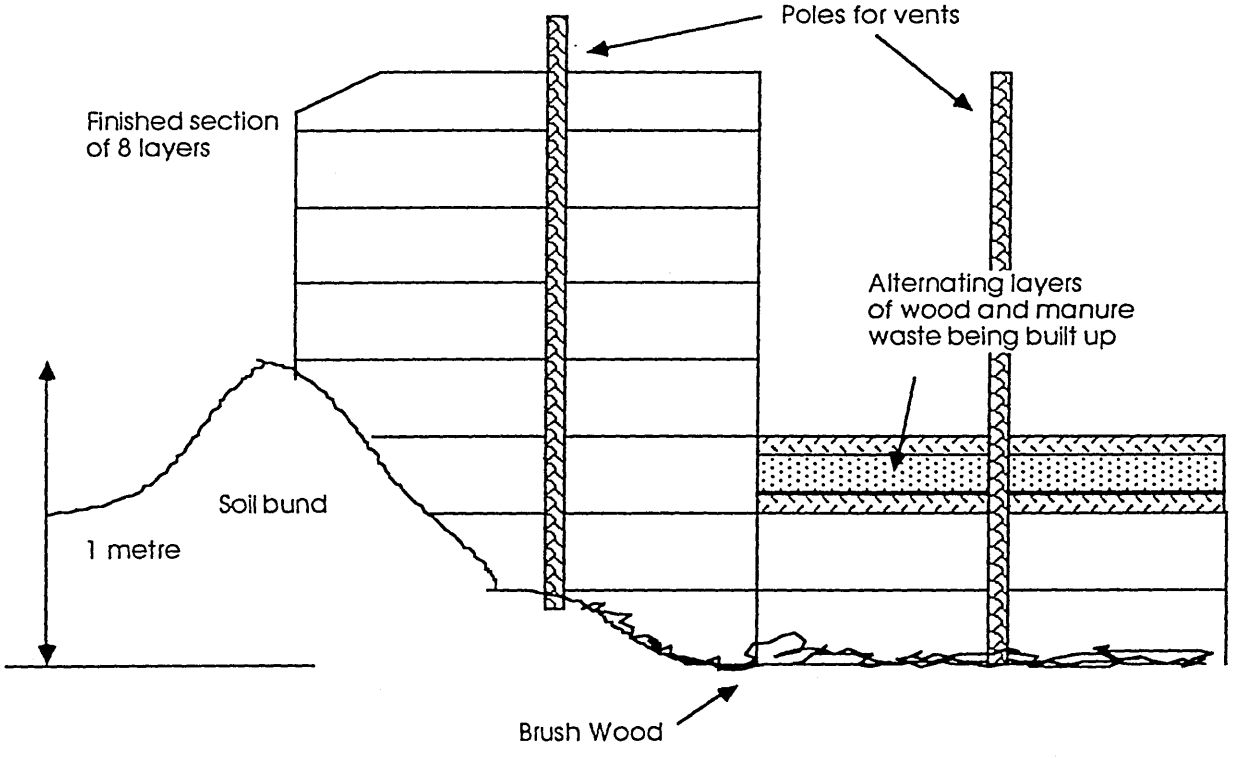
While large scale agriculture in Europe and North America was steadily and quietly abandoning composting as part of the soil-plant-nutrient cycle in favour of easy to use and readily available manufactured fertilisers, composting was still carried out on a local basis by subsistence farmers in the near and far East (as it had for centuries). Professor F.H. King of the United States Department of Agriculture, during visits to China, Japan and Korea in 1909, described the advantages of recycling human and other organic wastes by composting. These reports prompted the British economist and botanist Sir Albert Howard (who at the time was employed by the Colonial Indian Government) to study composting methods used by local Indian farmers. These observations ultimately lead him to develop the 'Indore' composting system (Fig. 2). The 'Indore' system used alternating layers of wood (carbon source) and animal manures (nitrogen source) with bamboo poles to aid aeration (a similar version to the Indore method, the 'Bangalore' process, was used extensively in East and South Africa) (Lopez-Real 1994). The prime object of the 'Indore' and 'Bangalore' composting systems was to treat and recycle human and animal waste into a humus for agricultural use. In Europe and North America, priorities were different, a growing shortage of suitable landfill sites, meant that alternative methods of disposal of organic material had became the main aim (Department of

the Environment 1995a).

Fig 2. Indore Composting System. Alternating layers of wood and animal manures are placed upon an initial layer of brushwood. Aeration is achieved by the use of hollow bamboo poles.


Air vents
1 metre
2 metres
unfilled
1
2
3
4
5

Plan view


Poles for vents
Finished section
of 8 layers
Alternating layers
of wood and manure
waste being built up
Soil bund
1 metre
Brush Wood

Mechanical Composting Systems

The Indore systems and their derivatives, have long processing and maturation period (up to a year per pile), before the material is suitable for use in agriculture. However, the prime requirement for composting as a waste management technique, is to dispose of organic waste as quickly and as economically as possible. This lead to the development of mechanically aided composting during the 1920's. Initially this involved simple mechanical pretreatment of the waste (shredding, crushing, etc.) in order to speed up the initial rate of biodegradation by increasing the surface area available for microbial attack. Later, forced aeration by means of air pumps and/or mechanical stirring or turning was introduced, in an effort to keep the system from becoming anaerobic.

A major development of this type of mechanised composting systems, was promoted by the Dano company of Copenhagen, Denmark, during the 1940's. This took the form of a drum or cylinder system (the DANO drum, Fig 3) which was filled at one end with a mixture of organic wastes; i.e. Mixed Solid Waste (MSW) and sewage sludge, and through a series of motors and gear wheels, rotated at 1 RPM. This caused the materials within the drum to collide with each other, the sides of the drum, and so gradually become reduced in size (homogenization). In later models air was injected to increase the biodegradation rate even further, thus incorporating shredding, mixing and aeration in one unit. There are three main models (based on drum size):

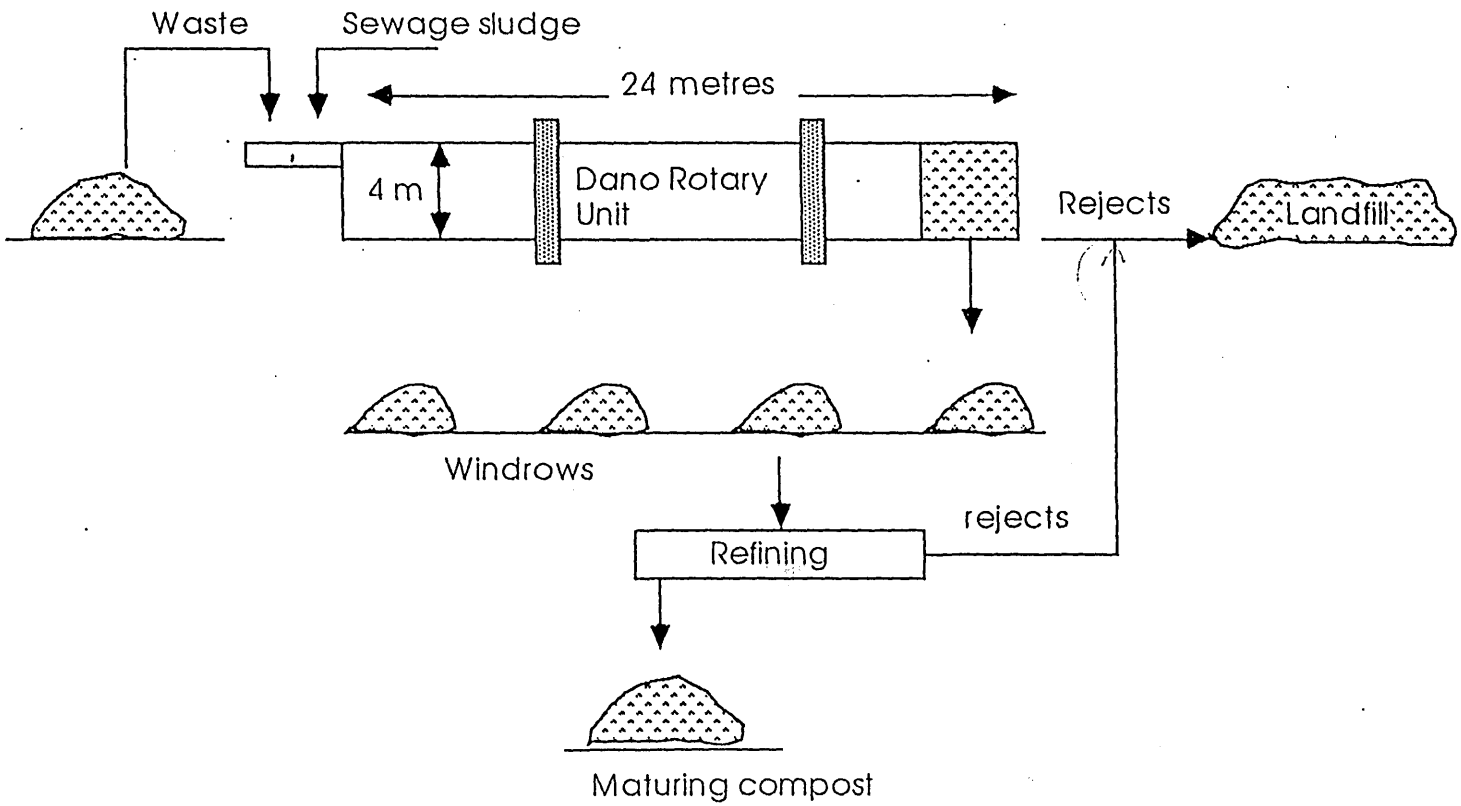
Type I: 3m x 11m drum

Type II: 3.8m x15m drum

Type III: 3.8m x 24m drum

The maximum running load (Type III) was around 175 tonnes (80% of total capacity) and fed at a rate of 35 tonnes per day. The normal retention time was five days and thermophilic temperatures (65°C) could be readily obtained. The residue having been substantially reduced in bulk, was normally taken to landfill.

Fig 3. The Dano System. Mixtures of solid waste (MSW etc.) is mixed with sewage sludge in a slowly rotating drum.


Waste
Sewage sludge
24 metres
4 m
Dano Rotary Unit
Rejects
Landfill
Windrows
rejects
Refining
Maturing compost

Anaerobic or Aerobic?

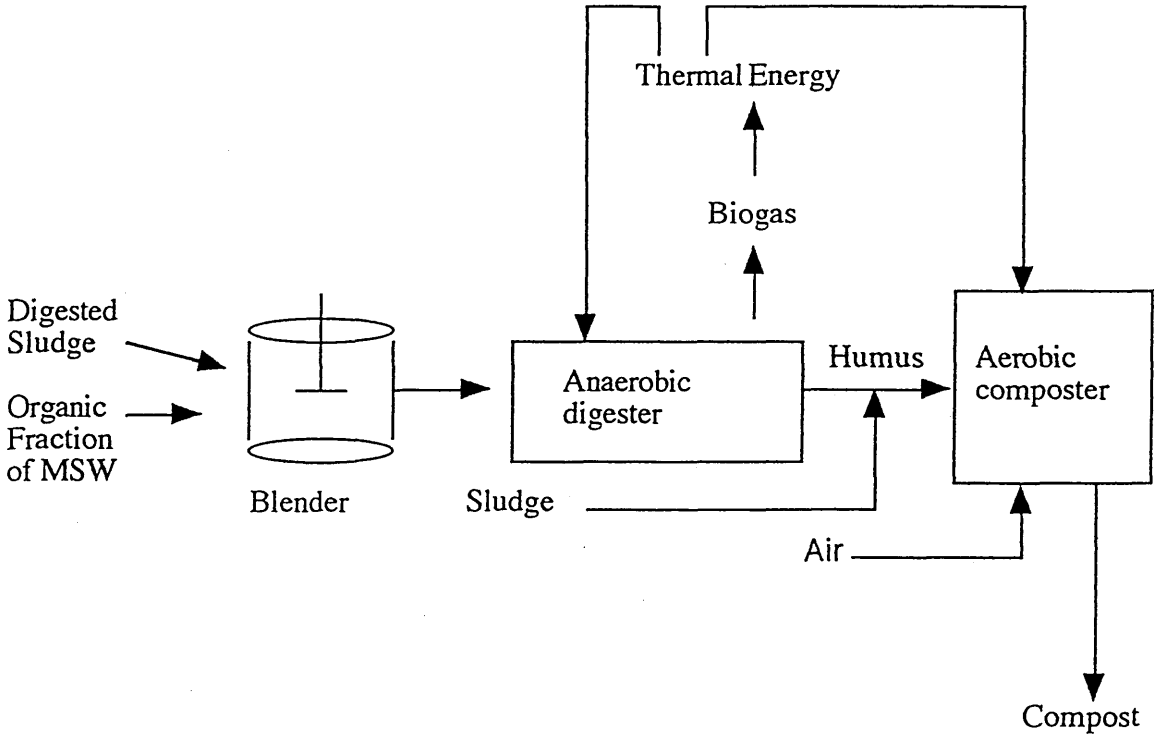
Early composting systems such as the Chinese static pile and the Indore systems, are aerobic only at the beginning of the process, relying on 'passive aeration', i.e. air trapped within voids and surface diffusion. As composting proceeds, microbial respiration causes the oxygen levels to drop and carbon dioxide levels to rise (Polprasert 1996). This results in the establishment of reducing conditions within the pile. Aeration can be assisted by regular mixing or turning, but as this is difficult with large heaps, most of the time these structures are anaerobic. Modern composting plants have developed a range of strategies to keep compost heaps aerobic, such as sucking or blowing air through compost heaps (aerated static pile) with the aid of pumps, sometimes combined with mechanical agitation with flails or screw devices. These system are often automatically controlled by the computerised sensing of one or a selection of composting parameter, i.e. temperature, moisture or oxygen demand etc. Other systems rely on simple turning with mechanical front end loaders (Stentiford 1985, 1992) .

In the anaerobic decomposition of organic matter, degradation of simple short-chain fatty acids, formic and acetic acids and some simple alcohols, e.g. methanol, may lead to the generation of methane (CH_4) by members of the family Methanobacteriaceae (Finstein *et al*, 1980). This has led to the development of large scale anaerobic digesters which utilise the production of methane as a source of energy ('Biogas'), as well as the production of humus. Early research into anaerobic digestion was carried out by the Natural Gas Service Co. and Dynatech during the 1950's and 1960's in North America, using a mixture of MSW and sewage sludge. Commonly these early digesters used a mixture of air-dried sewage and digested sludge, as an initial microbial inoculum. Early systems were single stage batch fed reactors. Most modern plants however, are multi-stage continuous feed reactors and some, produce methanol in addition to methane and humus (Fig. 4), (Kayhanian. *et al*, 1991).

Mechanical and Automated Aerobic Composting Systems.

Mechanical aerobic composting, or 'In Vessel' composting, is becoming an increasingly popular alternative to the more traditional 'Open Windrow' (Stenbro-Olsen & Collier 1994) processing. Some are relatively 'low-tech', consisting of a horizontal rectangular tank with air holes in the base, equipped with an endless conveyor belt mounted on wheels that move on racks placed on the bottom of the tank. As the belt moves, it picks up and mixes the composting material as required. Retention is normally around a week, before an extended maturation period in traditional windrows (Golueke 1991). The more sophisticated automated systems use a series of separate or linked enclosed chambers, in which the pre-treated (shredded etc.) materials are mechanically moved from chamber to chamber as each stage of composting is completed. Heat generation, moisture and oxygen demand etc. are continuously monitored and controlled by feedback systems. The advantages of these enclosed automatic systems, are that they use relatively little space when compared with the open windrow systems and need fewer operators. In addition, as the system is sealed, and the air used during the process is drawn through a series of 'bio-filters' to trap and eliminate odours, composting can be carried out in areas of high population densities (Edwards 1998; Wright 1998).

Fig 4. Multi-stage continuous feed composting system, incorporating an anaerobic digester to produce CH_4 (Biogas).


Thermal Energy
Biogas
Digested Sludge
Organic Fraction of MSW
Blender
Anaerobic digester
Humus
Aerobic composter
Sludge
Air
Compost

Legislative and Social Background

A New Beginning

Composting as a waste disposal technique, has been given a fresh impetus over the last ten to twenty years, by a series of reports, legislative measures and a change in the political and social climate. Prior to the 1970's, there was little or no legislative control in the management of waste. What controls there were, lay with the Alkali & Works Act (1906), Public Health Act (1936) and the common law of nuisance. And for the control of landfills, the Town and Country Planning Act (1947). However, during the 1960's and 1970's, two commissions, the Key (1961) and Sumner (1970), reported on the disposal of domestic waste and its potential for causing unacceptable water pollution. The Sumner (1970) committee concluded that more research was required into the way landfill sites were operated. This led to a series of legislative measures, aimed at the protection of the environment. The most significant being:

The Local Government Act (1972). This enabled the setting up of waste collection authorities in England and waste disposal authorities in Wales and Scotland.

Deposit of Poisonous Waste Act (1972). A 'stopgap' legislation, aimed at preventing indiscriminate dumping of toxic wastes (now repealed);

Control of Pollution Act (1974). The first attempt at a comprehensive pollution control legislation (substantially amended prior to 1990, when largely replaced by the Environmental Protection Act (Great Britain 1990; Battersby 1991; Hand 1997);

Refuse Disposal Amenity Act (1978). This Act made provisions for places where members of the public could deposit waste ('Civic Amenity' Sites) (Battersby 1991).

In 1974, the UK Government commissioned a White Paper entitled "War on waste: a policy for reclamation", which stated:

> "... the government believes that there should be a new
> national effort to conserve and reclaim scarce resources -

> a war on waste involving all sections of the community...
> the government will actively promote the reclamation effort
> at national level. In order to exploit the opportunities
> which now present themselves, we must henceforth adopt
> an integrated approach to the whole recycling chain,
> rather than engage in purely piecemeal efforts...".
>
> (Grayson 1991).

In the same year, the House of Commons Trade and Industry committee, examined recycling in detail, producing a report called: "The Wealth of Waste". It criticised the UK for not recycling with the same effort as its European neighbours. In the following year, a Royal Commission on Environmental Pollution, issued yet another critical report, this time calling for changes in the Control of Pollution Act, 1974 (COPA) (Great Britain 1974). The Commission recommended the setting up of a framework of best practice for waste management. This included reduction of waste at source. COPA, introduced a licensing system for waste disposal in an effort to counter potential environmental damage from unregulated dumping (fly tipping). It also required waste disposal authorities to prepare plans for the disposal of household, commercial and industrial waste, information on costs, disposal methods and to consider what arrangements could be made for reclaiming waste (recycling). However, much of this part of the Act was not implemented until the mid 1980's (a legal time limit for compliance was not included in the original legislation) (Battersby 1991). As a consequence, by the time the Environmental Protection Act (1990) came into existence, some Waste Disposal Authorities (WDAs) had still not published plans (Department of the Environment 1995b).

One Europe, One Environment.

The Single European Act (1986) (SEA), was perhaps the single most important event to date in European environmental policies. This Act (SEA) introduced environmental amendments to the Treaty of Rome by imposed legally binding measures on member states relating to the

protection and improvement of the environment (Article 130R). The SEA, also instigated the principle of 'the polluter pays' (since taken up by the UK government) (Department of the Environment 1995b) and the 'Integration Element', whereby all other policies of the European Commission (EC) must have an environmental protection component. Article 130R (130R(4)) enabled the 'subsidiarity principle', whereby the EC would take action, if it felt that environmental goals would be better achieved at a community level, rather than on an individual state basis. The powers of the EC to influence member states in environmental issue, was further strengthened at the signing of the Maastricht Treaty. Article 3(k) of Maastricht (or more correctly the Treaty of European Union (TEU)) confirmed that environmental consideration now forms an integral part of EC policies. The TEU council ruled that on matters of environmental policy, (apart from a few ill defined areas, and on issues under consultation prior to TEU), only majority voting was required, not unanimity as before (Battersby 1994).

The 1980's was also a decade of growing public concern about a range of environmental issues. Purity of drinking water, sewage dumping at sea and air pollution were some of their concerns. Issues that from time to time, occupied several column inches in both the 'popular' and 'quality' press. The UK had also (rightly or wrongly), gained a reputation as the "Dirty Man of Europe" (Grayson 1991), mainly as result of atmospheric emissions of SO_2 and NO_x (Smith & Warr 1991). This reputation, it has been suggested, was as a consequence of the Air Pollution Acts of the 1950's. The Clean Air Act (1956) was introduced in the wake of the great London 'smogs' and required all power stations (main producers of SO_2 and NO_x) to have tall chimneys. This had the (desired) effect of substantially reducing the local pollution from these emissions. Unfortunately, the SO_2 and NO_x, born on high altitude winds, fell as 'acid rain' elsewhere in Europe (Blunden & Reddish 1991; Smith & Warr 1991; Silverton & Sarre 1990). The cumulative effect from the various Commission reports, EC directives and legislation, Maastricht, public opinion and media attention, was to encourage the Government to 'reassess' its policy on environmental issues. One result of this, was the introduction of an Environmental

Protection Bill, in 1989. This Bill was given Royal Accent in 1990, to become the Environmental Protection Act 1990 (EPA 90). This Act modified and extended several existing acts including: Clean Air Act (1956), Radioactive Substances Act (1960) and the Local Government Acts of 1963 and 1972. The EPA 90 also re-enacted many of the provisions of the Control of Pollution Act of 1974, relating to waste on land, with modification with respect to the functions of the regulatory and other authorities concerned with the collection and disposal of waste.

The Environmental Protection Act 1990 (EPA 90)

The Environmental Protection Act fundamentally changed the waste disposal industry. It required a fundamental reassessment of many existing methods of waste disposal and through the Duty of Care Regulation (SI 1991 / 2839) under the Act (EPA 90, Part II Section 34), firmly put the onus on local authorities to control the disposal of most types of waste and to prevent contamination of the environment.

Extract from the Environmental Protection Act Part I Section 1:

(1) The following provisions have effect for the interpretation of this part.

(2) The 'environment' consists of all, or any, of the following media, namely, the air, water and land; and the medium of air includes the air within buildings and the air within other natural or man-made structures above or below ground.

(3) "Pollution of the environment" means pollution of the environment due to the release (into any environmental medium) from any process of substances which are capable of causing harm to man or any other living organism supported by the environment.

(4) "Harm" means harm to the health of living organisms or other interference with the ecological systems of which they form part and, in the case of man, includes offence caused to any of his senses or harm to his property; and "harmless" has a corresponding meaning.

Environmental Protection Act 1990.

The EPA 90 has two main parts:

Part I, deals with the control of pollution arising from certain industrial processes and introduces the foundation of integrated pollution control (IPC).

Part II, replaces much of the Control of Pollution Act (COPA); relating to waste on land and separates the regulatory and operational functions of local waste authorities, that were held under the old Act, with the creating of WRAs and WDAs (Waste Regulation and Disposal Authorities respectively). The new WDAs have responsibility (under WRAs) to arrange for the disposal of controlled waste (waste that came under the terms of the 'Duty of Care' regulations (Environmental Protection (Duty of Care) Regulation SI1991 / 28391 1991) (Battersby 1995; Hand 1997), but not to act as waste disposal contractors. Instead they employed private waste contractors, or set up more or less independent (from the local authority) new waste disposal companies, the Local Authority Waste Disposal Companies (LAWDCs).

In Scotland, the WDAs were not bound to set up LAWDCs, however some WDAs opted to employ private companies to manage waste disposal, i.e. Perth and Kinross Council, employed Northumbrian Water PLCs subsidiary NEM Ltd., to manage the landfilling of their domestic and commercial waste. Others (e.g. Dundee City Council) continue to operate landfill operations, recycling centres, transfer stations and composting plants independently of the private sector. In December 1994, the Department of the Environment (DoE) as part of their ongoing examination of environmental legislation, published for parliamentary debate, the Environment Bill. This Bill, would enable the setting up of the Environmental Agencies in England and Wales; incorporating the Pollution Inspectorate, National Rivers Authority and WRAs. In Scotland, the Scottish Environment Protection Agency (SEPA) with similar roles. The Environment Bill was passed by the UK Parliament in July 1995 (Great Britain 1995) and included a statutory framework for a national waste strategy and the recovery of packaging waste (Battersby 1995, Hand 1998).

The Control of Waste.

Part II of EPA90, strengthened the controls over the way waste is carried and disposed of, by extending the provisions under COPA and The Collection and Disposal of Waste Regulations 1988 (SI 1988/819), by creating different waste categories (Table 1). However, the majority of waste in the UK, is classed as controlled waste and comprises of: domestic household (including campsites and residential homes), educational establishments, industrial and commercial waste as well as materials not falling in the first category providing it can be disposed of without a disposal licence. (Battersby 1995). EPA90 also made it an offence for a producer of a controlled waste, not to ensure that waste is disposed legally, even if disposal was by a third party (Duty of Care Regulations 1991, SI 1991/2839), (Battersby 1994; Hand 1998).

Table 1. Definitions for wastes and regulations which govern them (Battersby 1994, 1998; Hand 1998).

Term	Definition	Legislative Derivation
Controlled	Household, commercial and industrial waste	EPA90 Controlled Waste
Municipal	Wastes that WCAs have a duty to collect	Regulation 1992 SI 1992/588
Directive	Materials which the holder Discards or is required to Discard	Waste Management Licensing 1994
Hazardous & Special	'Hazardous' waste is the term used by the EC of wastes that are dangerous to life. 'Special' is the UK term for the same	EC Directive 91/689 EEC. This replaces COPA (Special Waste) regulation 1980
Civic Amenity	Household waste which the WDAs have a duty to receive at collection points. This does not include wastes From commercial sources	Refuse Disposal Amenity Act 1978

Changing Attitudes, Changing Politics.

These legislative changes were thought by some commentators to be a reaction to the Government's belief that environmental damage would eventually led to a negative impact on the nation's economy. Others saw it simply as a political response to the growing public awareness of environmental issues (Grayson 1991). In Britain today (as in many other countries), attitudes towards environmental issues have undergone swifter changes than in the past. Today's society is much more informed of the human capacity to bring about changes to the global ecosystem (i.e. ozone depletion, greenhouse effect, toxic waste etc.), than previous generations (Silverton & Sarre 1990). This has led to the ascent of 'Green' political parties, often with policies and ideologies focusing on what have become known as, 'green issues'. These parties, as it turned out, had a profound effect on public (and government) attitudes to environmental issues. While the initial political success, of some of these new parties, was mixed, a notable early success, was a 'green' party called Die Grunen, in what was West Germany. This 'green' party, polled more than 5% of the popular vote across the country in 1983, sending (as a result of the proportional system of voting) 27 representatives to the Bundestag in Bonn. In the UK however, a lost deposit was the normal election result for a 'green' party candidate. This changed in 1989 with all 'green' parties improving their vote significantly, in most European countries during the European Parliament elections. Environmental awareness was now firmly on the political calendar. Culminating with the attendance of most world political leaders, at the Earth Summit meeting in Rio de Janeiro, Brazil, 1992. The 1980's also saw the emergence of a new type of environmental pressure group, the environmentalists.

Environmentalism.

An environmentalist, was defined in most pre-1970's dictionaries, as someone stressing the importance of social circumstances, rather than heredity to the development of a human personality. By the 1980's however, most people would identify environmentalists as: individuals or groups, concerned with preventing damage to the environment from polluters.

Similarly, the use of the word environment has taken on a new emphasis. Frequently becoming a word or expression used in the context of environmental protection e.g. "Our products are biodegradable to ensure there is no damage to the environment in either their production or disposal" (Body Shop Catalogue 1987), or on a packet of Persil detergent, under the heading Environmental Information: "... to safeguard the environment - Persil's surface active ingredients are biodegradable and break down rapidly by natural processes into harmless substances..." (Lever Brothers Ltd. 1995), rather than the literal meaning of the word i.e. the circumstances, objects or conditions by which one is surrounded, (Longman Dictionary of the English Language 1984). These individuals or groups had new, and sometimes quite radical ideologies, but more importantly, their ideologies were connected in some way to the nature of power within society (McLennan 1993). In other words, they served to sustain or undermine the legitimacy or the 'rightfulness' of a social-political order, and as such, is of great interest to politicians. (McLennan 1993).

The Genesis of Modern Environmentalism.

Modern day environmentalism, as is the case of most cultural phenomena, has its roots in the past. The first book of the Jewish and Christian Bible suggests the idea that the human race occupies a position of special privilege and responsibility, to safeguard the rest of nature. An example of this, is the story of Noah and his Ark in which Noah takes upon himself, the divinely ordained duty to save other species than man from an ecological disaster upon the Earth. This theme of a special responsibility of man and the likely effect of ignoring or flouting this responsibility, was symbolised in Samuel Taylor Coleridge's "The Rhyme of the Ancient Mariner", first published in 1798. In his poem, he tells the story of an Ancient Mariner who kills an Albatross, a bird of good omen, and, the ill luck that befall the ship thereafter:

> ..."how the Ancient Mariner cruelly and in contempt of the laws
> of hospitality killed a sea bird and how he was followed by many
> and strange judgements"...

At one level, the poem could be interpreted simply as a tale of superstition, but for the modern

environmentalists, it is a timely warning to the reader of the lamentable consequences for the Human race if, they fail to treat the lives of other living things (including the Environment) with respect. This view was also taken up by a leading nineteenth century social critic Thomas Carlyle (1795-1881), who wrote in the then influential Edinburgh Review in 1829:

> "that ... this age of ours... is the Age of Machinery,
> in every outward and inward sense of the word.
> We remove mountains, and make seas our smooth highway:
> nothing can resist us. We war with rude Nature; and,
> by our resistless engines, come off always victorious,
> and loaded with spoils".

Both Coleridge and Carlyle sought to illustrate mankind's special and privileged position, reflecting perhaps, the idea of defined responsibility to nature; the stewardship of nature. This was in stark contrast to an earlier attitude (as embodied in the writing of Frances Bacon (1561-1626) who is regarded as one of the founders of 'Modern Philosophy'), which maintained that nature had to be subdued (imperialism over nature). Frances Bacon postulated that the conquest of nature was the highest ambition a human being can have. Bacon's ideas and attitudes were associated with the rapid growth of science in the seventeenth century, and in his book, Novum Organum (New Instrument 1620), he explained how mankind can regain his rights over nature assigned to him by the gift of God. This perhaps reflected an earlier instruction by God to Adam in the Garden of Eden to, ..."replenish the Earth and subdue it: and have dominion over the fish of the sea and over the fowl of the air and over every living thing that moveth (sic) upon the Earth"...(Genesis 1:28), which could be taken as consistent with stewardship over nature, but has also been taken as a licence to treat everything in nature as subordinate to the needs of the human race.

Environmentalism vs. Conservation.

During the late nineteenth century, Canon Rawnsly, Octavia Hill, Robert Hunter and others, worked with various local pressure groups resisting the commercial development of the Lake District of England. They, as many others, believed that the only way to safeguard those landscapes from the ravages of the Industrial Revolution, was to own it. So in 1893 the National Trust in England was founded. The National Trust, had a brief to hold and own land and buildings in perpetuity (Silverton & Sarre 1990). These UK organisations and others, i.e. the Royal Society for the Protection of Birds (RSPB) and similar institutions in the USA, e.g. the Sierra Club and the Audubon Society, were formed mainly as conservation bodies (setting up national parks, restoring buildings etc.) or specialist interest organisation (caring for birds etc.) and to a large extent, still are. But for some individuals or groups, this was simply not enough. New forms of environmental pressure groups were formed; often with confrontational styles of campaigning. These were very different from earlier and existing so called Technocentric groups, i.e. recognising 'environmental problems' but believed that conventional economic reasoning (cost-benefit analysis etc.) is the best way to solve environmental problems. Some of the new groups however, were inclined towards the 'Ecocentric'position, i.e. lacking faith in all types of materialism and especially, large-scale modern technology. These groups campaigned for decentralised small-scale communities and encouraged everyone to: "Act Locally and Think Globally" (Silverton & Sarre 1990).

The militant environmentalism of the 1980's and 1990's.

Many of today's pressure groups, grew from the 'ecology' (a word coined by the nineteenth century German biologist Haeckel, during the 1860's) movement of the 1960's and '70's, and were heavily influenced by writings of individuals such as the anarchist Peter Kropotkin who, in his 1899 text, "Fields, Factories and Workshops", dealt with issues of social and environmental problems that are still as much of a concern today, as it was then (Smith & Warr, 1991).

While there still are a growing number of, sometimes quite diverse, groups, i.e. Deep Ecologists, Gaia followers, Green Alliance and the like; two groups, Greenpeace and Friends of the Earth (FoE) are probably the best known. Greenpeace was founded in Vancouver in 1970 and originally campaigned to prevent nuclear bomb testing in the Pacific. Other interest include the abolition of whaling. By 1989 Greenpeace had established itself as one of the best known environmental organisations with branches in 22 countries. FoE began as a splinter group of the Sierra Club also in 1970. By the late 1980's it had over 280 branches in the UK alone, and campaigns on a range of environmental issues, both local and global. FoE was instrumental in setting up 'Recycling City' awards during 1991 (to promote recycling). Both the FoE and Greenpeace have to some extent become 'Establishment' environmentalists with professional full-time researchers and sophisticated public relations infrastructures. Both organisations are frequently called upon to give an opinion on environmental issues and are quoted extensively by both politicians and media.

Summary.

The growing public perception of an imminent 'ecological crisis' (Silverton & Sarre 1990) as fostered by some environmental movements in the 1970's, and the realisation by the politicians (of all parties) of the power (perceived or real) of the new environmental ideologies, have led to an substantial change in official and governmental attitudes to environmental issues (especially to pollution). In the United States of America, an Environmental Protection Agency was set up, now mirrored in the UK by the Environment agency in England and Wales, and the Scottish Environment Protection Agency in Scotland. In Britain, a new government department the Department of the Environment (DoE) was set up in 1970 and later became part of the Department of the Environment Transport and the Regions (DETR) in 1997. From the relatively legislation free environment of the 1950's, todays society has to take note of nearly 500 UK and EC Acts and regulations, plus a host of international conventions.

Managing Waste

Managing Waste; Alternatives.

By 1990, approximately 435 million tonnes of waste from a range of sources (Fig.6) were produced in the UK. The principle disposal routes being landfill, incineration and some recycling (Fig. 5), (Department of the Environment 1995a; Hand 1997). The high level of landfilling (90% of household & 85% of commercial waste) was due to the relatively low landfill charges at that time (typically, less than £5 per tonne in rural Norfolk, to £30 in urban conurbations such as London and Manchester, at 1993 prices). The net result of which, direct landfilling, became the preferred method of disposal for domestic and commercial waste (Coopers & Lybrand 1993). In 1990, the UK Government published a white paper on waste and waste disposal titled: 'This Common Inheritance', in which it expressed the opinion that; unrestrained landfilling was no longer acceptable on environmental grounds. The white paper also called for WCAs & WDAs to give a higher priority to recycling. Maintaining that, given the right conditions and support, recycling, would reduce the risk of pollution, save energy in production and transport, and conserve natural resources. Thus making good economic, as well as environmental, sense.

Recycling; a Fresh Start.

The UK Government estimated that while 50% of domestic waste had the potential for recycling (Fig. 7& 8), only around 5% was actually being recycled (Department of the Environment 1992; Department of the Environment 1995b). So in an effort to increase this figure; the government placed a commitment on WCAs & WDAs, to draw up recycling plans under Chapter 43 Part II, Section 49, of the EPA90. The Government also set, albeit voluntary, recycling targets of half the recyclable segment of domestic waste (25% of total waste), (Fig. 6), by the year 2000 (Department of the Environment 1992). Section 45 (4) of the EPA90, enabled the WCAs to levy a reasonable charge on the collection of industrial and commercial waste and section 43 (3) of the Act, enabled the WCAs to specify which type of waste receptacle domestic householders should use, and where it should be placed. This is

arguably the most important change to effect recycling in recent times. It enabled economic source separation of waste by the use of separate wheeled bins for different types of wastes (i.e. compostable material in one bin, non-compostable material in another etc.). The net result of this, was the so-called 'kerbside collections' system; where households are required to place the receptacle at the 'kerbside' (i.e. at the side of a road), reducing collection costs by greater efficiency in the use of collection vehicles and as crews no longer needed to enter premises to collect waste, collection times were reduced. Some WCAs (e.g. Dundee City Council) require households to use 240 litre wheeled bin, for unseparated waste. The larger container needing, at least in theory, only be emptying twice a month with substantial savings in collection costs. However, most WCAs still collect on a weekly basis. While the EPA90 covers all of the UK, its administration within the UK differs, e.g. in England, the WCAs (Waste Collectors) have the statutory duty to arrange for the collection of household waste for the WDAs (the disposers) and produce recycling plans (in Scotland and Wales, the WCAs & WDAs are combined). Section 48 (EPA90) however, exempts the WCAs from delivering the waste to the WDAs if it intends that the waste should be recycled. The WCAs must however, inform the WDAs of its plans, in case the WDAs have already contracted to recycle the waste in question. In future, English WDAs must carry out their function through contractors i.e. the Local Authority Waste Disposal Companies (LAWDCS) or private contractors (Department of the Environment 1992).

Initially, there were doubts about the feasibility of local authorities to achieve this level of recycling, as few WDAs & WCAs had in place any form of recycling infrastructures. It was suggested that there would be little point in collecting materials for recycling if no proven demand for the recycled product existed (Great Britain 1994a). The Government response to these comments, was to clarify what it hoped could be achieved by the 25% recycling target. While maintaining that the targets are realistic, if challenging, the Government recognised that local circumstances may mean that some local authorities may find it difficult to achieve this rate of recycling. The targets are national ones; they carry no implication for the optimum rate

of recycling by any individual local authority. The Government also recognised that the perceived lack of markets for recycled materials would present a barrier to achieving targets and therefore commissioned a series of research projects into the markets for recyclable materials (Great Britain 1994b).

The Waste Flows and Management Hierarchy.

Waste Disposal can be portrayed as a series of flows that emanate from three main processes: Material Recovery, Biological Treatment and Thermal Treatment. (Fig 5). The disposal route a waste manager would chose, i.e. incineration or recycling etc., would be influenced not only by the treatments available, but by reference to the waste hierarchy (Fig 9).

Minimisation and Reuse.

The first priority for more sustainable waste management is to reduce the production of waste to a minimum. Waste minimisation and reuse are largely industry led and, itself being subjected to new legislation, aimed at reduction and recovery of packaging waste (Environment Act 1995, Producer Responsibility) (Battersby 1997; Hand 1998). However, Local Authorities still have a leading role. Some LAs, (Dundee City Council for example) set up EC funded training seminars for local businesses such as the EUROFORM Project (Brogan, *et al.* 1994), under Directive OJC 285 21.10.93 (Packaging & Packaging waste), to identify areas of potential waste minimisation. Other authorities give householders advice on methods they can implement to minimise the amount of waste they produce (i.e. home composting), (Department of the Environment 1992).

Materials Recovery or 'Recycling'.

Materials Recovery is better known today as 'Recycling', and differs from re-use or reclamation in that it involves a reprocessing step. Waste is collected in a separated form (i.e. separated from other waste at source or at a central depot), then either reprocessed back into its original form (i.e. waste aluminium cans back to aluminium cans), or to a different product, e.g. the

organic fraction of domestic waste, turned into a soil improver or substitute soil (composting is a prime example of this).

Fig 5. The waste treatment flows. The waste stream can be represented as an input with three principal treatments: biological, material recovery and thermal. Each treatment will support a number of intermediate management, i.e. composting, energy recovery etc. Note that only biological treatments have the potential to avoid landfill altogether. Some, e.g. thermal treatments have residues that are so highly contaminated with heavy metals etc., that at present, they can only be landfilled (Gaskell & Hindle 1995).

