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ASPECTS OF THE FUNGAL DEGRADATION
OF INTENSIVELY PRODUCED
FARM ANIMAL MANURES

by

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SUMMARY

Certain aspects of the biodegradation of intensively produced pig waste have been studied up to the process development stage.

A survey was first conducted to evaluate the problems of intensive waste management by studying present treatment systems and their limitations. Having established the problems a study of pig waste breakdown by micro-organisms was undertaken. A two-stage process was postulated consisting of a separation stage followed by a thermophilic biodegradation stage.

Parameters were determined for the acceleration and optimisation of the thermophilic biodegradation stage in terms of encouraging the establishment of a thermophilic fungal flora occupying a unique ecological niche which would make it easy to control in its activities. A normal composting process was studied to serve as a control.

Analyses of the biodegradation process showed that soil and end-product additions could enhance breakdown of cellulose and produce a more stable innocuous product.

A study of the growth of the thermophilic fungi on pig slurry showed it to be available as a substrate and not to inhibit activity. pH and carbon to nitrogen ratio optima were determined for the slurry.

An evaluation of the amino acid spectra for the products showed that adequate essential amino acids were present and the use of the processed waste as a feedstuff was suggested.

Two experimental pig pens were constructed to test the efficiency of straw in the separation stage of the process which would be the precursor to the thermophilic stage. Reductions from 10% to 1% solids contents were obtained, the filtrate being more amenable for further treatment. The design and operation of a pilot scale thermophilic stage is discussed which is to be linked to the filtration stage, and an evaluation is made of the process as a whole with regard to its establishment in intensive farm management.

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To Susie

C O N T E N T S

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CHAPTER 1

CHAPTER 1

AN INTRODUCTION TO INTENSIVE FARM MANAGEMENT AND FARM ANIMAL WASTE PROBLEMS

- 1.1 Introduction
- 1.2 The problems of waste management when animals become concentrated in small areas
- 1.3 The intensive farming method
- 1.4 The physical and chemical characteristics of farm animal wastes
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1.1 INTRODUCTION

All processes whether chemical, physical or biological produce by-products or wastes and such wastes may accumulate because Nature's or Man's economy may depend upon the useful products of the processes. The majority of, what may be termed, natural processes recycle the waste material to give carbon, nitrogen and other element cycles so that very little recalcitrant accumulation occurs. Man, with his technology, is able to accelerate and control certain natural processes to increase their efficiency thus increasing the by-product accumulation due to the inability of Nature's cycles to treat the wastes at the rate they are produced. As these wastes become more concentrated the cycles become attenuated due to the inhibiting nature of one stage on the latter stages when it must cope with very high concentrations of waste (Fulbrock, 1973). The eutrophication of lakes is an example. Phosphorus is essential for algal growth and fish will feed upon algae. Too much phosphorus increases algal growth causing oxygen depletion which in turn suffocates the fish and inhibits several elemental cycles within the lake. Thus the cycle has been short-circuited and, even though the algae may thrive, a wholly undesirable and unstable situation has occurred where there is a build-up of phosphorus and other polluting elements which would normally be dispersed throughout the cycle. Eutrophication may occur in lakes used for drinking purposes, making the water evil-smelling and bad tasting. In this instance efficiency in one process has affected another

process not directly related to it.

It is well documented that industrial pollution of rivers and waterways has been due to industrial concentration and the inability of natural breakdown and recycling processes to keep track of the amount of wastes being introduced into the cycles. Rigid legislation controlling waste removal to rivers has reversed the pollution which destroyed life in, and affected water supplies from, the rivers. Parallels may be drawn between the concentration of industry and the intensification of agricultural husbandry, in such a way that predictions as to the waste problem may be made. The increase in efficiency of the husbandry and the increase in animals per unit area of land means an increase in processes, in terms of waste producing animals, which gives an increased amount of waste. The inhibitory nature of the waste production may be seen in the decision of the river authorities to ban agricultural wastes from direct discharge to rivers - a decision which affected industry earlier and posed treatment problems. Thus, it seems likely that farm wastes will become an economic, social and health problem where intensification has occurred, and that this is due both to the toxic nature of the waste and the legislation which has been introduced to control removal methods which have, in the past, been considered normal on non-intensified farms.

Such wastes contain valuable nutrients for living organisms and instead of just rendering such intensive wastes innocuous, ways may be found to directly recycle and re-use these nutrients by means of controlled microbiological processes on the farm. These processes may be used to tackle the problem of waste treatment because they are continually present under natural conditions in the form of the various element cycles in which microorganisms play an important and vital part. It is necessary to select out a favourable group of microorganisms which are naturally present in the waste, and then determine their optimum parameters for growth upon the substrates. Having determined these parameters a process may be developed to encompass and encourage the conditions to accelerate the process. An accelerated process will be necessary to keep track with the intensified production so that a build-up and subsequent pollution may be avoided.

1.2 THE PROBLEMS OF WASTE MANAGEMENT WHEN ANIMALS BECOME CONCENTRATED IN SMALL AREAS

Wherever farm animals have been kept a variety of waste products, either direct or indirect animal by-products, have accumulated. Because systems of husbandry include the grazing of animals on meadow or fallow land, their wastes can be passively absorbed into the soil as a non-specific fertiliser or soil conditioner. The wastes are also deposited in

relatively low concentrations over the land as a whole. This means that the animals provide a cheap and convenient method of waste removal and disposal and, because of the low concentrations, the soil structure and its general condition are not affected, and there are no worries of pollution or any health hazards.

The application of animal manures is still considered to be important for the improvement of soils. In addition to providing a nitrogen and carbon source the manures are biodegraded in the soil to form a fine black acidic material known as humus. Humus will maintain the water-holding capacity, the permeability of the soil to water, its aeration and its temperature properties (Millar, Turk and Foth, 1958). It is thus important for plant growth, providing conditions which artificial fertilisers do not. In Table 1, however, the Ministry of Agriculture, Fisheries and Food's (MAFF) statistics show that, based upon the quantity of nitrogen in artificial compound fertilisers, the use of these has nearly trebled in the period 1952 - 1970. The ease of handling, the constant quality, and the known chemical composition of the artificial fertilisers have all contributed to this increase. Traditional farmyard manure may contain weed seeds, be of a variable chemical composition and, because of its handling characteristics, be very costly to spread on the land.

TABLE 1

	Nitrogen content (tons)*		
	1952 - 53 ⁺	1960 - 61	1969 - 70
Compound fertiliser	125,228	272,488	359,521

* The figures are from MAFF statistics tables
calculated from subsidies paid in by
farmers in Great Britain

⁺ The year runs from 1st June to 31st May

Where animals are continuously grazed on pasture land the problem of manure distribution and disposal does not exist. As the concentration of livestock increases on the same area of land the capacity of that land to safely handle and absorb the increased amounts of waste will be decreased, resulting in overloading and a breakdown of soil structure. Taken to its ultimate conclusion there is the situation where livestock is so concentrated that the wastes have to be mechanically handled and removed to other land for disposal. As the demand for artificial fertilisers has increased so has the problem of the disposal of animal wastes. It is not uncommon to see 5,000 pigs housed on 10 acres of land when their wastes might need up to 150 acres (calculated from Berryman, 1970)

for continuous safe disposal on grassland and in this respect it may be said that the microorganisms responsible for the breakdown and stabilisation of the spread waste need such large areas to carry out their activities. For disposal to land where corn is grown continuously, such as in Canada, Jones (1969) has estimated that the minimum area required to safely dispose of the nitrogen component of slurry from 1,000 pigs would be 100 acres. He has also quoted a minimum area of 50 acres where maximum application of nitrogen does not reduce corn yield or cause water pollution. Calculated from these figures 5,000 pigs would require between 250 and 500 acres of cereal crop area for safe disposal. Thus the farmer who concentrates his livestock must find a continuous supply of land for complete disposal of his animal wastes. The amalgamation of arable farms into large mono-culture systems demands that for a constant quality crop, a defined fertiliser is used. Large farming units utilise many acres of land and dress them with artificial fertiliser. Although the livestock farmer may be able to dispose of his wastes to arable farmers two to three times per year on a large scale, he must find alternative means of disposal or storage for the remainder of the time.

It has been estimated that 48.5 million acres of land are available in the United Kingdom for the disposal of farm animal wastes, and that the amount of land required to safely dispose of the annual output of waste in 1965 was 10.14 million acres. This would indicate that enough land is available to

accommodate animal waste disposal at the present and for some time to come. However, livestock and arable farming units are not evenly distributed throughout the country due to the climate and soil conditions, and the market availability and services. Thus a farmer who needs to continuously dispose of an animal waste may need to transport it when the neighbouring fields have been fully utilised. This may become a costly operation to be offset against any returns from the livestock.

The concentration of animals for rearing purposes has led to other problems of waste management which are now under legal control. The build-up of the wastes due to non-availability of land for disposal can lead to air and water pollution caused by odours and liquid run-off to streams respectively. The wastes are also a potential health hazard - more so in confined areas - and the siting of concentrated livestock areas near suburban settlements (Teller, 1970) because of the availability of services and marketing outlets emphasizes the problems. Legislation has been introduced (Bartrop, 1970; Fish, 1970) to protect the public from odours, public health hazards and the pollution of waters by animal wastes.

The amount and quality of farm wastes will depend upon rain-fall, type of feedstuff, the animal and whether the unit carries out milking, slaughtering or packaging on the

same site. The waste from the animal housing sheds will consist of mixtures of urine, faeces, undigested feed, bedding if used, and wash-water. The wash-water acts as a dilutant but increases the volume to be removed. The cost of waste removal from farms is often the limiting factor in intensive management, as farmers are loath to pay for disposal methods which bring no financial returns. The only outlets are to sell the solid waste as garden manure or as a potential compost for the mushroom industry, and to find fields on which to spray the liquid fraction. These outlets are seasonal and subject to demand. They also bring little financial return - the spraying equipment and labour may even need to be supplied by the unit with the waste problem.