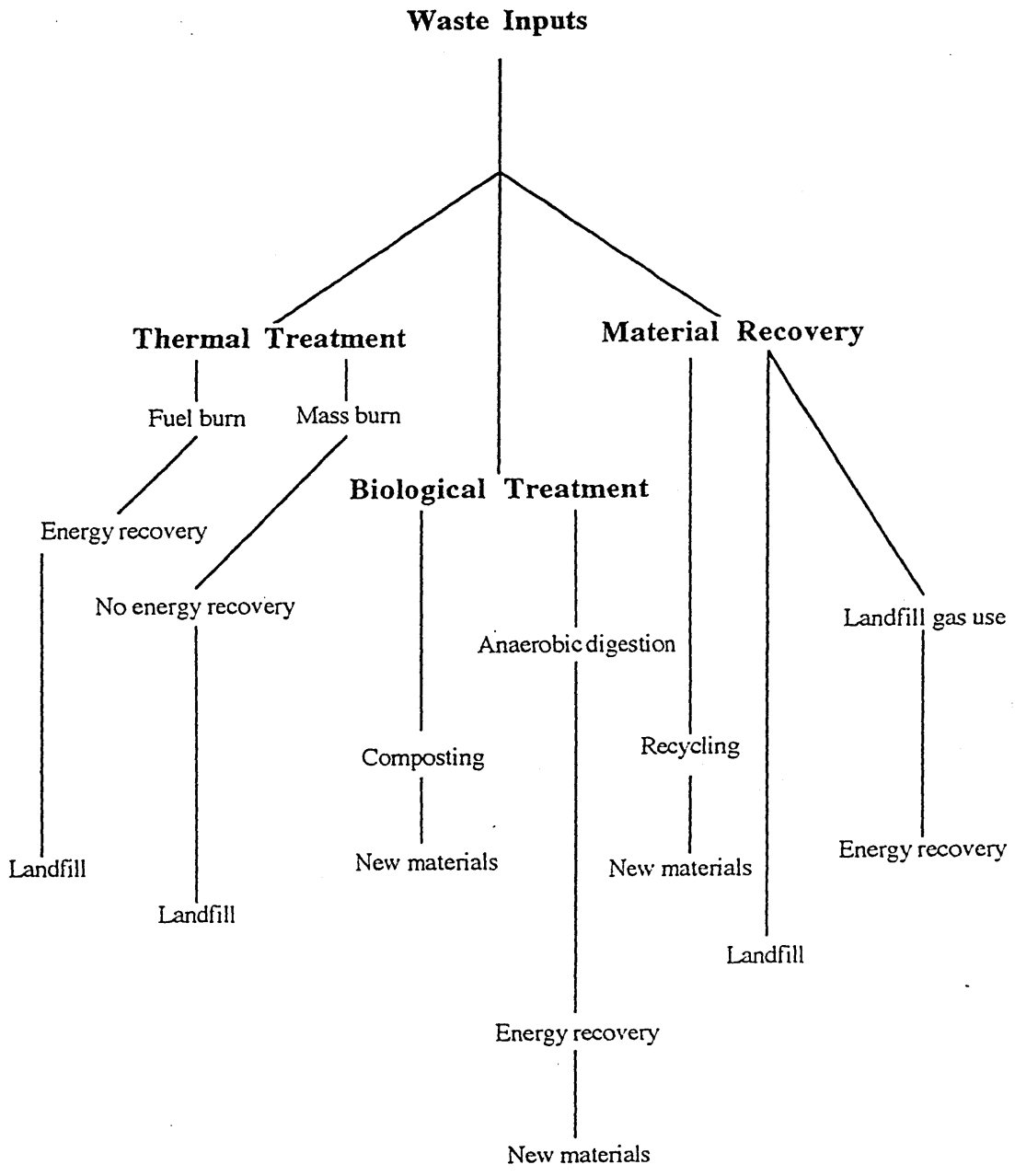

Waste Inputs
Thermal Treatment
Material Recovery
Fuel burn
Mass burn
Biological Treatment
Energy recovery
No energy recovery
Anaerobic digestion
Landfill gas use
Composting
Recycling
Landfill
New materials
New materials
Energy recovery
Landfill
Energy recovery
New materials

Fig 6. Fate of waste arising in the UK by sector and treatments. Household, includes civic amenity (Department of the Environment 1995a).

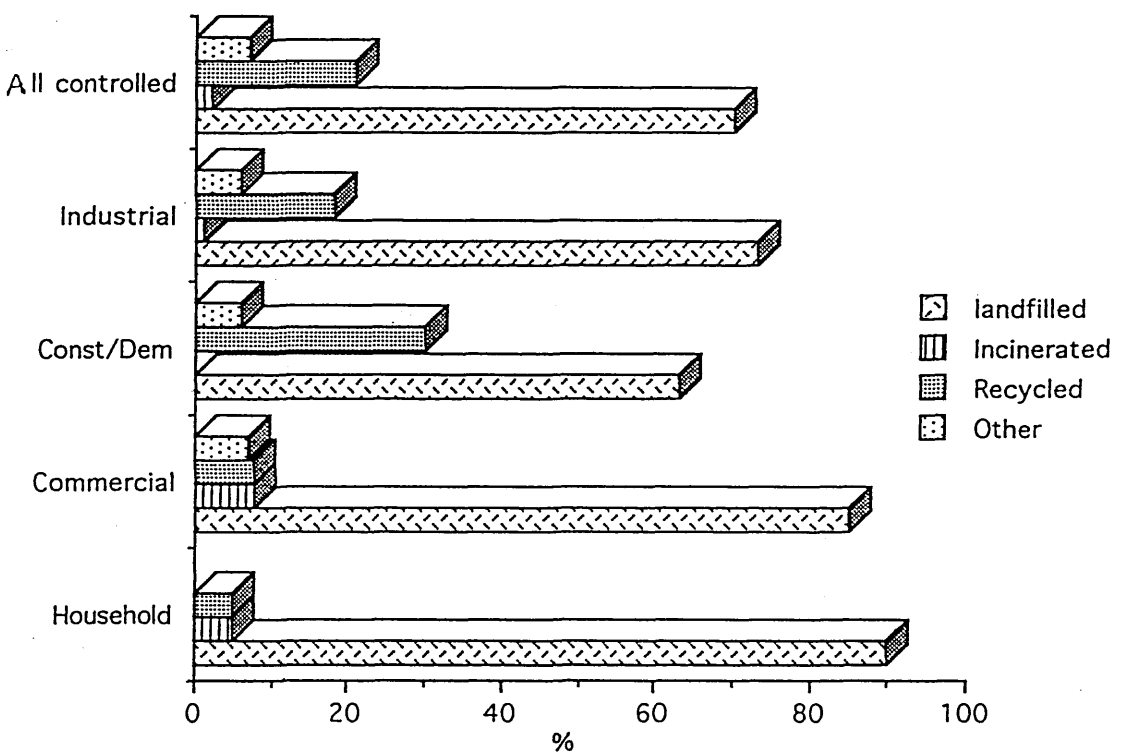
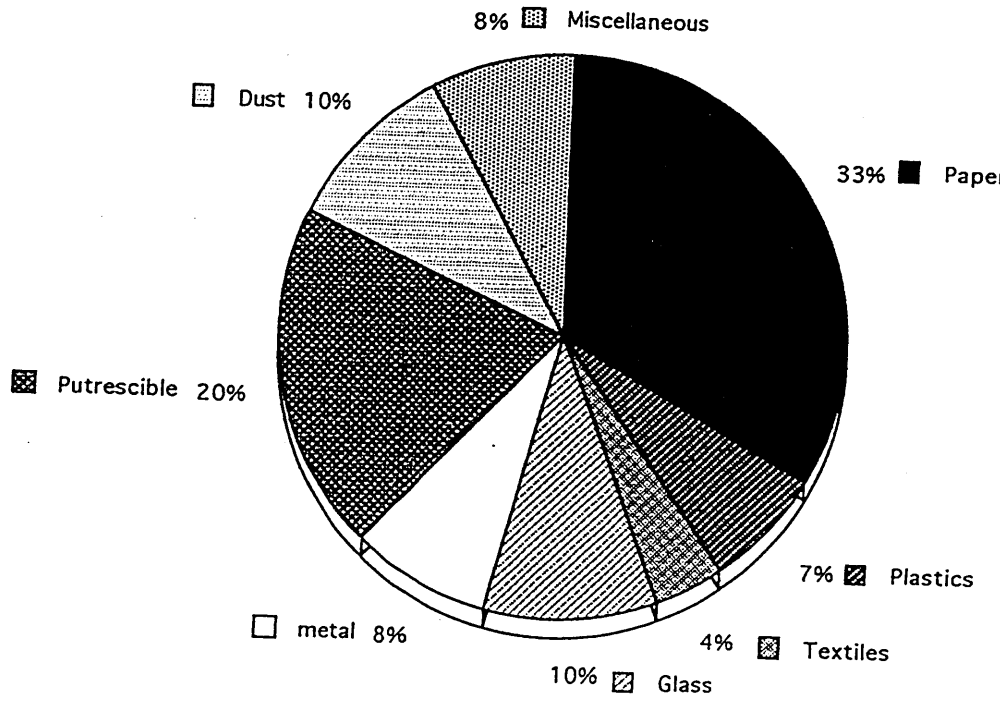

All controlled
Industrial
Const/Dem
Commercial
Household
0
20
40
60
80
100
%
landfilled
Incinerated
Recycled
Other

Fig 7. The typical composition of collected household waste in the UK, by percent. (Department of the Environment, 1992).


8% Miscellaneous
Dust 10%
33% Paper
Putrescible 20%
7% Plastics
metal 8%
4% Textiles
10% Glass

Energy Recovery.

Energy Recovery from the waste burned in incinerators, is often referred to as (albeit scientifically incorrect!) 'Energy Recycling'. The energy can be in the form of heat or electricity generation or as a Combined Heat and Power-station (CHP) (Lowe 1980). Fuel for this type of power-station can also be obtained from methane (CH_4) generated either through anaerobic digestion in bio-reactors, or from landfill gas extraction. The process however, is a 'once only' process, in that once the energy is used, it cannot be recovered. Incineration without energy recovery, is likely to find little support considering the UK Government policy of encouraging renewable energy sources for electricity generation (Department of the Environment 1995b).

Landfill.

At present, about 120 million tonnes of controlled waste are landfilled each year (Department of Environment 1995b). This is still the cheapest and most common waste disposal route in Britain (Battersby 1991, 1997; Hand 1998). A wide range of material is disposed of in this way, i.e. inert, solid organic, sewage-sludge, liquids and a restricted amount of special waste (low grade radioactive waste etc.). Landfill has served society far longer than any other disposal options, and many more areas of the UK have suitable geological and hydrological conditions than other North European countries. This tilts the balance away from other forms of waste disposal and if organised and managed correctly, landfill can be an environmentally efficient means of handling waste. In 1993 there were over 3500 licensed landfill sites in England and Wales (Department of Environment 1995b). In Scotland, the demand for landfill sites is still rising (ENDS Report 1994). Landfill sites are typically situated in old mineral workings or similar structures which meet a range of geological and other conditions. Increasingly land-raising is being used to increase void space. In the UK, two main types of landfill sites can be identified: dilute & disperse and containment types. Dilute & disperse sites rely on the natural conversion of the complex organic materials within the waste, into simpler molecules, i.e.: methane, carbon dioxide and water. These processes are often described as

attenuating processes. Dilute & disperse landfills are often in breach of the EC Directive on the protection of ground-water against pollution caused by certain dangerous substances (80/68/EEC (OJ) L20 20.6.80), as they are a source of ground-water pollution (micro-pollution) (Battersby 1991). These types of landfills are also believed to contribute significantly to the 'greenhouse effect' or 'global warming' by producing an estimated 30-70 million tonnes of methane (about 8% of total global emissions) each year (Smith & Warr 1991). Containment sites, isolate the waste from the environment until such time as they are no longer polluting. However, they are essentially only long-term storage and need active management to ensure that the waste or any leachate that may be produced, can not escape into the surrounding environment for at least 50 years after closure (Battersby 1991; draft EU Landfill Directive 1997). While recognising that there will remain a need for landfilling for the foreseeable future, EU and UK Governments policy is to encourage the move away from landfill, as demonstrated in a Community Strategy for Waste Management (COM(96) 399, the Draft Proposal for Council Directive on the Landfilling of Waste, and the imposition in October 1996, of a Landfill Tax (Department of the Environment 1995a).

The Landfill Directive.

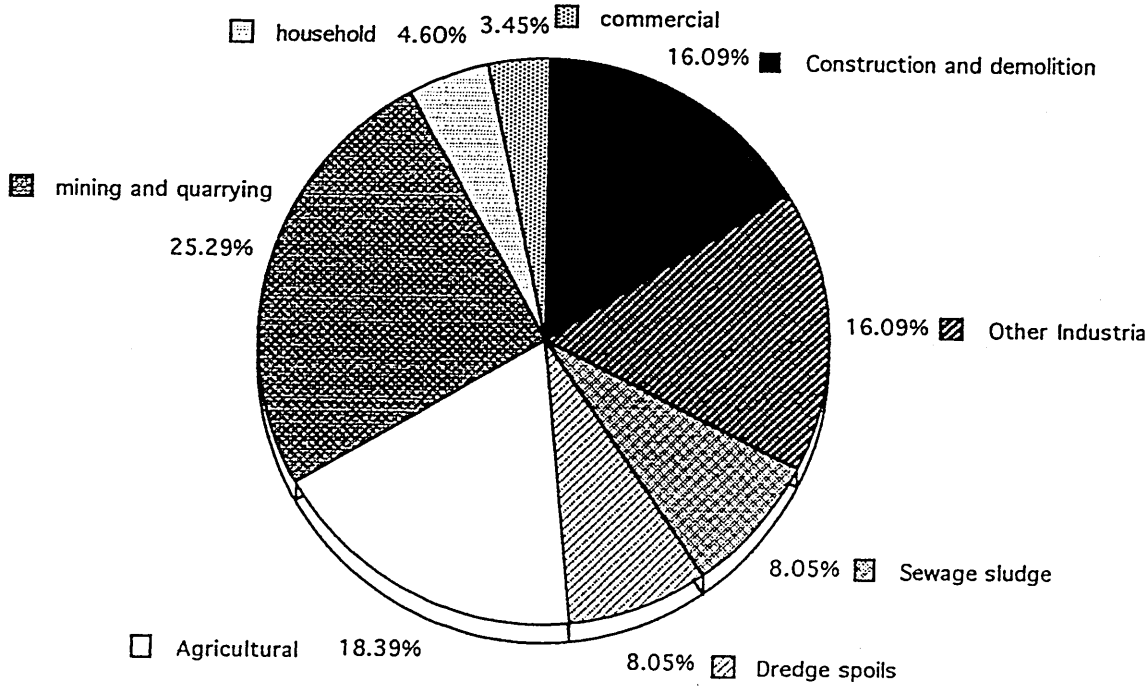
The Landfill Directive proposes a limit to the estimated 20 to 50% of biodegradable waste being landfilled and to ensure that the gases produced in new and existing landfills are collected, treated and used (Coopers & Lybrand 1996). Thus for the first time, a limit for the disposal of biodegradable waste has been introduced, significantly boosting the interest in composting as an alternative to landfill. The Draft Proposal introduced targets for biodegradable reductions that were initially set at a 25% reduction by 2002, 50% reduction by 2005 and 75% reduction by 2010 based on 1993 levels (Article 5). However, by 19th February 1998 some 30 amendments have been applied to this Draft Directive (Proffitt 1998) and the latest target for the UK (May 1998), is to reduce the quantity of biodegradable waste sent to landfill to 75% of the 1995 total by the year 2010, 50% of the 1995 figure by 2013, reducing to 35% by the year 2020 (Walker 1998).

The Landfill Tax.

Initially, the UK House of Commons Environment Committee Second Report on Recycling 1993-94 recommended that a landfill tax should not be imposed on grounds of cost (Great Britain 1994b). The UK Government however, rejected this recommendation as it felt that current prices for landfilling of controlled waste (Table 1), did not fully reflect the environmental impact associated with this particular waste management option. The landfill tax would therefore reflect this, as well as offering the most cost effective way of meeting environmental objectives (Great Britain 1994b). On the 1st of October 1996, the UK Department of the Environment under the provisions laid down in the Finance Act of 1996 (SI 1996 No. 1527) implemented the landfill tax (or levy) based on weight (Great Britain 1996; Hand 1997). This tax applies the 'polluter pays' principle, and is aimed at promoting a more sustainable approach to waste management by encouraging consumers to produce less waste, dispose of less in landfill and recover more through recycling. Allied to this, non profit making Environmental Trusts would be set up to promote sustainable waste management. These would primarily be financed from the landfill tax receiving up to a maximum of 20% of the levy in any one tax year (Barnes 1995; Battersby 1998). The new tax of £7 per tonne rising to £10 per tonne in 1999 (Proffitt 1998), applied to all taxable waste except those listed in SI 1996 No. 1528 and Customs and Excise Information Note 3/96, which had a lower initial tax rate of £2 per tonne (Hand 1997). It is argued however, as there are few alternatives to landfill at present, there would be little change in the quantities going to landfill. The tax would inevitably lead to a rise in waste-disposal costs (especially on Local Authorities), as the tax would be passed on by the landfill operators, to their customers (Coopers & Lybrand 1993).

Fig 8. The proportion by percent [1], of UK waste disposed by landfill, incineration or recycled by sector. Prior to the introduction of Landfill tax, half of construction and demolition waste went to landfill, and is used in landfill engineering (Department of Environment 1995a). However the £2 levy on this material has lead to 'stockpiling' of this type of material away from landfills (Dundee City Council 1998).

[1] Figures may not sum due to rounding.


household 4.60%
3.45% commercial
16.09% Construction and demolition
mining and quarrying
25.29%
16.09% Other Industrial
8.05% Sewage sludge
Agricultural 18.39%
8.05% Dredge spoils

The Waste Management Hierarchy, Dogma or Practical Options?

In December 1995 the UK Government issued a white paper entitled 'Making Waste Work'. This set out a national strategy for waste. It had 3 key objectives

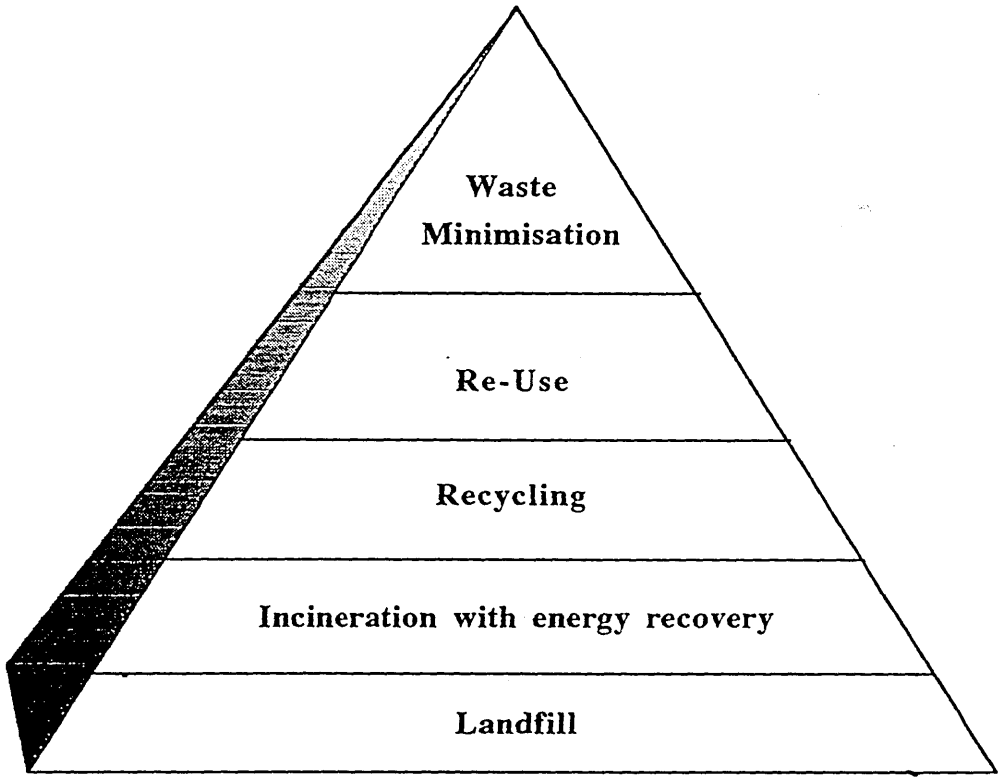
1 Reduce Waste

2 Make Best Use of Waste

3 Select Waste Managing Options That Minimise Risks to the Environment

Making waste work initially set out quantitative targets in the reduction of the proportion of controlled waste going to landfill by 60% and recover 40% of municipal waste by the year 2005. These targets were later modified by the EU Draft Landfill Directive (Battersby 1998; Proffitt 1998). To help achieve this, a waste management hierarchy has been evolved as a hierarchal pyramid (Fig 9). The top (waste minimisation) representing the preferred or best option, with the base (straight landfill) as the least favoured (Department of the Environment 1995b; Wilson 1995). However, the waste industry maintains that this leads towards the dogmatic, ignoring the need for flexibility. Industry need to consider other factors other than government preferences, i.e. costs, locations and degree of contamination. While in full agreement with the top option (waste minimisation) the position in the hierarchy of other methods, must reflect environmental, geographical, practical and financial circumstances, e.g. the cost of building a CHP incinerator or transporting waste away from landfill to an area which has a preferred method of disposal. With this in mind, the UK government advice is to consider the Best Practicable Environmental Option (BPEO). This suggests that the choice of waste management options for a particular waste stream will be guided by the economic costs and benefits of the different options (Department of the Environment 1995b).

Fig 9. The Recycling Hierarchy Pyramid. Simplified form of the waste hierarchy. The peak represents the preferred option. The energy recovery position is at present under review in light of the UK Department of the Environment decision to consider some waste to energy plants as qualifying under the Non Fossilised Fuel Options (NOFFO) (Scottish Renewable Order (SRO) in Scotland)), scheme (Department of the Environment 1995b).


Waste
Minimisation
Re-Use
Recycling
Incineration with energy recovery
Landfill

Conclusion.

It is clear that as a community, the EU has the technology to 'turn old lamps into new'. But, this has to be a systematic and careful approach with the so called 'BPEO' in mind. The campaign for greater effort in recycling *per se* is fine on environmental grounds, provided the cost in energy or money is not ignored. It is essential that all recycling schemes are evaluated to ensure that the environmental benefits claimed can be substantiated. There is always a danger that false promises of what can be achieved by recycling through legislation, will inevitably lead to the concept itself, being discredited (Great Britain 1994b).

AIMS OF THIS STUDY.

Composting as described, has been practised in one form or another, probably as long as agriculture has existed. The underlying principles of composting are known (if not fully understood) enabling generations of farmers to use compost as part of the plant-nutrient cycle. The decline of composting as a serious agricultural practice during the first part of the twentieth century, resulted in much of this knowledge becoming lost or at best, relegated to folklore. The changes in social attitudes, legislative restrictions and increasing disposal costs in Europe and North America, brought about fundamental changes in the way organic waste was treated and caused a heightened interest in composting as a waste disposal technique using a mix of 'traditional windrows' and fully automated systems. This new interest in composting, coincided with a move away from the use of peat as a horticultural medum by the general public and professionals alike, and brought waste derived compost under their scrutiny as a possible substitute. This presented the compost producer with a new set of problems: how to maximise the efficiency of the composting process for waste disposal, while at the same time producing a horticultural medium with sufficient quality and stability to satisfy a market used to using well researched mixes of peat based 'compost'.

This research aims to satisfy some of these questions by examining two main areas:

(i) examination of the microflora involved with aerobic composting.

(ii) public safety of the compost.

The principle goal will be to examine in detail the changes in microbial populations during composting and the parameters that effect those changes. This will potentially aid the producer in maximising the rate of composting from the point of view of waste disposal.

A further aim was to develop a fuller understanding of the nature of the microbial ecology of open windrow composting, by consideration of the effect of microbial population numbers and diversity, with changes in windrow temperatures.

GENERAL EXPERIMENTAL METHODS.

The following methods are common to a number of experiments

Temperature measurements.

Temperatures of windrows were taken with a 1.5 metre thermocouple probe attached to a Model 1000 portable thermometer -50 - +200°C with a resolution of ± 0.1°C (Industrial Pyrometer Co. Ltd. , Birmingham B5 6QY, UK). For routine temperature monitoring on windrows with high aspect ratios, a minimum of 16 readings were taken from each windrow from a minimum of 8 test points at one metre intervals along each length of the windrow and one at each end. For windrows with low aspect ratios, test points were situated as to give a reasonable spread. All test points were at a constant one metre height above ground. Once the temperatures readings had stabilised, they were noted at 20 and 80 cm. depths. The temperature mean and sample standard deviation of a complete windrow were calculated using the statistical programme of a Casio *f*x 570c electronic calculator and displayed using Lotus 123 spread sheet or Cricket Graph on a Macintosh Performa 400 personal computer.

Windrow Establishment

Windrows can be almost any shape, from circular to square, pointed or flat topped. Traditionally, compost windrows tend to be triangular or rounded in shape, with a maximum height of 2 to 3 metres, a base of 3 to 5 metres and any reasonable length. This type of windrow is sometimes called a 'delta' windrow and is the simplest to make, forming naturally, either directly from the shredder, or by using a mechanical shovel. Windrows constructed from shredders that eject material at high speed out of the rear of the machine, usually form triangular or rounded shaped windrows 1 to 2 metres high and 2 to 3 metres wide. Other types (i.e.'Tub Grinder' or 'Shredder Mixer' designs), which eject shredded material via an adjustable conveyer belt, can form a range of windrow shapes and sizes. These structures are usually further built up using mechanical shovels to form windrows up to 3 metres in height (maximum), 5 metres across the top and 8 to 9 metres at the base with a range of lengths.

In this study flat topped 'trapezoidal' shaped windrows were used with the initial windrow forming directly from the rear of a hammer-mill shredder and by use of a front end loader, adjusted to the desired shape and size. Pre-compostable material collected from households in the City of Dundee was added to a TIM SD 1000 shredder by the use of a mechanical shovel (front end loader). The composition and mixture of the pre-compostable material was altered by visual analysis and experience dependent upon the time of year and nature of the material. The TIM SD 1000 shredder was mobile and by the use of this facility it was possible to create windrows of a typical trapezoidal form of the dimensions 3m in height, 5m across the top, 8 to 9 at the base and up to 20m in length. Such windrows have a bulk density of between 400 kg m^{-3} and 600 kg m^{-3}, dependent upon season and exact mix, (Plate 1, Page 212). The front end loader is also used for turning operations. Turning was undertaken when a reduction in mean windrow temperature was observed for 2 or 3 consecutive days. Turning involved the physical collection, mixing and re-construction of an entire windrow into an adjacent site. Typical windrows of a trapezoidal form require 6 to 7h in order to undertake this process. The turning process necessarily involves the re-aeration of the composted material.

Air drying.

Air drying was carried out by spreading sufficient mixed sample to cover a 30 x 50 cm plastic tray to a uniform depth of 1cm and left overnight at room temperature.

Oven drying.

Mixed samples (100g +/- 0.1g) were distributed equally on four watch-glasses and transferred to a Carbolite ELF furnace (preheated to 106°C) (Carbolite, Sheffiield S30 2RR, UK) overnight.

Moisture determinations.

Moisture content was determined by weighing 25g (+/- 0.1g) of fresh sample, on an Acculab model 221 digital balance (Acculab, Newtown PA. USA). Samples were placed on a watch

glass of known weight and placed in the Carbolite ELF furnace (preheated to 106°C) overnight. The sample was allowed to cool to room temperature, weighed, then returned to the furnace. This was repeated until no further weight loss was observed. Percentage moisture was calculated by equation 1: where W_1 is initial weight and W_2 is final weight (both values adjusted for weight of watch-glass) calculations based on wet weight.

$$\% \text{ moisture} = \frac{W_1 - W_2 \times 100}{W_1} \quad [1]$$

Loss on Ignition (LOI).

A sample of oven dried material (approximately 1.0g) was placed in a ceramic crucible of known weight and placed in a preheated furnace at 600°C for 6 hours. The calculation for % LOI is given in equation 2: where W_1 is the initial weight and W_2 is the final weight.

$$\% \text{ LOI} = \frac{W_1 - W_2 \times 100}{W_1} \quad [2]$$

Organic Carbon .

Organic carbon (C_{org}) was determined by the application of equation 3, (Polprasert 1996):

$$\% C_{org} = \frac{\% \text{ LOI}}{1.8} \quad [3]$$

pH.

pH was measured with a Hanna HI 8417, (Hanna instruments Ltd. Bedfordshire LU7 8TZ UK) microprocessor automatic temperature compensated bench digital meter with an Ag/Ag Cl, combination glass electrode. Calibration: Before each measurement, the instrument is calibrated at pH 4.01 and 7.01 using reference solutions HI 7004 & HI 7007, (Hanna Instruments Ltd. Bedfordshire LU7 8TZ. UK).

An air dried sample (10ml +/-0.1ml) was added to deionised water (50ml), to give a sample to water ratio of 1:5 in a clean 100ml plastic container and shaken for 2 minutes to form a slurry. The mixture was left to stand for one hour after which, the pH electrode was immersed in the clear supernatant. Readings were noted when a constant value had been achieved, (Hendershot *et al* 1993).

Conductivity.

Conductivity was measured with a Hanna instruments HI 8820 digital ATC bench conductivity meter with 'built in' temperature compensation (Hanna instruments Ltd. Bedfordshire LU7 8TZ UK). Calibration: After rinsing the meter electrode in deionised water, the probe was placed into 25ml of calibration solution (HI 7031, Hanna instruments Ltd. Bedfordshire LU7 8TZ UK) standardised to 1413 µS at 25° C, and adjusted as per the manufactures instructions. An air dried sample, (10ml +/- 0.1ml) was added to 50ml of deionised water in a clean 100ml plastic container and shaken for 2 minutes to form a slurry. A calibrated conductivity probe was placed in the slurry and once a constant reading had been achieved, it was noted and expressed as $\mu S\ cm^{-1}$.

Determination of Viable Count.

Dilutions were performed using 9 ml sterile 25% Ringers' solution prepared by dissolving one Oxoid BR52 (Unipath Ltd. Basingstoke Hampshire UK) in 500 ml of deionised water. 9 ml of the solution was then distributed into screw top test tubes (lightly tightened) and placed in an autoclave and heated to 121°C, ($1.06kg/cm^2$) for 15 minutes. The sterilised solution was allowed to cool to room temperature before use. 1g (+/- 0.1g) of fresh composting material was aseptically added to 9ml of Ringers solution and mixed by shaking for 2 minutes. A series of dilutions were then performed up to 10^{-12}. Spread plates for enumerating viable cells were prepared (Collins & Lyne 1985) and 0.1ml aliquots from 10^{-2}, 10^{-3}, 10^{-6}, 10^{-9} and 10^{-12} dilutions (in triplicate), were aseptically removed using a micro-pipette and spread onto a labelled plate containing an appropriate growth media using a sterilised glass spreader. All plates were

incubated aerobically at ambient temperatures, 37°C, 55°C (+/- 2°C) and anaerobically at 37°C (+/- 2°C) for 24 hrs, apart from Malt Extract Agar, which was incubated aerobically at ambient temperatures for 48hrs. The number of colony forming units (CFU) were determined by the application of equation 4.

$$CFU\ ml^{-1} = n\ (1/df) \qquad [4]$$

Where; n = mean count of plate count
df = dilution factor

Statistical Methods.

Data collected was subjected to statistical analysis using the decision matrix, devised by Chambers and Parker (1989) and subjected to computer analysis using Statworks 1.2 (Cricket Software inc. Philadelphia, USA). Experimental data was derived from the mean of triplicated data sets.

Reagents and Consumables.

Preparation of Media.

Nutrient broth.

Nutrient broth (Oxoid CM1. Unipath Ltd. Basingstoke Hampshire UK.) powder, (13g) was added to a 1000 ml conical flask containing one litre of deionised water and mixed with a glass stirring rod. Once mixed, this solution was distributed into 100 or 250ml glass conical flasks that were lightly sealed with a foam stopper and aluminium foil and secured with autoclave tape. The containers were placed in an autoclave and heated to 121°C, (1.06kg/cm^2) for 15 minutes. The sterilised broth was allowed to cool to room temperature before use.

MacConkey Purple Broth.

For single strength, 40g (80g for double strength) of MacConkey purple broth (Oxoid CM5a Unipath Ltd. Basingstoke Hampshire UK.) powder was added to a conical flask (1000 ml)

containing one litre of deionised water and mixed with a glass stirring rod. Once mixed, solution was distributed into 20ml screw-top glass test tubes containing inverted fermentation tubes (Durham tubes). The containers were lightly sealed and placed in an autoclave which was heated to 121°C (1.06kg/cm^2), for 15 minutes. The sterilised broth was allowed to cool to room temperature before use.

Nutrient Agar.

Nutrient agar powder (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK.) (28g) was added to a conical glass flask containing one litre of deionised water. The mixture was placed on an heating block and boiled. The flask containing the melted agar, was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 121°C (1.06kg/cm^2) for 15 minutes. The sterilised mixture was then removed from the autoclave and cooled to 60°C. Once cooled, 20ml was aseptically poured into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

Malt Extract Agar.

Malt extract agar powder (Oxoid CM59. Unipath Ltd. Basingstoke Hampshire UK.) (50g) was added to a conical glass flask containing one litre of deionised water. The mixture was placed on an heating block and boiled. The flask containing the melted agar was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 115°C (0.69kg/cm^2) for 10 minutes. The sterilised mixture was then removed from the autoclave and cooled to 60°C. Once cooled, 20ml was poured aseptically into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

MacConkey Agar No. 3.

MacConkey No.3 agar powder (Oxoid CM115. Unipath Ltd. Basingstoke Hampshire UK.) (51.5g) was added to a conical glass flask containing one litre of deionised water. The mixture was placed on an heating block and boiled. The flask containing the melted agar was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 121°C (1.06kg/cm^2) for 15 minutes. The sterilised mixture was then removed from the autoclave and cooled to 60°C. Once cooled, 20ml was aseptically poured into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

Mannitol salt agar.

Mannitol salt agar powder (Oxoid CM85. Unipath Ltd. Basingstoke Hampshire UK.) (111g) was added to a conical glass flask containing one litre of deionised water. The mixture was placed on an heating block and boiled. The flask containing the melted agar was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 121°C (1.06kg/cm^2), for 15 minutes. The sterilised mixture was then removed from the autoclave and cooled to 60°C. Once cooled, 20ml was aseptically poured into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

***Pseudomonas* Agar Base with C-F-C Supplement** (Bridson 1990).

Pseudomonas agar base powder (Oxoid CM559 & SR103E. Unipath Ltd. Basingstoke Hampshire UK.) (24.2g) was added to a conical glass flask containing 500ml of deionised water and 5ml of glycerol. The mixture was placed on an heating block and boiled. The flask containing the melted agar was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 121°C (1.06kg/cm^2), for 15 minutes. The sterilised mixture was then removed from the autoclave and cooled to 50°C. Once cooled, C-F-C supplement, pre-prepared for use by mixing with 2ml of sterile water and ethanol (1:1), was added aseptically to the flask and mixed. 20ml of the resulting mixture was

poured aseptically into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

Legionella CYE Base with BYCE & BMPA Growth Supplement.

Legionella CYE (Charcoal Yeast Extract) base powder (Oxoid CM655 & SR118, SR111E. Unipath Ltd. Basingstoke Hampshire UK.) (2.5g) was added to a conical glass flask containing 90ml of deionised water. The mixture was placed on an heating block and boiled. The flask containing the melted agar, was lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape and placed in an autoclave which was heated to 121°C (1.06kg/cm^2), for 15 minutes. The sterilised mixture was then removed from the autoclave and cooled to 50°C. BCYE (Bacto Charcoal Yeast Extract) growth supplement was pre-prepared for use by mixing with 10ml of sterile water and BMPA (Bridson 1990) supplement was similarly pre-prepared with 2ml of sterile water. Both supplements were added to the flask and mixed. 20ml was aseptically poured into sterile glass containers or disposable polystyrene triple vented Petri dishes (Sterilin Ltd. England) and allowed to solidify.

Kliger Iron Agar.

Kliger iron agar powder (Oxoid CM33. Unipath Ltd. Basingstoke Hampshire UK.) (55g) was added to a conical glass flask containing litre of deionised water and placed on an heating block and boiled to dissolve. Once dissolved, distributed into glass containers which were lightly sealed with a foam stopper and aluminium foil, secured with autoclave tape, then placed in an autoclave which was heated to 121°C (1.06kg/cm^2), for 15 minutes. The sterilised agar was allowed to cool to room temperature as slopes with 25mm diameter and 30 to 50 mm deep butts before use.

Mobility Agar (Craigie Tube).

12ml of nutrient broth (Oxoid CM1) containing 0.1-0.2% technical agar (Oxoid No.3), was distributed into glass containers with 50 x 5mm glass tubing. The containers were lightly sealed

and placed in an autoclave which was heated to 121 °C, (1.06kg/cm^2), for 15 minutes. The sterilised 'motility agar' was allowed to cool to room temperature before use.

Microbial Identifications.

Grams Stain.

Approximately 50µl of water was placed onto a cleaned glass microscope slide and with a flamed wire loop, a colony from solid media was mixed and spread on the slide. The suspension was allowed to air dry, then fixed by passing through a Bunsen flame. The fixed slide was placed on a staining rack and flooded with ammonium oxalate-crystal violet stain for one minute. The excess was drained off and the slide flooded with Lugol's iodine for one minute. The slide was briefly washed under running water, then decolourised with 98% (v/v) acetone and quickly washed with water again. The decolourised slide was then counterstained with 0.5% (v/v) safranin for five minutes. The slide was blot dried before examination.

Spore Stain.

Approximately 50µl of water was placed onto a cleaned glass microscope slide and with a flamed wire loop, a colony from solid media was mixed and spread on the slide. The suspension was allowed to air dry, then fixed by passing through a Bunsen flame. The fixed slide was placed on a staining rack, flooded with 5% aqueous malachite green then steamed for 5 minutes (ensuring that the slide did not dry out). The slide was rinsed with water and flooded with 0.5% (v/v) safranin for 30 seconds, followed by water rinse and blot dry.

Catalase activity.

A test organism was cultured in Nutrient Broth with 1% glucose for suppression of pseudocatalase activity. After an overnight incubation, the culture was exposed to air for 30 minutes, then 1ml of 3-6% (v/v) H_2O_2 was added to the broth which was examined at five minutes, for the evolution of O_2 gas.

Rapid Identification Kits (Barrow & Feltham 1995).

(a) BioMerieux sa, (69280 Marcy-I'Etoile France) API 20 E for Enterobacteriaceae and other Gram-negative rods.

(b) BioMerieux sa, (69280 Marcy-I'Etoile France) API 50 CHB *Bacillus* identification

(c) BioMerieux sa, (69280 Marcy-I'Etoile France) Slidex Strepto D, sensitised latex suspension for the identification of Lancefield D ß-haemolytic Streptococci.

(d) BioMerieux sa, (69280 Marcy-I'Etoile France) Slidex Staph-kit, latex and red blood cell combination agglutination system, for the detection of *Staphylococcus aureus*.

All kits used as per manufacture's instructions and where applicable, using the APILAB (BioMerieux sa, 69280 Marcy-Etoile France) Personal Computer diagnostic programs for species or genus identification..

Biochemical Testing (Palintest Ltd. England).

The Palintest methods use as, an alternative to standard curve, a 'calibration chart' or 'look up tables'. This means that values are only approximate within the stated range. Concentrations leading to readings greater than the maximum value, require additional dilutions and an appropriate multiplier applied to the chart value (i.e. 5 times dilutions require the 'look up' value obtained to be multiplied by 5).

Nitrate Determination 0.1 to 25mgl^{-1} (Palintest Ltd. England).

A plastic sample container was filled with 50 ml of deionised water to which five Palintest extraction N tablets (1M ammonium chloride) were added and shaken until the tablets were observed to have dissolved. Compost (2ml +/ 0.01ml) was then added (Giving a volume mixture ratio of sample to water of 1:25). The container was sealed and the mixture was

shaken for one minute. Palintest Nitrate Test powder (0.27g +/- 0.01g), was then added and the mixture shaken for a further minute. The mixture was filtered into a 250ml conical flask using 40 grade ashless filter paper. Aliquots (10ml) of the filtrate were reacted to form a red azodye by the addition of one Palintest Nitricol tablet and percentage absorption at 570nm was compared with a control blank. Palintest 'look-up' tables were consulted to give nitrate values in mgl^{-1}.

Ammonia determination 0.3 to 75 mgl^{-1} (Palintest Ltd. England).

A plastic sample container was filled with 50 ml of deionised water and 5 Palintest extraction A tablets (1M potassium chloride) were added and shaken until the tablets were observed to have dissolved. Compost (10ml +/- 0.1ml) of was added to give a ratio of compost to water of 1:5 (volume). The container was sealed and the mixture was shaken for two minutes. The mixture was filtered into a 250ml conical flask using 40 grade ashless filter paper. Extract (1ml) was removed and the volume adjusted to 10ml with deionised water. The extract was reacted to form a green indophenol complex by the addition of one Palintest Ammonia No.1S tablet and one No.2S tablet. Percentage absorption at 640nm was compared with a control blank. Palintest 'look-up'tables were consulted to give ammonia values in mgl^{-1}.

TEMPERATURE AND ENERGIES IN OPEN WINDROW COMPOSTING.

Open Windrow Composting is an exothermic process, which under favourable conditions, can raise the temperatures within the windrows to 50°C (or more), above the ambient temperature for extended periods (Kelley *et al* 1998). The raw materials used to produce this level of metabolic microbial activity, are supplied by the waste materials in the form of amino acids, small peptides, high and low molecular weight carbohydrates (starch, lipids, cellulose, hemicellulose and lignin etc.), with variable moisture content (30-80% v/w) and fatty acids (Golueke, 1991). Chemoorganotrophic decomposers (e.g. *Bacillus* spp. etc.), quickly absorb amino acids, small peptides and low molecular weight carbohydrates, as a result of oxidative phosphorylation. Some materials however, i.e. high molecular weight polymers (the main constituents of 'green waste') are more difficult to degrade, requiring the combined action (co-metabolism) of a range of anaerobic, facultative anaerobic and aerobic microorganisms (e.g. *Aspergillus* spp., *Penicillium* spp., *Clostridium* spp. and *Bacillus* spp.). Many of these microorganisms, will use extracellular enzymes to degrade the material, i.e. amylases, (from *Bacillus* spp., *Pseudomonas* spp. etc.) and proteases (Crueger & Crueger, 1990) reducing the waste to amino acids, sugars, fatty acids and eventually to their constituent elements. The product of these processes, is the formation of humus, water, carbon dioxide and heat. It is this heat that is required for the elimination of microbial pathogens (Collier, *et al* 1994; Stenbro-Olsen, *et al* 1995b).