The concentration of farm animals has resulted from the desire for increased efficiency in livestock handling and the increased financial returns that accompany it. It is also necessary to discuss the need for the concentration of animals and why this situation will appear to continue and increase in the future. Four factors, apart from the economic one mentioned above, are important. These are:-

1. The increase in human population,
2. the decrease in available land,
3. the decline in the number of agricultural workers, and
4. the limited natural water resources.

The population of the United Kingdom has increased from 38 millions (1901), through 46 millions (1931) to 52 millions (1961) (Jones and Riley, 1970). It is anticipated that the increase will continue at about 4 millions every ten years so that the population will reach about 70 millions by the end of this century. An increased population will require more food and housing, and this must be made available using the fixed land resources or lowering the requirements of the population.

In 1964 the United Kingdom had a total of 48.5 million acres of crops, grass and rough grazings, made up of 18 million acres of arable, 12 million acres of permanent pasture, and 18 million acres of rough grazings (Jones and Riley, 1970). Agriculture loses 40,000 acres per year to non-agricultural developments of all kinds and this will continue even though reclamation of derelict land is implemented.

There are approximately 350,000 wholetime agricultural workers in the United Kingdom with an annual loss of 18,000 (Jones and Riley, 1970). This is expected to continue, but the rate will slow down as those remaining earn a higher wage. However, workers are becoming increasingly loath to work with liquid wastes and the influx of new labour is limited. In the U.S.A. a similar trend has been recorded (Smith, 1964). In 1855 practically 80% of the population lived on farms, and in 1963 85% lived in towns and cities.

However, mechanisation has meant that, as opposed to the farm worker only being able to support himself in 1855, in 1963 one farm worker could produce enough food to support himself and thirty others.

Our water resources have much to cope with, including treated domestic and industrial wastes; they also serve as reservoirs of drinking water and for recreation. All of these uses are increasing and the availability of water resources for open farming will become increasingly difficult.

The rating of concentrated livestock buildings situated within suburban areas has for long been an anomaly in the rating laws and has slowed down the increase in concentration, costing the farming industry about £1 million per year (Anon, 1971). Government action in the form of a Bill from the Department of the Environment should rectify this situation. In previous years the 1928 Act, which defines farm buildings, was adequate to exempt farms from rates, but now with the increase in concentration the definitions have become increasingly inadequate. The way is now open for a vast increase in the concentration of livestock in the United Kingdom.

1.3 THE INTENSIVE FARMING METHOD

Although intensification has only gained favour in the

last twenty years it is not a new innovation and was practised in London in George III's time (Harvey, 1970). The demand for pork by the navy and the availability of brewery waste as a feedstuff favoured such intensification. However, the increase in hygiene and general cleaning-up of London in the nineteenth century pushed farming back out into the country where no problems of waste disposal existed which were obvious at the time.

In the last twenty years livestock rearing has increased in efficiency and this efficiency has been reflected in new methods, most of which involve mechanisation (Mortimer, 1964) and an increase in the number of animals per acre of grazing land. Taken to its ultimate conclusion intensive units have arisen where the animals are kept in covered rearing sheds, all their feed and milking requirements being controlled by mechanised systems. The use of this type of confinement rearing and fattening leads to a rapid build-up of animal wastes, together with bedding waste, dairy waste (wash-water, whey and other creamery effluents), and waste water produced from cleaning the sheds and from rain guttering. Such intensive units are generally monocultures, being solely involved in dairy, meat or egg production, and thus there may be no arable land on which to spread the wastes. The MAFF has defined an intensive unit as one which contains more than one cow, four pigs or 100 chickens per acre of grazing land (MAFF, 1971). The increase in intensive farms based upon this definition is shown in Table 2. The figures

have been calculated from the annual MAFF census tables of 1965 and 1969 (MAFF Agricultural and Censuses and Surveys Branch, 1966 and 1970). The MAFF definition will be used throughout this thesis to define an intensive unit.

TABLE 2

Livestock	Intensive holdings (%)*	
	1965	1969
Dairy Cows	2.5	5.0
Pigs	14.7	23.0

* The figures are for England and Wales only

The intensive farming method is now practised throughout the world and there are varying degrees to which this has taken place. Two main categories involve intensification for some of the year:-

1. Summer grazed, winter housed,
- and 2. Zero grazed.

The first category is very dependant upon the amount of available land, the animal and the quality of the grass. Sufficient land must be available to allow for continuous

grazing and the quality of the grass much be such that milk or beef yields are not affected. On all but small farming concerns it is usually only the ruminants which are grazed, as pigs or chickens need to have their feed supplied which may be costly in terms of labour and wasted feedstuff.

Summer grazing which may be from April to November will be non-intensive and no problems of waste disposal will recur unless the animals are continuously polluting a stream.

From November to April the animals may have to be housed in sheds, not only for their own protection but to protect the land from over-grazing. This temporary intensification will cause problems for the farmer, but generally land is laid aside for disposal of the wastes and is oversown in the spring when manual disposal ceases. Because the intensification is temporary, solutions to the waste disposal problem have been solved by designing easily cleaned sheds, and by providing land for disposal.

The zero grazing system is used for ruminant, non-ruminants and poultry alike. It has varying degrees of refinement or efficiency. Pigs may be kept in styes where they have free movement and are fed on an ill-defined diet. Such systems are not generally applicable on large-scale units where maximum and constant quality is required. Thus the animals may be housed in sheds where feeding, milking and waste disposal operations are carried out automatically using a low labour force. Probably the greatest degree of intensification has been possible with poultry

where the birds may be caged on top of one another in a very small area, feeding and waste disposal of perhaps 10,000 birds being achieved by two men.

The arguments in favour of intensification revolve around economics. They involve public demand for a good quality meat product, and the decrease in land which is available to farmers and is situated near to markets on the edge of suburbia. The arguments against intensification probably involve two separate issues - the first involving the pollution aspects of grouping animals together, and the second involving the mental and physical health of such animals in close confinement. Those points in favour of intensification are certainly in evidence whilst those points against although present can be overcome.

The purpose of this thesis is to contribute to overcoming the pollution aspect of intensification which appears to be more evident than the adverse mental and physical condition of the animals which certain authorities have made comment upon.

Having stated that the intensive farming method is likely to increase and is desirable, it should be stated that a build-up of animal wastes causes many environmental and health problems. In particular, certain wastes are less likely to be amenable to treatment than others. To be able to compare the treatment possibilities of the wastes

it is necessary to discuss their physical and chemical characteristics.

1.4 THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF FARM ANIMAL WASTES

Before new methods of treatment and disposal can be considered it is necessary to know the properties and constituents of farm wastes so that problems will be fully understood and can be tackled. The physical properties of the waste will depend upon whether it is in the form of a slurry or a semi-solid state. Slurries have a high water content of between 85 and 90% because they usually contain non-faecal and non-urinary liquid such as spillage from watering devices; run-offs from rain gutterings and atmospheric water due to the mode of storage which is usually in an exposed site. The high liquid content of slurries aids their passive removal from the dunging areas as channels can be sloped towards a storage area or, as will be seen later, treatment can commence immediately.

Semi-solid wastes arise where straw or wood shavings are used as a bedding material (deep-litter bedding). The dunging is done on the bedding which absorbs the liquids and holds the solids to give a material with a moisture content of between 60 and 75%. Handling is necessary for removal. Wheat straw which has a cellulose content of 46%,

a hemicellulose content of 36% and a low lignin content (10%) according to Chang (1967) will serve to increase the cellulose content of waste found in slurry. It also provides more aerobic conditions for the colonisation of aerobic micro-organisms. Usually, however, it is compressed in the animal house, becoming water-logged and anaerobic. The anaerobicity is continued when the bedding is piled up in a heap creating odours and the associated fly problem. It is difficult to give even average figures for the composition of slurries and semi-solid wastes as they vary considerably according to the animal, the feeding regime and the amount of bedding used. Thus pigs will have large amounts of undigested cellulose in their faeces due to their inability to assimilate this polysaccharide. There will also be bacterial cells, cells of the pig, and urea from the breakdown of protein.

O'Callahan et al. (1971) have attempted to characterise the waste from pigs fed three defined feeding regimes and they have found that the characteristics do vary. Analysis of faeces and urine showed that these could be expressed as a percentage of meal and water consumed. The feeding regime also influenced biochemical oxygen demand (BOD), chemical oxygen demand (COD), total solids and pH.

Because of these variations any research conducted on animal wastes must state the type of feeding programme and the composition of the feedstuff as processes which work

TABLE 3

QUANTITIES AND CHARACTERISTICS OF EFFLUENTS
PRODUCED PER DAY BY ANIMALS AND MAN

	Weight of animal (Kg)	Quantity (Litres)	Moisture Content (%)	BOD mg/l	Organic Carbon (g)	Total Nitrogen (g)
	(a)	(a)	(b)	(c)	(a)	(a)
Cow	500	56.8	87	2000	1871	222
Calf	250	28.4	-	-	638	107
Pig	68	4.5	85	1200	-	23
Hen	2	0.13	75	35	-	2
Man	65	1.3	-	350	-	-

(a) Wheatland and Borne, 1970.

(b) MAFF. Farm Waste Disposal.
Short Term, Leaflet No. 67.

(c) Griffiths and Thompson, 1968.

with one characterised waste may be inadequate for another.

The amount of waste produced per animal per day is another variable factor which must be known for process calculations but it is very often difficult to ascertain. Over-estimation is probably very wise in such cases. Several authors have attempted to give averages as a guide to farmers for removal or storage requirements. These figures, together with moistures and solids contents are set out in Table 3. They do not include straw bedding in the solids content.

The polluting ability of an animal waste has many parameters. As for human wastes the following characteristics must be determined:-

1. The BOD₅ (the five day biochemical oxygen demand),
2. the nitrate and phosphate content,
- and 3. the pathogen content.

The inorganic nitrate and phosphate contents are very important in animal wastes as they are usually very high, causing oxygen depletion by the reduction of the nitrates and extensive algal blooms on waters caused by the phosphates. The BOD₅ of animal waste can be considerably higher than that of human waste, sometimes up to five times the strength. Thus when treatment is to be considered the processes may be longer and more costly. The strength of animal wastes

TABLE 4

POPULATION EQUIVALENTS (PE)

	Livestock (millions)	Fresh excreta (million tons)	PE for quantities (millions)	PE for BOD ₅ (per animal)
	(a)	(a)	(a)	(b)
Cows	3	45	30	5.5
Other cattle	9	50	50	-
Pigs	7	10	14	3.5
Poultry	126	6	12	0.1

(a) Jones and Riley, 1970

(b) Griffiths and Thompson, 1968

has been considered in terms of population equivalents (see Table 4) where one cow may be equivalent to five humans in terms of its BOD₅ and ten humans as regards its quantity. Such figures are important in determining costs for treatment when based upon domestic sewage schemes.

A discussion of the characteristics of animal wastes would indicate that all three wastes will create a problem under intensification. It has been shown previously that a higher percentage of pig intensive holdings (23.0%) than dairy cow holdings (5.0% were present in 1969) and that pigs were more likely to be kept in close confinement as their nutritional requirements could not be met from grazing. Thus, together with poultry, pigs may present the greatest immediate problem.

1.5 ANIMAL WASTE MANAGEMENT

Having considered the intensive farming method and how the characteristics of farm animal wastes can cause waste management problems, it is now necessary to discuss the methods of animal waste management. These will serve to indicate the following points:-

- a. The present situation regarding treatment and disposal methods,
 - b. the limitations of present methods,
 - c. methods which might lend themselves for incorporation into new and improved treatment systems,
- and d. the role which micro-organisms could play in treatment systems.

The management of animal wastes involves all procedures with the waste from when it leaves the animal until it is finally disposed of, or discarded by, the farmer. The methods of waste management may be classified according to the extent of treatment (see Table 5). There are four main headings.

1. No treatment.
2. Treatments to reduce physical bulk.
3. Treatments to reduce polluting ability as an end in itself.
4. Treatments to reclaim nutrients and upgrade the waste.

TABLE 5

CLASSIFICATION OF ANIMAL WASTE MANAGEMENT
SYSTEMS AND TREATMENT METHODS INVOLVED

Classification	Treatment methods	Micro-organism control
No treatment	Land spraying, discharge to waterways or sewers	None
Reduction of physical bulk	Drying, incineration, separation of solids	None
Reduction of polluting ability	Oxidation ditches, lagoons, activated sludge, anaerobic digestion	Limited
Upgrading of product and reclaiming nutrients	Composting, recycling, hydroponics	Very high

The progression from 1 to 4 shows an increase in the completeness of the treatment and thus more efficient management. Thus the management can range from direct disposal to land, waterways or public sewers up to an efficient recycling system where nutrients can be taken up by plants or micro-organisms to upgrade the product which may be recycled as a feed supplement. The need for any particular degree of treatment will depend upon the concentration of animals and the available land for the most economic form of management. Discussion of the overloading of land by animal wastes earlier may rule out this form of direct disposal as a continuous answer to intensive waste management. An investigation later in this section, into the types of treatments in present use will show how effectively they can be applied to intensive husbandry.

One of the purposes of this thesis is to investigate how micro-organisms may be positively used in farm animal waste management. In the light of the above four treatment classifications the role of micro-organisms can be briefly stated. Where there is no treatment the flora present in the waste, although utilising it, is not making any positive steps to efficiently reduce the polluting materials. Conditions prevail which may favour pathogens, oxidation of reduced nitrogen and phosphorus compounds to form inorganic nitrates and phosphates with a resultant high BOD. Anaerobic conditions resulting from oxidation processes will cause reduced carbon and sulphur compounds to be released

as methane, hydrogen sulphide and the lesser reduced thio alcohols, all causing odours. All these conditions will be increased if there is any storage prior to discharge. The second treatment system involves chemical and physical methods, micro-organisms playing no part in the treatment process. The last two headings involve the positive action of micro-organisms - heading 4. being more controlled than heading 3. The reduction of polluting ability may be achieved by a range of micro-organisms which have been selected out by providing basic physical controls such as aeration and agitation. The heading 4. involves the selection of a smaller specific group of micro-organisms which can be controlled by physical and chemical means to grow under their optimum conditions utilising the available nutrients and possibly recycling them in the form of a feedstuff - a recycling system quicker and more efficient than that provided by discharge onto land with its associated problems. Such a treatment system would involve several stages, the number of stages depending upon the quality of the product required and "the law of diminishing returns." As will be seen in the eighth chapter, animal wastes are a source of large amounts of nitrogen, phosphorus, potassium and other minerals which are available for micro-organism and plant growth. If the wastes cannot be returned to the land to be utilised for plant growth, which would be true for intensive units, it is important that their valuable nutrient content should be available for conversion and recycling through other means to prevent the overloading

mentioned at the beginning of this chapter.

It is now possible to postulate how micro-organisms might be usefully employed in such a positive conversion system and to select a group for investigation based upon their activities in relation to the nutrients present in animal wastes.

The nature of animal slurries with their high moisture content means that they may be highly anaerobic, causing odours and health problems. The low carbon to nitrogen ratio due to the high protein intake on intensified farms, particularly pig units, which causes large nitrogen wastage (Bunting, 1973) can also be a problem for microbial treatment. Although only mentioned briefly in the last section, straw is a very common agricultural waste which is associated with animal wastes and could be employed to provide extra carbon in the form of cellulose. Straw is produced in excess of 9 million tons per year with a low retail value of between £3.00 and £5.00 per ton, and of this amount 4 million tons are waste. A great deal of this waste is burnt because harvesting is too costly and it is widely believed that plant pathogens can colonise the straw and spread disease. Manures consist of admixtures of straw and waste, and this makes them amenable to composting, the natural microbial breakdown process. The use of straw in conjunction with the slurry could serve to increase the carbon content and decrease the moisture content in a way

which will be described later.

Cellulose can provide an energy source for many bacteria, fungi and actinomycetes. From these groups fungi may be the best choice for use in the process for the following reasons:-

- a. There are many cellulolytic species which are widely distributed, well characterised, and easily cultured. The cellulolytic bacteria are fewer in number, less well defined and more difficult to culture. Actinomycetes, like fungi, are widespread however their growth rates tend to be less than fungi.
- b. Fungi are found naturally in animal wastes.
- c. They are obligate aerobes producing no obnoxious odours.
- d. Their pH ranges tend to discourage bacteria (and actinomycetes) the former of which do contain pathogenic groups.
- e. With bacteria they are good sources of protein. However, fungi are favoured because bacteria also contain high levels of nucleic acid.
- f. They are able to tolerate lower moisture contents and would thus be more amenable to a semi-solid conversion process.

Hudson (1972) believes that cellulolytic fungi have an advantage over cellulolytic bacteria in that the mycelial habit of the former enables greater penetration of the substrate. This is due both to the production of extra-cellular enzymes and the ability of the hyphal tip to mechanically penetrate intact tissue. Bacteria, however, have neither the ability to penetrate intact tissue nor the powers of mechanical penetration. They rely upon the erosion of damaged tissue by enzymes. Thus the cellulolytic fungi are able to extensively colonise an intact cellulosic tissue such as the straw fibre and bacteria would be at a disadvantage, probably only establishing themselves after extensive breakdown has occurred due to fungal action. ✓
The fact that fungi are able to attack intact tissue is also important when considering the amount of pretreatment that a particular substrate needs in terms of mechanical breakdown. Grinding will break down the structure and allow both bacteria and fungi to utilise the substrate more quickly. However, this may be costly and time-consuming, and if fungi will act without this pretreatment then these two economic factors need not be involved in any costings for the complete process.

The fungi could be very useful in discouraging odours, health problems and reducing the cellulose bulk whilst utilising the nitrogen as a general nutrient source.

Particularly suited is a group of fungi which have

their optimum activities at or around 50°C, many of them being very cellulolytic. Having an optimum temperature of about 50°C offers the following advantages:-

- a. The range of fungi is drastically reduced to about 15 species which can be more easily controlled in their activities.
- b. Naturally occurring species have been isolated from animal wastes by many authors.
- c. Viable animal parasites and animal bacterial pathogens can be killed at this temperature.
- d. Conditions are such within animal wastes that if left in heaps they will tend to heat up to between 50 and 60°C. Thus, this energy could be put to good use to control points (a) and (c).
- e. The high temperature can be used to dry out the product.

It is unlikely that clear-cut lines can be drawn between the optimum conditions for the thermophilic forms of bacteria, fungi and actinomycetes and there will thus be some overlap. However, experimentation will indicate how great the overlap is and whether thermophilic fungi can be used as the dominant agents in the breakdown of farm animal wastes.

A discussion of the methods used in animal waste management practice and listed in table 5 will evaluate the ability of such methods to treat intensively produced wastes.

Waste management is usually left to the animal and the soil where the dunging is carried out. Owing to the grazing habit being carried out over large areas of land low concentrations of waste may be produced per acre of land. The housing of livestock during the winter requires that waste removal be done by manual or mechanical labour, the former being in plentiful supply before the presence of competitive wages and more attractive working conditions in other sectors of industry. All the manure can be stored to stabilise it and then spread on the land, again by manual or mechanical labour. Any odours arising are considered natural by the country people and thus no further management is usually necessary. Livestock numbers have increased and so have the numbers of intensive units. This means a larger amount of waste is produced per animal per acre of land. Couple this fact with the increase in the amount of artificial fertiliser being used, and a point is reached where the amount of waste produced exceeds the amount that can, or need be, applied to the neighbouring land for optimum fertility in a particular year. The waste will thus have to be kept in a highly unstable form leading to unpleasant odours, flies, public health and pollution problems.

Wheatland and Borne (1970) have estimated the total area of land required for disposal of animal wastes as manure in the U.K. (10 - 12 tons manure per acre is usually advised - MAFF Advisory Leaflet 435, 1961 - as the normal rate of dressing). For the total number of pigs, cows, calves and beef cattle, sheep and poultry they found that the total area of land required in 1965 was 10.14 million acres. The total area of available land was calculated at 48.5 million acres. This shows over three quarters of the available land is not used for disposal. However, the problems arise because the animal populations are concentrated into small areas and waste management is very important. The further the waste has to be taken for disposal the more costly is the operation and this in itself has caused several intensive units in Great Britain to close.