Wastes arriving from unknown sources are potentially contaminated with a wide range of potentially pathogenic organisms, many of which may be clinically significant, i.e. *Staphylococcus aureus*, *Bacillus cereus*, members of the family Enterobacteriaceae etc. (Barrow & Feltham 1995), as well as pathogenic fungi and plant pathogens (Golueke 1991; Lacey *et al*, 1992). Indeed many microorganisms involved with the biodegradation processes are themselves classed as pathogens as shown in Table 2, the range of isolates found during this

study. Studies have shown (Barrow & Feltham 1995), that vegetative cells of most clinically significant bacteria can be denatured at 55°C in 30 minutes. In principle therefore, the release of heat energy during composting, will eliminate most (if not all) potential pathogens (human and plants). However, the lethal temperatures for any particular microorganism, depends not only on that temperature, but also on exposure time. Some species, e.g. *Staphylococcus aureus, Streptococcus* spp. and some of the genus *Enterococcus* (e.g. *E. faecalis*), can survive temperatures of 60°C for 30 minutes (Barrow & Feltham, 1995) and there have been reports of *Salmonella* sp. surviving for extended periods at 55°C (Hay, 1996).

In order to determine the nature of the species and genuses of microorganisms associated with urban green waste composting, samples were taken from active windrows for analysis. In the case of fresh waste material or windrows, a minimum of 10 samples were taken at random and placed in sterilised 25ml universal bottles. Samples from equipment were obtained by using a sterile spatula to remove loose material from equipment surfaces. Aliquots (1g +/- 0.1g) of each sample material were aseptically added to 9ml of Ringers solution and mixed by shaking for 2 minutes. A series of dilutions was then prepared up to 10^{-12}. Spread plates for enumerating viable cells were prepared (Collins & Lyne 1985) and aliquots (0.1ml) from 10^{-2}, 10^{-3}, 10^{-6}, 10^{-9} and 10^{-12} dilutions (in triplicate), were aseptically removed using a micro-pipette and spread onto a labelled plate containing an appropriate growth media (Materials & Methods) using a sterilised glass spreader. All plates were incubated aerobically at the ambient temperature, 37°C, 55°C and anaerobically at 37°C for 24 hrs, apart from Malt Extract Agar, which was incubated aerobically at the ambient temperature for 48hrs. Isolated colonies were chosen for identification by reference to their colonial morphology. An attempt was made to select colonies typical of the range and variety of colonies present on the incubated plates. Identification of the colonies chosen was attempted by the techniques described in the Materials & methods section. The results are shown in Table 2.

Table 2. Genera and some species of microorganisms identified in composting operations in Dundee during this study.

Microorganism	Where Found
Enterterobacteriaceae spp.	Waste material, early composting stages
Bacillus cereus	Waste material, early composting stages
B.firmus	Waste material, early composting stages
B. licheniformis	Waste material, early composting stages
B.macerans	Waste material, early composting stages
B. megaterium	Waste material, early composting stages, equipment
B. pumilus	Waste material, early composting stages
B. lentus (Type 1)	Early composting stages
B.subtilus	Waste material, early composting stages
B. stearothermophilus	Waste material, all composting stages, equipment
B. amyloliqefaciens	Waste material, early composting stages
Azomonas macrocytogenes	Waste material, mid composting stage.
Pseudonorcardia thermophila	All composting stages and locations
Clostridium spp	Waste material, early composting stages.

In view of the importance given to achieving extended high temperatures during composting, experiments were designed to investigate some factors influencing:

(i) The amount of heat energy by microbial action that is required to raise the temperature of a windrow from ambient to pathogen elimination levels.
(ii) The heat energy contributed by individual microbial colonies (CFU).
(iii) The effect of windrow bulk density on temperature evolution and sustainability.

The Amount of Heat Energy generated by Microbial Action that is needed to Raise the Temperature of a Windrow from Ambient to Pathogen Elimination Levels.

The amount of heat required to raise the temperature of any material is called the specific heat capacity (*c*), (Armson 1980), (expressed as $MJkg^{-1}°C^{-1}$) and varies from one material to another, e.g. water has a *c* of $4200Jkg^{-1}°C^{-1}$. However, unlike water, windrows are not homogenous, they are a variable mix of organic and inorganic materials with a range of water contents. Therefore, to calculate the heat energy produced by microbial action within windrows, the specific heat capacity of each component (water, organic and inorganic), i.e. the heat energy required to raise the temperature of *n* kg of material (windrow) by *n* °C, must be determined.

In order to establish the water content of the different types of waste materials available for composting, material from various sources in June 1994 at the Marchbanks Materials Reclamation Plant, Harefield road, Dundee, were shredded using a TIM SD 1000 hammer-mill shredder to form 4 individual windrows (2m x 3m x 5m). A minimum of 10 samples (a total of 2litre) for moisture determination were taken from each windrow at random immediately after the windrows had been formed. Moisture was determined by the methods given in Materials & Methods, each comprised entirely of a single source material. Results are shown in Table 3. Samples from the composite windrow were used to establish the organic content

(Materials & Methods).

Results.

Table 3

Moisture content of some waste materials (as delivered to site)

Source Material	% moisture
Compostainer waste	58.60
Civic Amenity waste	56.00
Parks waste	56.79
Composite waste	48.32
Average content	**54.93**

Using the published value of *c* for water (4200 $Jkg^{-1}°C^{-1}$), the specific heat capacity of a fresh windrow (with average moisture content, Table 3), was calculated to be 54.93% of the water value, i.e.:

water content value $= 4200\ Jkg^{-1}\ °C^{-1} - (100 - 54.93)\%$

$= 4200\ Jkg^{-1}\ °C^{-1} - 45.07\%$ [5]

$= 4200 - 1832.94 = \mathbf{2307.06\ Jkg^{-1}\ °C^{-1}}$

The value of 2307.06 $Jkg^{-1}°C^{-1}$ represents only the water content of the material. Further adjustments are required to take into account the specific heat capacity of the organic and inorganic components of the windrow.

In order to calculate the weight of the organic and inorganic component, the moisture content was first subtracted:

For 1000 grams of fresh windrow material the average moisture content is 54.93% (from Table 3).

Therefore this value is subtracted from the total, i.e.

=1000g -54.93%

=1000g - 549.3g

Therefore, the dry weight of material = **450.7g.** **[6]**

The average organic content of fresh windrow material as derived from LOI measurements (Materials & Methods) on the composite windrow and was found to be 65% of oven dry weight. However, much of the organic fraction is in a soluble form and is used by microorganisms *in vivo* at variable rates, thus this value is subjected to errors. The *c* value for the soluble component of the windrow is therefore discounted, leaving only the inorganic component in the calculation.

By removing the soluble component:

dry weight = 450.7g - 65%

=450.7 - 292.96

inert weight = **157.75g.** **[7]**

The published specific heat capacity of inert material is 3350 $Jkg^{-1}\ °C^{-1}$ (Armson 1980). In the case of the 1000g sample of windrow material, after discounting the water and the soluble fraction, only 157.75g can be given a *c* value. By factoring this into the calculation, the result is:

the inorganic fraction = 15.775% of 1000g

=157.75g

=15.775% of 3350 $Jkg^{-1}\ °C^{-1}$

=528.446 $Jkg^{-1}\ °C^{-1}$ **[8]**

The products of calculation 5 and 8, was incorporated into calculation 9, giving an initial value of *c* for fresh windrow material as:

$$2307.06 + 528.446 \qquad [9]$$

$$= 2835.506$$

However, errors due to the unknown value of *c* of the organic fraction (292.96g or 30% of initial weight, calculation 7), the final value of *c* is calculated to be:

$$\mathbf{2836 Jkg^{-1}°C^{-1} \pm 15\%.} \qquad [10]$$

Using the value derived in 10, it is now possible to calculate (subject to errors identified), the heat energy required (*Eh*) to raise the temperature of a windrow from an ambient temperature of 12°C to maximum windrow temperature of 72°C (Table 4), by the application of formula 11:

$$Eh = mc\,(t_2 - t_1) \qquad [11]$$

Where *Eh* (in MJ) is the heat energy required to change the temperature of an object of mass *m* with a specific heat capacity *c*, from a temperature t_1 to a temperature t_2. (Armson 1980).

By application of formula 10, the energy (*Eh*), required to raise 1kg of windrow material from 12°C to 72°C, is calculated as:

$$Eh = 1\text{kg} \times 2836\text{J kg}^{-1}\text{°C}^{-1} \times (72\text{-}12)\text{°C} \qquad [12]$$

$$= 1 \times 2836 \times 60\ \text{J}$$

$$= 0.1702 \times 10^6\ \text{J}$$

$$= \mathbf{0.1702MJ}$$

However, this figure is subjected to the error identified in calculation 10:

$$= 0.1702MJ \pm 15\% \qquad [13]$$

$$= 0.1702MJ + (0.15 \times 0.1702)$$

$$= 0.1702MJ - (0.15 \times 0.1702)$$

The heat energy required to raise the temperature by 60°C from ambient is between:

0.145MJ and 0.196MJ

The Heat Energy Contributed by Individual Microorganisms.

By using the values derived from calculation 13, it was possible to calculate the heat energy generated by individual microorganisms. This was achieved by initially counting the number of colonies grown on Nutrient agar derived from 1 gram of fresh windrow material and relating that value to the heat energy required to raise windrow temperatures by 60°C.

Samples (10 x 10ml) were taken at random from the composite windrow established previously after verification that temperatures were 60°C above ambient. The samples were placed in universal bottles. Aliquots (1g +/- 0.1g) of each sample material were aseptically added to 9ml of Ringers solution and mixed by shaking for 2 minutes. A series of dilutions were then prepaired up to 10^{-12}. Spread plates for enumerating viable cells were prepared (Collins & Lyne 1985) and aliquots (0.1ml) from 10^{-2}, 10^{-3}, 10^{-6}, 10^{-9} and 10^{12} dilutions (in triplicate), were aseptically removed using a micro-pipette and spread onto a labelled plates containing Nutrient agar (Oxoid CM3, Unipath Ltd. Basingstoke Hampshire UK.) using a sterilised glass spreader. All plates were incubated aerobically at the ambient temperature, 37°C and 55°C for 24 hrs. Colony Forming Units (CFU's) were then counted and the average $CFUg^{-1}$ calculated.

Results.

It was established that windrow material would support an average of $2.29x10^{8}$ $CFUg^{-1}$. This is equivalent to $2.29x10^{11}$ $CFUkg^{-1}$ of windrow materia on Nutrient agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK). Using the figure of 0.145MJ to 0.196MJ, derived from

calculation 13, each microorganisms was calculated to produce heat energy in the order of:

$$\text{heat energy} = 0.145\text{MJ}/2.29\text{x}10^{11} \text{ (minimum value)} \quad [14]$$

$$\text{heat energy} = 0.196\text{MJ}/2.29\text{x}10^{11} \text{ (maximum value)} \quad [15]$$

This gives a value of heat production per CFU of between:

$$\mathbf{6.33\text{x}10^{-13}MJkg^{-1} \text{ and } 8.56\text{x}10^{-13} MJkg^{-1}}$$

Other researcers have reported similar values in experiments using asbestos and wool as a substrate (Poole & Hobson 1979). however, or the first time (accepting errors), a value can be assigned to the heat generated by individual microorganisms during the composting process.

The Effect of Windrow Bulk Density on Temperature Evolution and Sustainability

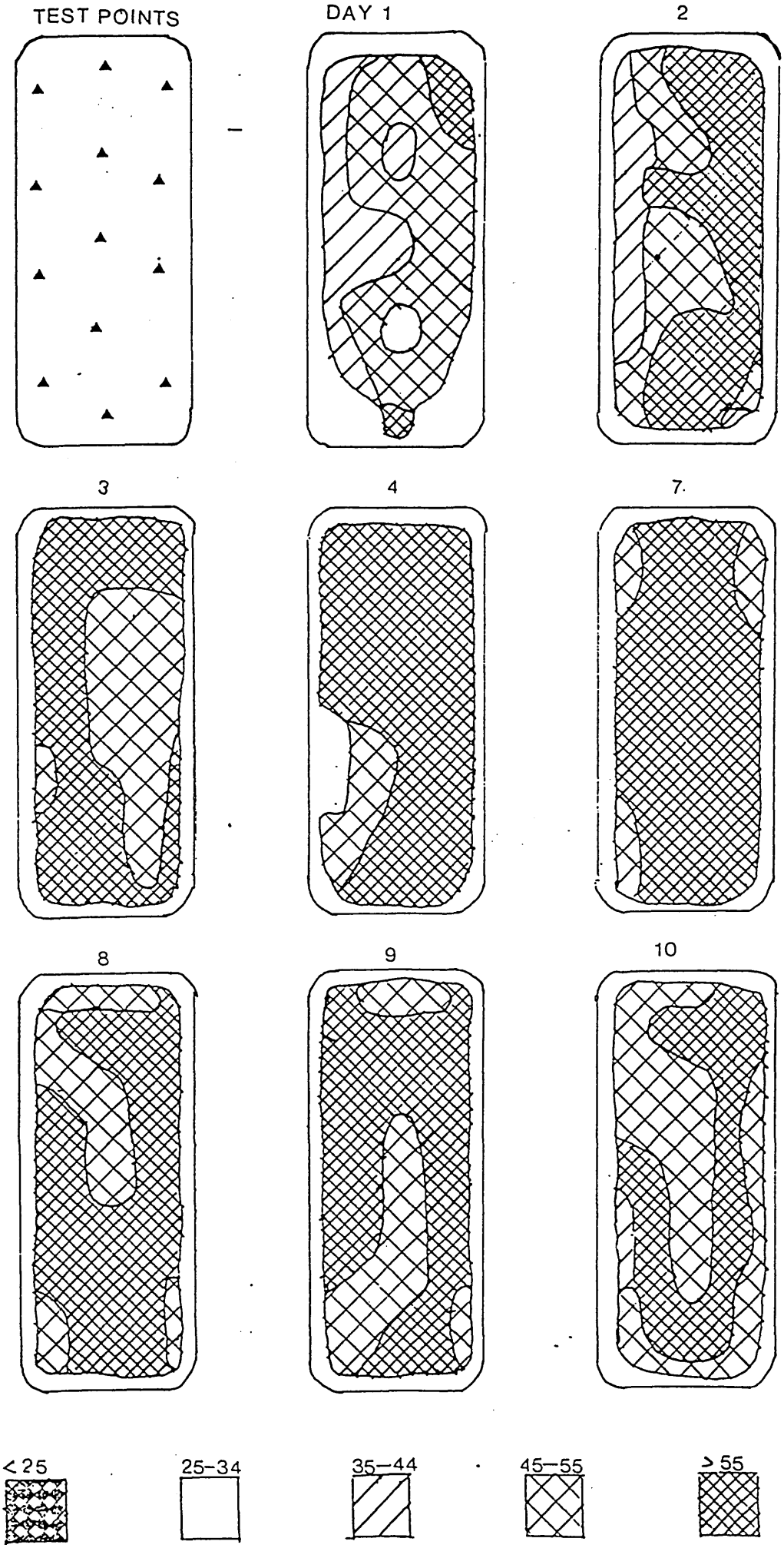
Most open windrows can sustain both anaerobic and aerobic microbial communities in a range of microhabitats, usually in the waste materials air/substrate interface. To be effective in pathogen control, composting must be predominantly an aerobic process and this obviously is conditional on having sufficient oxygen available. Oxygen, is made available for microbial use by a combination of diffusion through outer surfaces and internal gas void spaces. It is common however, for internal gas void spaces (especially in large commercial size windrows), to become compacted due to 'slumping', i.e. collapsing of the windrow under its own weight after initial construction. This inevitably leads to anaerobic conditions becoming established by the elimination and/or sealing of air void spaces. The effect of 'slumping' in open windrows, can be countered by mechanical aeration (turning), or the addition of material (in the construction of the windrow) that when shredded, produces oversized (up to 45cm in length) and partially crushed material. These confer upon the windrow, a degree of structural integrity by acting as internal physical supports so keeping the windrow 'open'. Nevertheless, microbial respiration will in time, lead to a decline in windrow oxygen concentration, with concurrent synthesis of carbon dioxide and other gaseous metabolites If this is left uncorrected, it is likely to change the windrow environment in favour of anaerobic and facultative anaerobic microbial

to change the windrow environment in favour of anaerobic and facultative anaerobic microbial species, which in turn will lead to a reduction in temperature due to their generally lower rate of metabolic heat production. This may also lead to a corresponding risk of clinically significant pathogens (e.g. *Clostridium* spp., *Salmonella* spp., etc.), becoming established (Finstein, 1992).

Traditionally, in order to confirm if microbial activity within a windrow is sufficient to raise temperatures to the levels required for pathogen destruction, several temperature readings are taken either at random, or from fixed points in the windrow (Hay 1996), averaged and displayed as a graph of maximum and minimum temperatures over time (van Roosmalen & van de Langerijt, 1989). Previous studies (not described in this study) detailed the changes in windrow temperatures by producing isothermal maps (Fig 10), which mapped temperature fluctuations within windrows (Stenbro-Olsen *et al*; 1995). However, that study did not identify (other than in general terms), reasons for those fluctuations. In this experiment, an attempt has been made to address that question, by examining the effect of bulk densities on temperatures within open windrows.

The average bulk density (i.e. weight for volume) of unprocessed green waste is estimated at 200 kg m^{-3} (HM Customs and Excise 1996). Several manufactures of shredders, offer equipment with specifications that enable operators to alter the characteristics of the shredded material, (that is, different particle size and thus bulk densities). This is achieved either by the addition of a screen or 'grate', placed directly behind the shredding mechanism, forcing the shredded material through a fixed size mesh to achieve a uniform size of particle, or a more variable shred by leaving the screen off. Other systems, use hydraulically adjustable 'sheargrates', with a range of clearances between fixed position pulverising hammers. After shredding (depending on type of shredder), this value can increase by a factor of 2 to 3, and is known as the compacted bulk density (*Db* ; CEN TC223, 1993).

Fig 10. Schematic diagram of a trapezoidal windrow, (solid triangles indicate approximate positions of the temperature sampling points) showing progressive temperature changes in a single winter compost windrow. Temperatures were taken at both 20cm and 80cm depths over a period of 25 days (Stenbro-Olsen *et al*, 1995).



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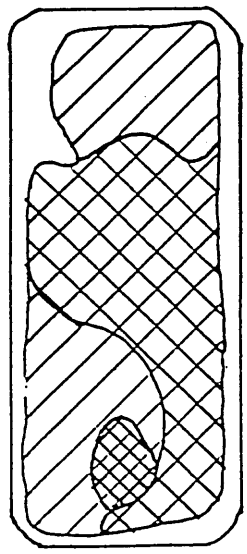

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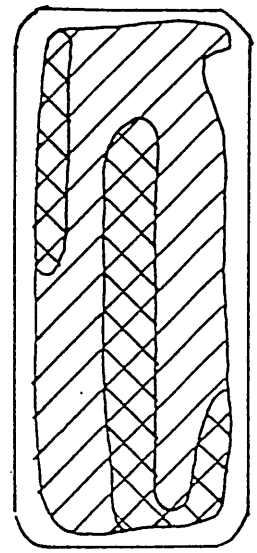

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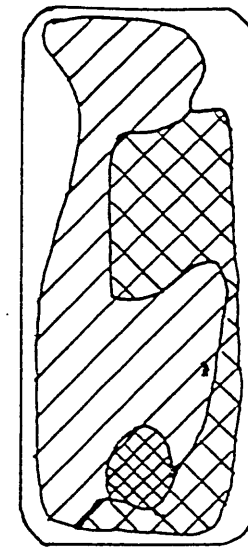

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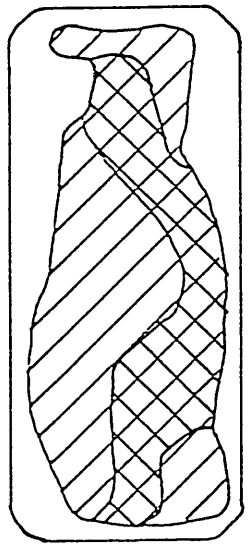

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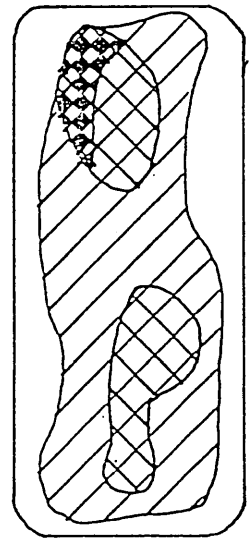

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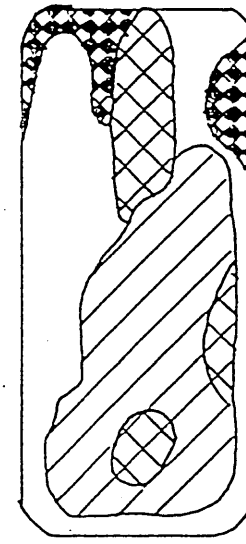


Initially, during the construction of a new windrow, waste materials (especially green waste) are subjected to a shredding phase in which the material undergoes mechanical fragmentation. Shredding, serves several functions: it increases the surface area for microbial attack, creates gas void spaces essential for aerobic composting and alters the bulk density of the material. A commercial size windrow of 3 x 3 x 20 metres ($180m^3$) for example (not described in this study) will after shredding, contain (depending on *Db*) on average 72 tonnes (*Db* of 400 kg m^{-3}) to 108 tonnes (*Db* of 600 kgm^{-3}) of waste material.

The amount of heat energy required to be generated (*Eg*) in order to raise the temperature of a windrow of $180m^3$ from the ambient temperature (12°C) to 72°C depends on its *Db* and *c* value and can be calculated by the application of formula 16:

$$Eg = W_{tot}\,(c_w) \qquad [16]$$

Where W_{tot} is the weight of the windrow in kg (*Db* x volume) and c_w is the c value as calculated in equation 12.

For a 72,000kg windrow, the energy generated is calculated as:

$$72{,}000\text{kg} \times 0.1702\text{MJ} \qquad [17]$$

$$= \mathbf{12{,}254MJ.\ \pm 15\%}$$

For a 108,000kg windrow, the energy generated is calculated as:

$$108{,}000\text{kg} \times 0.1702\text{MJ} \qquad [18]$$

$$= \mathbf{18{,}382MJ.\ \pm 15\%}$$

The heat generated by microbial action is not constant. Changes in nutrient levels (Gartland, *et al.* 1996) and fluctuation in microbial populations, temperature, oxygen and water etc., result in constant changes in the windrows environmental conditions. These fluctuations can be seen as variations in windrow temperature profiles. However, the potential adverse impact on

pathogen elimination due to these temperature changes, can be lessened by the windrows ability to retain heat. Most materials, can store heat (the heat storage capacity (*Eh/V*)) (Armson 1980) and the amount depends, in part, on the materials bulk density. This is represented by equation 19.

$$Eh/V = \rho c\,(t_2 - t_1) \qquad [19]$$

Where ρ is the bulk density, c is the specific heat capacity and t_2-t_1 the change in temperature. *Eh/V* can be expressed in more general terms as the storage density per unit rise in temperature, i.e. density (in kgm^{-3}) x specific heat (in $Jkg^{-1}°C^{-1}$).

For windrow material with a minimum bulk density of $400kgm^{-3}$, the storage density is:

$$400kgm^{-3} \times 2836\ Jkg^{-1}°C^{-1} \qquad [20]$$

$$= \mathbf{1.13\ MJm^{-3}\,°C^{-1} \pm 15\%}$$

For windrow material with a minimum bulk density of $600kgm^{-3}$, the storage density is:

$$600kg.m^{-3} \times 2836\ Jkg^{-1}\ °C^{-1}$$

$$= \mathbf{1.70\ MJm^{-3}\,°C^{-1} \pm 15\%} \qquad [21]$$

These figures display the linear relationship between heat generation and bulk density. A 50% increase in bulk density (400 kg m^{-3} to 600 kg m^{-3}) is reflected by a 50% increase in heat energy required to raise temperatures to a given level. Care therefore must be exercised by compost operators that sufficient oxygen, moisture and nutrients are available to sustain the extra heat energy needed in high density windrows to raise temperatures to pathogen kill levels.

Single Point (Test Point 6) Analysis of Windrows At 20 and 80cm.

In view of the potential importance of bulk densities to windrow temperature evolution, and the reliance compost operators place on temperature as the primary method of evaluating pathogen control (Kelley 1998), two trapezoidal windrows (2 x 2 x 8 metres) consisting of a

mixture of green waste (approximately equal loads of tree prunings and general mixed garden waste) were constructed during August 1995 at the Marchbanks Materials Reclamation Plant, Harefield Road, Dundee, using a Gannon Haybuster 'Tub grinder'shredder (Gannon UK Ltd. Lincolnshire, UK), with, and without, a 10cm screen. The windrow produced without the screen, consisted of particles between 5 and 30cm in length, with a maximum diameter of 16cm (10% oversize) with a compacted bulk density of 400 kg m^{-3}. This windrow, was designated as 'coarse shred'. With the screen fitted windrow particles were 5 to 30cm in length, with a maximum diameter of 10cm (2% oversize) with a compacted bulk density of 600 kg m^{-3}. This windrow was designated as fine shred. The compacted bulk density was calculated according to CEN 223 (1993). Once established, both windrows were marked with 12 test points, 6 test points at 1 metre above ground level equally spaced along one side and 6 equally spaced along the other side at the same height. These test points were designated Tp 1 to 12, with Tp 1 being opposite to Tp 12 on the other side of the windrow. Attempts were made to ensure test points were in the same location in each windrow. Temperature measurements at each test point were at 20 and 80 cm depths. The mean and standard deviation was calculated on individual temperature readings over 27 days. Statistical analysis from 1404 data points was carried out using Statworks 1.2 (Cricket Software inc. Philadelphia USA) Student's *t* Test for matched samples at 17 degrees of freedom, giving a *t* value of 2.567 at the 2% significance level (Chambers & Parker 1989). The windrows were turned once, after 8 days, as traditionally, temperature measurments are carried out on a single point in the windrow. Data from one test point (Tp 6) was used as a comparison to data obtained from all test points

Fig 11. Temperature of test point 6 (fine shreded windrow with a bulk density of 600 kgm^{-3}) at 20cm depth (fine) and 80cm depth (fine 80). Using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia USA).

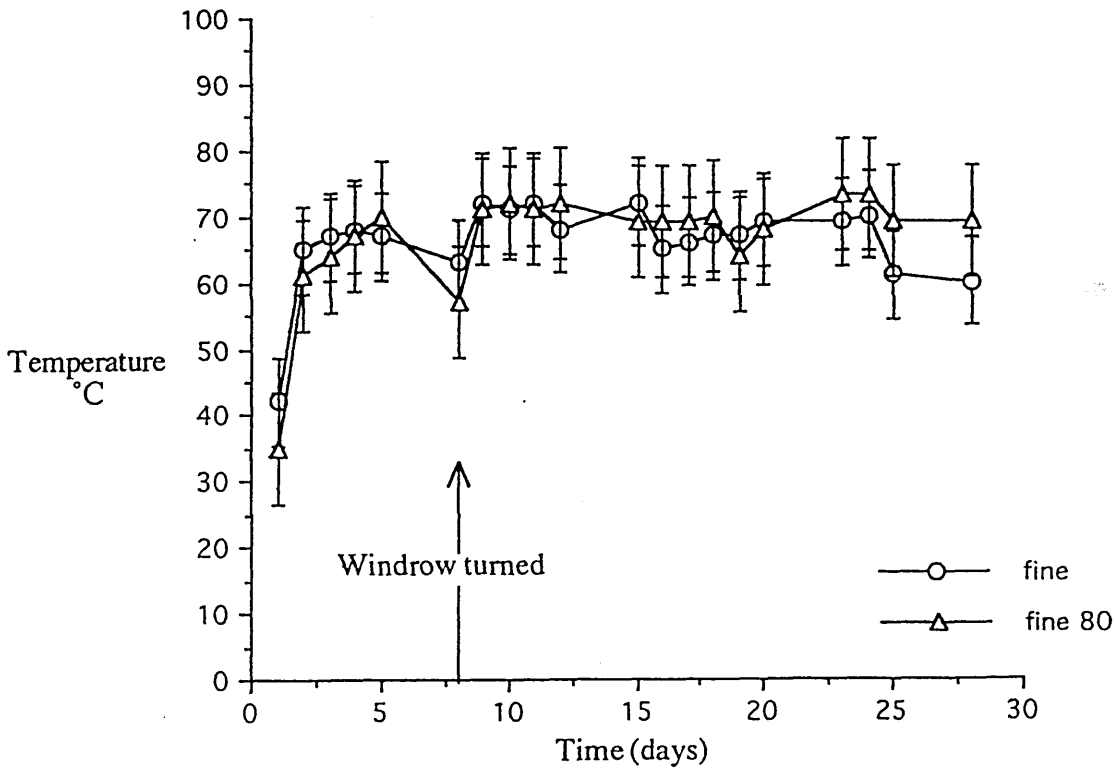
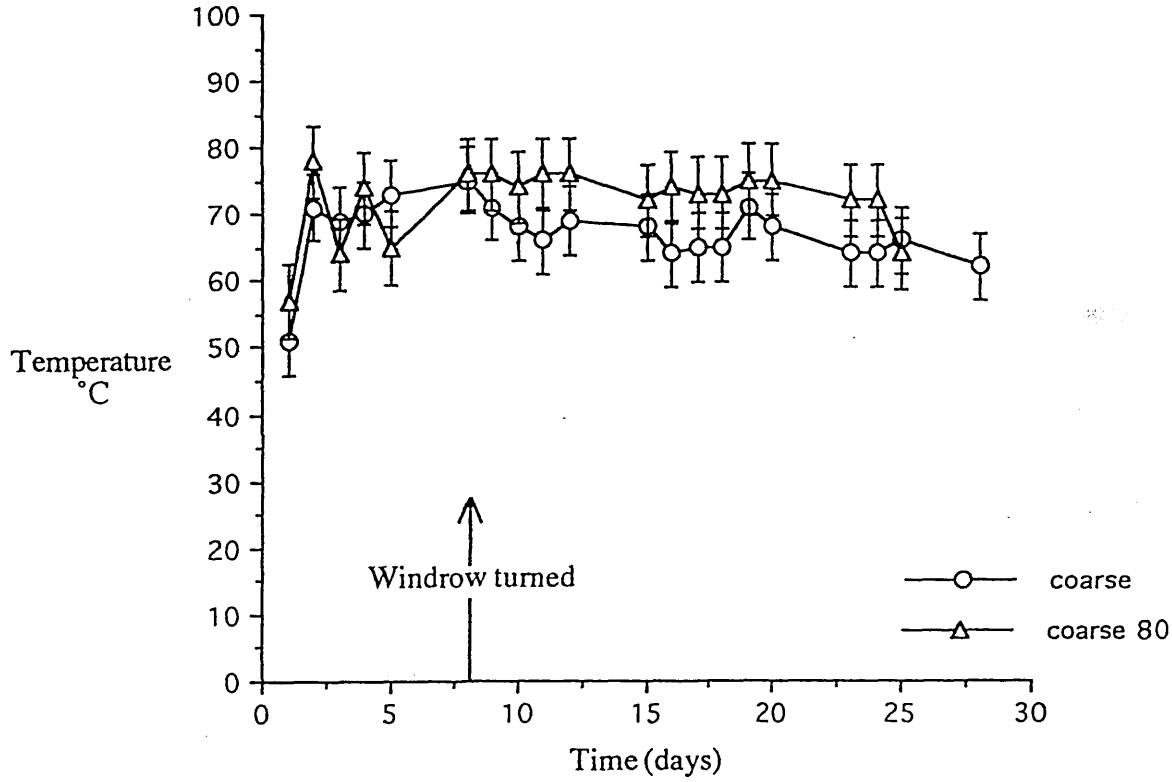

Temperature
°C
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (days)
Windrow turned
fine
fine 80

Fig 12. Temperature of test point 6 (coarse shreded windrow with a bulk density of 400 kgm^{-3}) at 20cm depth (coarse) and 80cm depth (coarse 80). Using Cricket Graph 1.3.2 (cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia USA).


Temperature
°C
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (days)
Windrow turned
coarse
coarse 80

An illustration of the possible influence of bulk densities on temperatures, is shown in Fig 11 & Fig 12. Fig 11 (Test point 6) shows the temperature profile of a windrow with a bulk density of 600 kgm^{-3}, while Fig 12 (Test point 6) a windrow with a bulk density of 400kgm^{-3}. The 600 kg m^{-3} windrow displays a more constant temperature over time, when compared with the 400 kg m^{-3} windrow. Therefore, based on the relationship between heat energy required and bulk densities (equation 20 & 21) and Test point 6 plots, there is a case for suggesting a hypothesis that; bulk densities are significant in open windrow temperature evolution. The single point temperature graph was constructed from data derived from test point 6 on the fine shred windrow (Fig 11). This shows that temperatures at 20 and 80cm depths, were broadly the same over most of the test period (difference in mean temperatures were 0.6°C between day 10 & 20), with clear differences only becoming apparent at the beginning and towards the end of the test period (before day 10 and after day 20). From day 20 to day 27, the 80cm depth temperature fell by 4°C, and the 20cm by 10°C. Statistical analyses (*t* test) of the difference between the average test point temperatures at 20 and 80cm over the whole test period, gave a *t* value of 0.24. This low *t* value suggests that; differences in temperatures in fine shred windrows, (single point measurements) between 20 and 80cm depths are statistically insignificant. As in the 'fine shred', single point analysis of the graph for the 'coarse shred' (Fig 12) shows clear differences in temperatures between 20 and 80cm depths after 8 days, with the 80cm temperature averaging 4.9°C higher than the 20cm depth the rest of the test period. Statistical analysis (*t* test) on the mean temperature differences over the whole test period, gave a *t* value of 4.2. This higher *t* value, suggests that unlike the fine shredded windrow, significant differences between the temperatures at 20 and 80cm are likely to occur.

Analysis of the data which compared single test points from the fine and coarse shred windrows, initially suggested that a fine shred windrow may produce a more consistent temperature spread throughout the windrow than one made with coarsely shredded material. However, these differences were based on a single test point. Expanding statistical analysis to include data from all the test points and differences between windrows, showed that relying on

a single data point (in this case Tp 6) to give information on the overall temperature spread within the windrow, may be misleading.

Table 4

Mean Temperature Data for Test Points over 27 days (°C).

Test point 6	20cm	fine	66.05	Sd	6.60	coarse	67.00	Sd	5.06
	80cm	fine	66.65	Sd	8.49	coarse	71.89	Sd	5.45
All test points	20cm	fine	65.00	Sd	7.20	coarse	68.42	Sd	4.71
	80cm	fine	66.50	Sd	9.60	coarse	71.83	Sd	4.71

A Sd of 6.60 to 9.60 for the fine shredded windrow and a Sd of 4.71 to 5.45 for the coarse shredded windrow, suggests that individual readings taken from the fine shred windrow deviated further from the average than was the case for the coarse shred windrow. This is diametrically opposed to the conclusions drawn from the average temperature data. The standard deviation figures suggest an alternative hypothesis, that is, coarse shred windrows produce a more constant temperature within the windrow than fine shred windrows.

Applying *t* test analysis to temperatures taken at the same depth (i.e. one test point average compared with another at the same depth), shows that in the case of the fine shred windrow, 14 out of 240 comparisons at 20cm (5.8%; Fig 13), and 20 out of 234 at 80cm (8.5% ; Fig 14), gave a *t* value above the 2% significance level. For the coarse shred windrows, 12 out 216 at 20cm (5.6%; Fig 15) were significantly different, but only 2 out of 216 were different at the 80cm (0.9%; Fig 16) level. Comparisons were also made between temperatures at the same level in different windrows (coarse & fine), which demonstrated that, no significant differences could be measured between coarse and fine shred windrows at the 20cm level. However, 7 out of 12 readings (58.3%) were significantly different at 80cm (Fig 17). This, and data from

average temperatures, suggested a new hypothesis, that is, 'that when individual test points are compared, coarse and fine shred windrows will have similar temperature characteristics at 20cm and where differences are seen, they are most significant at 80cm depth. However, when comparisons are made between depths (20 & 80cm; Fig 18), differences are only significant in coarse shred windrows'. For the fine shred windrow, 14 out of 240 comparisons at 20cm gave a *t* value >2%. This indicates that these 14 readings deviated significantly from the overall mean at 20cm

Detailed physical examination of the windrow structure, showed that the coarse shred windrow was in the main, poorly sorted (wide range of particulate sizes) with a large number of oversize fragments (greater than 20cm in length). The compacted bulk density, was confirmed to be 400kg m^{-3}. The material tended to be crushed and split longitudinally, rather than simply cut (chipped). It was also observed that a large proportion of void spaces were interlinked. The 'fine shred' material was moderately to well sorted (even particulate sizes), with a 'chipped' appearance. As in the coarse shred material, oversize fragments were noted, although none were over 20cm in length. The material had a compacted bulk density of 600kg m^{-3}, with fewer crushed areas. The void spaces were physically smaller in diameter and had proportionally fewer interlinks. These contrasting physical attributes may be the key to the observed temperature differences, that is, a greater temperature difference between 20 and 80cm depths in windrows with a fine shred matrix than was observed in coarse shred windrows.

Fig 13. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA), of the temperature differences in fine shredded windrows at 20cm depth. Each open circle represents the calculated *t* value per test point.

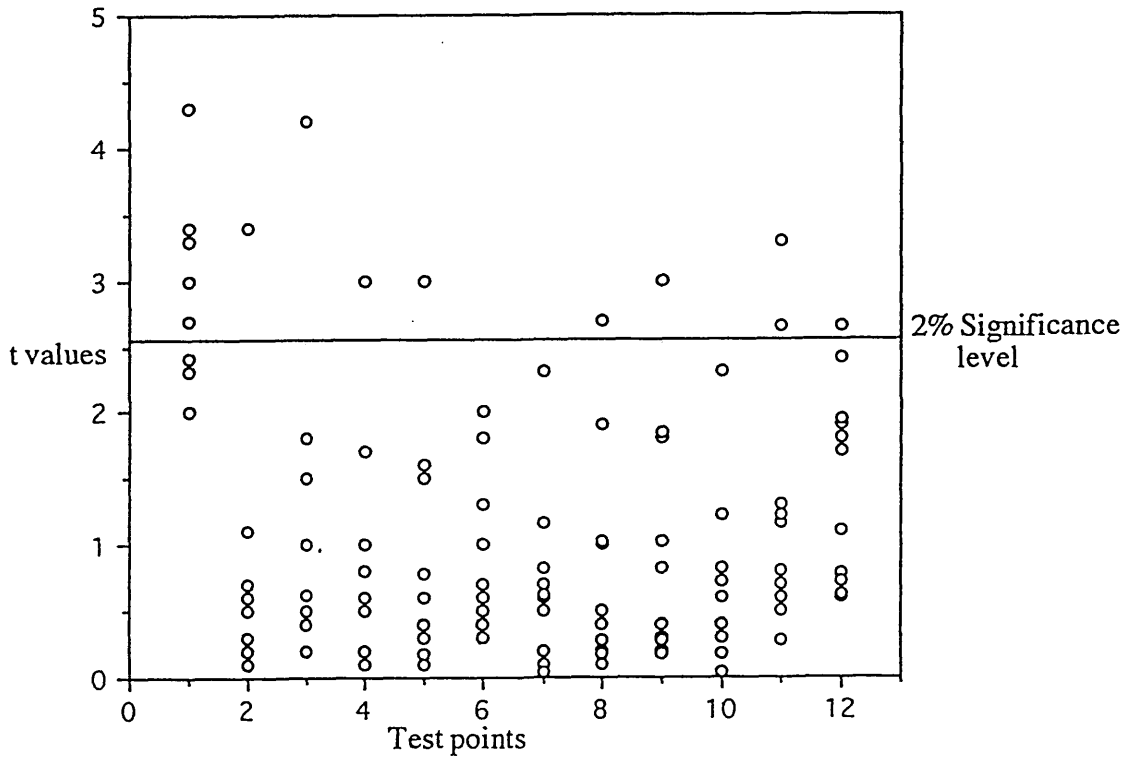

5
4
3
2
1
0
t values
0
2
4
6
8
10
12
Test points
2% Significance level

Fig 14. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA) of the temperature differences in fine shredded windrows at 80cm depth. Each open circle represents the calculated *t* value per test point.