Waste management embraces one or more of the following steps:-

1.5.1 The removal of waste from the site of production.

1.5.2. The treatment of the waste.

1.5.3. The disposal of the waste.

1.5.1 The removal of waste from the site of production

The liquid manure can be removed using slatted floors

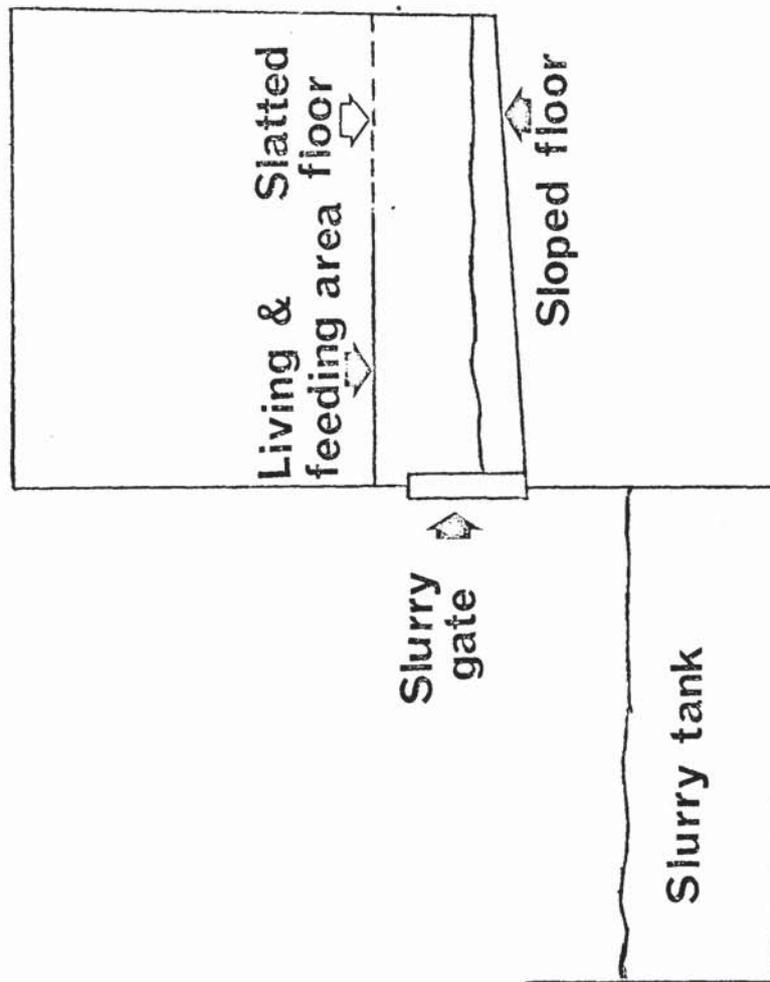


FIGURE 1

DIAGRAM OF SLATTED FLOOR SYSTEM

coupled with a sloping pit (see Figure 1 and Plate 1). This is generally used as it involves minimum labour and no machinery. The use of large quantities of wash water is used in particular for dairy cattle and involves larger quantities of liquid removal usually done by designing a sloping floor towards the storage area, as for the Nettled Project described later. Before washing, the floors are generally scraped by a tractor with a scraping attachment. This will decrease the polluting quality of the wash-water in terms of BOD (Notes on Water Pollution No. 17, 1962, Water Pollution Research Laboratory). However, this is not always consistent (Wheatland and Borne, 1964) and depends upon climatic conditions and the effectiveness of scraping.

Slatted floors generally provide an instant way of waste removal from the immediate site of production and are fairly efficient if they are not allowed to become blocked and the waste is removed from beneath the floor quickly. Solid floors, usually covered by a layer of straw, (see Plate 2) can be used efficiently if the animal is prevented from moving into the dunging area and soiling itself by the use of especially designed pens. Availability of capital also plays an important part in the choice of systems.

An advisory guide to dairy herd management (The User's Guide to Modern Milking IV, NAAS booklet 1968) has been issued by the National Agricultural Advisory Service and



PLATE 1

PIGS ON A SLATTED FLOOR MADE FROM 'WELDMESH'
WITH A SLOPING PIT BENEATH



PLATE 2

PIGS ON A DEEP LITTER STRAW BEDDING
Note liquid run-off from pen

this compares the slatted floor system with the scraping and washing method. The slatted floor system in Sweden has been supplemented by adding a water lock between the storage and the animal areas, and the wash-water system has been replaced to a certain extent by the design of mechanical scrapers which push the waste from the centre of the cow shed to one end (Meek, Merrill and Pierce, 1969). In Denmark the continuous flow principle is used (Meek, Merrill and Pierce, 1969) whereby there is little or no storage below the animals, the waste being drawn into a separate area using channels. The solid waste removal problem usually involves a tractor which removes the solid by pushing it in front of a scraper to the storage area (Plate 3). 'Mucking out' may still have to be done manually in small pens but tractor attachments can usually successfully substitute for manual labour.

A recent patent by a Lancashire farmer (Anon., 1970) includes the use of an auger situated beneath a grid and running the whole length of the cowshed. The faeces and urine drop directly into the auger in the channel which automatically removes them to one end of the shed. The system has improved the cleanliness of the cows as they do not walk in their own dung spreading it onto their bedding. Hosing down of the concrete completes the process.

Solids and liquids are usually present on a particular unit and thus two systems of removal have to operate. The



PLATE 3

STORAGE OF SOLID WASTES AND LIQUID RUN-OFF
FROM BASE OF HEAP

A stream was situated to the left of this
heap and all the liquid trickled into it
unless checked

advantages and disadvantages of separate handling have been outlined (Slurry Handling. Short Term. Leaflet No. 44 MAFF, 1966) as a guide to farmers.

1.5.2. Treatment methods

Treatment methods are designed to reduce the polluting ability of the waste whether it be solid, or liquid. It should be noted at this point that not all farming units will institute a treatment process. In Loehr's (Loehr, 1971) review of the systems practised in waste management three out of nine systems do not involve any form of polluting ability reduction before disposal. Reduction of polluting ability is usually defined by the reduction in the biochemical oxygen demand (BOD), the suspended solids (SS) content and the chemical oxygen demand (COD), the odour removal, and the reduction in inorganic nitrogen. All these parameters are legal thorns in the side of the intensive unit owner. The following treatment methods have been devised to reduce the parameters above. They can be divided into aerobic and anaerobic methods.

1.5.2.1. Aerobic methods

Aerobic methods may be classified as follows:-

1.5.2.1.1. The oxidation ditch and its modifications.

1.5.2.1.2. Activated sludge processes.

1.5.2.1.3. The lagoon system.

1.5.2.1.4. Composting.

1.5.2.1.1. The oxidation ditch and its modifications

The oxidation ditch (see Figure 2) was devised in Holland by Pasveer who found that long retention times (three days) combined with high oxygen levels caused a greater reduction in BOD in shorter time than short retention times (six hours) (Scheltinga, 1968). The basic form of the system is a 'race track' shaped circuit. A rotor or large brush provides aeration and circulation, and is situated on one of the long sides of the ditch. Work by Scheltinga (Scheltinga, 1968) has revealed that in this system the daily loading of BOD is more important than the long retention time for more complete mineralisation of the sludge. The lower the loading the more likelihood is there of the stationary phase of bacterial growth being maintained.

A modification of the oxidation ditch by Scheltinga (Scheltinga, 1969) involves the routing of part of the ditch beneath the slatted floors so that the waste is removed and treated immediately. Wheatland and Borne (1970) have found that foaming occurs through the slatted floors and that a

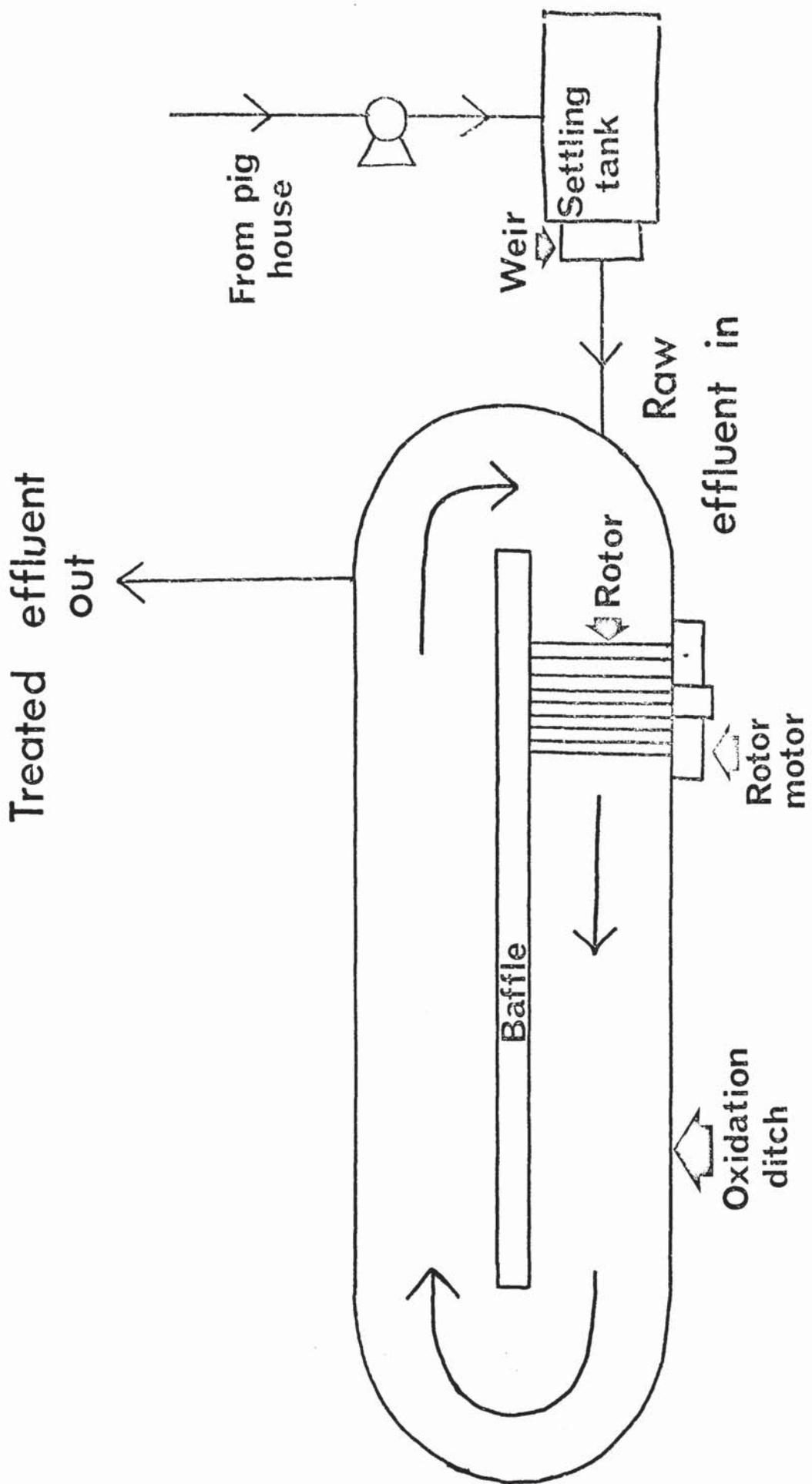


FIGURE 2

DIAGRAM OF TYPICAL OXIDATION DITCH

solution may be to provide a relatively shallow channel below the slatted area so that slurry may be flushed out to an oxidation ditch. American workers (Miner, 1971; Jones, Day and Converse, 1969) have also noted the dangers of foaming in connection with anaerobiosis and ammonia production within the pig houses.

Robertson (1972a) has further modified the oxidation ditch by adding a secondary inner ditch, a separator and a clarifier. However, it has been found that these additions only reduced the odour - the BOD and foaming still being a problem. The solids content of the ditch built up, as the separator only filtered out coarse material. The author suggests that with additional filtration equipment and anti-foaming devices the BOD could be reduced for release into water courses. However, this would increase the costs considerably and might involve extra labour for the farmer.

Taiganides and White (1971) describe a further modification to the oxidation ditch, that of the addition of an integrated aerobic digester which is fed with the solids separated initially from the incoming waste. The digester has a retention time of between 14 and 22 days after which the solids are released for land spreading. It is too early to assess the viability of such a system and whether it would be accepted by the farmer.

Gowan (1972) has expressed some reservations about the use of oxidation ditches. He believes that although they will remove odours (by creating aerobic conditions) they cannot be used as complete treatment methods for strong wastes. The Dutch originally designed the Pasveer ditch to cope with domestic washings and sewerage of low strength, and they are now looking at other forms of waste treatment. Thus it becomes expensive if the oxidation ditch with its modifications can only partially reduce the BOD.

1.5.2.1.2. Activated sludge processes

Wheatland (Wheatland, 1968) has worked with activated sludge processes and biological filtration as used for domestic sewage. He has found that adequate BOD reduction can be attained by modifications such as recirculation and an active flora present. Extended aeration and contact-stabilisation processes which are modifications of the activated sludge process, both serve to increase the activity of the flora (sludge). Aeration is of the variety of types used in domestic sewage treatments (Abson and Todhunter, 1967). Biological filtration through a filter bed could be improved by using a high rate filtration tower with plastic as the bed material (probably ICI 'Flocor'). This gave better reduction but still needed the secondary biological filter and the recirculating of strong wastes.

The owner of a 1,200 pig herd has developed a process

involving a 14 inch industrial centrifuge which causes a rapid decrease of up to 75% BOD (Lovelidge, 1971). The liquid is relatively inoffensive and can be sprayed onto fields. The separated solid is removed by an arable farmer. The system has been in operation for two years and no complaints have yet been received.

Although activated sludge processes are applicable to human wastes their efficiency in dealing with much stronger animal wastes still needs to be demonstrated. It is unlikely that such processes could achieve the same BOD reductions whilst remaining economically viable propositions.

1.5.2.1.3. The lagoon system

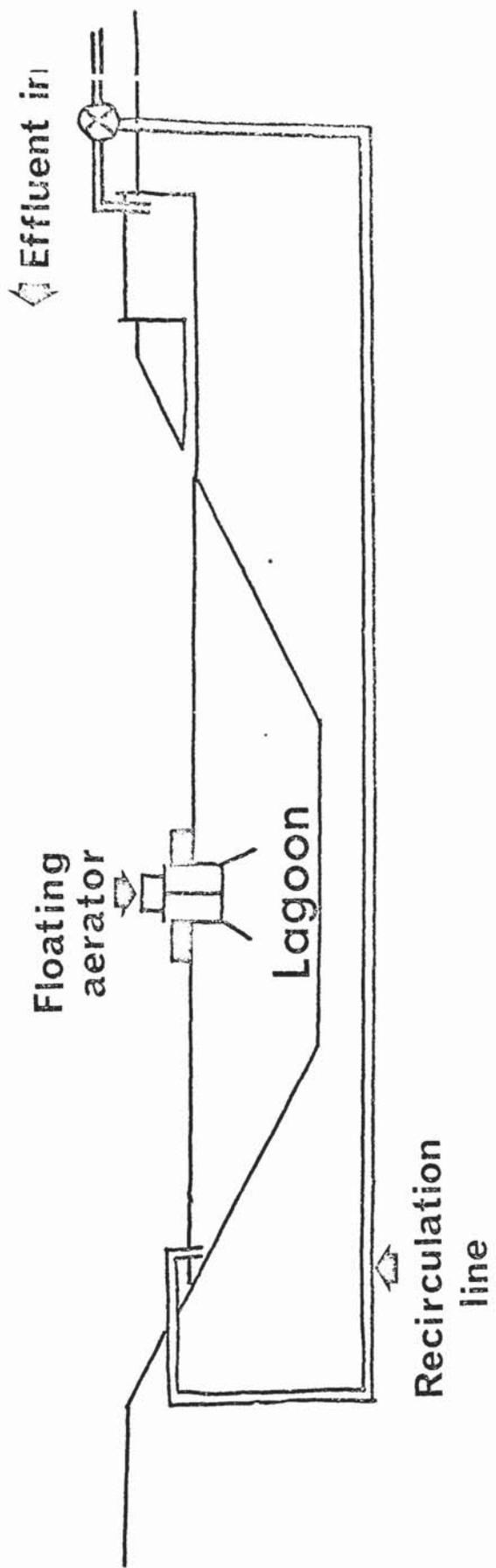
Lagoons are shallow lakes over a wide area of land so that a large surface is available for oxygen transfer. They are used as storage areas for the treated liquid waste, and in this case need not be too shallow. In the Nettlebed Project (Sayce, 1970) a series of six lagoons is used, each one carrying out a different treatment process by having different conditions or dimensions. The aerobic treatment involves a shallow turbulent lagoon and the anaerobic stage a static lagoon with a longer retention rate. Aerobic lagooning as opposed to oxidation ditch methods usually involves the settling of solids to the bottom of the

lagoon for reduction and then rising up under gas pressure to be treated aerobically, either by a floating aerator or submerged sparge pipes (see figure 3). In either case odourless operations have been reported (Dale et al., 1969) and depths of the lagoons may be up to 20 feet (Miner, 1971) (3 to 5 feet for non-aerated lagoon).

Thus, lagoons require large amounts of land for long periods of time. They also need to be dredged if they are to last for many years. The reduction in capital equipment and running costs achieved by using lagoons is probably offset by the loss of valuable arable or grazing land. The intensive farmer with limited land available would not be able to find room for a lagoon system.

1.5.2.1.4. Composting

So far the aerobic methods discussed have applied only to liquid waste. Aerobic systems with solid wastes have been attempted on a laboratory scale. Poultry waste has been successfully stabilised on a laboratory scale using a semi-solid system with aeration (Bell, 1969). Temperature increases to about 60°C were recorded, and the final product was described as a compost. However, the large-scale operation (Bell and Pos, 1971) was not as successful. The composter used was a large drum with a rotor inside and aeration sparges. Trouble was experienced in turning the



Aerated lagoon system

FIGURE 3

DIAGRAM OF AERATED LAGOON

rotors and mean temperatures did not remain above 45°C for pathogen removal. Ammonia was produced and the resultant product was not completely stabilised. Willson (1971) has composted dairy cow manure on both a bench scale and pilot scale and achieved a stable odourless product with a pH of between 7 and 8. The process took about 22 days on the bench using heating elements and between 26 and 44 days on the pilot scale. This variation depended upon whether the manure was chopped or not. It appeared that chopping increased the rate of breakdown and thus completion of the process.

The process of composting has received much attention as a possible solution to the profitable treatment of certain components of town refuse. Composting occurs naturally when solid waste from bedding is piled up and left. It has been recognised for many centuries that the black humus resulting from the composting process is of high soil conditioning value. Because of this cities and towns throughout the world have attempted to compost their town waste for profit. Examples of the systems used are:- Windrowing (Wylie, 1960), the Dano Stabiliser (Wylie, 1960) and Multi-stage composting in vertical silos (Wylie, 1960). Gray (1966) has attempted to increase the efficiency of the process by controlling its parameters of temperature, aeration and nutrition to give an accelerated compost process. Reviews by Gray, Sherman and Biddlestone (1971), Kershaw (1968) and Wylie (1960) cover the conditions for the composting

of town and animal wastes. Mills (1973) has studied the ecology of composting town refuse in order to determine optimum conditions. The process of composting animal wastes is an important one in providing the soil with a cheap stable fertiliser. Research is urgently needed to optimise conditions for the production of an animal compost consistent in its composition. Uncontrolled composting which is normally practised on the farm takes time, may take up space, attract flies and be subject to a liquid run off which is high in polluting ability. Accelerated composting processes are possible and these may be used to improve the natural process.