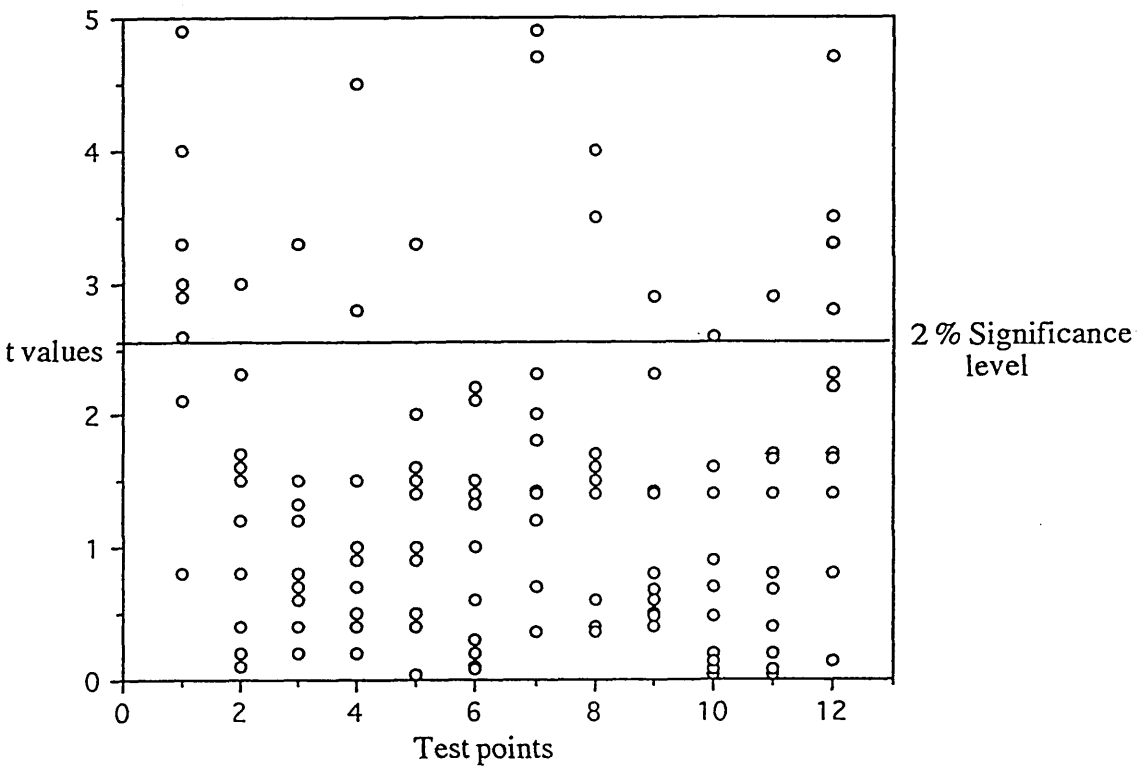

5
4
3
t values
2
1
0
0
2
4
6
8
10
12
Test points
2 % Significance level

Fig 15. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA), of the temperature differences in coarse shredded windrows at 20cm depth. Each open circle represents the calculated *t* value per test point.

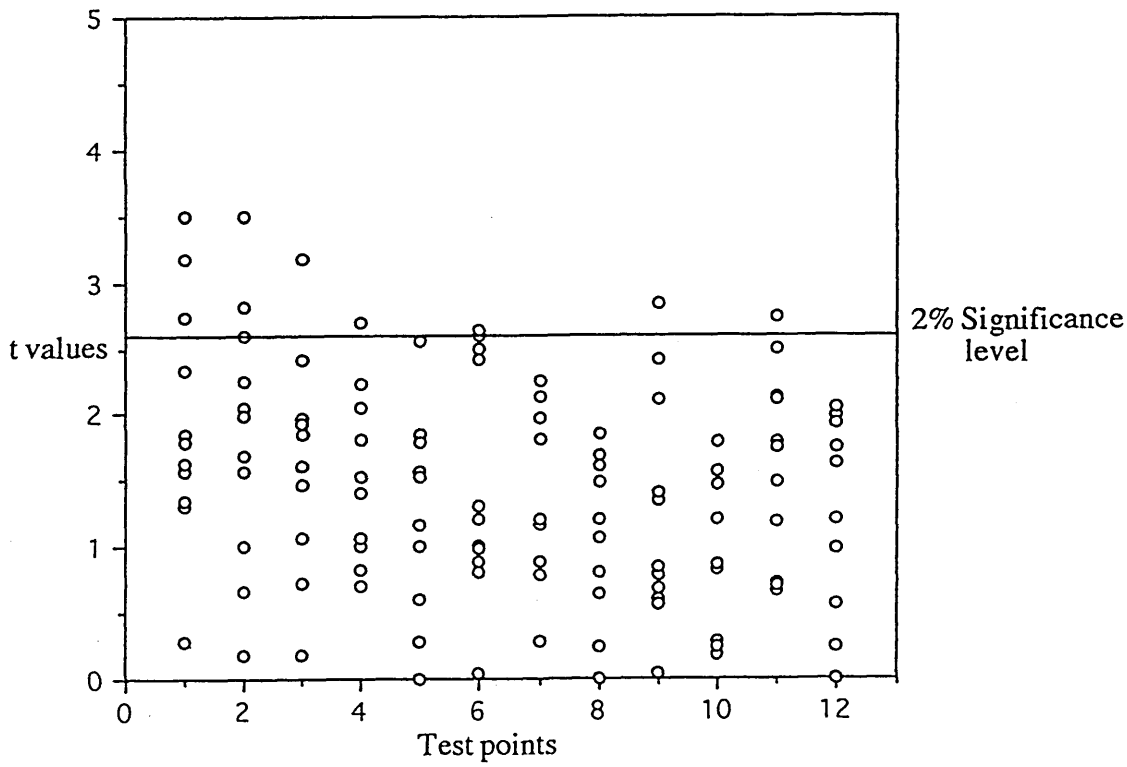

5
4
3
2
1
0
t values
0
2
4
6
8
10
12
Test points
2% Significance level

Fig 16. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA) of the temperature differences in coarse shredded windrows at 80cm depth. Each open circle represents the calculated *t* value per test point.

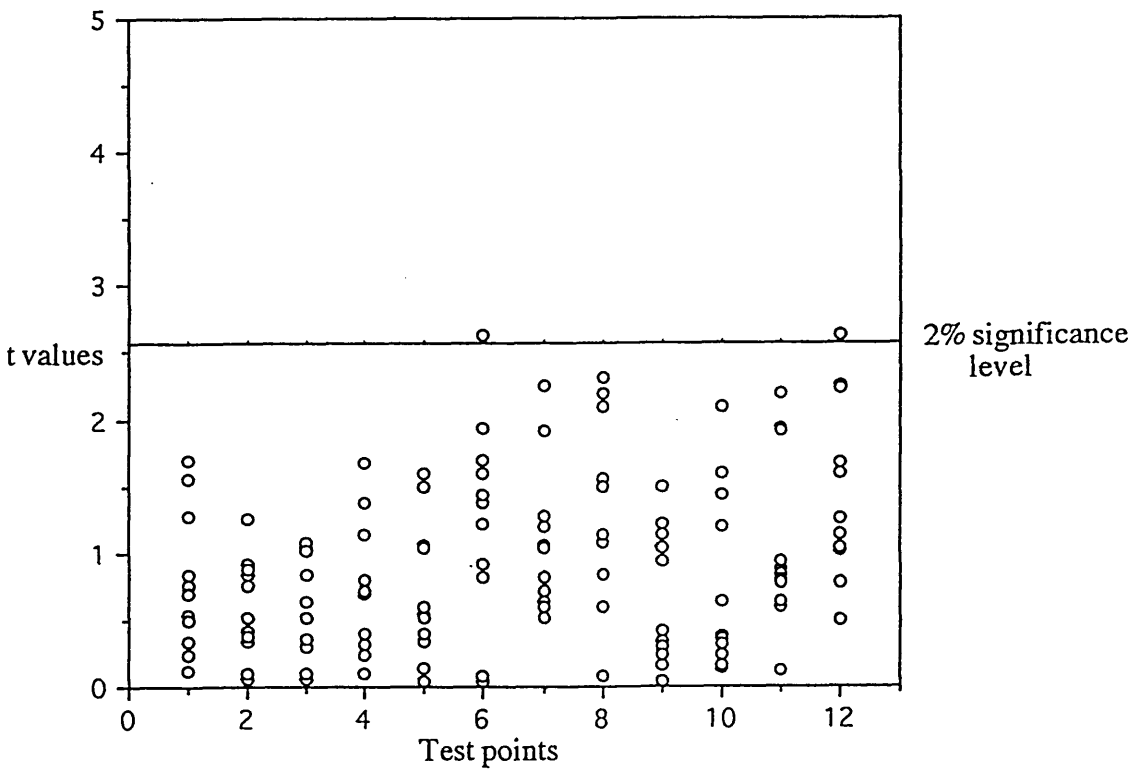

5
4
3
t values
2
1
0
0
2
4
6
8
10
12
Test points
2% significance level

Fig 17. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA) of the temperature differences at the same depth (80 & 20cm) on coarse and fine shredded windrows. Open circles show the differences between fine and coarse at 20cm, open triangles the differences at 80cm.

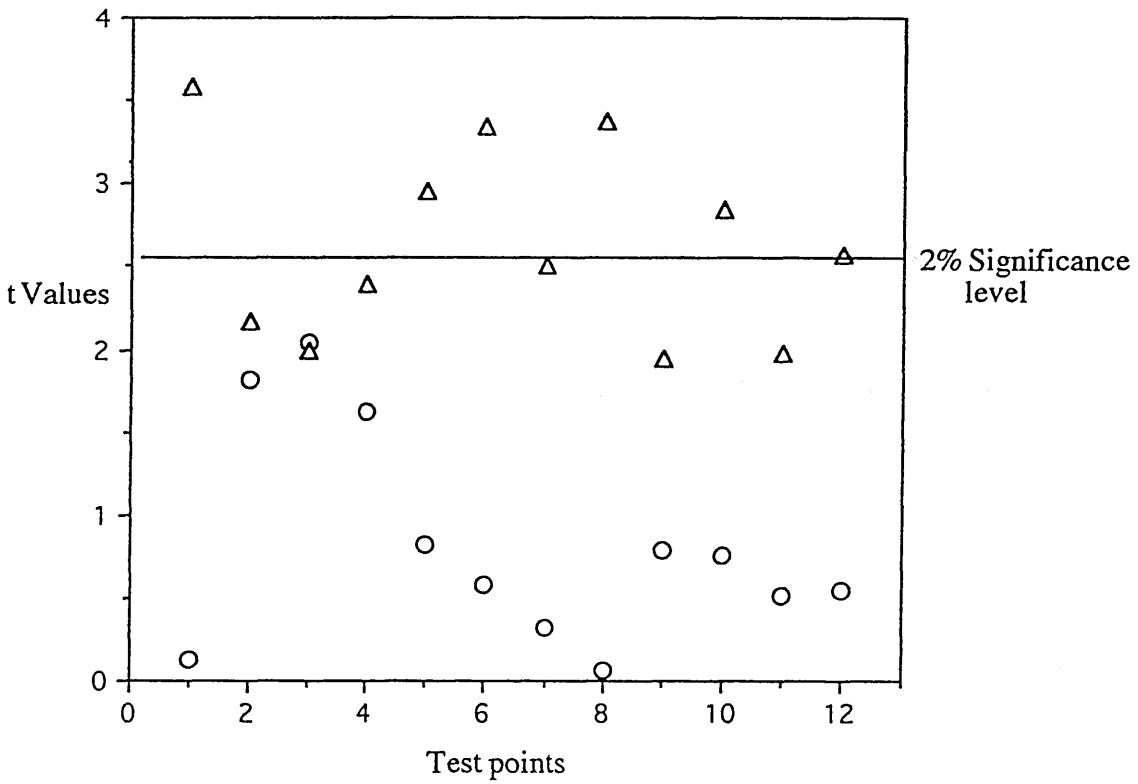
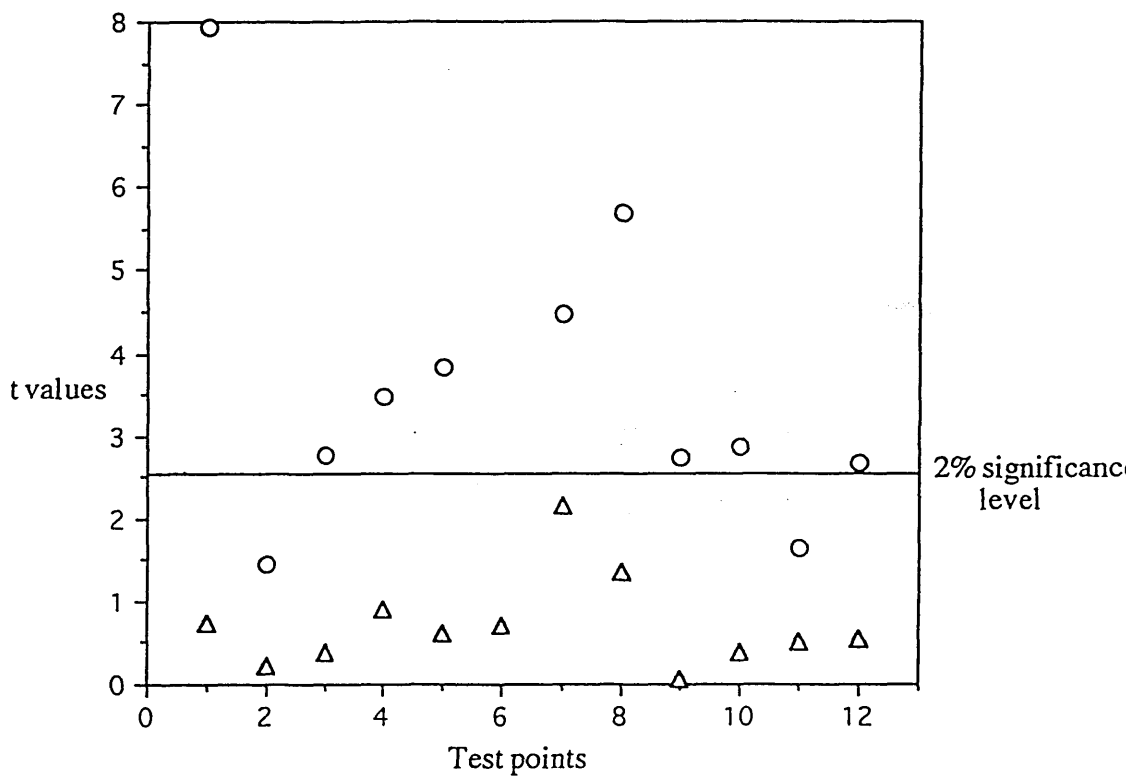

4
3
2
1
0
t Values
0
2
4
6
8
10
12
Test points
2% Significance level

Fig 18. Results from Student's T test for matched pairs, using Statworks 1.2 (Cricket Software inc. Philadelphia, USA) of the temperature differences in coarse and fine shredded windrows at 20 and 80cm depths. Open circles represents the differences in coarse shredded windrows between 20 and 80cm, open triangles the differences in fine shredded windrows.


8
7
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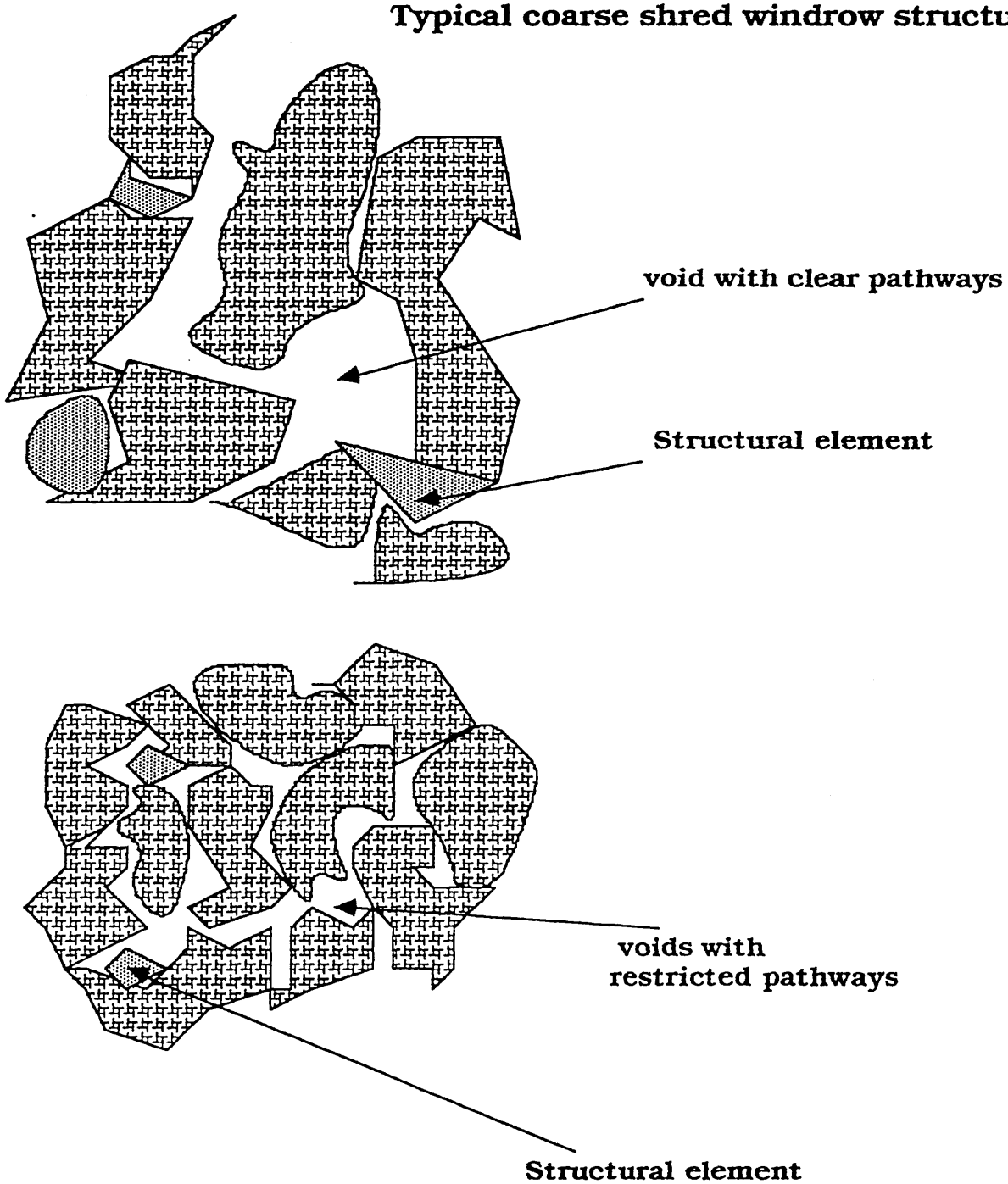
t values
0
2
4
6
8
10
12
Test points
2% significance level

In fine shred windrows, individual void spaces take on greater importance than in coarse shred windrows, in that the smaller voids have fewer interconnecting channels when compared with the poorly sorted coarse shredded material (Fig.19). This limits the amount of oxygen able to enter the windrow by passive diffusion and similarly, slows down the secondary metabolites (carbon dioxide, water, ammonia etc.). therefore, the windrow environment is likely to change from aerobic to anaerobic, leading to an early reduction in microbial activity. Logically, this should happen first in the lower windrow layers (as there are a lower number of voids connected directly to the atmosphere and subjected to compaction due to 'slumping'etc.), and analysis of temperature graphs of both fine and coarse windrows (Fig 11& Fig 12), to some extent, supports the switch from aerobic to anaerobic conditions. It shows that after a initial rise (corresponding with early aerobic conditions), a drop in temperature can be observed in both windrows at the 80cm level. This drop is greatest in the fine shred 80cm layer (a drop of 24°C as apposed to 15°C in the coarse) by day 8. Similarly, in the coarse windrow, the 20cm level shows a small increase in temperature over that period, while the fine shred windrow exhibits a small decrease suggesting that aerobic activity is reduced first in the fine shred windrow. Supporting evidence of this phenomena, can be seen in the temperature rises of both windrows at all levels. After turning (aeration), a longer time period (24 hrs) is observed in the fine shred windrow, before the previous high temperatures are regained. This may indicate that anaerobic conditions (with a reduction in aerobic microbial activity), have become more firmly established in the fine shred windrow by day 8, than in the coarse shred windrow. Consequently aerobic microorganisms would need to out-compete the greater numbers of established anaerobic populations in the fine shred windrow, before regaining the 'upper hand'. The coarse shred windrow, with its lower bulk density and greater structural integrity (less likely to suffer further compaction due to windrow collapse) and greater number of interconnected voids, results in a 'slower drift' towards anaerobic conditions. Thus aeration on day 5, had less of an impact than it had in the case of the fine shred windrow. However, the coarse shred windrows lower heat storage capacity (calculation 20), coupled with a greater number of voids connected to the atmosphere in the top layers, leads to greater latent heat loss

in those regions. This in turn may lead to greater temperature differences between upper and lower layers and a more rapid fall in temperature at the lower levels when conditions become anaerobic. As composting proceeds, the organic fraction is reduced and the windrow will inevitably suffer from 'slumping', as the structural elements become less effective due to loss of strength from the various biodegradation processes. Experiments (The Composting Process; Fig 21) in which changes in organic fraction were measured (LOI method), demonstrated that during composting the organic fraction can reduce by over 33% (from 45% (v/w) to under 15% (v/w) (Fig 21)), in less than 25 days. Therefore, the compacted bulk density in windrows increases with time (usually to a maximum of approximately 780 kg m^{-3} (CEN TC 223 1994)). This increases the heat storage capacity (calculation 21), enabling windrows (despite lower microbial activity) to retain heat for extended periods.

Fig 19. Typical differences between the structure of fine and coarse shred material. In a fine shreddded windrow, pathways become progresivly tortuous and thus increase transfer time.

Typical coarse shred windrow structure



Typical Fine shred Windrow structure

Conclusions.

This experiment have shown that microbial activity will generate sufficient heat for pathogen elimination and that windrows with compacted bulk densities of between 400 and 600 kg m^{-3}, will sustain temperatures above 55°C for at leased 20 days. Fine shred windrows (600 kg m^{-3}), with their greater heat storage capacity, produce a more even temperature distribution between depths, but greater variability between individual test points at the same depth. Shredding to produce lower compacted bulk densities (400 kg m^{-3} coarse shred windrows), with the greater number of interconnected void spaces, results in more uniform spread of temperatures deep within the windrow (80cm depth).

Coarse shredding, with its potential for longer periods of high microbial activity deep within the windrow, may mean fewer windrow turnings (to ensure aerobic composting), over a given period. Coarse shred windrows also require less heat energy to be generated in order to achieve high temperatures (Calculations 17 & 18). However, their lower heat storage capacity (Calculation 20 &21), may mean a faster temperature drop (especially if ambient temperatures are low) particularly in the surface layers of the windrow, which may lead to incomplete composting of the material. This is undesirable, as the high organic compost which results from incomplete composting, tends to be phytotoxic (de Bertoldi *et al* 1983) and thus limits its usefulness as a growth medium. However rapid falls in temperature due to nutrient depletion (provided sufficient time for pathogen elimination has occurred), can be an advantage over slow cooling (fine shred windrows), as it will allow early re-colonization by mesophilic and psychrophilic microbial populations many of which (Actinomycetes and Basidiomycetes etc.), are essential in breaking down the residual highly resistant materials such as lignin by oxidative ring cleavage thus reducing maturation periods. Highly compacted bulk densities on the other hand, produce fewer large fragments (over 32cm in length) of resistant material, hence requiring less microbial effort to degrade it totally. This could mean higher yields of compost after final processing (screening to remove non-composted material and inert waste).

Composting, is about compromises. Coarse shredded windrows may result in better aeration thus requiring less labour intensive turning, but under some circumstances, may lead to incomplete composting with large amounts of waste. Fine shredded windrows on the other hand, could produce less waste, but require careful monitoring for temperature spread and may need more frequent turning to ensure aerobic conditions. The method adopted (i.e. fine or coarse shred windrows), very much depends on the final destination of the composted material. If the compost is destined for horticultural use, pathogen removal takes priority, therefore compacted bulk densities of 400 kg m^{-3} will assist in maintaining sufficiently high temperatures to achieve pathogen kill, without the need of constant monitoring. If in the other hand the compost is a simple bulk reduction method and the compost is destined for landfill or low grade non agricultural land restoration, then windrows with compacted densities of 600 kg m^{-3} will ensure low waste but with an uncertain level of pathogen removal.

THE COMPOSTING PROCESSES

Composting as a waste management process must continue at optimum efficiency so that time, space, labour and equipment etc., are used as economically as possible. In order to achieve this, the elements required for the biological processes (water, oxygen, nutrients etc.), need to be readily available in the waste material. In order to observe the dynamics of moisture content, organic content and temperature within the composting process, a windrow was established. The windrow (2 x 2 x 5 metres) was constructed using a TIM SD1000 hammer-mill shredder from a mixture (approximately 1:1) of Compostainer waste and civic amenity 'green' waste with a compacted bulk density between 400 and 600 kg m^{-3} at the Marchbanks Materials Reclamation Plant, Harefield road Dundee, during July 1995 and samples were taken on a regular basis for up to 25 days. The windrow was turned on day 6 and day 15.

Once established, the windrow was marked at 9 individual test points on one side of the structure. 3 test points were situated at the base of the windrow (20cm above the ground level), 3 were situated at 1 metre above ground level and the final 3 were situated 20cm from the top of the windrow. All test points were at equal distances along the length of the windrow. At each test point 3 samples (a total of 300 ml) were taken and mixed in a clean 3 litre container. Samples (3 x 50g) were removed from the container for analysis and the mean value of the 3 samples calculated and plotted. Temperatures were measured at 1 metre height above the ground and at a depth of 50cm, at 9 test points evenly distributed around the windrow. The mean and standard deviation of each days readings from all test points were calculated and plotted.

Water.

From the perspective of most microorganisms, compost windrows are semi aquatic environments. This means that the water content of the waste material (which is variable, Table 3), is of prime importance to the composting process, as without water, most microbial

metabolic processes will either not start, or will rapidly cease. Water also plays a significant role in determining the microbial community structure in both the species and the 'habitats' they occupy. Waste with low water content (≤ 20% w/v), may induce environmental conditions that reduce the competitive ability of many bacteria (competitive exclusion principle) (Begon & Mortimer 1987), denying them an initial 'footing' in the waste, selecting instead for species that can subsist in dry conditions far better than most bacteria, i.e. most fungi (i.e. *Aspergillus* spp., *Penicillium* spp. etc.). These microorganisms (and some Actinomycetes), are able to use their mycelium to transport water from wetter parts of the windrow, to the growing apex (Lynch 1979). Dry conditions at the start of the composting may slow the composting process by both limiting metabolic activity and restricting bacterial populations. Lack of moisture, may also encourage some pathogenic fungal species, e.g. *Aspergillus fumigatus*, etc., to become established and so should be avoided if possible (Sigsgaard *et al*, 1994).

Higher water contents (30-45% w/v), lead to the formation of a film of water on the surface of the waste material (comparable to the microbial cell size), which produce environments that favour *r*-strategist microorganisms (i.e. *Bacillus* spp. etc.), (Begon & Mortimer 1987), these microorganisms rapidly colonise the waste, creating the large bacterial populations needed to raise temperatures from ambient to thermophilic levels. The rapid colonisation and the heat generated by large numbers of bacteria will eventually exclude most mesophilic populations (including most fungi), reducing microbial diversity. Windrows with a high water content (≥ 60% w/v), may fundamentally change the composting process from one that is mostly aerobic, to one which is analogous to digestion. Open windrows depend largely on passive diffusion for oxygen availability and should windrows become water saturated (i.e. void spaces become filled with water (Fig 19)), the normal exchange of respiration gases will become severely restricted. This inevitably, will lead to anaerobic conditions. Should such conditions become firmly established, there is a risk that the lower temperatures associated with anaerobic windrows, coupled with selection pressures that favour obligate or facultative anaerobic species (some of which are potentially pathogenic, i.e. *Clostridium* spp. *Klebsiella* spp. etc.), then the

compost produced may be regarded as hazardous.

Waste materials delivered to the site for composting, will have variable moisture contents (Table 3), and may loose or gain moisture from local environmental conditions (evaporation, rainfall etc.), during storage. In view of the importance moisture has on microbial metabolic activity and populations, adjusting the water content to achieve optimum microbial growth is desirable. However, the type of organisms to be encouraged depends on the stage of composting. At the start of the process, moisture contents of between 35 and 45%, are recommended, as this encourages the rapid colonisation and high levels of activity usually associated with bacterial populations early in the process, thus reducing composting time. If on the other hand, the process is at the maturation stage (Collier *et al* 1994; Stenbro-Olsen & Collier 1994), drier conditions (≤ 35% w/v), will encourage the growth of species (Fungi & Actinomycetes etc.), that assist the maturation process by breaking down residual lignin and other resistant material. Drier conditions (≤ 35%) are also preferable during mechanical screening operations, as they prevent losses of finished compost by the compost aggregating or 'balling', reducing yields and increasing waste. Effective adjustment of the water content of waste is not practical once the windrows are built as this would involve dismantling of the structure. Any water adjustments must be made before windrow construction and this is normally achieved by mixing waste with differing water contents during the shredding phase. Maturing windrows will be encouraged to loose water by natural evaporation by turning, or else moved under cover to prevent being affected by precipitation. The overall water content of a windrow will inevitably reduce during composting by a combination of evaporation, leaching and microbial use. The loss of water by evaporation is usually due to high windrow temperatures (≥65°C), which are generated during the composting processes (although some losses may be balanced by the production of metabolic water because of microbial activity), and some loss will occur during turning operations. The free water content of the windrow will (due to gravitational forces), eventually form areas of high moisture at the foot of the windrow, where it contributes to the formation of anaerobic conditions commonly found in those

locations. During turning processes, some of the water that had accumulated at the foot of the windrow, will become redistributed, temporarily increasing the moisture content from 18% to 40% in the upper layers of the windrow. These phenomena are displayed as increased average moisture levels in samples (Fig 20) taken from the upper level of a windrow, immediately after turning operations.

Most waste material destined for composting, will contain sufficient moisture not only to achieve a rapid initial microbial colonisation of the waste (thus instigating the composting process), but also to sustain the microbial activity needed (despite losses from temperature induced evaporation), and to keep windrow temperatures at 65°C or more, for several weeks. While changes to temperature, oxygen etc., will alter microbial populations and thus the process, changes to the windrow water content on the other hand, may halt the process entirely.

Carbon and Nitrogen.

Waste materials for composting, will normally be the sole source of nutrients required to sustain microbial activity and the most important are the organic forms of carbon and nitrogen. Carbon can be obtained from organic acids, carbohydrates, fats and proteins, while nitrogen is derived from proteins, amino acids, nucleotides, uric acid, urea and ammonia. In 'green waste', most of the carbon is in the form of cellulose and lignin, of which around 35% is used in the production of microbial biomass and humus (compost). The remainder is usually oxidised (through microbial respiration), to form carbon dioxide and water (Slater 1979). The amount of carbon available for microbial use in composting, is often called the $\%C_{org}$ a value calculated from Loss on Ignition (LOI) measurements (Materials & Methods). The value of $\%C_{org}$ is highly variable, depending on the nature of the waste. Studies have found that the $\%C_{org}$ of MSW can range between 18% and 40% of dry weight (Garcia *et al* 1991), while sewage sludge was in the region of 32% to 57% (Brunnmair *et al* 1996).

The Carbon Problem.

Composting is a dynamic process involving complex feedback networks both chemical and microbial in nature (i.e. microbial activity will degrade or assimilate the organic content of the waste), which alters nutrient and other parameters (carbon, nitrogen, moisture etc.). This will, in turn, alter the microbiology of the system, which again influences the nutrient levels. In order to examine the changes in the carbon content during the early stages (approximately the first three weeks) of open windrow 'green waste' composting, an experiment was designed to measure the change in the value of $\%C_{org}$ during that time and observe any changes in the microbial population.

In order to observe the dynamics of organic content, a windrow was established. The windrow (2 x 2 x 5 metres) was constructed using a TIM SD1000 hammer-mill shredder from a mixture (approximately 1:1) of Compostainer waste and civic amenity 'green' waste with a compacted bulk density between 400 and 600 kg m^{-3} at the Marchbanks Materials Reclamation Plant, Harefield road Dundee, during July 1995 and samples were taken on a regular basis for up to 25 days. The windrow was turned on day 6 and day 15.

Once established, the windrow was marked at 9 individual test points on one side of the structure. 3 test points were situated at the base of the windrow (20cm) above the ground level), 3 were situated at 1 metre above ground level and the final 3 were situated 20cm from the top of the windrow. All at test points were at equal distances along the length of the windrow. At each test points 3 samples (a total of 300 ml) were taken and mixed in a 3 litre clean container. Samples (3 x 50g) were removed from the container for analysis and the mean value of the 3 samples calculated and plotted. The mean and standard deviation of each days readings from all test points were calculated and plotted.

Fig 20. Changes in % Moisture content in an open windrow, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA). Arrows indicate Windrow turning

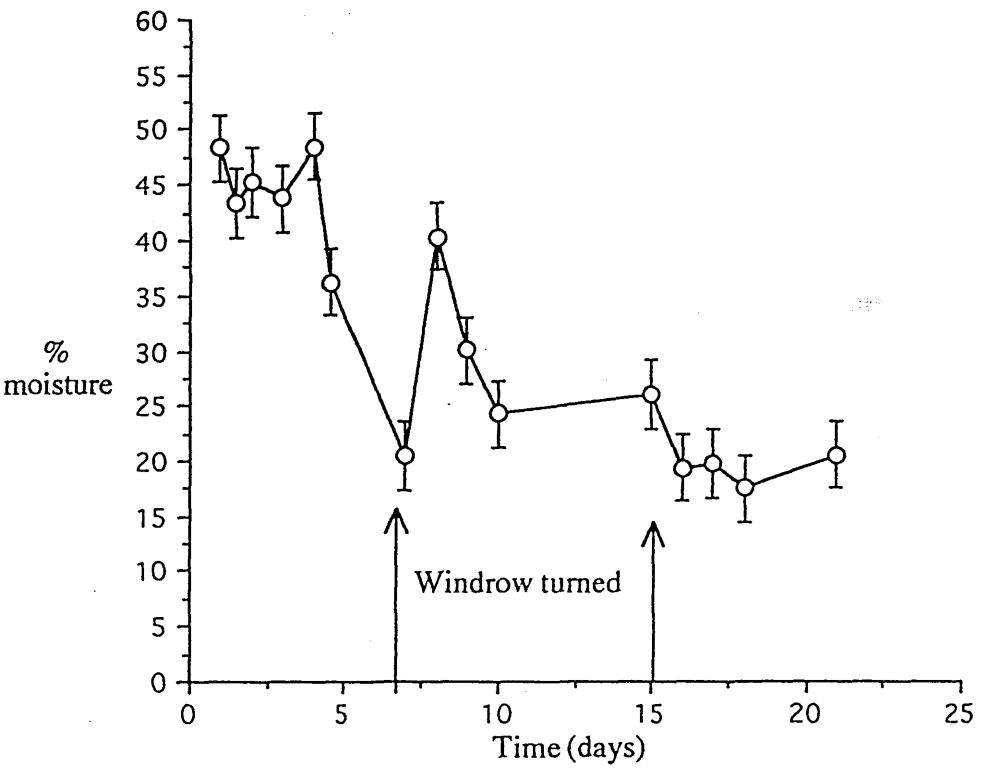

60
55
50
45
40
35
30
25
20
15
10
5
0
% moisture
Windrow turned
0
5
10
15
20
25
Time (days)

Fig 21. Changes in organic content in an open windrow over time. As measured in Loss on Ignition (LOI). Using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA. Peaks are as a result of sample variability and arrows indicate windrow turnings.

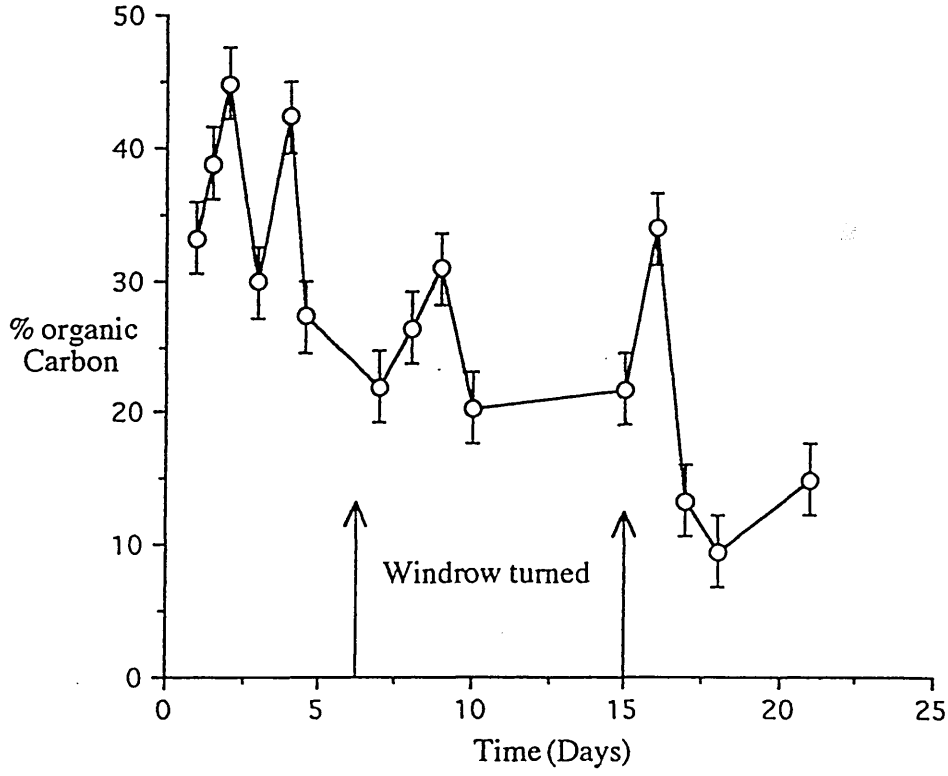
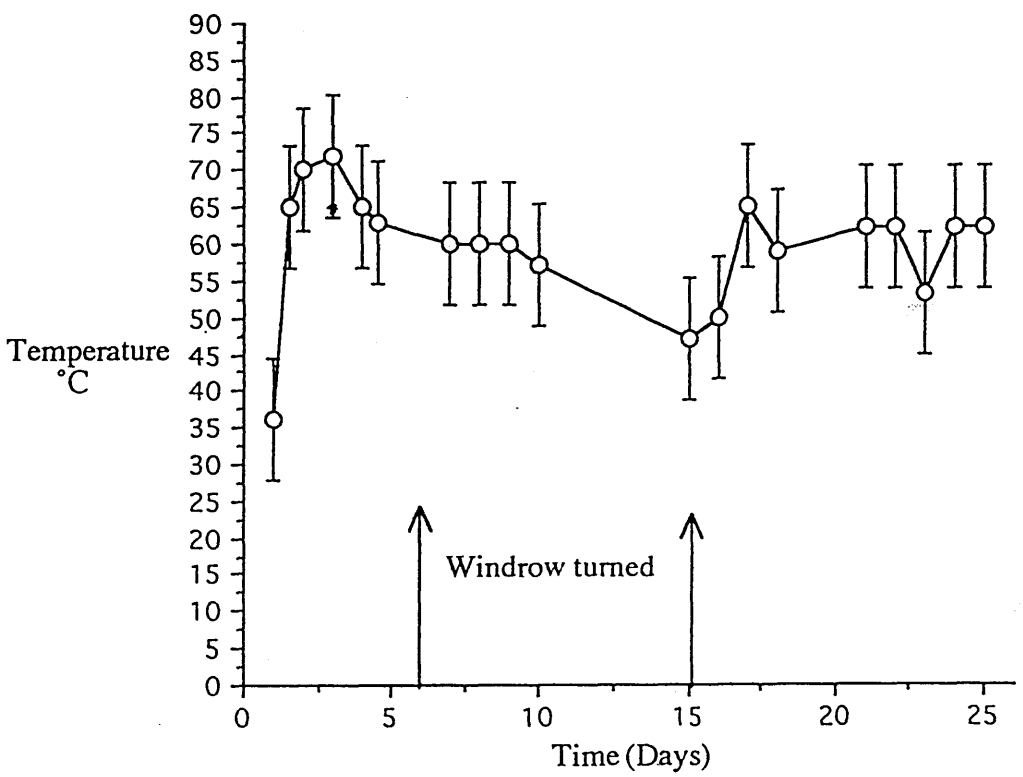

50
40
30
20
10
0
% organic Carbon
Windrow turned
0
5
10
15
20
25
Time (Days)

Fig 22. Changes in temperature in an open windrow with time. Using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA). Arrows indicate windrow turnings.


Temperature
°C
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
5
10
15
20
25
Time (Days)
Windrow turned

Following 17 days of composting, the initial value of $\%C_{org}$ (44.8%), fell to 13.3%, (Fig.21), a threefold reduction in the carbon content of the windrow. Moisture during this time, fell from the start value of 48.32% (w/w), to a final value of 19.74% (w/w), (Fig 20). The temperatures (mean 59°C, SD of ± 9.9°C), were plotted as in Fig 22. The reduction in the $\%C_{org}$ value (approximately 3.7 times) and the moisture content by a factor of around 2.4, is of particular interest to operators who use composting as a weight reduction technique before disposal in that, charges for waste disposal (including Landfill Tax) are normally on a weight basis. Therefore, any change in the overall weight of the waste, will have a major influence on composting economics. Applying the weight loss data (calculated from the experimental windrow), to a hypothetical windrow of similar material having a start weight of 100 tonnes, the carbon content will initially represent approximately 44.8 tonnes and the moisture another 48.3 tonnes. A total of 93.1 tonnes. After 17 days composting, these values may have been reduced to 13.3 tonnes for carbon, and 19.7 tonnes for water. A combined reduction in weight of 63.8%. Such a reduction in weight, represents a significant potential savings on any subsequent disposal costs.

Microbial Analysis.