The control of odour by aerobic treatment is a very important factor in all the above methods and has received attention because of the legislation which classifies smells as a nuisance. Robertson (1972b) describes methods for controlling piggeries' smells. These include the use of chemicals which will either provide unfavourable conditions for microbial action (such as pH increase using lime) or act as powerful oxidising agents (chlorine and ammonium persulphate). Aeration is the other

method of odour reduction but, as has been mentioned above for oxidation ditch operation, ammonia can still be produced. The control of ammonia production can be done by keeping the pH of the waste at around or below neutrality. An increase to between 8.5 and 9.5 will cause the release of gaseous ammonia from solution - a method used for the ammonia stripping of domestic sewage (Culp and Culp, 1971).

1.5.2.2. Anaerobic methods

There is much controversy as to the advantages of aerobic and anaerobic treatments over each other. Wheatland (1968) praises aerobic treatment because the products of metabolism are carbon dioxide, water and cell protein and thus no odours or anaerobic bacteria are found. However, aerobic treatment involves large amounts of biomass being produced and settlement is needed before large BOD reductions can be recorded. The biomass may be kept to a minimum in most cases by keeping the process in the stationary phase (minimum BOD loading, causing limited substrate).

Anaerobic processes involve the reduction of proteins and nitrates to mercaptans (thio alcohols), skatol, hydrogen sulphide, ammonia and methane. All of these compounds create the obnoxious odours which characterise animal wastes. However, processes exist which control these odours by complete enclosure. Anaerobic digestion is a method of waste reduction and stabilisation with economic returns for

the operator. The digestors are of the same design as for those used in municipal sewage treatment, but more methane gas is produced and thus more is available than just to keep the digester in operation. Optimum digestion is obtained at about 36°C (Taiganides, Baumann and Hazen, 1963) and this is maintained using heat exchangers operating on methane gas liberated (Pohland, 1968). The resulting sludge can be drawn off and dried, and the supernatant sprayed onto fields. As for domestic sewage treatment, anaerobic digestion follows the settlement of sludge from aerobic treatment. However, this is not always so when consideration of nitrogen conservation for fertiliser value is considered. Lagooning systems such as the Nettlebed Project (Sayce, 1970) system encourage a long retention lagoon in which solids are reduced in bulk and stabilised. The process is akin to anaerobic digestion but is slower and liable to gas leakage should the sludge on the bottom of the lagoon become disturbed, as it would be when removed for disposal.

Indian workers have recently succeeded in increasing the production of methane from cow dung (Laura and Idnani, 1971) under anaerobic conditions by supplementing it with urea, casein, cane sugar and dry leaves. The application is for the small farmer who wishes to make his digester self-sufficient whilst providing more fuel for other processes such as heating and electricity production.

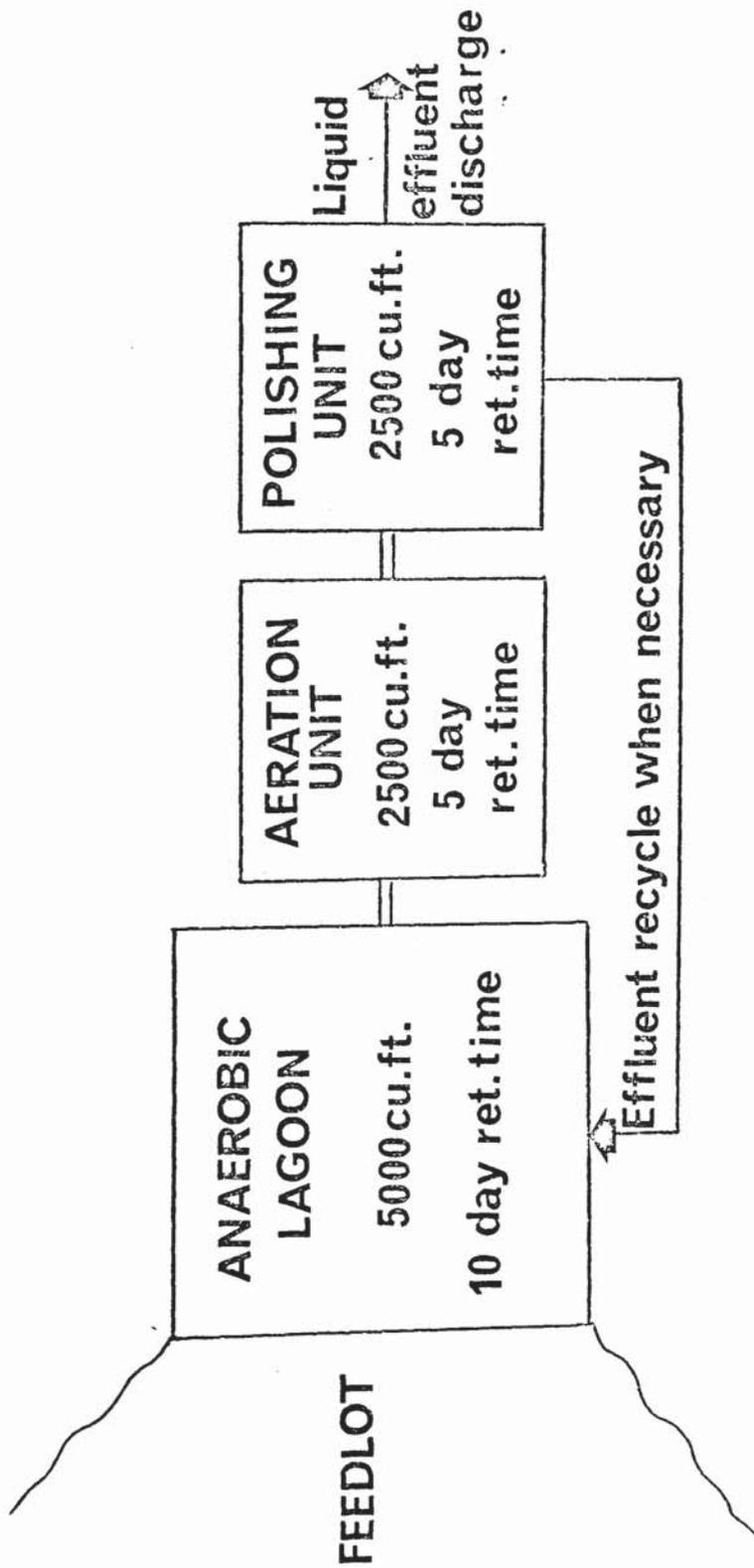


FIGURE 4

OF LAGOONS

Combinations of aerobic and anaerobic processes (Agnew and Loehr, 1966) are generally found in comprehensive treatment processes as reported above (see Figure 4). Each has its own advantages and disadvantages as described previously and each method is limited.

The need for new areas of research increases as land availability decreases. To summarise the above treatment systems the following may be said as to their limitations. Lagooning systems need large amounts of land as storage may be for up to five years. Although much work has been concentrated on oxidation ditches as a possible solution at the present time for treatment methods, there is a growing concern for their inadequacies as demonstrated by Gowan (1972). The composting of solid wastes takes space and time both of which are not available to the intensive unit owner. Accelerated composting may solve these problems but control mechanisms and machinery will be required for efficient operation.

1.5.3 The disposal of the waste

Due to the concentration of livestock rearing and loss of available land, disposal is the rate determining step in the process. Complaints from suburban dwellers usually result from disposal methods and pollution of natural waters appears when the effluent is disposed of.

Liquid wastes from swine and cattle units are usually sprayed onto adjoining land. This may be done from a tanker and tractor (Culpin, 1969) or direct from the storage area using a high pressure spray. The amount of liquid which can be sprayed onto an acre of land is limited. Exceedingly large doses will cause a breakdown of soil structure and a colloidal clogging of pores. Thus plants cannot grow because of the low oxygen content which results. Worms will also be prevented from penetrating into the subsoil. Berryman (1970) suggests that liquid dressings should not exceed 750 - 1000 gal/acre at three to four week intervals as this allows time for breakdown of materials and stops the clogging of the surface. Seepage of heavy dressings may occur into water courses causing pollution, and odour picked up by the wind may bring complaints from local residents. The fertiliser value is also emphasised by Berryman (1970) who states that the total potassium and phosphorous requirement can be supplied from intensive slurry production together with a large proportion of the nitrogen requirement. It is very important that inorganic nitrogen as nitrate should be reduced to a non-toxic level in the treatment method. Nitrate poisoning in animals has been reported (Webber and Lane, 1969) as well as the impairing of the health of infants drinking water containing more than 10 ppm nitrogen as nitrates (Walton, 1951; Webber and Lane, 1969).

The effects of copper, which is used as a fattening and anti-microbial agent in feedstuffs, for ruminants grazing on slurry spread fields, have been discussed by Batey, Berryman and Line (1972). They treated grassland with copper-enriched pig slurry over a period of three years and found that the copper content of the herbage increased from 9.1 ppm to 21.2 ppm. They concluded that this level would offer little risk to grazing animals, the only risk being from the slurry on the herbage which might cause disease. However, they recommend a limit of 9.5 kg/ha of copper per year as the maximum dressing until more is known on the availability of copper in slurry to crops and grass. Copper can be stored in the liver until a toxic level is reached, when it is released into the blood stream causing sudden death (McParland et al., 1972). It is thus important not to overload herbage and crops with slurry unless the amount of pollutants contained is known and can be monitored to prevent a build-up.

Disposal procedures increase in difficulty the nearer the unit is situated to a town because the waste may have to be transported continuously to spraying areas. Liquids are easier than solids to transport from both a mechanical and labour viewpoint but may be produced in larger amounts where straw or sawdust is used to a minimum as deep litter.

The disposal of solid wastes is generally by land application. It is usually ploughed into the land in

autumn and again in late winter. Thus application is seasonal. For the remainder of the year it is usually heaped in piles and is a potential pollutant. Seepage through the pile, from rain water and the waste liquors, can trickle into streams which may empty into reservoirs (see Plate 3). During the summer, when horses are field grazed, horse manure is in short supply for use as mushroom compost. Mushroom growers have been utilising pig manure as a substitute. When the horses are stabled the manure is in greater supply but the increase in demand for mushrooms dictates the continual use of other manures.

Alternatives to land spraying of liquids consist of drainage into domestic sewers for municipal treatment. It is estimated (Taken for Granted. Report of the Working Party on Sewage Disposal. London: HMSO, 1970) that as many as 10,000 farms empty untreated farm animal waste into the public sewerage system. It has been shown above that farm waste is very much stronger than that of humans and it is thus impractical for this practice to be increased. Charges (Simpson and Hibberd, 1970) may be levied upon farmers who dispose this way, assessed according to strength and quantity of the waste. Gowan (1972) believes that local authorities should handle agricultural wastes but because of the high costs a pre-treatment plant be installed.

The potential of recycling should not be overlooked. Recycling of animal wastes (Evans et al., 1968; Anthony,

1969; Gallop, 1970) as feed additives has had limited success in the past few years and may help the farmer to economically treat his waste and dispose of it. Poultry faeces have been dried and fed successfully to ruminants and back to poultry. Levels of up to 40% manure (Durham et al., 1966) produced no differences in mortality or egg production but on re-feeding cattle waste beyond 10% the egg production was slightly reduced. Paunch manure from ruminants is recovered from slaughter houses and commercially sold as feed for fattening cattle (Miner, 1971). Orr (1971) found that up to 22% of dried swine faeces incorporated into a corn-soy feed depressed the rate and efficiency of body weight gain but had no effect on appetite or flavour of meat. It was suggested that the depressed body gain could be restored to normal by adding fat. Poultry wastes have been digested and the supernatant liquid used to produce algae which are dried and recycled to the poultry (Dugan, Golueke and Oswald, 1972). The nutritional aspects of recycling will be discussed more fully in a later chapter.

Other novel methods of waste treatment and disposal involving recycling have been investigated. American workers (Calvert, Morgan and Eby, 1971) have assessed the value of house-fly larvae as stabilisers of hen manure. They found that the odour disappeared after four days and that the fly pupae and adult flies just emerged provided a protein source equal to soya bean meal. The hen manure product did not compare with soya bean but had a slightly higher nutritive

value than cellulose, and it is suggested that by substituting it for corn in the diet of the chick, normal growth could be obtained.

The other method involves the use of the common earthworm Lumbricus terrestris (Fosgate and Babb, 1972). Worms have been introduced into beds of dairy cattle wastes for periods of between six months and one year during which time more waste was added at intervals throughout the year. Samples of worms were removed, dried, ground up and used as a feed for domestic cats. The feed was found to be palatable and acceptable to the cats. The resultant earthworm dirt was found to be more porous than, and weigh one half of, normal potting soil mixture. Growth trials with plants resulted in better root systems with the earthworm product.

Incineration as a disposal method for municipal refuse is practised widely and developments have led to units which do not emit any particles into the atmosphere. However, this practice is undesirable with animal wastes unless a high density population requires it. On incineration vital fertility 'elements' such as organic phosphate and nitrates, are lost. The removal of these important 'elements' by incineration means that there will be a deficiency in the soil. This can be made up with fertiliser, but the cost of incineration, together with that of artificial fertiliser, is not economical to the farmer. Incineration may be batch or continuous, the latter being more expensive, but the

former needing more labour for loading purposes. Solid wastes with low moisture contents are best suited, being able, once ignited, to support combustion.

Drying methods have been applied to poultry wastes (Ryder, 1968). These generally have a low moisture content and drying can lead to recycling producing returns for capital input of machinery.

Recent developments (Anon, 1971) by a manufacturer of incinerating equipment has led to the production of machinery which will reduce the moisture content of chicken waste to 50% and then dry it using a micro-wave oven which also sterilises the waste. The product is odourless and of a sufficient amino acid content to be useful as a ruminant ration. It is not likely, however, to be applicable to larger animals because of the increased costs due to higher BOD's, moisture contents and no available outlet for the product.

This review has shown that poultry waste lends itself to easier treatment and disposal than does pig waste. It will be seen in later chapters that the nutritional and fertiliser values of poultry wastes are greater, and this will also contribute towards its easy disposal.

It can be seen that waste management will have to play

an increasing part in the operation of intensive units. Agriculture is an industry which has, like most industries, tended to automation and development of continuous operations. Industrial effluents are having to be rigorously treated before discharge. So, too, will effluents from the 'factory farms' of the future. The working party on sewage disposal (Taken For Granted, HMSO, 1970) recommends that farmers should not be encouraged to press authorities for enlargement of domestic sewage plants to take agricultural wastes - the waste should be returned to the land wherever possible. It also suggests that grants be available for treatment plants. Waste treatment and disposal in the future should become a profit-making industry like the component industries to car-making firms have become. Thus, the farmer will have no worries about the disposal or treatment problems, and his waste may even bring him financial returns.

1.6 THESIS PROPOSALS

From the above review of the characteristics of wastes and the methods by which they are managed on the farm it can be seen that there are problems which no one system is able to control. These problems may be classified as follows:-

1. Odour production.
2. Disease hazards.
3. Pollution of waters.
4. Large bulk for storage.
5. Handling problems.
6. Cost of treatment equipment.
7. Large areas of land required for safe disposal.
8. Low value of waste as fertiliser or re-saleable product.

In the past disciplines concerned with waste treatment have been mainly the engineering and agricultural sciences. Much of their work has involved designs which do not necessarily create optimum conditions for the agents which treat the wastes - the micro-organisms. There is now an increasing realisation that by catering for the micro-organisms improvements on treatment processes can be made.

The objects of this thesis will be to investigate the stabilisation of farm animal wastes by the use of thermophilic fungi, and to develop a cheap simple process which can be incorporated into present intensive management with very little change in routine operations. Certain disposal methods discussed above have indicated that wastes may be recycled either back to the animal which produced them or to another type of animal to prevent deficiencies arising. For example, the digestibility of waste vegetable matter in the form of cellulose from pigs would be greater in cows where a cellulolytic flora exists, than back to pigs where it does not. The action of the thermophiles could be to convert and enhance the waste in its nutritional value.

The increase in intensive pig units and the associated increased treatment problems have pointed towards pig waste as being in most need of research and it is proposed to concentrate on this for the thesis.

For the reasons discussed earlier thermophilic fungi would seem to be suited to solving the eight points mentioned above. Experimentation will investigate the efficiency of this group on the stabilisation of pig waste.

An outline of the practical work will serve to indicate the pattern of the research to be carried out. It was thus proposed to incorporate the following stages:-

- a. An isolation programme to qualitatively and quantitatively determine the occurrence of fungi in intensively produced pig waste.
- b. A determination, on the laboratory scale, of the optimum parameters for the biodegradation of the waste using equipment designed to simulate large-scale operation.
- c. A comparison of the above results with a control compost heap to determine whether the parameters investigated do in fact increase the rate of biodegradation over the natural composting process.
- d. An investigation of the nutritional value of the products from the laboratory-scale work.
- e. An examination of separation techniques for the wastes to ease handling problems, reduce polluting ability, and reclaim valuable cellulose substrate for fungal breakdown.
- f. An 'on farm' pilot-scale plant designed and operated according to the parameters determined in the laboratory. This would then make an economic assessment possible.

It is proposed to develop a two stage process which involves firstly separation of the cellulose fibre from the slurry and secondly thermophilic breakdown and upgrading of the separated solids. Methods for treatment of the liquid fraction will also be investigated. Agriculturalists have been increasingly aware of the advantages of early separation of the solid and liquid fractions of the waste. The MAFF (Short Term Leaflet No. 44) states that solids can block pumps and pipelines and that the separated liquid is easier to spread on the land. Slurry separators are now being marketed in the form of vibrating screens, centrifuges and presses. The vibrating screen can reduce poultry manure to 28% (Anon, 1972) dry matter leaving the liquid to undergo further treatment and the solids either to be composted or spread on the land directly. As slurries can contain up to 95% moisture it is important from a handling viewpoint that this liquid be separated so that it can be handled more easily. Also, the solids fraction is lessened in bulk and is more amenable to further treatment. By reducing the moisture content of the solids to between 60 and 70% and increasing the natural air flow through the material which might have been prevented by waterlogging, microbial, and particularly fungal, activity can be encouraged. In order to prevent odours it is necessary to separate the solid and liquid fractions as quickly as possible so that each fraction can be kept aerobic. Thus, the separation process will probably be an 'in house' type utilising straw as the filtering medium.

In this way it is hoped to create conditions which require very little labour with negligible cost for any conversions required.

The parameters governing the thermophilic stage will be investigated on a laboratory scale to determine the optimum conditions, and then an 'on site' pilot-scale plant will be constructed to test these conditions under small-scale operation in conjunction with the separating stage. It will then be possible to make an economic assessment of the full-scale possibilities.

CHAPTER 2

C H A P T E R 2

THE ROLE OF MICRO-ORGANISMS IN THE BIODEGRADATION OF FARM WASTES AND THEIR OCCURRENCE

- 2.1 The role of micro-organisms in the biodegradation of farm wastes.

- 2.2 The isolation of mesophilic and thermophilic fungi from slurry and semi-solid bedding.
 - 2.2.1 Materials and methods
 - 2.2.2 Results and discussion

2.1 THE ROLE OF MICRO-ORGANISMS IN THE BIODEGRADATION OF FARM WASTES AND THEIR OCCURRENCE

The involvement of micro-organisms in the breakdown of materials has been recognised for over a century although their role has not been fully studied until recently when it was realised that micro-organisms could adversely affect economically important materials such as timber and textiles. This has prompted much research and the word biodeterioration is used to define such research. Hueck (1968) defined biodeterioration as the biological breakdown of economically important materials being a pragmatically negative action of organisms. Results from the study of micro-organisms involved in biodeterioration can be valuable in the study of biodegradation, as in many cases it is often only the value of the material and its siting which determines whether biodeterioration or biodegradation be used to describe the process. In both cases, however, the economic value of the material is important - in biodeterioration the value of the material is decreased and in biodegradation it is enhanced, both actions being brought about using a common knowledge of the growth parameters and requirements of the micro-organism. However, in the case of biodeterioration conditions are created to inhibit growth, whereas for biodegradation the growth parameters are selectively encouraged. Thus biodegradation may be termed as the converse of biodeterioration, and information gained from biodeterioration studies may be used for biodegradation studies. A definition of biodegradation will show how closely inter-related the two

subjects are. Thus, it may be defined (Seal and Eggins, 1972) as the useful breakdown or conversion of those materials subject to biodeterioration (usually waste products) to a higher nutritive value, an economically enhanced or a more aesthetically pleasing product, and in this connection it may be termed as a pragmatically positive action of organisms.