Microbial analysis of the waste material identified a minimum of 14 separate species or genera which are displayed in Table 2. Further analysis of the microbiology of composting was carried out (on the windrow used for the water, organic content and temperature analyses) at the beginning and end of the experimental period by taking samples (10 x 20ml) at random and placing them in sterilised 25ml universal bottles. Aliquots (1g +/- 0.1g) of each sample material was aseptically added to 9ml of Ringers solution and mixed by shaking for 2 minutes. A series of dilutions was then performed up to 10^{-12}. Spread plates for enumerating viable cells were prepared (Collins & Lyne 1985) and 0.1ml aliquots from 10^{-2}, 10^{-3}, 10^{-6}, 10^{-9} and 10^{-12} dilutions (in triplicate), were aseptically removed using a micro-pipette and spread onto a labelled plate containing an appropriate growth media (Materials & Methods) using a sterilised glass spreader. All plates were incubated aerobically at the ambient temperature, 37°C, 55°C and

for 24 hrs, apart from Malt Extract Agar, which was incubated aerobically at the ambient temperature for 48hrs. Isolated colonies were chosen for identification by reference to their colonial morphology. An attempt was made to select colonies typical of the range and variety of colonies present on the incubated plates. Identification of the colonies chosen was attempted by the techniques described in the Materials & methods section. The results are shown in Table Table 5 and 6 .

Table 5

Microorganisms identified in an experimental windrow (day 1).

Bacillus spp.	*B. lentus.*
B. licheniformis	*B. macerans*
B. stearothermophilus	*Vibrio* spp.
Salmonella spp.	*Proteus* spp.
Staphylococcus aureus	*Serratia rubidea*
Nocardia spp.	*Pseudonocardia thermophila*
Penicillium spp.	

Of the 13 microbial species and/or genera identified at day 1, 5 were found at day 17 (Table 6).

Table 6

Microorganisms identified in an experimental windrow (day 17)

Bacillus spp.	*B. licheniformis*
B. stearothermophilus	*Nocardia* spp.
Pseudonocardia thermophila	

Table 2 and 5 suggests that, 'green waste' composting features both prokaryote and eucaryote microorganisms. However, as this type of waste is usually high in cellulose, the direct microbial biodegradation of cellulose will be restricted to a relatively narrow range of microorganisms

(i.e. those that can produce the hydrolytic enzyme cellulase). These will include the Actinomycetes, most fungi, some *Bacillus* sp (e.g. *B. circulans*), *Pseudomonas* spp. and, under anaerobic conditions, *Clostridium* spp. It may be reasonable to conclude therefore, that representatives of these species would be heavily represented at day seventeen. However, the temperatures produced within the windrow during composting (Fig 22), may exclude several of these species, especially mesophilic organisms such as *Pseudomonas* along with Actinomycetes and most fungi.

Table 6 shows that out of the 13 genuses and/or microbial species identified on day 1 (Table 5), on day 17, positive identifications were reduced to *Bacillus* spp. (*B. licheniformis & B. stearothermophilus,* including an unknown thermophile), and an Actinomycete from the genus *Nocardia* subgroup 2: *Pseudonocardia thermophila* (Holt *et al* 1994). The surviving microorganisms were all thermophilic or moderately thermophilic species, reflecting the change in the composting environment from ambient temperatures to over 60°C. However, while representatives of both microbial genuses were found in most samples, *Pseudonocardia thermophila* (a thermophilic Actinomycete), was the dominant species, its mycelium giving a 'greyish white' colour to the surface of the samples. One reason for this, may be the change in moisture levels during the 17 days (Fig 20). As the moisture content declined, species that rely on water films for spreading (i.e. *Bacillus* etc.), may be at a disadvantage against the more xero-tolerant *Pseudonocardia thermophila.*

This experiment exhibited the changes in the carbon and water content of a windrow, during composting. It also indicated that in material with high cellulose content (i.e. green waste), thermotolerant microorganisms with the ability to degrade celluose (i.e. *Pseudonocardia thermophila*) will dominate at the end of the composting period.

The Nitrogen Problem.

In a natural ecosystem (forest floors etc.), the biodegradation of organic matter can be slow (sometimes taking years), however, for the use of composting as a waste management process, this must be achieved in days or at most, weeks. The microbial ability to achieve this is dependant on the availability of nutrients and it is the nitrogen content of those nutrients that governs the rate at which composting proceeds (Bell *et al* 1986). The microbial break down of proteins and other nitrogenous material in the waste (Equation 22), usually involves the enzyme glutamate dehydrogenase. This means that much of the available nitrogen needed to establish the composting process, will initially, be present as ammonia (Postgate 1978; Bell *et al* 1986).

Nicotinamide-adenine dinucleotide + glutamate dehydrogenase + water ⇌ nicotinamide-adenine dinucleotide + glutamate dehydrogenase + ammonia

$$NAD^+ + HOOC.CH_2.CH_2\ CHNH_2\ COOH + H_2O \rightleftharpoons HOOC.CH_2.CH_2\ CHNH_2\ COOH + NH_3 + NADH \quad [22]$$

In order to establish if the likely nitrogen content of a particular waste is sufficient for efficient biodegradation (Hawker & Linton 1971), it is usual to class waste materials by their carbon to nitrogen ratios (C:N), (Table 7).

Table 7.
Typical C:N ratio of:
Selected Waste Material (adapted from Charlsworth, 1995)

Garden Weeds	12:1
Kitchen Waste	30:1
Lawn Cuttings	12:1
Straw	80:1
Wood Prunings	100:1
Newspaper	200:1
Cardboard	500:1

The optimum C:N ratio for composting, is between 20:1 and 40:1.
Higher ratios (up to a maximum of 60:1) are acceptable.

The C:N of green waste, is highly variable (Table 7), depending not only on the time of year,

but also the source of material (Gartland, *et al.* 1997). Winter conditions will see large increases in carbon content from the end-of-season tree prunings etc., while during spring and early summer, high nitrogen containing materials (grass cuttings etc.) are more usual. Food wastes (especially waste vegetables and fruit), arrive intermittently throughout the year and will skew the ratio (usually towards a low C:N).

Because of this seasonal variability, composting times will vary (e.g. waste with high levels of carbon (C:N 100:1), will lead to slow composting because of the inevitable reduction in microbial activity from the decrease of readily available nitrogen). This may mean a long composting period, requiring several microbial generations, before total biodegradation is achieved. In a waste management situation, this can be problematic, as this will increase the physical space required for active composting (i.e. a longer period before final processing and removal from the site). Slow composting may also lead to material being removed from the site before composting has been completed, which may result in a final product that has excessive levels of carbonaceous material (usually as lignin and cellulose), (de Bertoldi, *et al* 1983). Agricultural use of high carbon composts (e.g. as a soil conditioner) may be problematic, especially if introduced into a nutrient depleted soil, as secondary metabolites from renewed microbial action (in order to break down the partially composted material by soil born microbes), can lead to soil phytotoxicity (Garcia *et al,* 1991).

Waste materials with a low C:N ($\leq$ 12:1, often containing monosaccharides such as glucose), are rapidly degraded. This can result in a level of microbial activity that is sufficient to raise windrow temperatures to over 70°C, within 24 hrs. While this is desirable for microbial pathogen elimination, it is suggested by some researchers, that such high temperatures will lead directly to loss of nitrogen from the windrow, through ammonia volatilization (Schwab *et al,* 1994). Other studies (Bernal, *et al,* 1993), found that during the composting of low C:N materials (i.e. poultry wastes (10:1), pig slurry (5:1) etc.), up to 50% of the nitrogen was lost from the windrow, through ammonia volatilization. The increases in pH that are often observed

in composting, have also been quoted as a factor in the loss of ammonia (Abbès *et al*, 1993). Notwithstanding its role in microbial metabolic functions, ammonia is an important plant nutrient (Iglesias-Jimenez & Alvarez, 1993), and therefore, its conservation during composting is desirable. In an effort to examine the factors surrounding the loss of ammonia during composting, a series of studies were initiated. Taking advantage of the large amounts of low C:N material (usually as grass cuttings) which are common during the summer months (Gartland *et al* 1997)

A windrow (2 x 2 x 5 metres), was constructed from approximately 60% grass cuttings (C:N $\leq$12:1), and 40% shredded tree prunings (C:N $\geq$100:1, primarily structural elements) with a compacted bulk density between 600 and 750kgm^{-3} using a TIM SD1000 hammer-mill at the Marchbanks Materials Reclamation Plant, Harefield road Dundee, during July 1995 and samples for pH and ammonia were taken on a regular basis for up to 25 days. The windrow was turned on day 5 and day 16.

Once established, the windrow was marked at 9 individual test points on one side of the structure. 3 test points were situated at the base of the windrow (20cm) above the ground level), 3 were situated at 1 metre above ground level and the final 3 were situated 20cm from the top of the windrow. All at test points were at equal distances along the length of the windrow. At each test points 3 samples (a total of 300 ml) were taken and mixed in a 3 litre clean container. Samples (3 x 50g) were removed from the container for analysis and the mean value of the 3 samples calculated and plotted. Temperatures were measured at 1 metre height above the ground and at a depth of 50cm, at 9 test points evenly distributed around the windrow. The mean and standard deviation of each days temperature readings from all test points were calculated and plotted

Results.

The levels of ammonia in the windrow, were observed to fall by approximately 60.5% (190 mg l^{-1} to 75 mg l^{-1}), and windrow temperatures rose from an initial 35°C to 70°C (Fig 24), during the first 24hrs. During the following 72 hrs, ammonia levels fell to values of between 60 and 75 mg l^{-1}, with temperatures averaging 60°C. In line with normal composting procedures, the windrow was turned and mixed on day five. Comparisons of measurements of ammonia levels taken directly before and after this procedure, exhibited an increase in extractable ammonia of 482% (from 67 mg l^{-1} to 390 mg l^{-1}). The windrow was left undisturbed for a further 11 days, in which period ammonia levels fell to 62 mg l^{-1}. The windrow was then mechanically turned and mixed and ammonia levels were seen to increase after turning (from 62 mg l^{-1} to 410 mg l^{-1} (561% increase)). After peaking at 550 mg l^{-1}, ammonia levels fell to approximately 200 mg l^{-1} at the end of the experiment.

The Rise and Fall of Ammonia.

At the start of the composting process, there is a phase of high microbial activity during which, windrow temperatures can exceed 70°C. Fig 23 shows ammonia levels falling by approximately 60%, during the first 24 hrs. Other studies (Bernal *et al* 1993), reported similar reduction in ammonia, which were largely attributed to ammonia volitilzation due to high temperatures. However, in our experiment, despite average windrow temperatures remaining above 60°C for a minimum of three days, the levels of ammonia (after the initial fall), remained relatively constant. If the Bernal (1993) hypothesis is to be accepted (i.e. high temperatures will lead to loss of ammonia through volitilzation), the ammonia levels would have been expected to continue to fall during the period of high temperatures. However, Fig 24 and Fig 25 exhibit ammonia levels rising with increasing windrow temperature and reducing with falling temperatures. In order to sustain the initial microbial activity needed to raise windrow temperatures above 60°C, the windrow must contain sufficient nitrogen to initiate the process (cellulose for example, will not be degraded if the nitrogen content of the waste falls below 1.2% w/v). Microbial decomposition processes begin with the death of the plant and there can

be a delay of up to two weeks (more in some circumstances) before the waste material becomes available for composting. This means that the waste material will already contain readily available nitrogen (usually as ammonia (Postgate 1978)), derived from those decay processes. Ammonia is relatively easily assimilated by microorganisms and thus, rapid microbial growth and activity are usual at the start of the composting process (this can be seen as a rapid increase in windrow temperature, as seen in Fig 24). Because of the increase in microbial activity, easily assimilated sources of nitrogen such as ammonium ions, will rapidly become depleted. However, as ammonia is at the same time being produced, there will be a point where production and consumption are balanced (i.e. an equilibrium is achieved), resulting in levels of ammonia that follow the temperature (microbial activity), pattern of the windrow.

Fig 23. Changes in ammonia content in an open windrow, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Data point A was measured prior to windrow turning and data point B directly after turning. Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA). Arrows indicate windrow turnings.

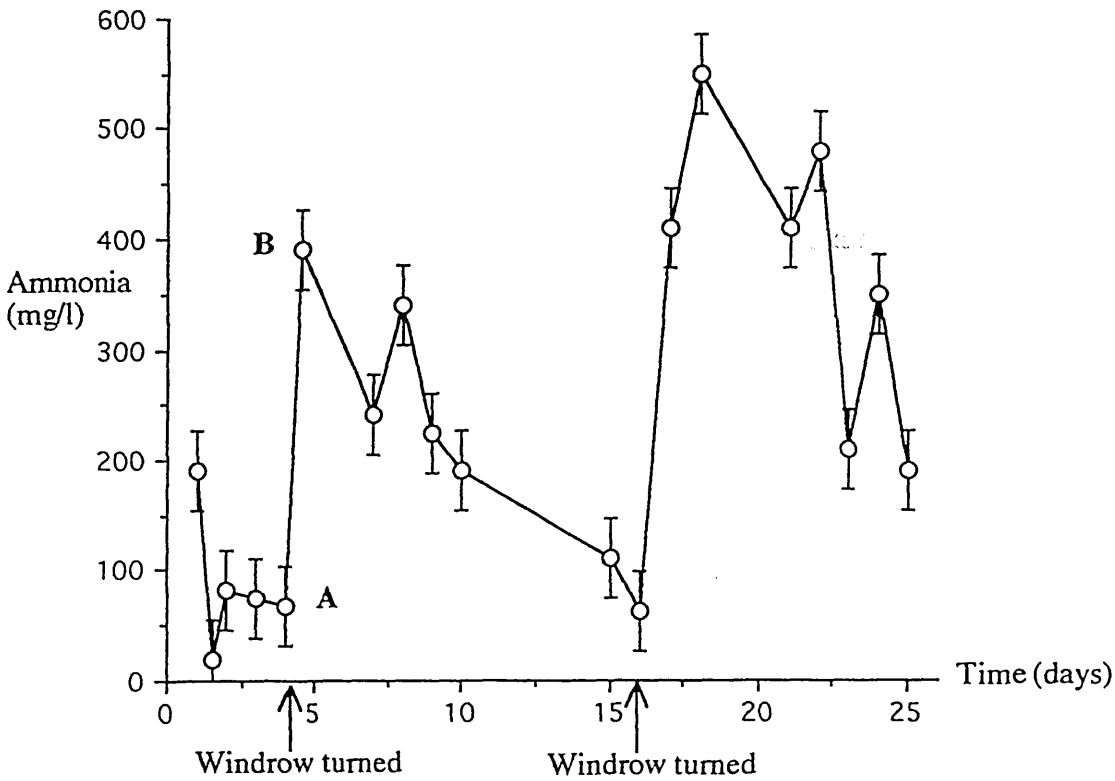

600
500
400
300
200
100
0
Ammonia
(mg/l)
B
A
0
5
10
15
20
25
Time (days)
Windrow turned
Windrow turned

Fig 24. Changes in ammonia content in an open windrow against temperature over four days, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA).

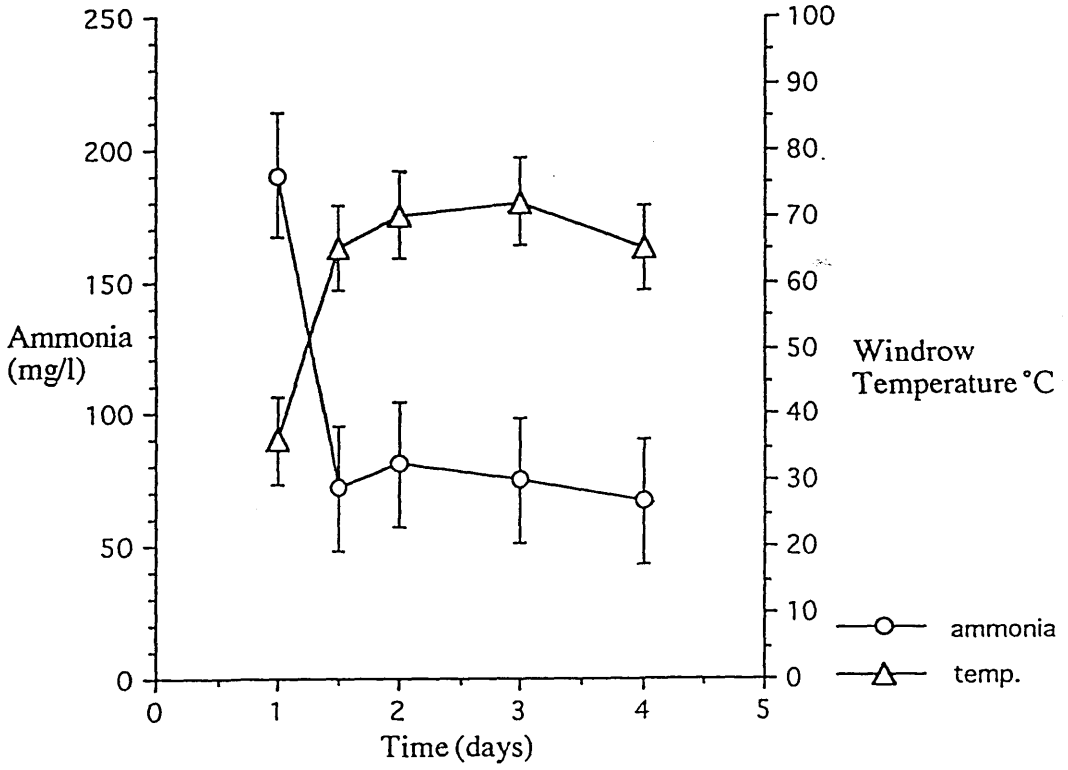
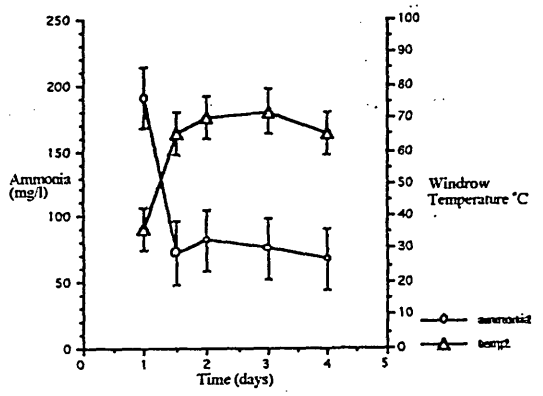

250
200
150
100
50
0
Ammonia
(mg/l)
0
1
2
3
4
5
Time (days)
100
90
80
70
60
50
40
30
20
10
0
Windrow
Temperature °C
ammonia
temp.

Fig 25. Changes in ammonia content in an open windrow against temperature over twenty-five days, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA). The first 4 days (Fig 24) is the inset.


250
200
150
100
50
0
Ammonia (mg/l)
100
90
80
70
60
50
40
30
20
10
0
Windrow Temperature °C
0
1
2
3
4
5
Time (days)

Inset Fig 24.

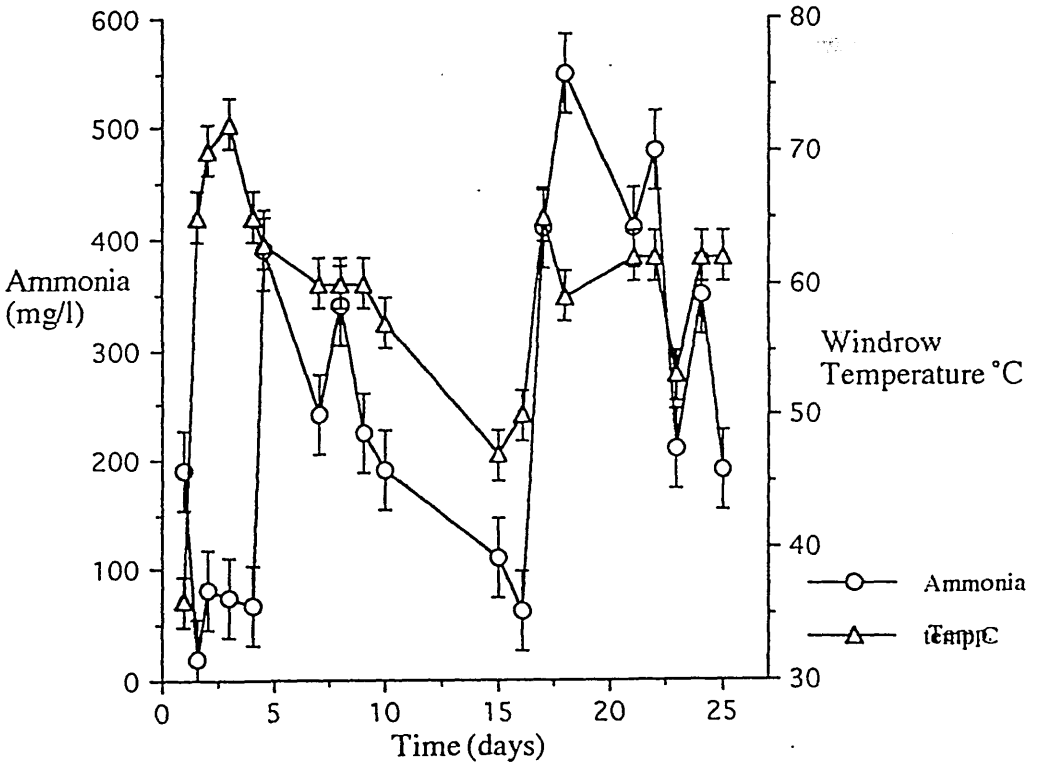
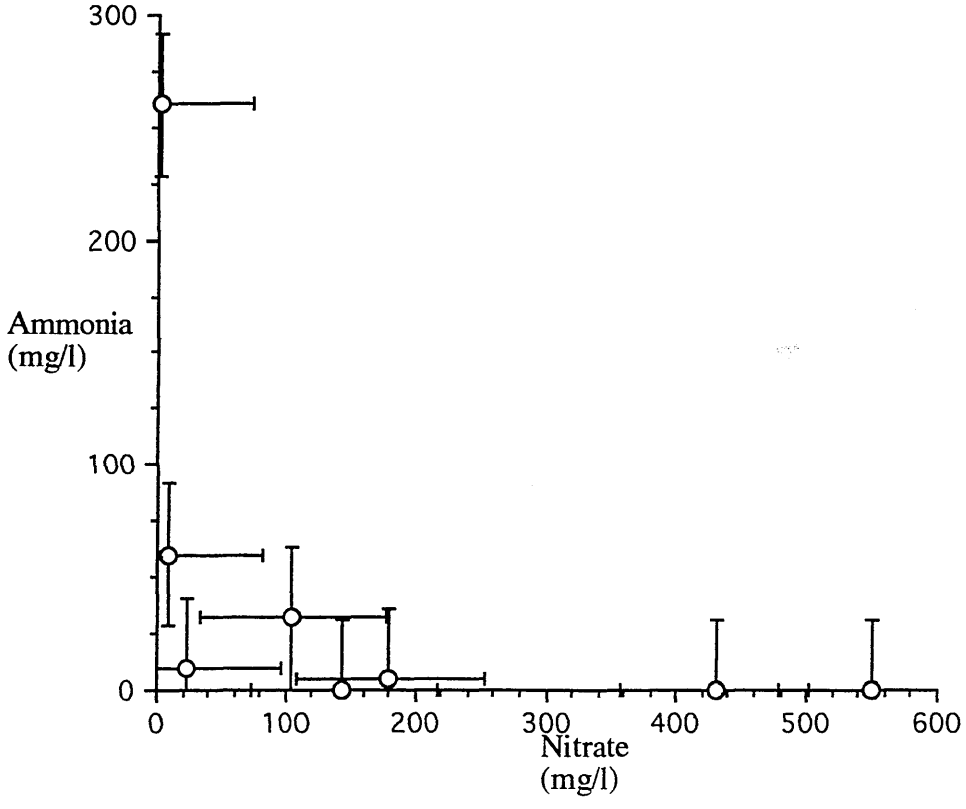

600
500
400
300
200
100
0
Ammonia (mg/l)
80
70
60
50
40
30
Windrow Temperature °C
0
5
10
15
20
25
Time (days)
Ammonia
Temp°C

Fig 26. Changes in ammonia content in an open windrow against the nitrate content, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA). Data obtained from separate experiments not described in this study.


300
200
100
0
Ammonia
(mg/l)
0
100
200
300
400
500
600
Nitrate
(mg/l)

The Rise of Ammonia.

Windrows constructed primarily from low C:N materials such as fresh grass, tend to collapse or 'slump' resulting in anaerobic conditions within the windrow. Anaerobic conditions are usually detected by a fall in the windrow temperature, which can be seen to occur at around day 4. The normal action by the operator is to turn and mix the windrow so that aerobic conditions can be re-established. In this experiment, this was done on days 5 and 16. Samples taken for ammonia analysis directly before and after turning, indicated an 8 fold increase in ammonia concentration after the turning process. This may be the result of an accumulation of soluble ammonium ions at the foot of the windrow where the anaerobic conditions may have prevented the oxidation of ammonia to nitrite by autotrophic organisms (i.e. *Nitrosomonas* spp. and *Nitrobacter* spp. (Nodar *et al* 1992; Mahimairaja *et al.* 1994). By turning and mixing the windrow, accumulation of soluble ammonia ions at the foot of the windrow become re-distributed and subsequently show up as an increase in ammonia levels. The effect of the increased availability of ammonia and oxygen, can be seen in a 20°C increase in windrow temperature at the second turning episode.

Fig 27. Changes in pH in an open windrow using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA).

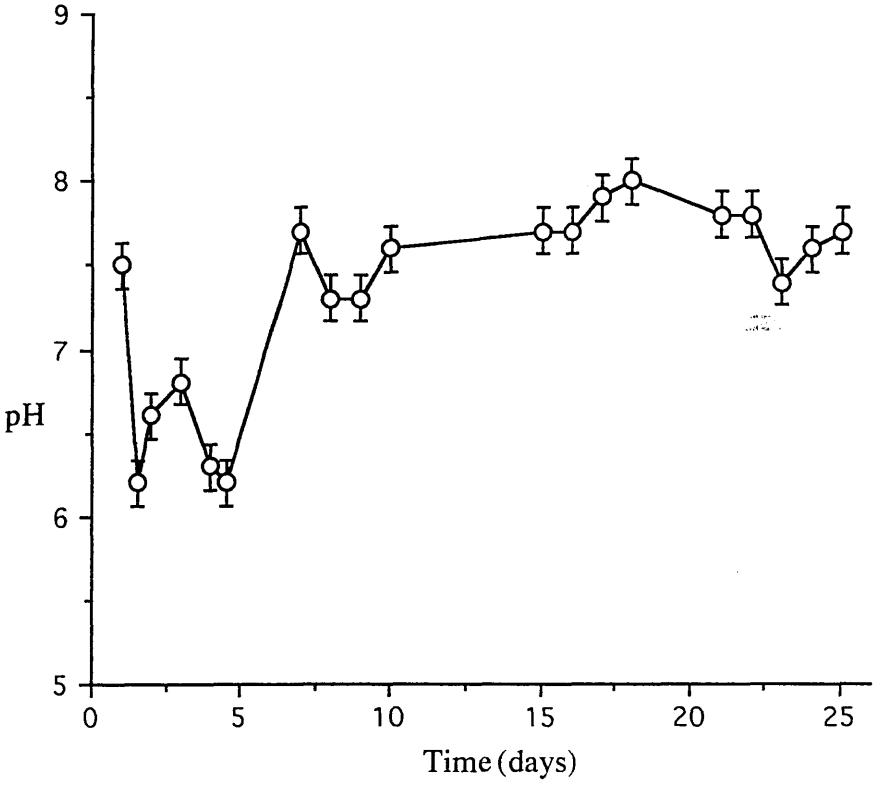

9
8
7
6
5
pH
0
5
10
15
20
25
Time (days)

Fig 28. Comparisons in pH against the ammonia content in an open windrow, using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA).

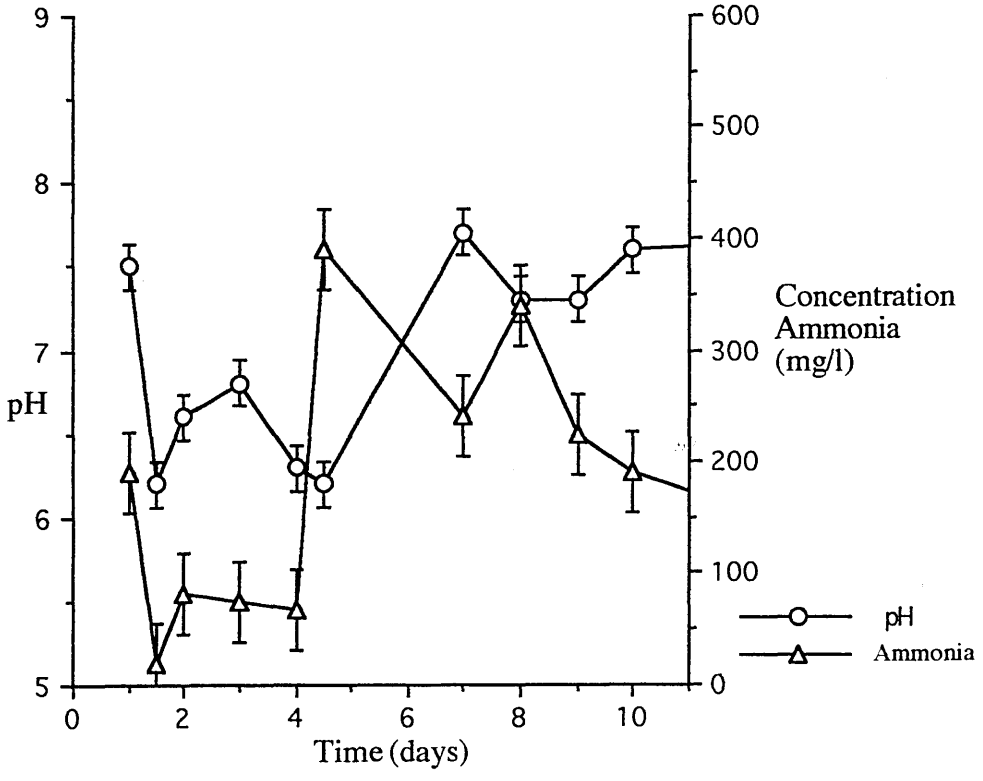
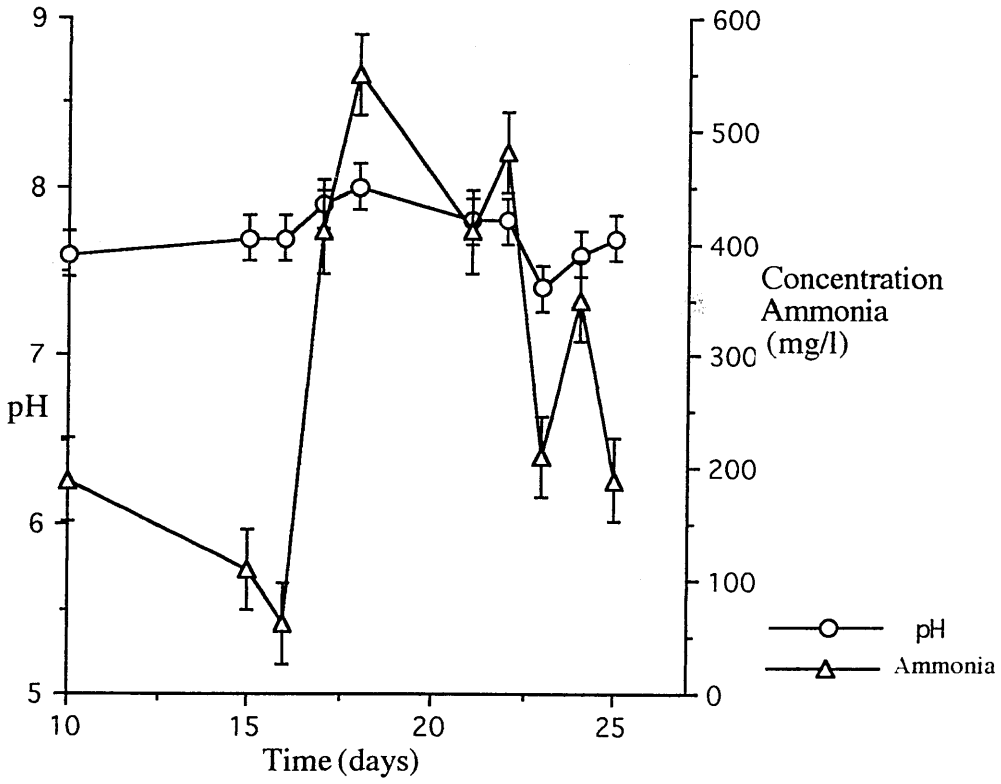

9
8
7
6
5
pH
0
2
4
6
8
10
Time (days)
600
500
400
300
200
100
0
Concentration
Ammonia
(mg/l)
pH
Ammonia

Fig 29. Comparisons in pH against the ammonia content in an open windrow from ten to twenty-five days. Using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA). Error bars are derived from the standard error of the data set, using Statworks 1.2 (Cricket Software inc. Philadelphia. USA).


9
8
7
6
5
pH
600
500
400
300
200
100
0
Concentration
Ammonia
(mg/l)
10
15
20
25
Time (days)
pH
Ammonia

The Fall of Ammonia.

Several microorganisms will produce nitrite from ammonia (nitrification), which will itself become reduced to nitrates and as part of the nitrogen cycle, back to ammonia (Postgate 1978). However, some studies have shown that high levels of ammonia may inhibit the nitrification process (Schwab *et al*, 1994), thus preventing the formation of nitrate. Evidence for this was seen in analysis of the relative levels of nitrate and ammonia within a range of windrows at different ages (1 week to 9 months, Fig 26), which showed that when high levels of ammonia are detected, nitrate levels were low and when nitrate levels were high, ammonia was low. The fall in ammonia and increase in nitrate levels, has been used as an indication of compost maturity on the premise that: as the 'raw material' (in the form of organic matter), is usually rich in nitrogen thus enabling ammonification, the formation of nitrate indicates that ammonification has by and large has ceased, the compost therfore, can be regarded as 'mature' (Iglesias-Jiménez & Garcia 1989).

Schwab *et al* (1994), reported that as well as increase in temperature, a rise in pH will contribute to the loss of ammonia. While changes in temperature and pH were detected (Fig 25 & Fig 27) over the experimental period, and a good correlation between pH and ammonia levels were observed (significant at the 1% level, Spearman rank correlation, Chalmers & Parker 1989), the data suggests that rather than ammonia becoming lost because of an increase in pH, pH is directly *proportional,* to the amount of ammonia measured in the windrow (Fig 28 & Fig 29). Efforts to modify the composting processes in order to reduce the loss of ammonia have included the addition of peat (Abbès *et al* 1993), chopped straw or natural zeolite (*Zeolite clenoptilolite)* (Bernal, *et al* 1993). However, such modification adds cost to the process and, may be counter-productive in plant nutrient terms, as high levels of ammonia have been shown to inhibit the formation of nitrate. Nitrate in common with ammonia, is an important plant nutrient but unlike ammonia, is not easily absorbed into the soil and thus remains available for plant use over a longer period (Jakobsen 1995).

Conclusions.

These experiments have examined the changes in water, carbon and nitrogen levels during the composting of 'green waste'. It has shown that the loss of ammonia during composting may in addition to losses due to volatilization as reported by some researchers (Bernal, *et al* 1993; Schwab *et al*, 1994), but also as a result of microbial metabolic use. It is also concluded that attempts to conserve ammonia by using additives such as peat or zeolite, may not be needed as high levels of ammonia may inhibit the production of nitrate, which is also an important plant nutrient. These experiments also found that composting, by reducing the organic fraction and water content of the waste, significantly reduces the mass of the waste material. This has significant economic implications, for if composting is used simply as a bulk reduction process prior to disposal by means of landfill, then the reduction in weight would see a corresponding reduction in Landfill Tax payable (Great Britain 1996).

THE WINDROW ECOSYSTEM

The Microbial Community.

The collecting together of a whole range of (often dissimilar) organic materials and placing them into a compost windrow, will inevitably result in the creation of an interactive community of microorganisms, often known as an ecosystem. As ecosystems go however, a compost windrow is small, being similar in scale to that of a decaying log, which is rated 12 on the G scale. The G scale, is based on successive subdivisions of the Earth's surface area by the power of 10 (Table 8, Bell 1986). Organisms within an ecosystem are, on the whole, reasonably self-sustaining. Ecosystems contain most of the physical and chemical requirements for population growth and have minimal dependency on external inputs and few outputs. A fish farm lagoon for example, would not be described as a self sustaining ecosystem since all inputs (fish food) and outputs (waste removal) are performed by humans. Remove any one of those functions, even for a short period and the system ceases to be viable. A lake however, can be counted as a self-sustaining ecosystem, as a lake will besides fish, contain water weeds, snails etc. and, as an ecosystem, requires only an input of light. Such ecosystems are often described as closed systems (Finstein *et al* 1980). Ecosystem viability however, does not always depend on it being a closed system. Rivers, streams and marshes for example, often receive large inputs of organic and inorganic matter, from the surrounding land, subsequently exporting it downstream or into the sea. Viability is not also guaranteed, there are factors (edaphic factors), that will from time to time, directly threaten the ecosystem's viability. Edaphic factors are placed into five main categories and are detailed in Table 9.

Table 8

The Division of Biological, Climatic and Geomorphological Features of the Earths Surface. Based on the successive subdivisions of the Earth's surface area by the power of 10 (adapted from Ridge & Varly 1986).

G scale	Biological Division	Characteristic Geological Unit
1-3	biographical realms on land and sea	tectonic plates and areas of land and sea.
2-4	biomes, e.g. a tundra tropical rainforests	very large structures, i.e. a mountain systems
3-5	biotic provinces	rift valleys, mountain belts European tundras
6-9	associations, all communities of one type within a biotic province	river valleys, deltas
8-9	local community e.g. a particular Oakwood	corries, moraines
10-12	microstands, e.g. dead log with its flora and fauna.	Small scale landforms. Compost windrows

Table 9

Edaphic Factors (Bell *et al* 1986).

Factor	Comments
Stability	Not washed or blown away. A collapsing of structure may effect the amount of oxygen available by sealing or destroying void spaces
Physical Structure	Particle size. Different size particles will not only change the insulation factor, but also effect oxygen availability
Chemical Composition	Nutrients, moisture etc.: the type and amount of nutrients will influence directly the microbial community. Moisture, as well as its metabolic role, significantly effects microbial motility.
Aeration	Changes in oxygen content of a windrow, can fundamentally change populations or processes.
Temperature	Psychrophilic or thermophilic populations.

The success of microbial communities within an ecosystem, depends not only on edaphic factors, but also upon the strategies microorganisms have evolved to deal with competition for space, nutrients, moisture etc. from other individuals. While each species may have evolved a range of different strategies, they inevitably fall within two main headings; intra-specific and/or interspecific competition (Begon & Mortimer 1987).

Intra-specific Competition.

Individuals within a population will affect, and are affected by, other individuals within that population as intra-specific competition. Intra-specific competition has four basic characteristics:

(I) The Ultimate Effect. Where the effect of competition is a potential decrease in the contribution individuals make to future generations, when compared with the contribution the individual may have had without competition.

(ii) Competition for Resources. In compost windrows, resources (nutrients, water, oxygen etc.) are strictly limited, thus individuals will compete directly with each other for access to those resources.

(iii) Reciprocity. While it is perfectly possible for intra-specific competition to be one sided, e.g. a strong early seedling shading a later arrival, thus the late arrival is disadvantaged by less light. This does not mean that any individual has an implicit advantage over another, as roles might have been reversed. The net result is; competing individuals within a population are inherently equivalent. This is in contrast to a predator-prey relationship, where the predator, is the natural 'inflictor' (of an adverse effect) and the prey is the natural 'receiver'.

(iv) Density Dependence. The probability of an individual being adversely affected is proportional to the numbers of competitors. This is sometimes described as 'Scramble or Contest'. Where Scramble is a situation where all individuals can gain access to a resource. Contest on the other hand, means that individuals have to compete with each other for resources. In a (hypothetical) ecosystem with a population growth threshold that if exceeded, would lead to resource limitations and if, in this ecosystem, all individuals require the same amount of the resource to function, then during low population densities, all individuals have access to as much of the resource as they need (Ridge & Varley, 1986). Thus, the percentage

mortality is zero. However, should the population density threshold be exceeded, Scramble and Contest factors come into play. In a Scramble situation, all the individuals still get an equal share of resources, but this is now less than they require. Therefore, the percentage mortality is 100 percent, i.e. they all eventually die. In a contest situation, individuals will fall into two classes, those that are still able to get sufficient resources and those that cannot. Those that are able, will survive, those that cannot will perish. The key difference between Scramble and Contest is, that in a Contest situation, there will always be survivors (Begon & Mortimer 1987). It is unlikely however, that any ecosystems will exist in an exclusive Scamble or Competition 'mode'. 'Real' ecosystems are likely to be a mixture of both (Begon & Mortimer 1987). As well as this, microbial populations in compost windrows do not normally consist of a single species, but several. In the Dundee composting system, results have shown that at least 34 microbial species or genera have been identified (Table 10). Other researchers have identified as many as 68 thermophilic species or genera (Strom 1985). This suggests that, in an average windrow ecosystem, microbial populations will, besides the pressures of intra-specific competition, be subjected to the effects of interspecific competition.

Table 10

Microorganisms Identified in Compost Windrows in Dundee During this Study

Bacillus spp.	*Salmonella* spp.
B. stearothermophilus	*Pseudomonas* spp.
B. subtilis	*Vibrio* spp.
B. cereus (type 1)	*Pasteurella* spp.
B. macerans	*Erwinia* spp.
B. pumilus	*Serratia rubidaea*
B. lentus	*Enterobacter* spp.
B. licheniformis	*Achromobacter* spp.
B. firmus	*Proteus* spp.
B. megaterium	*Aeromonas* spp.
Clostridium spp.	*Nocardia* spp.
Escherichia spp.	*Staphylococcus aureus*
Aspergillus niger	*Pseudonocardia thermophila*
Aspergillus fumigatus	*Neurospora crassa*
Penicillium spp.	*Mucor* spp.
Yeasts.	

Interspecific Competition.

While the process of interactions between members of different species will occur in a myriad of ways (fighting in animals, shading in plants etc.), ultimately, there are only three basic effects. (1) One species can either cause (or be the cause of) an increase (+), or (2) a decrease (-), or (3) have no effect (+/-) on the survival or growth of another species.

Population Growth in a Limited Environment.

The number of microbial cells observed in compost windrows depends on several factors (i.e. type of waste, environmental conditions, degree of biodegradation etc.) and the stage of the process (i.e. mesophilic or thermophilic phases). Notwithstanding those factors, population sizes are usually found between 10^2 and 10^{12} microbial cells per gram (dry weight) of material (Cooper & Gouleke 1978; Kearney *et al* 1993; Schwab *et al* 1994; Keeling *et al* 1994; Beffa *et al* 1995). Given a completely favourable environment, with unlimited quantities of resources, no intra- or interspecific competition, microbial populations will grow exponentially without a limit (Slater 1979). However, in the enclosed ecosystem of the compost windrow, exponential growth is only possible for a limited period. Inevitably, because of the accumulative effect of competition, build up of metabolic waste products, depletion of essential nutrients or changes to one or more edaphic factors, growth will become limited. Population growth in a closed environment is therefore, a self-limiting system (Poole & Hobson, 1979). In most comparable ecosystems, (i.e. the decaying log), unless there are catastrophic occurrences (e.g. fire, flood etc.), changes in edaphic factors that influence the survival or growth in populations, usually occur gradually. This gives sufficient time for the various microbial communities to adapt and develop optimum survival strategies. Compost windrow ecosystems however, can within hours of being formed, experience an edaphic change that causes fundamental and sometimes irreversible changes to the resident microbial populations. One such edaphic change is the rapid increase in temperatures that results from microbial exothermic metabolic activity (Cooper & Gouleke 1978; Strom 1985; de Bertoldi 1992; Stenbro-Olsen & Collier 1994).

Windrow temperatures are rarely constant, consisting instead of a series of repeated troughs and peaks. Such temperature changes have been described by some researchers as part of a process leading to 'microbial mass suicide'. The logic being, that high temperatures (above 55°C), will kill, or severely reduce, most if not all of the psychrotropic and mesophilic microbial populations (the so called 'composting microorganisms'), eventually eliminating them entirely (de Bertoldi *et al* 1983; de Bertoldi 1992). The metabolic activity responsible for the heat rise in the first place will now become reduced, followed by a subsequent reduction in temperature. The reduced windrow temperature will allow the psychrophilic and mesophilic microbial populations to re-establish themselves in the population depleted areas, 'migrating' from the cooler regions of the windrows and thus restarting the heating process. These phenomena have in the past, been used to justify the advice given to compost producers, that they must try to limit windrow temperatures (Stentiford 1992). However, there is a flaw in this logic, as it largely ignores or discounts any contribution to the composting process by the thermophilic microbial population.

Ecological Niches Within the Composting Process.

Windrow temperatures have been observed to remain at 65°C and above (well into the temperature range of thermophiles) for several weeks. This suggests that microbial activity must be at a relatively high level during this period, in order to sustain those temperatures. Windrows in the early stages of the composting process (first two weeks), are usually observed to undergo a reduction in the overall windrow temperatures (from ≥ 70°C to ≤ 35°C). If however, the windrows are aerated (by mechanical means), the windrow rapidly regains the previous high temperatures. A microbial population eliminated or severely reduced, would be unlikely to recolonise the windrow and expand their population to the extent that they could increase temperatures from mesophilic to thermophilic levels within such a short time period (approximately one hour). This suggests that microbial activity have not been limited by reduction in the psychrophilic and mesophilic microbial populations. Others (Finstein *et al* 1980), have also commented on this, observing that while windrow diversity falls at high

temperature, microbial activity remains high. Microorganisms that inhabit compost windrows, may do so in a number of ecological stages or temperature niches, i.e. psychrotrophic (15 to 25°C), Mesophilic (25 to 40°C) and thermophilic (55 to 75°C) etc. As windrow temperatures below 10°C are rarely encountered, psychrophilic (-5 to 15°C) populations are usually discounted as a significant 'player' in active composting, being more relevant to the cooler 'maturation' phase at the end of the composting process.

The Ecological Niche.

When data from previous a study (McDougall, 1997) on the general microbial count when incubated for 24 hrs at 37,44 and 55°C from windrows composed of a mixture of sewage-sludge and green waste (from which material would eventually be used to determine plant growth characteristics of composted sewage-sludge / green waste mixes (Irvine *et al* 1996) were plotted as a scatter gram (Fig 30), the data exhibited wide differences in population counts (up to 9 $\log_{10}$ cycles) between individual test points. The samples incubated at 44°C (Fig 31), exhibited a more constant population count between samples. While samples incubated at 55°C (Fig. 32), gave results that were similar to those seen at 37°C (Fig. 30). As the main differences in incubation environment between the samples was temperature, (the same batch of Nutrient Agar was used (Materials & Methods) throughout), the differing growth characteristics exhibited must therefore be related to how the microbial populations in the samples react to temperature.

Studies have shown that the optimum growth for most mesophiles is found between 35 and 40°C and, at temperatures over 56°C most mesophilic bacteria in the vegetative state are killed (Barrow & Feltham 1995). Therefore, at 37°C, observing a wide range of growth characteristics giving a similar wide range of data could be expected. As temperatures approach 55°C, the windrow environment starts to shift in favour of thermophilic species which, as temperatures reach a 'threshold' (i.e. a temperature below which population growth or activity is limited in thermophiles) become active. The population now is able make full use

of the available nutrients, enabling them to expand their populations to the maximum, thus giving a similar data spread to that seen in Fig 30 (37°C). It is as if the population has now entered an exclusive high temperature 'ecological niche'. This means that the combined effects of 'intra' and 'interspecific' competition are exhibited in the samples, which can be seen as a wide 'range' of population numbers. However, the 'bunching' in population numbers as observed in samples incubated at 44°C (when compared with those seen at both 37 and 55°C), suggests that there may exist a previously undescribed intermediate or transitional composting phase at around 44°C.

The Transitional Phase.

When windrow temperatures increase (from ambient to around 40°C), the psychrotrophic and mesophilic microbial populations will start to fall in number or even become eliminated. However, the temperatures are not yet sufficiently high (55°C) for the majority of, 'true' thermophiles to become established, leaving only a reduced number of, 'top' i.e. thermotolerent mesophiles with some 'bottom' thermophiles making up the remainder of the population. This phase is likely to be short lived (perhaps only a few hours). Thus, microorganisms that favour such conditions are likely to be low in numbers. This is exhibited in Fig 31 where each sample has a similar, relatively low, number of viable cells when compared with the 37°C and 55°C samples. The Transitional phase is characterised as a temporary reduction in the overall population levels. The timing of this decline in numbers will depend however, on the initial microbial species composition and on local windrow conditions (material, structure nutrients etc.) as seen in Table 11 where microbial numbers temporarily fall at around 60°C.

Fig 30. Scatter Gram of microbial populations derived from sewage-sludge/green waste mixture (experiment not discribed in this study). Using Statworks 1.2 (Cricket Software inc. Philadelphia, USA). Each open circle represents a replicate population count from samples incubated at 37°C, from test points 1 to 6. Five replicates were taken at each sample point. Where fewer than five replicates are seen this indicates the presence of replicates with identical values.

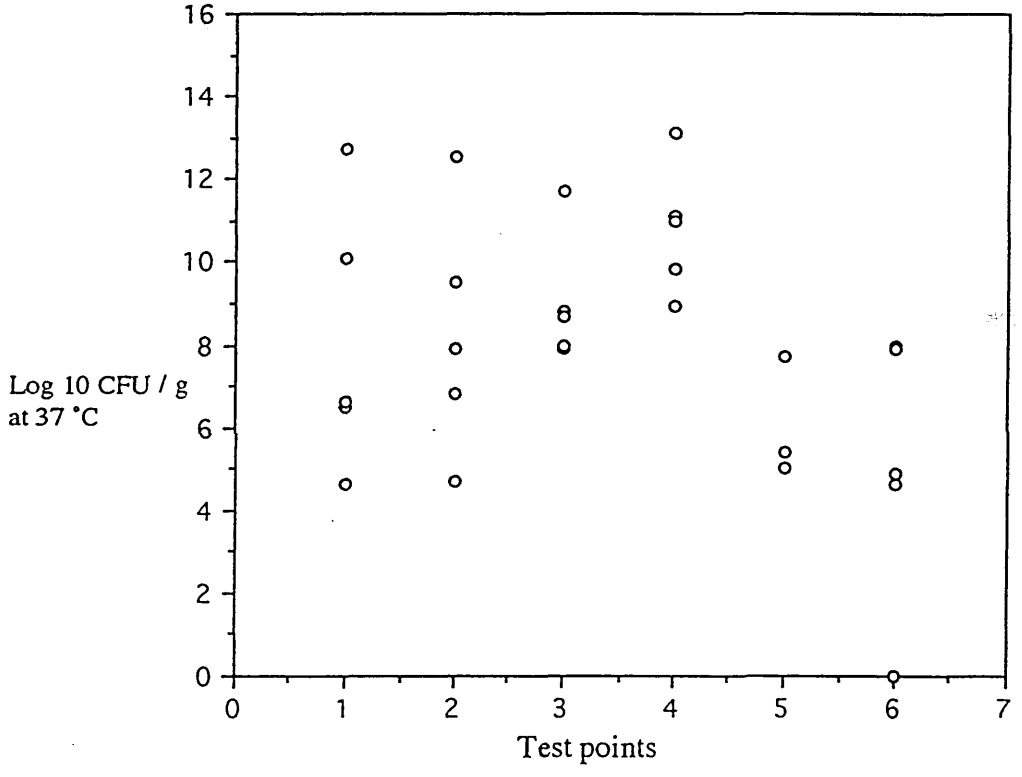

Log 10 CFU / g
at 37 °C
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Test points

Fig 31. Scatter Gram of microbial populations derived from sewage-sludge/green waste mixture (experiment not discribed in this study). Using Statworks 1.2 (Cricket Software inc. Philadelphia, USA). Each open circle represents a population count from samples incubated at 44°C, from test points 1 to 6. Five replicates were taken at each sample point. Where fewer than five replicates are seen this indicates the presence of replicates with identical values.

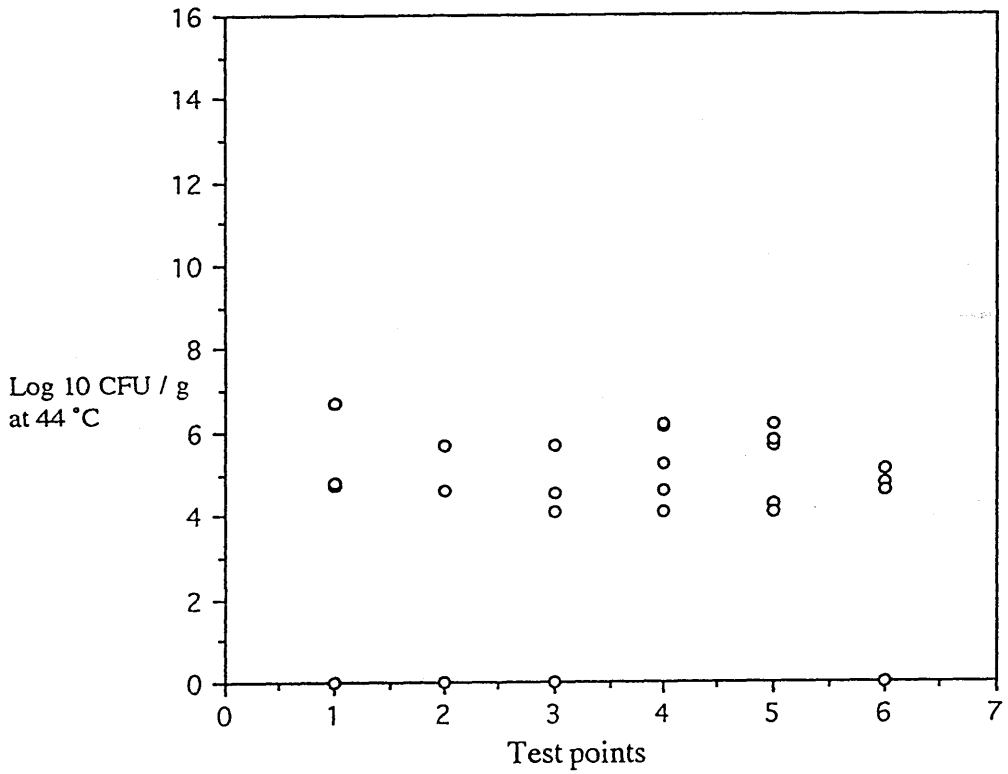
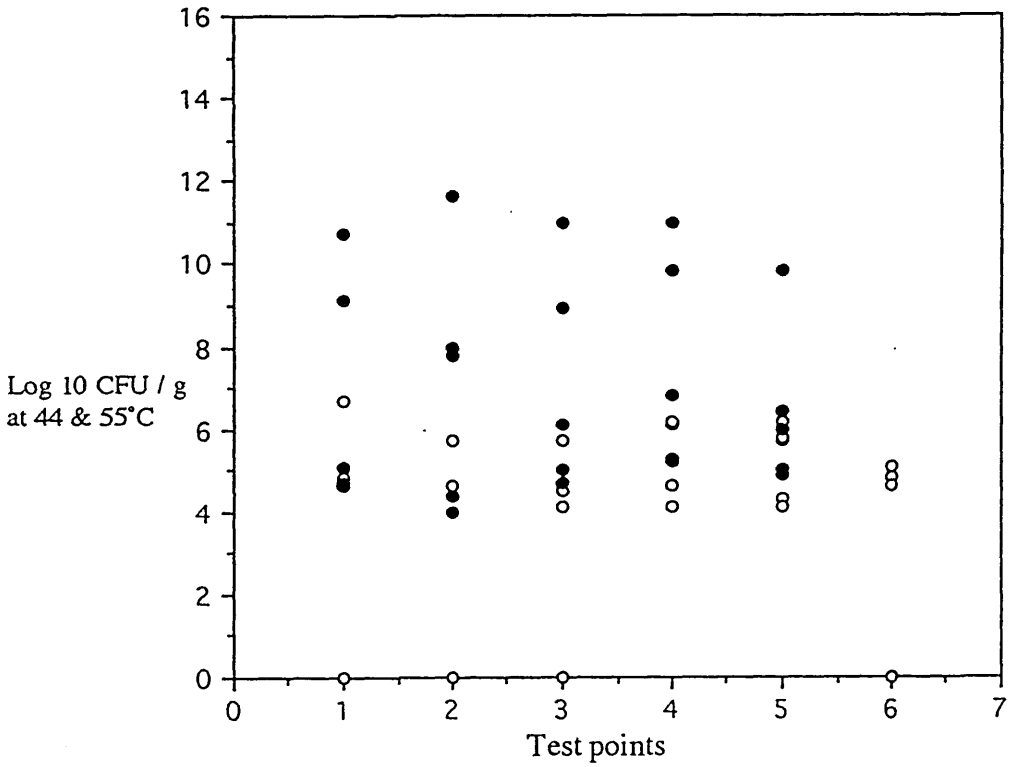

16
14
12
10
8
6
4
2
0
Log 10 CFU / g
at 44 °C
0
1
2
3
4
5
6
7
Test points

Fig 32. Scatter Gram of microbial populations derived from sewage-sludge/green waste mixture (experiment not discribed in this study) incubated at 55°C (closed circles), overlaid with the results from Fig 31 (open circles). Using Statworks 1.2 (Cricket Software inc. Philadelphia, USA). No data for 55°C (closed circles) on Tp 6 due to overgrown plates. Five replicates were taken at each sample point. Where fewer than five replicates are seen this indicates the presence of replicates with identical values.


16
14
12
10
8
6
4
2
0
Log 10 CFU / g
at 44 & 55°C
0
1
2
3
4
5
6
7
Test points

The screening of composted material while windrow temperatures are relatively high (> 60°C), is a normal procedure for operators of composting plants, (removing contaminants and oversize materials) as cool windrows can rapidly become waterlogged, thus difficult to screen due to 'balling'. In order therefore, to study the effect of temperature on microbial communities and interactions, prior to and post screening (when temperature falls towards ambient), a windrow was constructed (3 x 8 x 20 metres) using a TIM SD1000 hammer-mill shredder from a mixture (approximately 1:1) of Compostainer waste and civic amenity 'green' waste with a compacted bulk density between 400 and 600 $kgm^{-3,}$ at the Riverside composting site Wright avenue Dundee, during July 1996. Samples (3 x 30ml) were selected from windrow locations where the temperature at a 20cm depth within the windrow, was measured to be at 14°C (ambient), 42, 60 and 70°C.

Samples from each location were then divided into two (sample 'A' & 'B'). Aliquots (1g +/- 0.1g) of sample 'A' material was aseptically (within two hours of removal from windrows) added to 9ml of Ringers solution and mixed by shaking for 2 minutes. A series of dilutions was then performed up to 10^{-12}. Spread plates for enumerating viable cells were prepared (Collins & Lyne 1985) and 0.1ml aliquots from 10^{-2}, 10^{-3}, 10^{-6}, 10^{-9} and 10^{-12} dilutions (in triplicate), were aseptically removed using a micro-pipette and spread onto a labelled plate containing an appropriate growth media (Materials & Methods) using a sterilised glass spreader. All plates were incubated aerobically at the ambient temperature, 37°C, 55°C. Sample 'B' was stored aseptically at room temperature (20°C), for 24hrs before being subjected to aseptic serial dilution to 10^{-12} (Materials & Methods) and spread on nutrient agar (Oxoid CM3, Unipath Ltd. Basingstoke Hampshire UK).

Sample 'A' Population Analysis.

The overall microbial populations are represented by Figs 33 to 36 at ambient temperatures (14°C, Fig 33), the total microbial population was observed to be in the order of 4.2×10^{8} CFU g^{-1} of fresh compost. Within this population, psychrotrophic organisms were

predominant, accounting for 78.6% (3.3 x 10^8 CFU g^{-1}) of the total population. Mesophiles were the second largest group with approximately 17.9% (7.5 x 10^7 CFU g^{-1}) followed by the thermophiles with 3.6% (1.5 x 10^7 CFU g^{-1}). At the higher windrow temperature of 42°C (Fig 34), the overall microbial population was observed to increase by 678.6 % to 3.27 x 10^9 CFU g^{-1} when compared with the samples taken from the 14°C areas. Psychrotrophic organisms were still the highest in numbers (1.8 x 10^9 CFU g^{-1}) they now, however, represented only 55.0% of the overall population with mesophiles now accounting for 44.3% (1.45 x 10^9 CFU g^{-1}) of the overall population. Thermophiles, while maintaining their numbers (1.5 x 10^7 CFUg^{-1} fresh weight), now accounted for only 0.46% of the population. The reason for the 'true' thermophiles only maintaining their populations and not increasing them (as seen in the mesophilic and psychrotropic populations at lower temperatures), may be a combination of circumstances that involve temperature and the effect of interspecific competition. At 42°C, the windrow temperatures may not be sufficiently high enough to create an exclusive thermophilic ecological niche for the thermophiles to occupy, as many 'top' mesophiles will be in direct competition with the 'low' end thermophiles for space and nutrients. This intense competition may restrict microorganisms close to the edge of their 'operating envelope'.

At 60°C (Fig 35) the overall population fell to 2.85 x 10^9 CFU g^{-1}. The psychrotrophic population fell by approximately 79% (3.7 x 10^8 CFU g^{-1}) when compared with the population at 42°C and now only accounted for 13% of the overall population. The mesophilic population similarly, fell by 52% to 8.6 x 10^8 CFU g^{-1} and now accounted for approximately 30.2% of the population. The thermophilic population on the other hand, at 60°C increased by approximately two orders of magnitude, to 1.62 x 10^9 CFU g^{-1}. The thermophiles were now clearly in the majority at 56.8%. This decline in overall population numbers at 60°C, is similar to that observed in the transitional period or 'phase', noted in the sewage sludge/green waste mixture (Fig 31). However, in this case the temperature at which it occurred was higher. This may be due a large number of 'top' mesophiles (i.e. *Bacillus licheniformis* (Table 10)) found in the windrow. The transitory nature of this 'phase' was demonstrated when windrow

temperatures reached 70°C (Fig 36) and populations again increased in numbers, culminating at around 6.4 x 10^9 CFU g^{-1} fresh weight. At the highest temperature recorded (70°C), thermophiles increased their population numbers by over 100% to 3.35 x 10^9 CFU g^{-1}. However, they still only represent 52% of the total population.

Overall examination of the microbial population found that in the sample 'A', the psychrotrophic and mesophilic populations were highest in numbers between 14°C and 42°C. Both declined in numbers as the windrow temperatures reached 60°C. Thermophilic populations were relatively constant up to 42°C, and then increased significantly (by 2 orders of magnitude) as windrow temperatures climbed towards 70°C. Compost samples taken from 70°C regions of the windrow, were shown to have the largest population numbers. However, this does not mean that the population was exclusively thermophilic. While 48% of the overall microbial population could be classed as psychrotrophic or mesophilic (being able to grow at between 25°C and 37°C), some microbial species, e.g. *B. licheniformis,* while usually referred to as a mesophile, have a wide range of growth temperatures, including the ability to grow at 50 to 55°C (Barrow & Feltham 1995). Conversely, several microorganisms that are usually called thermophiles (i.e. *B. stearothermophilus*, Table 10), have been observed to have a wide range of growth temperatures, including growth at 30°C (Yanagita 1990).

Fig 33. Microbial populations in sample 'A' plotted using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), at different incubation temperatures. Samples were obtained from a 14°C windrow source. Error bars were derived from 10% data set error.

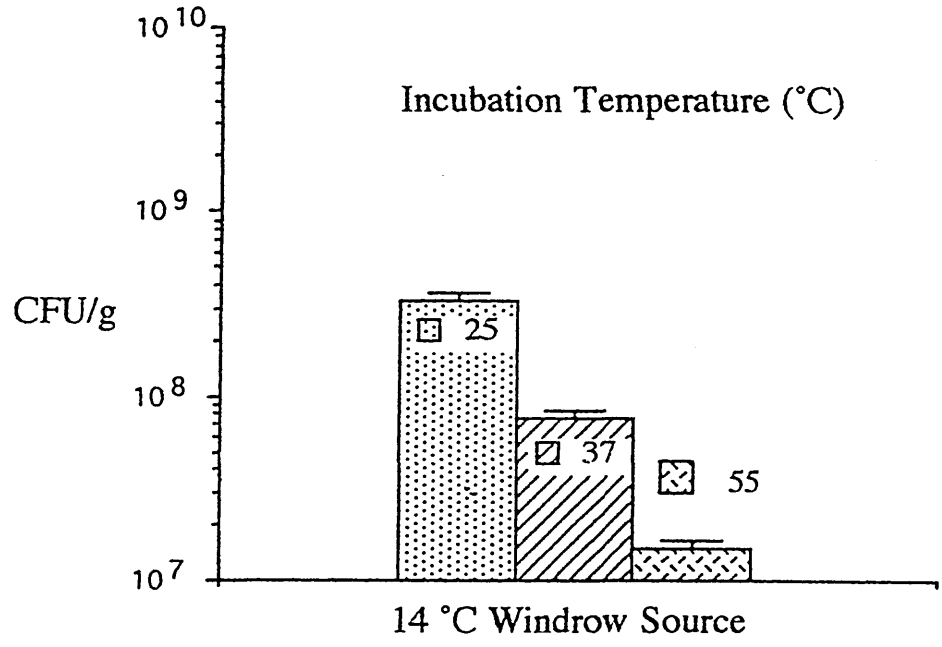

10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
Incubation Temperature (°C)
25
37
55
14 °C Windrow Source

Fig 34. Microbial populations in sample 'A' plotted using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), at different incubation temperatures. Samples were obtained from a 42°C windrow source. Error bars were derived from 10% data set error.

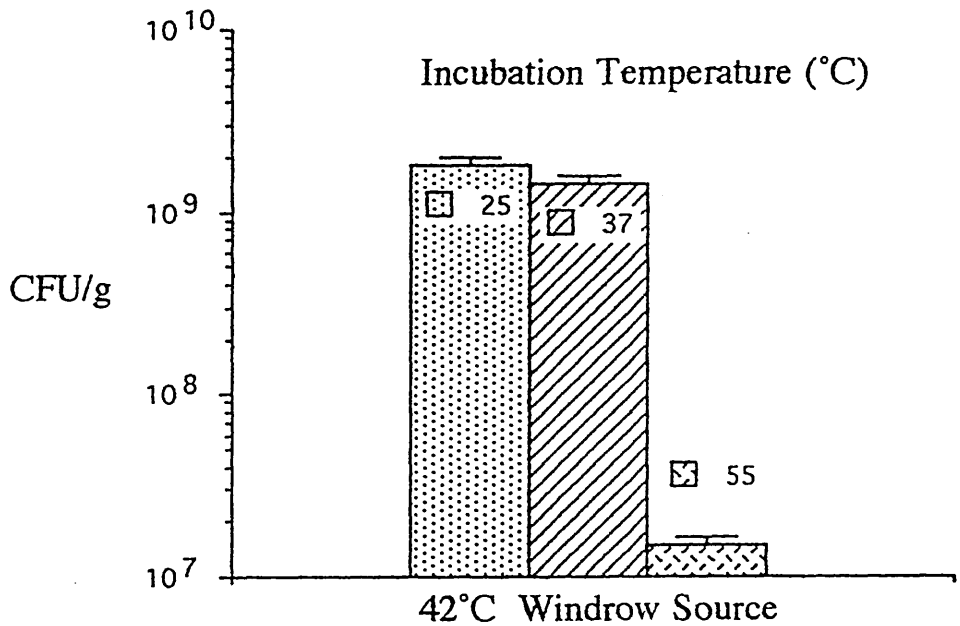

10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
Incubation Temperature (°C)
25
37
55
42°C Windrow Source

Fig 35. Microbial populations in sample 'A' plotted using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), at different incubation temperatures. Samples were obtained from a 60°C windrow source. Error bars were derived from 10% data set error.

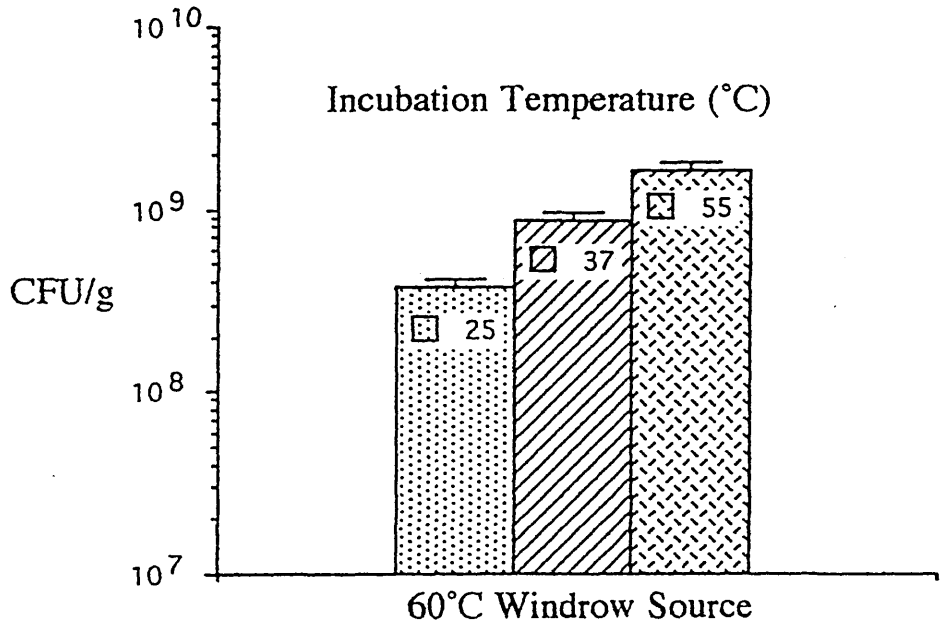
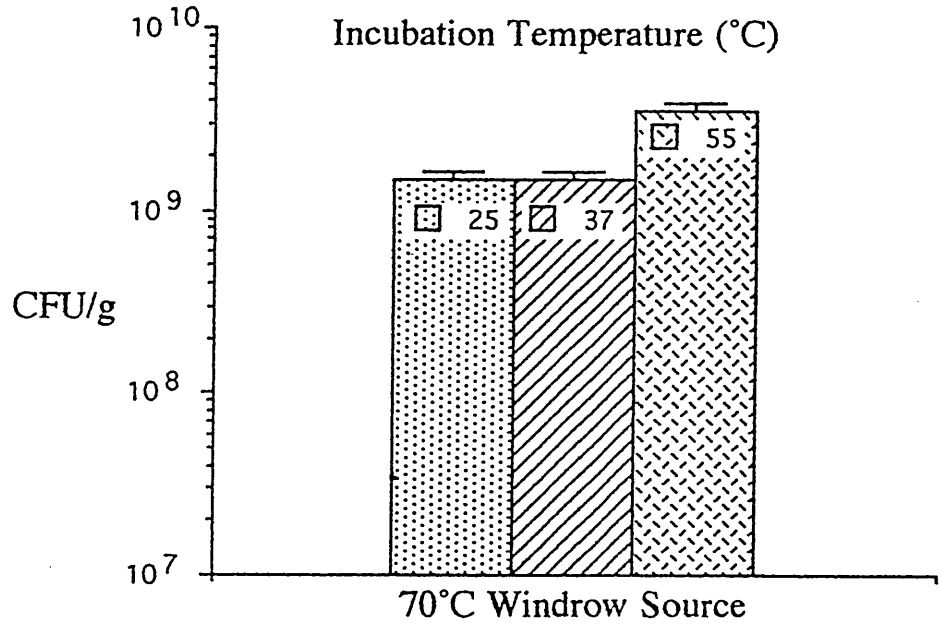

10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
Incubation Temperature (°C)
25
37
55
60°C Windrow Source

Fig 36. Microbial populations in sample 'A' plotted using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), at different incubation temperatures. Samples were obtained from a 70°C windrow source. Error bars were derived from 10% data set error.


Incubation Temperature (°C)
10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
25
37
55
70°C Windrow Source

Sample 'B' analysis.

Sample 'B' taken from 42, 60 and 70°C (Table 11 & 12) areas of the windrow and incubated at temperatures of 25, 37, 55°C (±2°C), thus simulating windrow material which is allowed to cool over 24 hrs to ambient (or near ambient) temperatures after mechanical screening. In the case of samples from the 42°C and 70°C areas of the windrow, a net decrease in overall population numbers was noted, while the 60°C samples, a net increase in population was observed. The 14°C 'B' samples were found to be overgrown with *Proteus* spp., and were not used in any subsequent analysis.

Table 11

Populations at Different Windrow Temperatures in sample 'A' compared with 'B' When grown on Nutrient Agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK.) **Incubated at 25, 37, 55°C (±2°C)**

Windrow Temperature (°C)	Population Numbers (x 10^9 CFU g^{-1})	
	Samples	
	A	B
14	0.42	no data
42	3.27	2.0
60	2.85	6.1
70	6.4	0.5

Table 12

% Changes in Microbial Population in sample 'B' when compared to sample 'A'

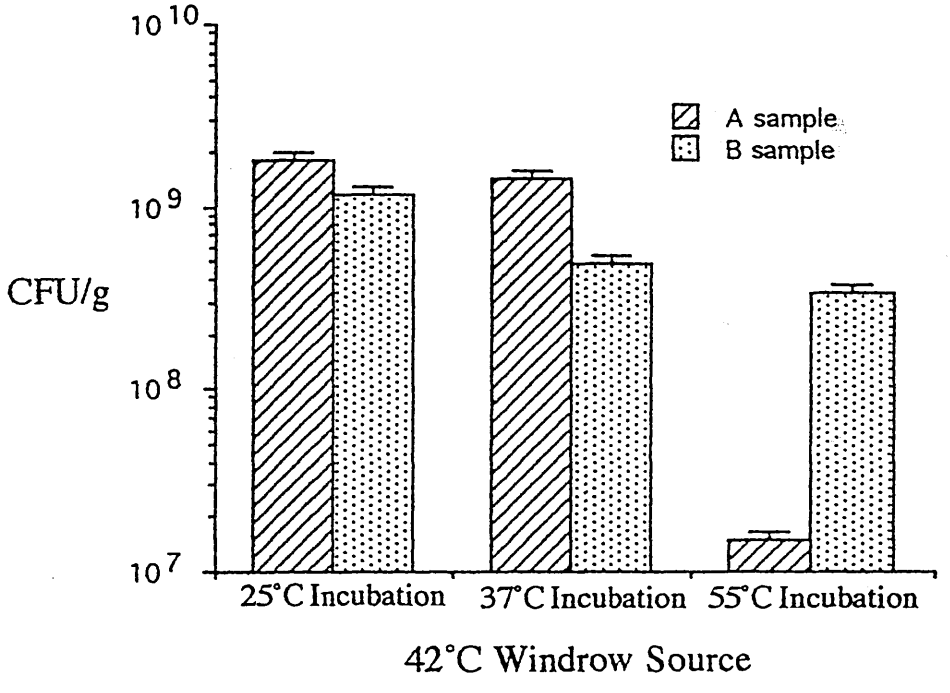
Windrow Temperature (°C)		Incubator temperature (°C, ±2°C)		
	Sample A poulation (10^9) CFU		Sample B poulation (10^9) CFU	
42	1.8		1.2	- 33
60	0.37	25	3.1	+837
70	0.005		0.42	+8400
42	1.45		0.485	-66
60	0.86	37	2.6	+302
70	0.005		0.04	+800
42	0.015		0.335	+2233
60	1.62	55	0.38	-76.5
70	3.5		0.055	-98.4
				Sample 'B' % Increase or decrease

Table 11 demonstrated that in the 'B' samples, the overall microbial populations appear to have declined. The main losses were seen in samples taken from the 70°C regions of the windrow. On further analysis (as represented by Table 12) however, a more complex situation can be seen. The 'B' samples taken from the 42°C regions of the windrow and incubated at 25 and 37°C, exhibited a net fall in populations, while the same samples incubated at 55°C saw a net increase. With the 60 and 70°C windrow samples, the opposite was observed. Increasing in population at 25 and 37°C incubation, and falling at 55°C incubation. In order to explain these phenomena, consideration must be given to the changes in microbial populations during the 24hrs at ambient temperature prior to incubation.

The 42°C 'B' Windrow Sample Incubated at 25, 37 and 55°C.

It is likely that the initial resident microbial population (represented by the 42°C Windrows sample 'A') would consisted mostly of 'top' mesophiles and 'bottom' thermophiles, with lower numbers of psychrotrophs. During the cooling period (24 hrs) however, the temperatures that sustained high numbers of 'top' mesophiles and 'bottom' thermophiles, dissipated. As a result, these microorganisms, would now be in direct competition with the surviving psychrotrophs. This increase in intra- and interspecific competition, may lead to an intense 'Scramble and Contest' situation, translating into an overall lowering of the microbial population. This is particularly noticeable at 37°C incubation, with a fall in population numbers of 66.6%, compared with 33% at 25°C incubation. 37°C is the temperature at which many mesophilic microorganisms produce their maximum growth, thus intense mesophilic / psychrotrophic competition is probable. At 25°C incubation, psychrotrophic microorganisms would predominate with the possibility of less interspecific competition thus, a smaller change in overall population numbers. At 55°C incubation however, most psychrotrophic and mesophilic microorganisms would become suppressed, and therefore, less able to compete with the thermophiles. The net results in this case (Fig 37) indicate an increase in thermophilic growth, leading to an overall increase in population size.

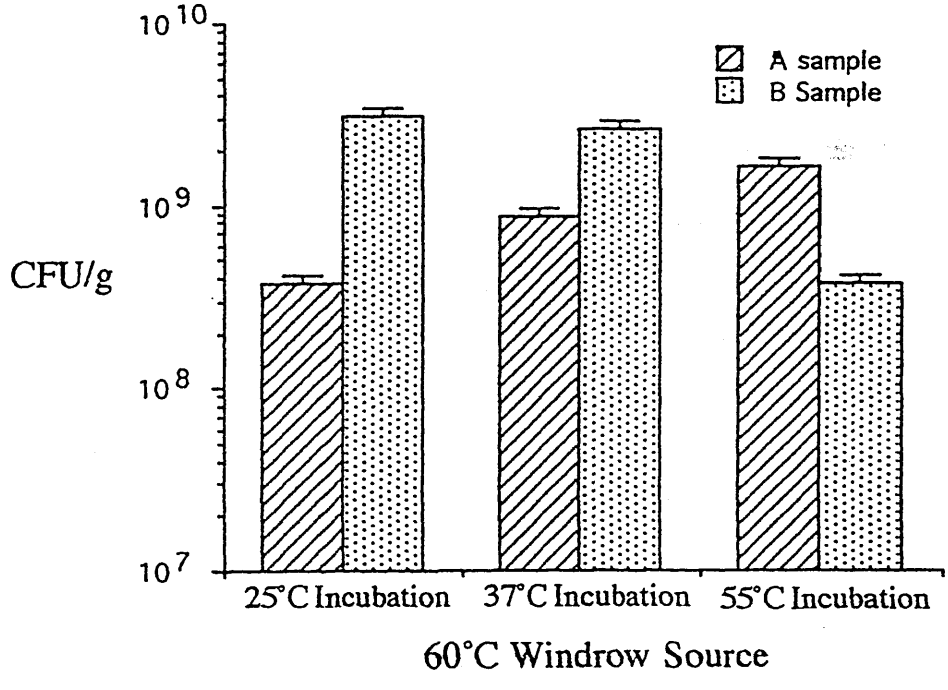
Fig 37. Comparisons using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), of the differences in microbial population numbers when incubated at 25, 37 and 55°C, between samples 'A' and the 'rested' (for 24 hrs.) sample 'B' derived from 42°C windrow source. Error bars were derived from 10% data set error.


10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
A sample
B sample
25°C Incubation
37°C Incubation
55°C Incubation
42°C Windrow Source

The 60°C 'B' Windrow Samples, Incubated at 25, 37 and 55°C.

At 60°C, the original 'A' sample would have had high numbers of thermophiles. However, these samples also contained significant numbers of thermotolerent mesophilic and psychrotrophic populations (Fig 35). During the 24hr cooling period, these microorganisms (the mesophilic & psychrotrophics) would become re-established. This is shown in Fig 38, where the samples incubated at 25 and 37°C show large population increases when compared with the original sample 'A'. However, during the 55°C incubation, populations were seen to fall by 76.5%. These phenomena may be the result of the special conditions that exist during the 'Transitional Phase'. The microorganisms that give rise to this phase would, during the 'cooling down' period, be out-competed by the surviving mesophilic and psychrotrophic population. The effect is seen in Fig 38, as large increases in sample 'B' populations at 25 and 37°C incubation. However, as in the 42°C samples, at 55°C incubation, this large increase in psychrotrophic and mesophilic populations would be negated by the higher incubation temperatures, leaving only the now much reduced, 'Transitional Phase' microorganisms. The net result is, a reduction in population (Fig 38).

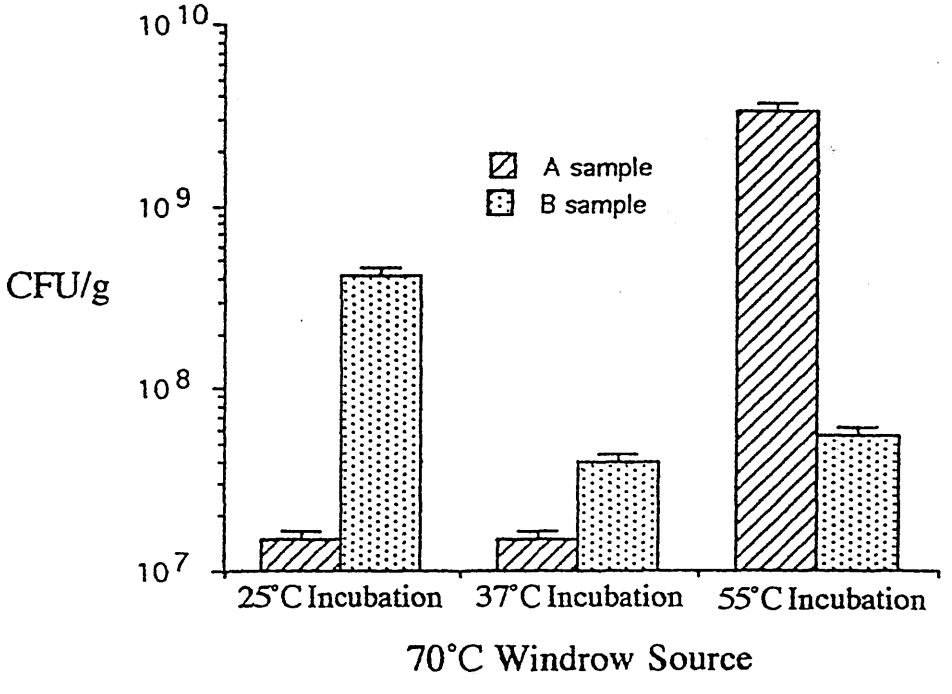
Fig 38. Comparisons using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), of the differences in microbial population numbers when incubated at 25, 37 and 55°C, between samples A and the 'rested' (for 24 hrs.) sample B derived from 60°C windrow source. Error bars were derived from 10% data set error.


10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
A sample
B Sample
25°C Incubation
37°C Incubation
55°C Incubation
60°C Windrow Source

The 70°C 'B' Windrow Samples, Incubated at 25, 37 and 55°C (Fig 39).

At 25 and 37°C incubation similar large increases in population were observed in the 'B' sample (8,400% at 25°C and 800% at 37°C). At 70°C 'true' thermophiles predominate. However, by incubating the samples from the high temperature regions of the windrow at 25°C and 37°C, surviving psychrotrophic and mesophilic and possible spores (many of which, can survive 70°C, Barrow & Feltham 1995), may germinate in the cooler conditions. In addition, vegetative cells, that may have survived the high temperatures in the windrow would start to increase their populations. As most thermophiles would find incubation temperatures of 25°C and 37°C too low for active growth, they would be at a competitive disadvantage and would be suppressed in favour of psychrotrophic and mesophilic populations. While both mesophilic and psychotropic microorganisms expanded their populations, the psychrotrophic (incubated at 25°C) population displayed the greatest increase in sample 'B' (8,400%). The reason for this could be that mesophilic populations usually require a higher temperature for optimum growth (i.e. 37°C). Thus, they may have been at a competitive disadvantage with the psycroptrophs when incubated at lower temperatures.

Fig 39. Comparisons using Cricket Graph 1.3.2 (Cricket Software inc. Malvern, USA), of the differences in microbial population numbers when incubated at 25, 37 and 55°C, between samples A and the 'rested' (for 24 hrs.) sample B derived from 70°C windrow source. Error bars were derived from 10% data set error.


10^{10}
10^{9}
10^{8}
10^{7}
CFU/g
A sample
B sample
25°C Incubation
37°C Incubation
55°C Incubation
70°C Windrow Source

In the original 'A' sample the mesophilic population stood at 3.35 x 10^9 CFU g^{-1}. After cooling, it fell by 98.4% (Table 12, 70°C 'B' sample). This is further evidence that either significant survival of 'low' temperature microorganisms, or post sampling 'immigration' of psychrotrophic and mesophilic populations has effectively suppressed the thermophilic population during the 'cooling down' period. Even when the incubation temperatures are restored to thermophilic levels, this suppression has left the thermophilic population in a condition where they are unable to respond effectively.

Conclusions.

Most microorganisms will adopt a strategy for competition and survival. These are often referred to as 'K' (Fig. 40) or '*r*'(Fig 41) strategies, where '*r*' strategists tend to maximise their 'fitness' for survival, by reproducing rapidly in an uncrowded environment. 'K' selective individuals on the other hand, maximise fitness by making a large proportional contribution to a population that remains close to its carrying capacity (Begon & Mortimer 1987). While such a simple dichotomy is not always adequate to describe life-history strategies of all microorganisms, it can be regarded as typical of the 'gains and losses' to which compost windrow microbial communities are subjected. The overall data suggests that most microorganisms have evolved an '*r*' strategy, responding quickly to a change in the environment which has lead to a reduction in competition for space and resources. While this may herald a change in the type of microbial population, maximum microbial numbers however, tend to be similar to those of the original (now displaced) population carrying capacity.

The data also suggests that the ecological niche inhabited by thermophiles is usually exclusive, in that few other microorganisms can tolerate these environmental conditions. This means that, in respect of interspecific competition, thermophiles, once established in their niche, will have little if any effect on psychrotrophic and mesophilic populations and *vice versa* (i.e. their overall effect is +/-). However, at temperatures of 60°C or below, the data suggests that some

thermophiles are not efficient competitors when faced with re-emerging mesophilic populations. Under these circumstances the net effect on the thermophilic population is negative. The reason for this phenomenon may be that thermophiles are only truly active in environmental conditions that are hostile and unsuitable for most psychrotrophic and mesophilic microorganisms. Thus, no effective competitive strategy to deal with non-thermophilic microorganisms has been evolved by thermophilic organisms. Consequently, in temperatures where psychrotrophic and mesophilic organisms are able to grow, they may restrict or even exclude the majority of thermophiles that could be active under the same conditions, or if not active, prevent them from becoming re-established when windrow temperatures increase. This series of experiments has also shown that as temperatures increase, microbial populations go through a transient phase where the bulk of the psychrotrophic and mesophilic populations are eliminated or are no longer active, but temperatures are not yet at a level where 'true' thermophiles are able to expand their populations. In these situations overall populations tend to fall (Table 11). The data also shows high composting temperatures (60 & 70°C) for short periods of up to 48 hrs, will not entirely eliminate the viability of microorganisms regarded as psychrotrophic or mesophilic. It has also been shown that high temperatures do not reduce the overall microbial population, as has been previously suggested. But may, because of intra- and interspecific competition between microorganisms, actually increase it.

Fig 40. Lifestyles typical of populations producing 'K'-selecting individuals in 'K'-selecting environments. 'K'-selected individuals maximise fitness by making a large proportional contribution to a population that remains close to its carrying capacity. (Adapted from Begon & Mortimer 1986).

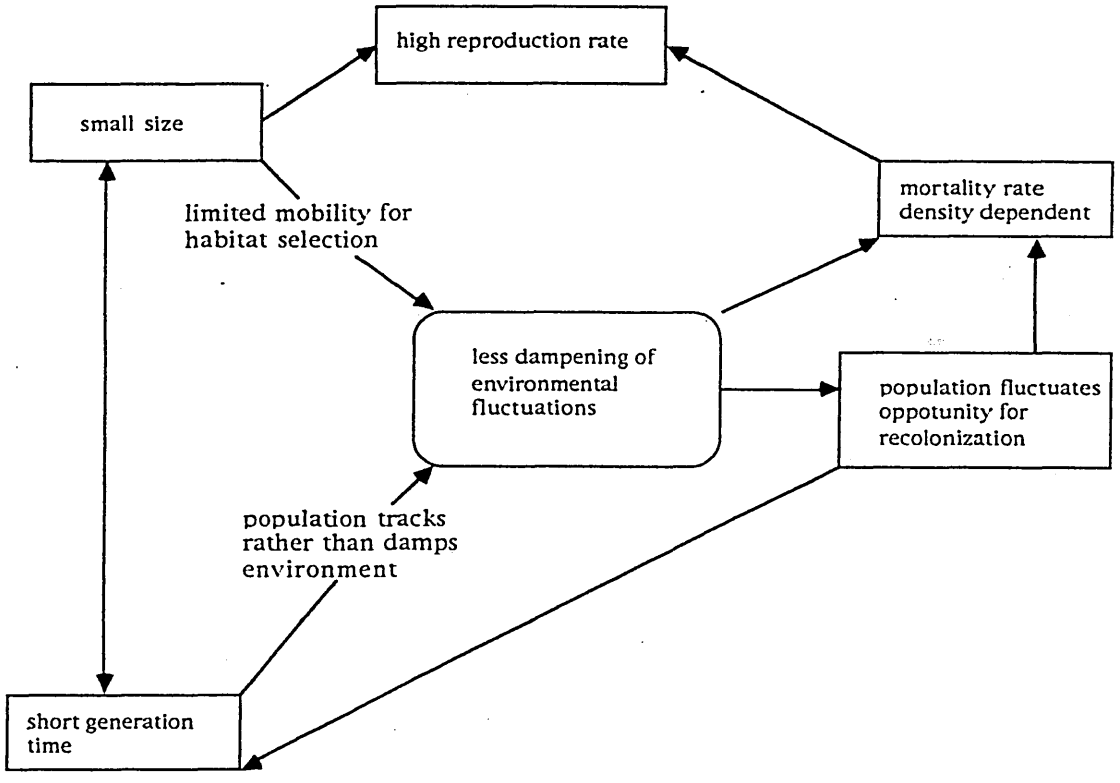
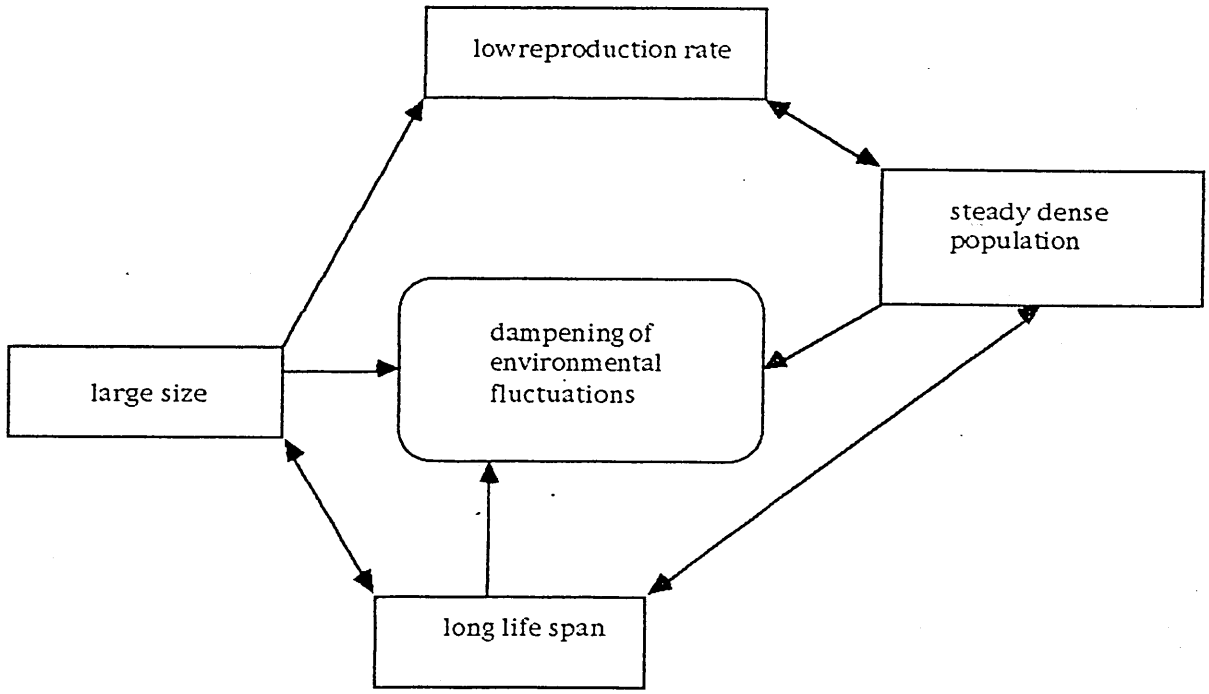

high reproduction rate
small size
mortality rate
density dependent
limited mobility for
habitat selection
less dampening of
environmental
fluctuations
population fluctuates
oppotunity for
recolonization
population tracks
rather than damps
environment
short generation
time

Fig 41. Lifestyles typical of microbial populations leading to '*r*'-selecting individuals in '*r*'-selecting environments. '*r*'-selecting individuals adopt a strategy that tends to maximise fitness by enabling them to reproduce rapidly in an uncrowded environment (Adapted from Begon & Mortimer 1986) .


low reproduction rate
steady dense population
dampening of environmental fluctuations
large size
long life span

THE ISOLATION IDENTIFICATION AND CHARACTERISATION OF A NOVEL THERMOPHILIC COMPOST BACTERIAL SPECIES

The general advice given to operators of large scale composting systems, is to try to ensure that windrow (or composting vessel) temperatures are kept below 55°C. Higher temperatures, it is assumed, reduce microbial numbers and diversity, resulting in a reduction of composting efficiency (Stentiford 1992). However, microorganisms that are active at high temperatures are not unusual. Several species have been identified whose normal high temperature habitats include geothermal environments etc. (Llarch *et al* 1997). The advice advocating keeping temperatures relatively low largely ignores the observed ecology of composting and in particular, the microbial successions within active windrows. Studies have shown that it is not only possible to isolate high numbers of viable microorganisms (10^5 to 10^9 g^{-1}) at high temperatures, but also a large variety of species (>72) (Strom 1985). This number includes several species that have a minimum growth temperature of 40°C (Beffa *et al* 1995). Analyses of the microbial population of windrows in Dundee found similar numbers of microorganisms at windrow temperatures of 70°C (Table 11) . Upon observing this, an experiment was designed to expand further, the knowledge regarding microbial populations in green waste composting at high temperatures.

A recently established windrow (3 x 8 x 12 metres) using a TIM SD1000 hammer-mill shredder containing 'urban green' waste with a bulk density of approximately 600 kgm^{-3}, was chosen at random from the Dundee City Council composting site (Wright Avenue, Dundee). Using a 1.5 metre thermocouple probe attached to a Model 1000 portable

thermometer (-50 to +200°C with a resolution of +/- 0.1°C Industrial Pyrometer Co Ltd., Birmingham BE 6QY, UK) confirmation was obtained of the existence of high temperatures (>than 65°C) within a windrow which had been established for approximately 7 days. This was achieved by taking 16 temperature readings at a 1 metre height above ground and at 20 and 80 cm depths at equal intervals along the windrow's length (12 metres) and one at each end. A note was taken of the readings at each depth and averaged using the statistical programme of a Casio Fx 1250 electronic calculator. The region of the windrow where average temperature was observed to be in the range of 65 to 75°C, was designated as a 'high temperature area'. From this area, 10 samples (a total of 250ml) were aseptically removed from a depth of 1 metre, within $1m^2$ of the chosen high temperature area. The samples were aseptically mixed within a Class 2 laminar flow cabinet (Envair Q-Flow 915, Rosendale Lancs. BB4 4HX. UK.) in a sterilised 3 litre container.

A sample (1 x 10ml) of the mixed windrow material was aseptically added to 90ml of sterile 0.9 % saline solution and mixed (by shaking) for 2 minutes. After a further 2 minutes at rest, the mixture was subjected to serial dilution sequence to 10^{-3}, 10^{6}, 10^{9} and 10^{2} dilutions and were plated in triplicate (plus 3 negative controls), onto nutrient agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK.). The plates were incubated for 24hr at 55°C. Single colonies of similar morphology were isolated and replated in triplicate (plus 3 negative controls) on to nutrient agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK.) and incubated at 60, 65 and 70°C temperatures. In order to establish the maximum temperatures at which the isolates could grow, colonies observed to grow at 70°C, were further subjected to increasing incubation temperatures (3°C steps) until no growth was

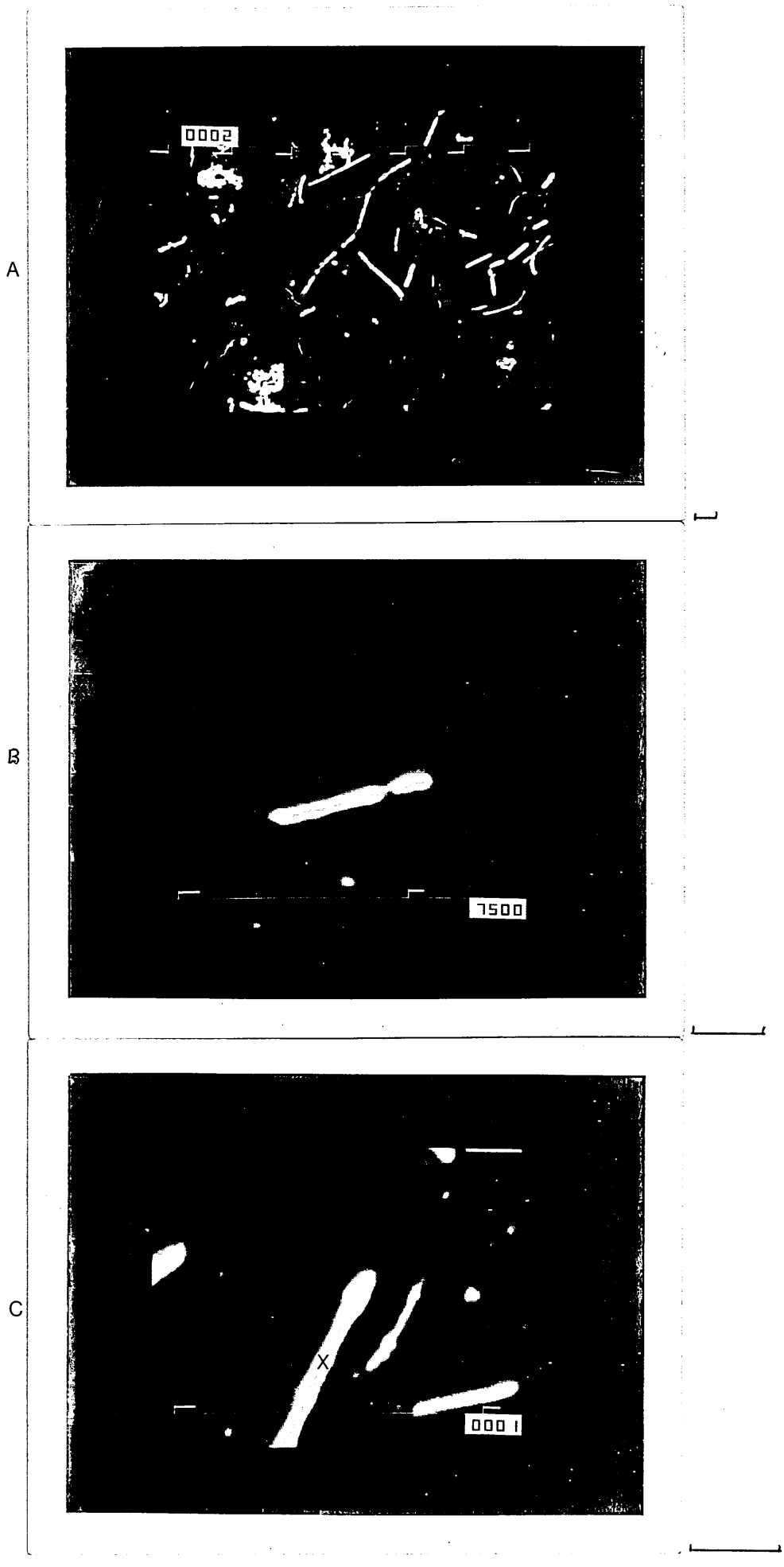
apparent. The maximum temperature at which growth was observed in this way was 73°C (+/-2°C). An isolated colony grown at this temperature was subsequently identified as isolate BB001. This isolate was characterised by a typical and widespread morphology. Colonies of this type were the large majority of colonies present on these plates, indicating that this particular isolate was the most common microorganism active at these elevated temperatures. Upon Gram staining and subsequent microscopy the cellular morphology of these colonies was shown to be consistent.

General Characteristics of Isolate BB001

Under a phase contrast microscope (x1000 magnification) and scanning electron microscope up to x10,000 magnification, isolate BB001 appears as rod shaped bacterial cells with oval spores within swollen terminal sporangia (Barrow & Feltham 1995). The length was measured at 0.8 x 15 μm (Fig 42) and tested Gram-positive (Barrow & Feltham 1995). The temperature range for growth was determined by incubating on nutrient agar (Oxoid CM3, Unipath Ltd, Basingstoke Hampshire UK.) at a minimum of 20°C (ambient) and then observing growth at increasing incubation temperatures (5°C steps to a maximum 80°C+/-2°C). In this way the growth range was determined to be a minimum of 40°C, with a maximum of 73°C. As plates were readily ovegrown at 65°C, optimum growth temperature was assumed to be 65°C (+/- 2°C). At this temperature the isolate was observed to form short branched chains of up to five cells (Fig 46), with some occurring as individual cells. When grown at 70°C (+/- 2°C), on nutrient agar (Oxoid CM3, Unipath Ltd. Basingstoke Hampshire UK.), isolate BB001 produced pale yellow irregular raised or flat colonies, with 'fern' or 'rhizoid' like structures on mainly lobate or undulating margins. Despite noting

that a number of general characteristics (shape, temperature range, etc.) were similar to previously described thermophilic microorganisms (Beffa *et al* 1995; Llarch *et al* 1997), no firmly expressed identification to species level could be made at this stage. However, as isolate BB001 was observed to be aerophilic, thermophilic, rod shaped, spore forming and testing Gram positive, it is reasonable to suggest that, BB001 belongs to the Genus *Bacillus*.

Fig 42. Scanning electron microscope (JROL T100, JROL, Tokyo, Japan) micrograph of unknown *Bacillus* BB001, at x 2000 (A), 7500 (B), and 10,000 (C). In micrograph C, X indicates BB001. Bars represents 10μm.


A
2000
B
7500
C
X
1000

In order to test the hypothesis that isolate BB001 is a member of the genus *Bacillus*, it was compared with a number of other members of the Genus *Bacillus* which had been positively identified to species level, (i.e. *B. stearothermophilus, B. subtilis, B. megaterium, B. licheniformis,* and *B. macerans*) in terms of: carbohydrate utilisation, enzyme production, reaction to temperature and salinity of substrates etc.(Holt *et al* 1994, Llarch *et al* 1997).

Biochemical (Carbohydrate) Analysis

Where possible, biochemical analyses were carried out by using rapid identification kits API 50 CHB and API 20E (BioMerieux sa, 69280 Marcy Etoile, France), used according to manufactures instructions (Materials & Methods), (Barrow & Feltham 1995). Results were noted at 6, 12 and 24 hr intervals and interpreted with APILAB (BioMerieux sa, 69280 Marcy Etoile, France) computerised diagnostic programs. The results are displayed in Table 13. Data for comparison microorganisms were obtained from published data. (Holt *et al* 1994)

Table 13

Comparisons Between Isolate BB001 and others of the Genus *Bacillus*.
Carbohydrate Use.

Test / Isolate	BB001	*B. stearothermophilus*	*B. subtilis*	*B. megaterium*	*B. licheniformis*	*B. macerans*
Glycerol	-	-	+	+	+	+
Erythitol	-	-	-	-	-	-
D arabinose	-	-	-	-	-	-
L arabinose	+	-	+	+	+	+
Ribose	+	-	nd	+	+	+
D xylose	-	-	-	+	+	+
L xylose	-	-	-	-	-	-
Adonitol	-	-	-	-	-	-
methyl-Dxyloside	-	-	-	-	-	+
galactose	+	-	-	-	+	+
Glucose	+	+	+	+	+	+
Fructose	+	+	+	+	+	+
Mannose	+	+	+	-	+	+
Sorbose	+	-	-	-	-	-
Rhamnose	+	-	-	-	-	+
Dulcitol	-	-	-	-	-	-
Insitol	-	-	+	-	+	+
manitol	+	+	+	+	+	+
Sorbitol	-	-	+	+	+	+
Methyl-D mannoside	-	-	-	-	-	-
Methyl-D glucoside	+	-	-	-	+	+
N Acetyl Glucosamine	+	-	-	+	+	+
Amydgdalyn	+	-	-	+	+	+
Arbutin	+	-	+	+	+	+
Aesculin	+	+	+	+	+	+

Table 13

Comparisons Between Isolate BB001 and others of the Genus *Bacillus*

Isolate / test	BB001	*B. stearothermophilus*	*B. subtilis*	*B. megaterium*	*B. licheniformis*	*B.macerans*
Salcin	+	+	+	+	+	+
Celiboise	+	+	+	+	+	+
Maltose	+	+	+	+	+	+
Lactose	-	-	-	+	+	+
Melibiose	+	+	-	+	+	+
Sucrose	-	+	+	+	+	+
Trehalose	+	+	+	+	+	+
Inulin	-	-	+	+	-	+
Melezitose	-	-	-	+	-	+
Raffinose	-	-	nd	+	+	+
Starch	+	+	+	+	+	+
Glycogen	-	-	+	+	+	+
Xylitol	+	-	-	-	-	-
Gentibose	+	-	+	+	+	+
D-Turianose	+	-	-	nd	+	+
D-Lyxose	+	-	-	-	-	+
D-Tagatose	+	+	-	-	+	-
D-Fucose	+	-	-	-	-	-
L-Fucose	+	-	-	-	-	+
D-Arabitol	-	-	-	-	-	-
L-Arabitol	+	-	-	-	-	-
Gluconate	-	-	-	-	-	-
2-Keto Gluconate	-	-	-	-	-	-
5-Keto Gluconate	+	nd	-	-	-	-

+ reaction. - no reaction, nd no data

Table 13

Comparisons Between Isolate BB001 and others of the Genus *Bacillus*.

Test / Isolate	BB001	*B. stearothermophilus*	*B. subtlis*	*B. megaterium*	*B. licheniformis*	*B. macerans*
Gram Reaction	+	+	+	+	+	+
Anaerobic growth	+	-	-	-	+	+
Catalase	+	+d	+	+	+	+
Cytochrome Oxidase	+	+d	+d	+d	+d	
Sporangium swollen	+	+	-	-	-	+
Growth at 30°C	-	-	+	+	+	+
Growth at 40°C	+	+	+	+d	+	+
Growth at 50°C	+	+	+d	-	+	+d
Growth at 55°C	+	+	-	-	+	-
Growth at 65°C	+	+	-	-	-	-
Growth at 70°C	+	+	nd	nd	nd	nd
Growth at 75°C	-	+(Yanagita 1990)	nd	nd	nd	nd
Growth at pH 5	-	-	nd	nd	nd	-
Growth at pH 6.8	+	+	+	+	+	+
Growth at pH 5.7	+	-	+	+d	+	+
Growth at 4.5% NaCl	+	nd	+	nd	+	-
Growth at 5% NaCl	-	+d	+	nd	+	-
NO3 reduced to NO2	-	+d	+	+d	+	+
NO2 reduced to N2	+	-	-	-	+d	-
Gas from KNO3	+	nd	nd	nd	nd	nd
Gas from Glucose	-	-	+	-	some	+
Formation of Dihydroxyacetone	+	-	nd	nd	nd	-

+ reaction. - no reaction, nd no data, +d 11-89% +ve

Table 13

Comparisons Between Isolate BB001 and others of the Genus *Bacillus*.

Test / Isolate	BB001	*B. stearothermophilus*	*B. subtlis*	*B. megaterium*	*B. licheniformis*	*B. macerans*
Citrate utilisation	-	+d	+	+	+	+d
Casine hydrolysis	-	+d	+	+	+	-
Lipase hydrolysis	-	nd	+d	nd	+d	nd
Tween hydrolysis	+	-	-	+d	-	-
Indole production	-	-	-	-	-	-
Acetoin production (VP test)	+	-	+	-	+	-
pH in VP	6.3	<6	<6	<6	<6	<6
Tyrosine degraded	-	-	-	+d	-	-
Phenylalanine deamination	-	-	-	-	-	-
NaCl & KCI required	-	-	-	-	-	-
Allantoin required	-	-	-	-	-	-
Arginine dihydrolase	-	nd	-	-	+	nd
β-Galactosidase	-	nd	+d	+	+	nd
Chitinase	+	nd	nd	-	nd	nd
Lysine decarboxylase	-	nd	-	-	-	nd
Acetate	-	+	nd	+d	nd	nd

+ reaction. - no reaction, nd no data, +d 11-89% +ve

Table 13

Comparisons Between Isolate BB001 and others of the Genus Bacillus.

Test / Isolate	BB001	B. *stearothermophilus*	*B. subtlis*	*B. megaterium*	*B. licheniformis*	*B. macerans*
Glutarate	-	nd	nd	nd	nd	nd
Glycine	-	nd	nd	nd	nd	nd
Lactate	+	nd	nd	nd	nd	nd
Malate	+	nd	nd	nd	nd	nd
Malonate	+	nd	nd	nd	nd	nd
Manitol	+	nd	nd	nd	nd	nd
Pyruvate	+	nd	nd	nd	nd	nd
Succinate	+	nd	nd	nd	nd	nd
Tartrate	+	nd	nd	nd	nd	nd
Mobility	+	nd	nd	nd	nd	nd
Gelatinase	+	nd	nd	nd	nd	nd
Asparate	+	nd	nd	nd	nd	nd
Glutamate	-	nd	nd	nd	nd	nd
Gelatinase	+	nd	nd	nd	nd	nd
Orithine decarboxylase	-	nd	nd	nd	nd	nd
Urase	-	nd	nd	nd	nd	nd
Tryptothane deaminase	+	nd	nd	nd	nd	nd
Casine hydrolysis	-	nd	nd	nd	nd	nd
H2S production	-	nd	nd	nd		

+ reaction. - no reaction, nd no data

Numerical Taxonomic Analysis.

Numerical taxonomic analyses examine the similarities between different isolates by observing the number of individual tests which give the same or similar results (Priest & Austin 1993). However, in comparing some differences between isolates, a degree of subjectivity is inevitably introduced, i.e. colony colour, shape etc. In these cases a judgment has to be made in assigning a similarity score (eg.1 for not similar and 5 for identical or 2, 3 and 4 for degrees of similarity). While diagnostic tests that attempt to quantify subjective data are available (Gower's coefficient, Priest & Austin 1993; Barrow & Feltham 1995), it was felt that such analysis relied too heavily on individual observer's adjudication of results. In order to avoid this, only non-subjective data from Table 13 was used in tests.

There are several different discrimination coefficients in use that attempt to quantify non-subjective data (i.e. negative or positive reactions). In many of these coefficients, matches that are positive (+) are given the letter **a**, negative (-) the letter **d**, and non-matching or dissimilar characteristics, i.e. isolate 1 + and isolate 2 - or isolate 1- and isolate 2 +, **b** & **c** respectively. Each test is then 'scored' and a percentage 'relativeness' calculated. The two numerical analyses most often employed in bacterial work are (Preist & Austin 1993):

Jaccard discriminating coefficient $S_j = a / (a+b+c)$ (23)

Simple Matching discriminating coefficient $S_{sm} = (a+d) / (a+b+c+d)$ (24)

In Jaccard's system, negative scores (d) are discounted; i.e. only if both isolates react positively to a diagnostic test or one isolate is positive and the other negative, will a score be made. The rationale being that, only tests that elicit a positive response between isolates can be measured. In Jaccard's system the range of values are from 0 (for total dissimilarity) to 1 (total similarity). The simple matching dicriminating coefficient on the other hand, measures positive and negative matches as a proportion of the total number of characteristics. As in Jaccard's system the range of values are 0 (for total dissimilarity) to 1 (total similarity).

Results (Numeric Taxonomy).

The scores for each category (a,b,c,d) were obtained by comparing isolate 1 (BB001) with a number of known isolates (isolate 2) from the data obtained from Table 13. As a control, *B. megaterium* was compared with *B. subtilis*. The results are summarised in Table 14.

Table 14
BB001 Comparisons with other Isolates data.

Coeficient Data

BB001 Compared to:	**a** both +	**b** Isolate 1 + Isolate 2 -	**c** Isolate 1 - Isolate 2 +	**d** both -
B. stearothermophilus	22	23	5	26
B. subtilis	22	20	13	20
B. megaterium	23	20	7	18
B. licheniformis	33	13	11	18
B. macerans	31	13	12	17
Control	27	4	6	32

a = number of times both isolates were positive for a test. **d** = number of times both isolates were negative for a test. **b**= isolate 1 positive and isolate 2 negative. **c** = isolate 1 negative and isolate 2 positive.
Control *B. megaterium* to *B. subtilis*

By applying formula 23 (S_j) or formula 24 (S_{sm}) to the data obtained from Table 14, a percentage similarity index can be obtained. These values are presented in Table 15.

Table 15
BB001 Percentage similarity to other *bacillus*

Percentage similarity index

BB001 Compared to:	**Jaccard S_j % index**	**Rank**	**Simple matching S_{sm} % index**	**Rank**
B. stearothermophilus	44	4	63	3
B. subtilis	40	5	56	5
B. megaterium	46	3	60	4
B. licheniformis	56	1	68	1
B. macerans	55	2	66	2
Control.	73		86	

Table 15, indicates that the numeric values giving a percentage similarity, depend to some extent on which system is used. However, both the Jaccard and Simple Matching systems agree that *B. licheniformis* is the closest species match (ranked 1) with *B. macerans* in second place. However, convention rules that when using numerical taxonomy, for an unknown isolate to gain genus status, the score (percentage of similarity) must be greater than 65%. To reach species status, this score must be greater than 95%. Only in the Simple Matching index is genus status achieved with regard to isolate BB001. In both systems, the controls (*B. megaterium* to *B. subtilis*) are confirmed as belonging to the same genus.

Pattern Differences (PD).

Jaccard and Simple Matching systems simply note that there are degrees of similarity between isolate BB001 and comparison microorganisms. The percentage similarity however, gives no real clue or indication of which of the several tests employed were similar (e.g. it may be important to know that the Gram reactions were different). PD tries to address this point by the adoption of polar diagrams (Crothers 1987). PD displays data in clusters or natural groupings, i.e. if two isolates were able or unable to use a particular carbohydrate (e.g. Glucose), but only one of the isolates was able to use sucrose, then two distinct data points would have been created. Each data point (as in Simple Matching Coefficients) is then assigned a numerical value. The numeric values in themselves are not significant, however, their positions are, as areas of the PD will be exclusive to a range of results, e.g. carbohydrate results. In this way a pattern of matches and non-matches is created with dark (match) and light (non-match) areas.

The PD Graph.

Like most graphs the PD graph has an X and Y axis. In this case, The X axis refers only to the number of individual tests and forms a full 360° circle and the Y axis has only two values, 5 if there is a no match between isolates and 20 if a match has been found. If two isolates give identical results on all tests, e.g. all tests either matched or did not match, then, in both cases, the graph would display just one value, i.e. 20 in the case where all tests gave the same result, or 5 in the case where all results were different. However, such a circumstance would be unusual. A more likely result would be a mixture of 5s and 20s. which would be displayed as light (score 5) and dark (score 20) pattern (Fig 43 & 44). Therefore, the greater number of matches, the darker the pattern on the polar graph.

Further information can be gained by grouping the tests under major headings (e.g. carbohydrate utilisation, temperature, enzyme production etc.) and placing them on a dedicated part of the X axis (Fig 43). This enables further information to be obtained on the difference between individual isolate's activity in that area, e.g. if two isolates have similar responses to a range of carbohydrate tests, it would score a large number of 20s in that part of the X axis reserved for carbohydrates, which would result in a predominantly dark pattern in that area. Fig 43 shows the basic lay out for the PD diagrams used in comparing BB001 with a range of microorganisms belong to the Genus *Bacillus*. The 0° to 90° section is reserved for carbohydrate utilisation, the 90° to 180° section for temperature etc.

Fig 43. Polar Pattern Graph (PD). General layout of the graph. Matches (score 20) results in a dark area from the centre to the edge of the graph (20 points on the central scale). Non matches (score 5) results in a dark area extending only to the 5 point on the central scale.

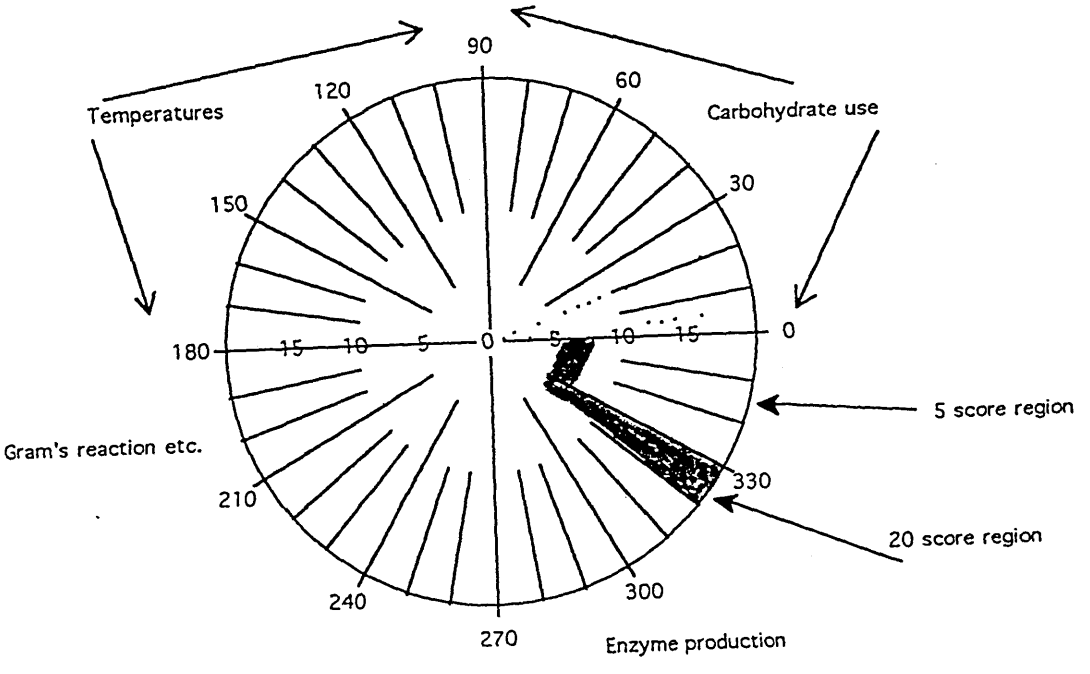

90
60
30
0
330
300
270
240
210
180
150
120
15
10
5
0
5
10
15
Temperatures
Carbohydrate use
Gram's reaction etc.
Enzyme production
5 score region
20 score region

Fig 44. Polar Pattern (PD) Graph of comparisons between BB001 and:

A: *B. stearothermophilus*

B: *B. megaterium*

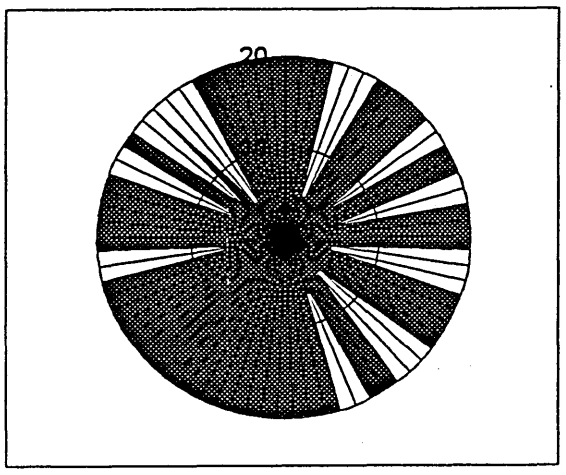
C: *B. subtilis*

D: *B. licheniformis*

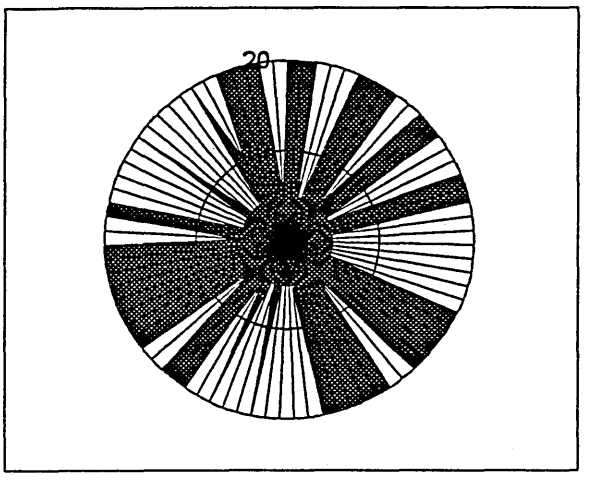
E: *B. macerans*

Control: *B. megaterium* compared with *B. subtilis*

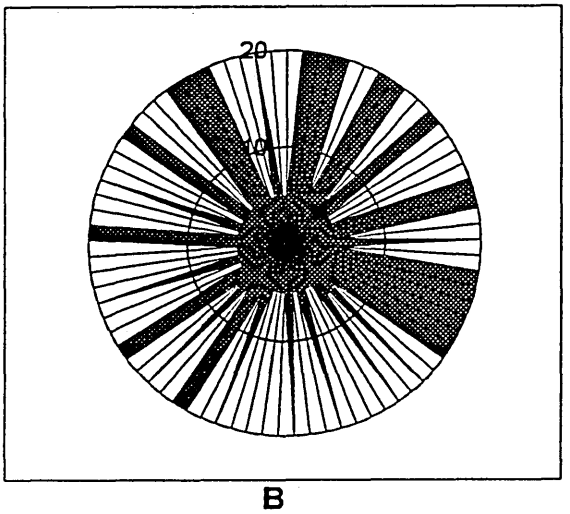
Dark areas relate to matches, light areas non-matches.



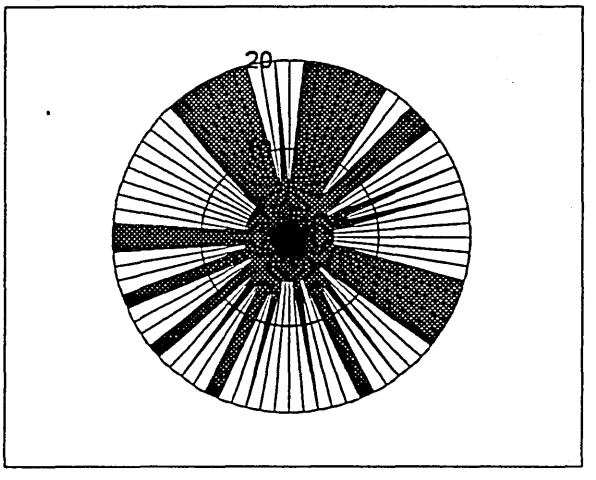
Control



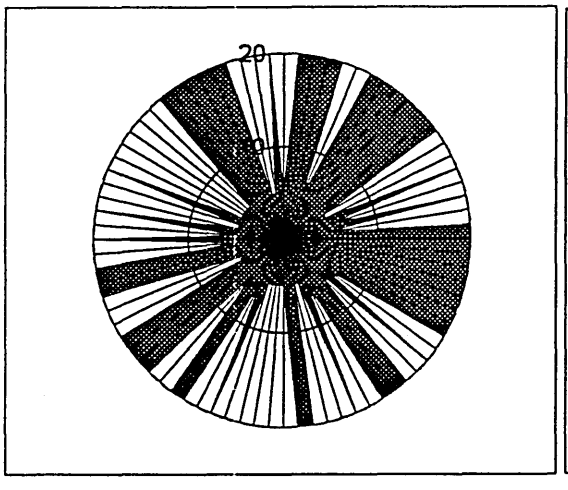
A



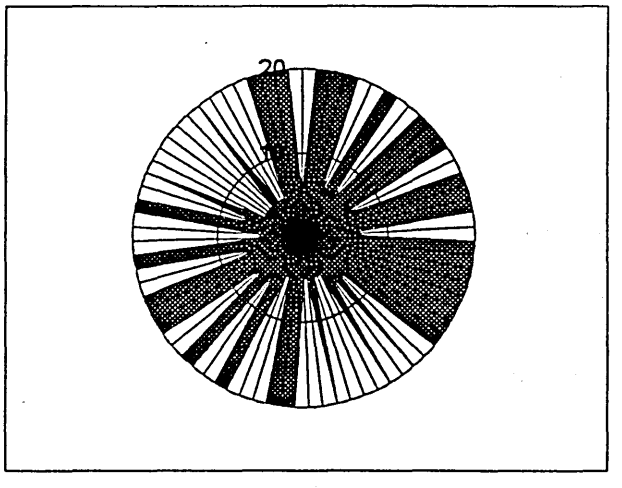
B



C



D



E

Results of Matching Bb001 to Other Members of the Genus *Bacillus* Using Pd.

When compared with the control (*B. megaterium to B. subtilis*), none of the comparisons (A-E; Fig 44) appear to be a good match. However, it can be observed that in the area of carbohydrate use (0-90°) and enzyme production (270-0°) larger number of matches (dark areas) can be seen, while in the temperature range (90-180°) a more varied picture emerges. In this way, differences and similarities between isolates and where those differences may lay are easily seen, supplementing the results from other systems. In conclusion, while isolate BB001 shares many of the characteristics with regard to carbohydrate use and enzyme productions, it is sufficiently different to be regarded as a novel bacterial species.

Isolate BB001's 'Ecological Niche'.

Environments found within a composting windrows can be highly variable especially in regard to temperature. As temperatures within windrows range from ambient to well over 70°C (Fig 22) and, that the microbial populations within those windrows change significantly (Fig 33, 34, 35, & 36), an attempt was made to ascertain the ecological position or niche of isolate BB001 within the observed temperature regimes. Besides temperature changes, moisture content within windrows is subjected to change (Fig 20) which in turn is reflected in alterations in the overall salinity. This in turn, can effect the osmoregulation processes within microbial cells. The microorganism's ability to contend with these changing environments, may give information about the 'ecological niche' of BB001 and identify further its roles within composting.

Temperature.

Identifying organisms by the temperature at which they grow is common practice. As BB001 has a relatively restricted range of growth temperature (42 to 73°C, Table 13) an attempt was made (by using the system devised by Yanagita 1990) to categorise BB001's 'temperature classification'.

Table 16

Classification of Thermophilic Microorganisms (Yanagita 1990).

Growth range (°C)	Division
32-100	Facultative Thermophile
55-78	Moderate Thermophile
55-100	Obligate Thermophile
78-100	Extreme Thermophile

With a temperature range for growth of 42 to 73°C, using this classification, isolate BB001 was confirmed as a moderate thermophile, thus able to tolerate the high temperatures commonly found in compost windrows.

Salinity.

In a small closed ecosystem such as a windrow, the water content can vary significantly (from less than 20% to over 50%, Fig 20) which can in turn affect the 'salt' levels within the windrow. Changes in 'salt' contents of windrows can be assessed by measuring their electrical conductivity. This may be achieved by mixing an air dried sample (10ml) (Materials & Methods) of windrow material with 50ml of deionised water in a clean 100ml

plastic container (giving a sample to water ratio of 1: 5). This was shaken for 2 minutes in order to form a slurry. A calibrated (Materials & Methods) conductivity probe (Hanna Instruments HI 8820 digital ATC bench conductivity meter with 'built in' temperature compensation; Hanna instruments Ltd. Bedfordshire LU7 8TZ UK) was then placed in the slurry and the reading noted and expressed as micro-Siemens (μS) cm^{-1}. The soluble salt content of a windrow can be calculated by equation 25 (Palintest England):

$$\text{soluble salts (mg } l^{-1}) = \text{conductivity } (\mu S\ cm^{-1}) \times 3.5 \qquad [25]$$

Isolate BB001's Salt Tolerance.

In order to ascertain isolate BB001's salt tolerance, i.e. its ability to grow in a range of salt concentrations, growth media with differing salt content were prepared, into which isolate BB001 was inoculated. The minimum NaCl content of standard Nutrient Agar CM3 (Unipath Ltd. Basingstoke Hampshire UK.) is 0.5% w/v (Bridson 1990).This concentration was used as the NaCl 'baseline'. A number of Nutrient Agar CM3 mixtures were prepared in which the NaCl content was incremented by 0.5% (w/v) to a maximum of 10% w/v by the addition of laboratory grade NaCl (Sigma ACS reagent S-9888, Sigma chemical's Co, St.Louis USA.). Each modified growth medium with the additional NaCl, was prepared (Materials & Methods) and poured into triple vented plastic disposable Petri dishes.

Each modified growth medium was inoculated from the same BB001 culture. Once inoculated, the media was placed in an incubator set to 68°C (+/- 2°C) for 24hrs. Once the point had been reached where no growth was observed after 24hrs a note of the NaCl

concentration was taken and incubation was continued for another 48hrs or until such time that no growth was observed with a maximum time of 72hrs. Once this point had been reached (i.e. no growth observed), the previous growth medium in which growth was observed, was deemed as the maximum salt concentration of Nutrient Agar which could support growth of isolate BB001. In this way the maximum value of NaCl content in the growth media at which isolate BB001 is able to sustain growth was found to be 4.7% w/v (0.7M).

A system of classification for microbial growth in a range of NaCl concentrations, has been devised by Yanagita .

Table 17

Classification of Bacteria According to NaCl (Yanagita 1990).

Type	NaCl Concentration (M)
Non Halophile	< 0.2
Slight Halophile	0.2-0.5
Moderate Halophile	0.5-2.5
Extreme Halophile	2.5-5.2 (saturated)

Using Yanagita's (1990) classification, isolate BB001 is classed as a moderate halophile. The conductivity of compost windrows can range from <10 to 4100μS cm^{-1} (Iglesias-Jiménez & Alvarez 1993) and thus, may at some stages become slightly toxic to cells either through dehydration, or precipitation of intra-cellular proteins. Halophilic microorganisms

are much less affected by increased levels of sodium (Lynch & Fletcher 1979). Therefore, being a moderate halophile may give isolate BB001 an ecological advantage within the windrow over other microorganisms, especially in areas of high conductivity.

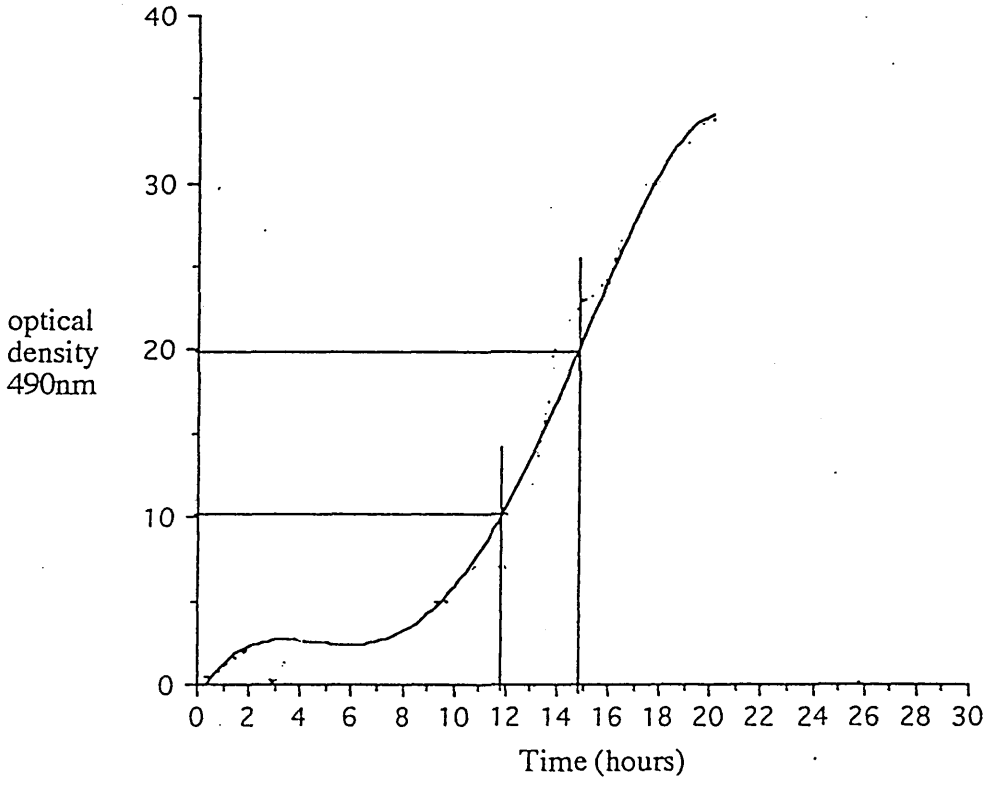
Composting windrows while subjected to the described environmental conditions, are not normally environments of extreme (i.e. very high or low temperatures, very high salinity etc.), in which case, isolate BB001's 'status' as both a moderate thermophile and halophile would mean that it can tolerate most of the conditions found within an average windrow, and can also tolerate conditions where temperatures and salinity are somewhat higher than normal. Thus, this organism enjoys a competitive advantage when conditions move towards the extremes.

BB001 Growth Characteristics (*in vitro*).

In order to examine isolate BB001 growth rates, the population doubling time was measured. This was achieved by mixing 1 ml of BB001 microbial suspension (Materials & Methods) with 250 ml of nutrient broth (Oxoid CM1 Unipath Ltd. Basingstoke Hampshire UK.) in a 500ml conical flask. The broth containing the microbial suspension was then incubated at 68°C (± 2°C) for 24hrs. Aliquots of culture (10 ml) were aseptically removed every hour and the optical density (OD) was measured at a wavelength of 490 nm after which the aliquot was returned aseptically to the culture. A growth curve was plotted from the readings of OD 490nm against time. The growth curve obtained was typical of that found in many thermophiles (Yanagita 1990), in that it displayed a long lag phase (approximately 8 hrs) followed by logarithmic growth with a doubling time of approximately 3 hrs (Fig 45).

Test for motility were carried out by the Craigie Tube method (Materials & Methods), in which the exposed glass tubes were incubated with microbial suspension (100μl) of isolate BB001 and incubated at 65°C overnight. Motility was determined by observing whether or not the microorganism had migrated out of the glass tube into the surrounding growth medium. Isolate BB001 was observed to have migrated out of the inoculation tube after 12 hrs of incubation suggesting that the cells are motile.

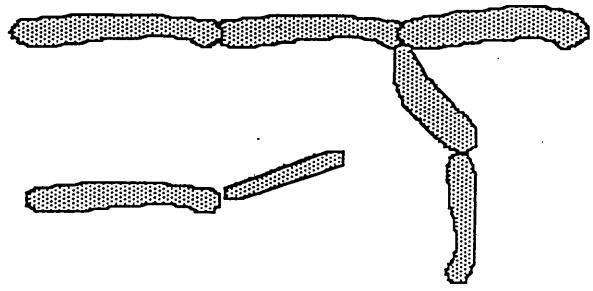
Fig 45. Growth rate of isolate BB001 over 24 hrs as measured at 490 nm, using Palintest model 5000 photometer (Palintest Ltd. Gateshead, Tyne & Wear, NE11 0NS KK). The doubling time (t_d) was 3 hrs.


optical
density
490nm
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30
Time (hours)

In order to further examine isolate BB001's growth characteristics, Nutrient Agar (Oxoid CM3, Unipath Ltd. Basingstoke Hampshire UK.) filled Petri plates (3 plus control; plastic disposable 75mm triple vented) were inoculated (100μl) at a single point, with isolate BB001. Without disturbing the inoculation, the plates were aerobically incubated at 65°C (+/- 2°C) for 16 hrs (overnight). The colonies formed elongated 'finger' like structures approximately 1cm in width, growing in parallel lines away from the inoculation point towards the margins of the Petri dish. A further 8 hrs of incubation at 65°C resulted in plates being overgrown. Light microscope analysis of cells taken (after 16 hrs) from the colony's leading edge showed single cells with little evidence of the formation of chains. Analysis of those cells found within the 'body' of the colony (close to the inoculation point) recorded short chains of three to four cells which were strongly Gram-positive. The general cellular morphologies of both samples were similar. Consideration of these phenomena, suggest that the BB001 is able to modify its selection / survival strategy by initially adopting an '*r*'-strategy when faced with no competition. This would enable it to rapidly colonise uncrowded substrates with highly motile cells. However, as the population increases, the BB001 maximises its fitness by adopting a K-selection strategy and thus, stabilising its population at, or near the population carrying capacity (Begon & Mortimer 1987). In this way the isolate ensures the best use of available resources. An analogy of this type of microbial behaviour can be seen in microbial populations that inhabit the roots of plants. Plant root apices are a rich source of microbial nutrients (i.e. mucilage and sloughed off plant cells). However, root growth can be rapid which may mean that the microorganisms that initially inhabited root apices, could be left behind. The result of this is that population density at root apices tend to be low, thus favouring those microorganisms that can adopt

'*r*'-strategies of rapidly building their population. Those far from the root tip (those left behind), grow less densely under the limitations set by the K -election mechanism (Yanagita 1990).

Fig 46. Typical colonial morphology of BB001 isolate when grown on nutrient agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire) at 65°C (+/- 2°C). Typical chain lengths were in the region of 3 to 4 cells.



Typical chain

During aerobic composting, energy metabolism (oxidative phosphorylation) usually depends upon oxygen as the final electron acceptor. Cultures of the BB001 have been grown *in vitro* under anaerobic conditions (using Anaerocult C mini; E Merk Microbiology D64271 Darmstadt, Germany) on Nutrient Agar (Oxoid CM3. Unipath Ltd. Basingstoke Hampshire UK.), suggesting that this isolate is a facultative anaerobe and thus, able to tolerate reduction in oxygen tension. This is a useful attribute for a composting organism, as oxygen levels within the windrow are likely to fall as a result of the build up of waste metabolites and the collapse of void spaces. This attribute may allow a degree of competitive advantage, especially at high temperatures where oxygen tension falls rapidly. The adoption of an '*r*'-survival strategy under such circumstances would reinforce BB001's competitive advantage at high temperatures. The morphology of BB001 colonies grown under anaerobic conditions, were markedly different from those grown under aerobic conditions. While total numbers of cells were comparable, many were Gram-variable and the 'fern' or 'rhizoid' like structures observed from aerobic cultures, was missing. Cells were also much smaller (1-2 μm in length as opposed to 12-15μm when incubated aerobically), growing either as single cells or short straight chains of two or three cells. Most of the aerobic attributes were however, reinstated when anaerobic cultures were subsequently regrown under aerobic conditions.

One reason for these changes during anaerobic growth, may be that while the isolate can grow and undertake metabolic functions under anaerobic conditions, it may find itself under 'stress' and hence, trigger survival strategies similar to those observed in microorganisms growing under nutrient depleted conditions. Compost windrow ecosystems are inherently

unstable in that they are subjected to large fluctuations in temperature, changing oxygen and water content and alterations to nutrient type and availability, as well as being subjected to regular mechanical disruptions. In such unfavourable conditions, several survival strategies have been observed. Some microorganisms will reduce all metabolic processes to a dormant or near dormant state, while others will concentrate their activities on the production of specific proteins, ATP, RNA and increasing membrane transport activity. This means that if environmental conditions improve, the cell will be in a better condition to respond immediately and thus, have a competitive advantage over those microorganisms that opted for dormancy (Yanagita 1990). A reduction in cell size is also common in microorganisms, as a response to adverse environmental factors, as this permits the cells to be more efficient in obtaining what little energy is available (Gilbert *et al* 1990). There is much evidence to suggest that the BB001 adopts just such a strategy (i.e. reduced cell size), when faced with reduced oxygen tension in the windrow. This enables the organism to respond almost immediately when aerobic conditions are restored. Evidence for this can be seen in windrows where the temperatures have been falling for some days, exibit a rapid rise in temperatures after being subjected to aeration processes (Fig 22).

Carbohydrate Assimilation.

The main source of nutrients in 'green' waste for microbial growth and metabolic activity, are usually carbohydrates and in particular, polysaccharides such as starch and cellulose (Ouellette 1997). For microorganisms to hydrolyse these compounds directly, extracellular enzymes are normally required. In the bacteria found most frequently in compost windrows (i.e. *B. stearothermophilus, B. licheniformis, B. amyloliquefaciens* etc.), the most

commonly produced enzyme is amylase (Crueger & Crueger 1990). Cellulase activity in bacteria associated with composting is however, relatively rare, and is more commonly associated with fungi (Gould & Corry 1980). Of those cellulase producing bacteria that have been found in compost windrows, Actinomycetes predominate and in particular, *Pseudonocardia thermophila*. Cellulase producing microorganisms take on an important role in composting by hydrolysing cellulose to a form more amenable for other microorganisms (Coghlan 1996). This is particularly important with regard to isolate BB001. While BB001 is able to use a range of carbohydrates both by oxidation and fermentation, including some complex carbohydrates such as disaccharides (ribose and maltose) and oligosaccharides (D-fucose), and displays some evidence of weak amylase activity, most of the carbohydrates utilised were monosaccharides. This suggests that BB001 may only have a limited ability to hydrolyse the major constituent of green waste directly, cellulose. This might be regarded as a significant liability to the overall composting process. However, as previously discussed, a compost windrow can be viewed as a self-contained ecosystem. Decomposition reactions within that windrow, depend not only on the numbers of organisms, their physiological state and characteristics, but also the microbial community and especially, the process of microbial succession.

The Microbial Succession within the Compost Windrow.

Initially, a newly formed windrow will contain high levels of easily available nutrients (simple sugars etc.) and windrow temperatures will be at ambient levels. This means that much of the early microbial activity will be carried out by the psycrotrophic and mesophilic microorganisms that were originally present (i.e. *B. subtilis, B. macerans, Pseudomonas*

spp. etc. and fungi such as *Aspergillus* spp.;Table 2). Isolate BB001 at this point (as temperatures are below its 'working level'), remains relatively inactive and contributes little if anything, towards the composting process. However, as composting proceeds, the abundant supply of readily available nutrients, means that microbial activity will be high, both in biodegradation processes and expanding populations. The net result is that windrow temperatures rise. In most correctly formed windrows, the temperature will reach thermophilic levels (>55°C) within 24hrs. This rise in temperature will rapidly kill (or incapacitate) the vast majority of psychrotrophs and probably most of the mesophiles (Fig 35 & 36). In a 'normal' ecosystem, such an event will inevitably trigger a negative feedback response, in which the reduction in microbial activity will result in a general cooling down of the system (Begon & Mortimer 1987). Once this happens and conditions return to 'normal', renewed microbial activity by surviving psychrotrophs and mesophiles, will restart the process. In the case of a composting ecosystem however, windrow temperatures tend to remain high for long periods (Fig 11 & 12), suggesting that this type of feedback system is not operating. This is partly due to the heat insulation effect of windrows causing temperatures to lag behind microbial activity and, the effect of the microbial population moving from a predominant psychrotrophic and mesophilic mix, to an almost exclusively thermophilic one. The BB001 isolate is in the vanguard of these changes.

Table 13, shows that BB001 is able to hydrolyse only a limited number of complex carbohydrates when compared with other microorganisms found in windrows, which could be considered an evolutionary disadvantage to the isolate. However, this need not be the case if the biological interactions taking place within a windrow ecosystem and, in

particular, synergisms are taken into account. This means that the organisms that are able to break down the relatively insoluble polymeric plant cell wall carbohydrates into soluble oligo-, di-, and monosacchirides, (e.g. a number of fungi including *Aspergillus* spp.) could in turn provide substrates for other microorganisms such as BB001 (Hawker & Linton 1971, Lynch 1979). Furthermore, as most microbial cells contain significant carbon (up to 50%) and nitrogen (10-15%) (Hawker & Linton 1971), the nutrients required could be obtained from the dead cells of the previous population (killed by the rapid rise in windrow temperature). With sufficient nutrients and rising temperatures, isolate BB001 may also adopt an '*r*' survival strategy (rapid colonisation), which may explain the rapid growth in the thermophilic populations (Fig 35 & 36) observed. The depleted mesophilic and psychrotrophic populations, would allow isolate BB001 to take full advantage of the available nutrient supply, thus becoming dominant within the windrow. The net effect of this is that composting continues at high temperatures, usually reaching a maximum windrow temperature of around 70 to 75°C. Therefore, isolate BB001 is in every sense, a vital part of the composting ecosystem. The survival strategy adopted by thermophiles such as isolate BB001, ensures that even in the event of the suppression (or elimination) of whole populations of psycrotrophic and mesophilic microorganisms from an ecosystem, the microbial succession ensures that the windrow ecosystem remains viable. Such occurrences are indeed vital in the composting process, as the maintenance of high temperatures is essential in ensuring that pathogens have less of a chance of surviving the process.

Conclusions.

As discussed in the introduction, composting has been practised for centuries in one form or another. In recent times however, there has been a tendency to adopt a reductionist philosophy especially regarding mechanised composting. Using highly sophisticated computer aided devices based on algorithms derived either from mathematical formulae based on laboratory models (Schwab *et al* 1994), or from modifications of similar processes, mechanised composting seeks to control all aspects of composting (aeration, pH, water content, temperature etc.), as separate functions. This type of approach is often called, 'engineering with microbes' (Haug 1993). Much of this engineering effort however, ignores the fundamental premise of composting: that it is a natural process involving living organisms in a self-contained ecosystem. The attempt to control one or more of the parameters (i.e. temperature) of such an ecosystem in isolation, indicates a misunderstanding of the basic underlying principles of composting. The isolation of isolate BB001 has expanded our knowledge regarding microbial life in compost windrows, especially at thermophilic temperatures. A previously unknown thermophile (belonging to the genus *Bacillus*) assimilating the detritus formed by the death of psychrotrophic and mesophilic microbial populations as windrow temperatures rise, has been described. This microbe (with other thermophiles), form an essential part of the composting process. It has also been shown that high temperatures during composting do not necessarily lead to a reduction in microbial numbers, only a change from a predominantly mesophilic to a predominantly thermophilic populations. This knowledge may spur designers of composting systems (especially the in-vessel types where environmental control systems are common) into evaluating ecological aspects of composting alongside engineering ones which may in time,

lead to better and more efficient designs.

DISCUSSION

The growth of industrialised farming practices since 1945, has meant that large amounts of organic material, the traditional source material for plant nutrients, is now regarded as a waste problem, to be managed by landfilling or burning (Walker, *et al* 1997). Coupled to this, has been the general increase in the amount of waste being produced from both industrial and domestic sources (Coopers & Lybrand 1996). This has meant that the disposal of waste is now becoming a serious environmental problem. In the UK, the traditional disposal route for this ever growing amount of waste, is landfill. However, concern over the production of 'greenhouse' gases, i.e. methane, from organic waste in landfill, has lead to pressures from special interest groups (both global and local, e.g. Friends of Earth, Greenpeace etc.) and public opinion, to reduce the amount of waste going into landfill. These pressures have prompted governments into producing a growing volume of environmental legislation (i.e. the 1990 Environmental Protection Act 1990 and the Environment Act 1995 (Great Britain 1995)). All these pressures among other things, seek to reduce the environmental impact of waste. Add to this a series of UK Government targets for recycling and waste reduction, a succession of EU directives, some of which, are directly aimed at reducing the amount of organic material destined for landfill (draft Landfill Directive; Battersby 1994), and for the first time, the taxing of material going for landfill disposal (Great Britain 1996) means that landfill is becoming much less viable as a waste disposal route, and may in time (in a way that is analogous to the process of evolution), even become extinct.

The search therefore, for alternative methods of organic waste disposal has become a matter of urgency. Composting has for some time been regarded as having the potential for a long term alternative to landfilling (Fig. 9) and is rapidly becoming the first choice in the disposal of organic waste for many waste disposal companies. However, despite composting's long history, many of the processes that occur within the windrows are poorly understood, and in particular, the role of the bacterial communities. If composting is to realise its full potential,

detailed examinations of these processes will be needed. Composting is predominantly aerobic and initially, an exothermic process in which each microbial CFU as single cells will contribute between 4.98 x 10^{-13} $MJkg^{-1}$ and 8.52 x 10^{-13} MJ kg^{1} of heat energy (calculation 14 &15). This heat energy can raise the temperature of a windrow from ambient to temperatures in excess of 70°C (Fig. 22). This feature of composting is the prime means of controlling or eliminating pathogens in the original waste material. As pathogen reduction and temperature are time linked (Barrow & Feltham 1995), a key area of this study was the examination of the various characteristics of windrows that may influence temperature. During this examination, it was found that particle size and bulk densities of the windrow significantly affected distribution of heat within the windrow. Windrows with a particle size giving a bulk density of 600 kg m^3 produced a more even temperature distribution between various depths within the windrow, but greater variability between individual points at the same depth. This could mean that they are more likely to be subjected to 'cool spots', i.e. regions of cooler or ambient temperatures. A coarser shred material with a bulk density of 400 kg ^{3} on the other hand, results in more constant spread of temperatures especially deep within the windrow (80cm), but with cooler surface areas (20cm depth). These findings may have an impact on the economics of windrow preparations as coarse shredded windrows require fewer turnings (to aid aeration) than the finer shredded material. However, as always, a balance has to be met, in that fewer turnings allow water within the windrow to gravitate to the lower layers (foot) of the windrow, with the possibility of producing anaerobic conditions in those sites.

Water is of prime importance to the composting process, as without water, most microbial metabolic processes will either not start, or will rapidly cease. This study found that water was lost (by gravitational forces) from the upper layers (20cm) to the foot of the windrow. It was found that turning and mixing the windrow, helped to redistribute the displaced water. This study concluded that operators should regularly monitor the water content of windrows and aim to balance bulk densities at between 400 and 600 kg ^{3} and be aware that windrows with high bulk densities, may be subjected to 'cool spots'.

The use of composting as a waste management process, means that biodegradation must be achieved in days or at most, weeks and as space is always at a premium in any composting site, the most important part of this process is the reduction of bulk. Microbial abilities to achieve rapid reduction in bulk, is dependant on the availability of nutrients, the amount and types of which are well documented (Abbés 1993; Bernal 1993). This study into the microbial activity during composting, concluded that with an ideal carbon to nitrogen balance (20-40:1) and water content (30-60:1), a windrow could loose up to 64% of its weight within 17 days of composting, through a combination of water loss and microbial activity producing CO_2. This is of economic significance, as most composting areas are exempt from Landfill Tax only if the material is removed from the site within one year of its arrival (HM Customs & Excise 1997). Any material left on the site after that time even with the most rudimentary of composting, would only attract a fraction of the tax payable had it been left unprocessed.

Microbial activity, as has been shown in this study, raises temperatures in windrows from ambient to more than 70°C within 48 hrs (Fig.22). It has been suggested by some researchers (Bernal 1993) that while this increase in temperature is desirable in the control of pathogens, high temperatures will lead to the loss of nitrogen from the windrow through ammonia volatilization and thus, limiting the plant nutrient value, of the compost produced. However, while noting that the loss of ammonia from windrows may be linked to temperature, this was not interpreted as a loss principally through volatilization. An alternative hypothesis was proposed suggesting that, nitrogen is lost through several pathways: including volatilization, microbial metabolic use and, the conversion of the ammonia to nitrate (Fig.26). Evidence to support this hypothesis was seen in windrows constructed from materials with a low C: N ratio (<10:1) and having a poor physical supportive structure. Under these conditions, the windrow collapses causing anaerobic conditions, usually around 4 days after construction. This is always followed by a fall in the windrow temperature. Samples taken from a collapsed windrow are often found to be low in both ammonia and nitrate. However, after turning and mixing the windrow (so that aerobic conditions can be re-established), it was observed that samples taken

for analysis exhibited no increase in nitrate levels, however, an 8-fold increase in ammonia concentration was observed. This was as a result of an accumulation of soluble ammonium ions in the lower layers of the windrow, where the anaerobic conditions would have prevented the oxidation of ammonia to nitrite by autotrophic microorganisms, thus interrupting the nitrogen cycle. By turning and mixing the windrow, oxygen as well as ammonium ions from the foot of the windrow become redistributed throughout the windrow. The net effect of this is to make the ammonia available for aerobic microbial metabolic functions. As a result, temperatures rose within hours of turning and mixing. It was also noted that ammonia levels, as previously observed, started to fall in value, but this time with a simultaneous increase in nitrate concentrations (Fig 26). The conclusion is that ammonia was lost during composting, some through volatilization, but mostly from microbial use (both for metabolic functions and nitrate production). Both ammonia and nitrates are important plant nutrients, nitrates however, are the more valuable plant nutrients (Jakobsen 1995). Providing aerobic conditions can be maintained, facilitating nitrate production, high plant nutrient value composts can be achieved.

Traditionally, it is believed that composting operates at optimum efficiency if carried out at temperatures below 55°C (Golueke 1991; Finstein 1992). The rationale for this hypothesis is that psychrophilic and mesophilic microorganisms are the main 'composting microorganisms' and, if temperatures were allowed to rise significantly above this level, those populations would become reduced or even eliminated. Thus, causing the composting process to slow, which is undesirable in a waste management situation. However, this study showed that, while confirming that the psychrotrophic and mesophilic microbial populations were smaller in samples taken from windrows at temperatures above 55°C (Fig. 35 & 36) than compared with those found in samples of compost windrows below 55°C (Fig. 33 & 34), no significant effect on the composting process itself was observed. Not only did the composting process continue, but windrow temperatures up to 70°C were sustained for up to 25 days. This suggests an alternate hypothesis: that temperatures up to 70°C do not effect the composting process. Evidence to support this alternative hypothesis was to be found in examining the dynamics of

the microbial populations associated with various windrow temperature profiles. Thermophilic temperatures, it was observed, could be established from ambient within 48hrs. Examination of the microbial population during this period, showed that it rose from an initial 0.42 x 10^9 $CFUg^{-1}$ at ambient, to 3.27 x 10^9 $CFUg^{-1}$ at 42°C. As the temperatures continued to rise, a reduction in the psychrotrophic and mesophilic populations were noted at 60°C, to 2.85 x 10^9 $CFUg^{-1}$. However, the temperature continued to rise, and at windrow temperatures of 70°C, population numbers rose to 3.3 x 10^9 $CFUg^{-1}$ a value close to that observed at 42°C. This confirmed that microbial populations are not 'eliminated' at thermophilic temperatures. The reduction in population numbers at 60°C, is attributed to the effect of the 'transitional phase'. The 'transitional phase' is an interval where the microbial population is subjected to a rapid temperature increase. The effect of this phase is that the microbial population moves from a predominantly mesophilic population, to an almost exclusively thermophilic population. (Fig. 31). This phenomenon has not previously been reported.

The Scottish Environment Protection Agency (SEPA), as part of a composting site licence regulation, require that temperatures within the windrow should be allowed to rise above 55°C for a minimum of 48hrs, in order to eliminate or control mesophilic pathogens, (e.g. *Salmonella* spp., *E. coli* etc., SEPA 1997). By this action, it is claimed, most (if not all), pathogens will be eliminated. However, this study showed that this assumption by SEPA is flawed. If analyses of compost material exposed to thermophilic temperatures are carried out immediately after sampling (as is normally the case), a reduction in viable psychrophilic and mesophilic microbial populations is observed (Fig. 35 & 36). However, it was found that if the samples had been examined after being stored for 24hrs at ambient temperatures, then significant increases in both the mesophilic and psychrotrophic populations were observed (Table 12). This suggests that once ambient windrow temperatures are re-established after a relatively short period (48hrs), psychrotrophic and mesophilic microbial populations, while initially reduced during composting, may again become dominant within 24hrs once temperatures fall. Temperatures of 55°C sustained for only 48hrs may not have any significant impact on the

survival of potential pathogens. Studies have shown (Beffa *et al*, 1995) that bacterial species that would normally be regarded as mesophiles, have evolved thermotolerant spores and strains that can survive what would normally be regarded as lethal temperatures. Another factor in the survival of mesophilic populations is the insulation effect within windrows. While ensuring that the heat generated by microbial metabolic activity is retained, the insulation effect may also create 'cool spots' within the windrow which are insulated from the rising temperatures, allowing individual mesophilic microbial colonies to survive in what otherwise would be a lethal environment. This study concludes therefore, that as temperatures of 55°C did not prevent the regrowth of mesophiles after 24hrs (Fig. 38), the average minimum temperature of windrows should be raised from 55°C to 65°C, and maintained for at least 72hrs.

The key to understanding many of the dynamics of the microbial population during composting, is to regard a compost windrow as a self contained and fully functional ecosystem. However, unlike most natural ecosystems (Table 8), the microbial populations will not only be subjected to all the pressures brought on by inter and intra-specific competition for space and nutrients, but they will also be subjected to an environment that undergoes rapid and often fundamental changes. Temperature (Fig. 22), pH (Fig. 27) and moisture (Fig. 20) will all change in addition to fundamental structural disruptions (turning and mixing), on a regular basis. This means that if microbial populations are to expand their populations or even survive within such an ecosystem, they will need to evolve survival strategies that enable them to expand their populations rapidly when conditions are right. This study of the population dynamics of composting suggests that while overall microbial population numbers do not become significantly reduced at windrow temperatures up to 70°C, they have an impact on microbial diversity. At the start of composting, 13 separate microbial species or genera have been identified (Table 5). After 17 days of composting, only 5 species remained (Table 6) and of those, the genus *Bacillus* dominated. Species belonging to the genus *Bacillus*, are regarded as a group of microorganisms that, in the main, have adopted an *r*-strategy for survival (Fig. 41) and several are thermotolerant or true thermophiles (Holt *et al* 1994). As such, they are

able to adapt to the changing environments by rapidly expanding their populations, often at the expense of other species. In environments that undergo fundamental and often rapid changes in temperature, the microorganisms were observed to have evolved ecological niches based on temperature. Once in their particular niche (i.e. temperatures over 55°C) thermophiles adopting an *r*-strategy for survival (i.e. *Bacillus* spp.), while subjected to the usual pressures of interspecific competition, will experience a rapid population growth. However, as high temperatures would effectively exclude most mesophiles, thermophiles appear not to have evolved to any extent, a survival strategy for dealing with 'serious' competition from mesophilic microorganisms. This means that when temperatures in a windrow reduce to a point (circa 60°C) where surviving thermotolerant mesophiles are able to expand their populations, then it is observed that, the thermophiles are out-competed. This was shown clearly in samples taken from areas of a windrow where the temperature was 60°C, and allowed to 'rest' for 24hrs (under aseptic conditions) confirming that the psychrotrophic and mesophilic population expansions were almost entirely at the expense of the thermophiles (Fig 38).

Temperatures within a windrow, it was noted, were routinely measured at more than 70°C. On examination, the microbial species that 'inhabit' such high temperature regions were thermophiles commonly associated with composting, i.e. *B. stearothermophilus* and *Pseudonocardia* spp., (Strom 1985; Unaogu *et al* 1994; Beffa *et al* 1995). The windrows however, were also extensively populated by colonies of a novel, large (up to 18μm in length), rod shaped, spore forming bacteria (identified as isolate BB001 Fig. 42). Further examination of isolate BB001 using a series of standard identification methods, and polar diagrams (Fig 44), placed the novel isolate provisionally in the genus *Bacillus*. However, no firm identification of species was possible. BB001 was found to be a facultative anaerobe, moderate halophile and thermophilic (Yanagita 1990), thus able to survive conductivity values over 4000 $\mu S\ m^{-1}$ and temperatures between 70 and 75°C. BB001 did not however, grow at temperatures below 43°C. These ranges of temperatures coincide with the 'normal' spread of temperatures found in active compost windrows. This suggests that not only will isolate BB001 occupy an

ecological niche within the windrow that would be denied to most mesophiles, reducing the effect of competition from those populations, but also that it may be unique to composting. However, competition for space and nutrients from other thermophiles would still be apparent, but BB001's high temperature tolerance and its ability to adapt to low oxygen tension means that it will be at a competitive advantage over other thermophiles within the windrow (i.e. *B. stearothermophilus*). From the results of carbohydrate utilisation (Table 13), it was found that isolate BB001 was relatively limited in the range of carbohydrates it is able to assimilate, relying on the simple carbon sources (i.e. the monosaccharides). This suggests that the prime constituent of 'green waste', complex polysaccharides such as cellulose and lignin, could not be utilised by BB001. However, the high diversity of microorganisms found at the start of the composting process (Table 2 & 5), many of which can produce extracellular cellulases (Crueger & Crueger 1990), means that the process of breaking down the more complex carbohydrates into simpler compounds, is swiftly initiated. However, the rapid rise in windrow temperature that follows, means that the mesophiles that were responsible for the initial breaking down of complex carbohydrates, quickly become suppressed or killed. Leaving the thermophiles such as BB001, to inherit an environment rich in simple sugars and low on competition, thus enabling them to dominate.

Conclusions

The study charted and examined, the way social pressure and legislation surrounding the disposal of organic waste, has lead to a growing interest in composting. Composting however, is at present subjected to a good deal of 'folklore' and, as such is regarded by many as an art rather that a science. This study attempts to alter this balance towards the science, but without eliminating the art.

In pursuit of these goals, the study examined the microflora involved in aerobic composting in open windrows and their impact on public safety. The study showed that during the construction of a windrow, special attention must be given to the particle size of the shredded waste and bulk densities of windrows, as these can significantly effect the temperature. The study also examined population numbers and microbial diversity at the start and during the composting process. The investigations showed that microbial diversity was high at the initiation of the process (Table 5) but fell rapidly as temperatures increased to only 2 genera after 17 days of composting; *Pseudonocardia* spp. and *Bacillus* spp. (Table 6). The study also isolated a bacteria not previously described, possibly belonging to the genus *Bacillus.*

In examining the role of temperature, the study showed that while microbial diversity fell, overall population numbers did not, contradicting traditionally held beliefs that high temperatures lead to low population numbers. The study also showed that nitrogen (as ammonia) loss may not only be lost through volitization, but also through microbial metabolic use and conversion to nitrate. The study also explained that psychrotropic and mesophilic microbial populations do not become eliminated after 48hrs despite being exposed to temperatures in excess of 55°C. This means that the recommended duration for windrow temperatures over 55°C for 48 hrs (SEPA 1997), may not be sufficient to eliminate all pathogens. These investigations lead to the suggestion that a compost windrow should not be viewed simply as a vehicle for a biodegradation process, but as a self-contained ecosystem in which the resident microbial communities have evolved survival strategies based on the

pressures of inter- and intra-specific competition for the availability of nutrients and temperature, in a series of temperature linked ecological niches. Each having its own distinct microbial population. This suggests that the composting process in essence, simply consists of a series of microbial successions based on temperature. Composting has for many years been relatively unregulated with as yet no meaningful standards either for the operation of a site or the final product. However, this is changing (Walker, *et al* 1997) and if composting is to become the principle organic waste disposal method in the next millennium, then a greater understanding of the process and in particular, the microbiology, is required. This thesis is part of this process.

SUGGESTIONS FOR FURTHER WORK

A major and significant finding of this study is the large, thermophilic bacterial species present in high numbers at top composting temperatures. This microorganism has been initially identified as a member of the genus bacillus. It would be of great interest to compost microbiologists to fully identify and classify this microorganism and fully characterise it's role in the exothermic composting process. To that end I suggest that the following methods should be applied:

(1) A full classical taxonomic identification and classification using Bergey approach.

(2) A numeric taxonomic classification based upon the characteristics determined for the Bergey approach.

(3) 16s rRNA and C+G analysis in order to determine the taxonomic position of this microorganisms within the genus Bacillus.

(4) Pyrolysis Mass Spectroscopy (PyMS) of both the isolate and several (minimum 12) other members of the genus Bacillus in order to determine the percentage relatedness of all test strains.

(5) the full biochemical, genetic and physiological characterisation of the thermophilic isolate in order to determine it's contribution to the exothermic composting process and to determine whether or not it has commercial value due to it's thermophilic enzyme complement.

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Plate 1. Composting operation during August 1998, Wright Avenue Dundee.

(A) Fresh waste

(B) TIM SD1000 Shredder

(C) Typical Windrow (approximately 3 months old)

A

B

C