A review of the micro-organisms which have been found to be involved in the biodeterioration of cellulose-based materials, and the biodegradation and breakdown of animal, plant and other cellulose-based wastes will serve to indicate the potential of such micro-organisms in the biodegradation of farm animal wastes.

The isolation of fungi and actinomycetes in mouldy self-heated hay and various manures has led to the conclusion that fungi, and in particular thermophilic fungi, may play an important role in the breakdown of animal wastes (Cooney and Emerson, 1964). Rege (1927) first studied the role of thermophilic fungi in the breakdown of cellulosic material. Waksman, Umbreit and Cordon (1939) found that a culture of what was probably Humicola insolens Cooney and Emerson removed nearly 40% of the dry matter in stable manure and reduced the cellulose content from 19.7% to 12.6% at 50°C in 42 days. Waksman and Cordon (1939) obtained similar results with straw and alfalfa decomposition, and a third paper (Waksman, Cordon and Hulpoi, 1939) suggested that the composting temperature was one of the most important factors in the decomposition

rate, 50°C being the optimum for thermophilic fungi and actinomycetes.

Using horse dung, organic compost, various "muck soils" and composted mint hay Crisan (1959) isolated four thermophilic fungi and the thermotolerant Aspergillus fumigatus Link. His species were confined to the genera Mucor Micheli, Malbranchea Saccardo and Humicola Traaen. This was probably because the isolation temperatures (40 or 45°C) would select out the above species, and also because only one isolation media was finally chosen.

Even though the first isolation of Mucor miehei (described by Cooney and Emerson) in self-heated hay by Miede (Cooney and Emerson, 1964) was at the turn of the century it was not until 1964 that Cooney and Emerson (1964) published the first book on the thermophilic fungi. The authors state that little work has been done on the physiology and role of the thermophiles in thermophilic composting. Prior research carried out by Crisan (1959) and Eggins and Coursey (1964) who isolated thermophilic fungi from composts and palm kernel stacks respectively indicated that such fungi had minimum, optimum and maximum growth ranges outside the normal ranges for mesophilic or psychrophilic fungi. Crisan (1959) defined thermophilic fungi according to the optimum temperature for growth which should lie at or above 40°C. Cooney and Emerson (1964) used maximum and minimum growth ranges as they found that these would exclude

those thermotolerant mesophilic species with a minimum growth range well below that of the true thermophiles. They thus defined a thermophilic fungus as "one that has a maximum temperature for growth at or above 50°C and a minimum temperature for growth at or above 20°C.". More work on the physiological and biochemical activities has been undertaken to characterise the group as important agents in breakdown and conversion processes.

Fergus (1964) investigated the thermophilic and thermotolerant actinomycetes and fungi found in 'Phase II' (controlled temperature of 50 - 60°C) of the composting horse manure for mushroom production. His investigation was qualitative, isolating eleven thermophilic actinomycetes, nine of which had optimum growth temperatures of between 55°C and 60°C. Eight thermophilic fungi were isolated, including a new species Stilbella thermophila Fergus sp. nov. The strongly cellulolytic Humicola Traaen and Chaetomium Kunze species and the thermotolerant Aspergillus fumigatus Link constituted five of the eight fungi isolated. On the whole it was found that the actinomycetes isolated were able to grow at a higher temperature (55 - 60°C) than the fungi (50 - 55°C) isolated. In 1969 Fergus further determined the cellulolytic activities of the thermophilic fungi but not in terms of the ability to degrade native cellulose - only the ability to hydrolyse filter paper and carboxymethyl cellulose was tested.

Chang and Hudson (1967), however, studied wheat straw composts. They made ecological studies of the occurrence of thermotolerant and thermophilic fungi in the compost and related this to the variations in temperature. They thus determined a succession pattern consisting of three groups. The first group consisted of those fungi found on the straw after harvesting and up to about two days after the beginning of composting. By this time the temperature had reached about 40°C. Most of these fungi were primary sugar utilisers which are replaced by other fungi when the simple sugars are exhausted. Group two consisted of the thermophilic species and these were dominant when the compost reached its plateau of about 50°C (this occurred after a maximum of about 70°C was reached in the centre of the compost, killing off most of the viable population). Thermophilic temperatures persisted until about 28 days after the beginning of composting. As the temperature began to drop the group three fungi began to appear beginning with two thermophilic fungi at the end of the plateau and then the mesophilic species. Though not viable the group two fungi were still present.

The biochemical studies of Chang (1967) showed that the three groups defined above were responsible for the degradation of different substrates within the compost. The Group one fungi utilised all the soluble sugar, Group two were responsible for the cellulose and hemicellulose utilisation and Group three utilised cellulose to a small extent but also the soluble sugars produced by Group two

fungi. Gollmich (1967) working with chopped plant material, also determined three groups of fungi based on their cellulolytic activity. Chang (1967) in particular showed that Chaetomium thermophile La Touche was able to break down over 40% of the total weight of straw in three weeks. This was almost equivalent to the amount of breakdown by the remainder of the microbial population.

Malik (1970) has isolated eleven thermophilic fungi and the thermotolerant Aspergillus fumigatus from pasture land soil using soil enrichment and perfusion techniques (Malik and Eggins, 1969). Using the perfusion technique he was able to determine patterns of colonisation, optimum pH values and cellulolytic activities of the isolated fungi on cellulose paper strips. Optimum pH values for the thermophilic cellulolytic fungi were found to be between 6 and 7 when measured as the extent of the biodeterioration of a cellulose strip by weight loss. The patterns of colonisation elucidated cellulolytic fungi as primary colonisers followed by the "secondary sugar fungi" (Garrett, 1963) such as Humicola lanuginosa (Griffon and Maublanc) Bunce, which are thought to utilise the simple sugars produced from the breakdown of the cellulose (Chang, 1967). Cellulolytic activities were also found to be at their optima between pH 5 and 7. The work of Malik has provided much information on the growth parameters of the thermophilic fungi, for biodeterioration studies, particularly the cellulolytic species, and this information may well be

important in the study of animal waste biodegradation at thermophilic temperatures.

Cooke and Matsura (1969) isolated 212 species of mesophilic fungi and yeasts from a human waste stabilisation pond. They found that fungi and yeasts were abundant in number in the raw sewage, increasing in number as the waste was left. Most of the fungi and yeasts arose from the surrounding soil and air.

The presence of actinomycetes in farm wastes was first shown by Tsiklinsky (Cross, Walker and Gould, 1968). She isolated a thermophilic actinomycete from decaying straw and manure and named it Thermoactinomyces vulgaris Tsiklinsky. Waksman, Umbreit and Cordon (1939) isolated a similar actinomycete and named it Micromonospora vulgaris. However, it is now known that these actinomycetes were both the same, being T. vulgaris and having the ability to withstand boiling and high temperatures by forming endospores very similar to those found in the family Bacillaceae (Cross, Walker and Gould, 1968). Waksman et al. (1939) also isolated actinomycetes from horse manure and soils. Corbaz, Gregory and Lacey (1963) isolated eight species of actinomycetes at 40°C and 60°C from mouldy hay. The incidence of up to 10⁹ spores per gram of hay had previously been determined by Gregory and Lacey (1963). Fergus (1964) isolated eleven species of actinomycetes from mushroom compost at its peak temperature and followed this up (Fergus, 1969) by the

determination of the cellulolytic activity of thermophilic actinomycetes. He concluded by saying that the actinomycetes were much less capable of degrading cellulose than the thermophilic fungi. Williams (1966) considers the competitive ability of the actinomycetes. He states that they grow more slowly than fungi and do not reproduce as quickly as bacteria. However, their ability to produce antibiotics, to colonise complex compounds, and to penetrate difficult substrates such as wood using the combined characteristics of high cellulolytic enzyme production with much thinner hyphae gives them an advantage in natural substrates. Fergus (1967) also tested the thermodurability of thermophilic actinomycetes and found that their range of resistance at 100°C in sucrose solution was from 10 minutes (Pseudonocardia thermophila Henssen) to 4 hours (Thermoactinomyces vulgaris and Thermomonospora curvata Henssen). The resistance to dry heat at 100°C was longer - 6 hours for all species excepting P.thermophila. This work is in agreement with Erikson (1955) who found that T. vulgaris could survive up to 4 hours at 100°C.

Malik (1970) isolated thermophilic actinomycetes from soil using the antifungal antibiotic pimafulcin which was unaffected in its activity at 50°C. Penicillin and streptomycin were used as antibacterial antibiotics. When these antibiotics were incorporated into the isolation medium maximum isolations of actinomycetes were obtained as adverse competition from fast growing fungi and bacteria

was eliminated. Four cellulolytic species were identified by the method of Cross and MacIver (1966) and their cellulolytic activity determined using the perfusion technique. Maximum percentage weight loss of the cellulose strip of 33% was obtained after four weeks. Thus, even in pure culture the activities of the thermophilic cellulolytic actinomycetes were relatively slow. Stutzenberger (1971) has found that Thermomonospora curvata, isolated from municipal waste, is highly cellulolytic and states that he believes this actinomycete to be important in the composting process. However, Poincelot (1972) states that such research in pure culture cannot be extended to processes where antagonisms and competitions exist.

The importance of actinomycetes in farm waste disposal, especially the thermophilic group, is shown by their range of occurrence and ability to utilise cellulose as a pure substrate and in the form of hay and straw. They have also been shown to be able to grow at higher temperatures and may be of use where temperatures of between 50°C and 60°C may be required for removal of pathogens.

Sie, Sobotka and Baker (1961) found that the factor determining thermophilism in bacteria could be transferred from thermophilic to mesophilic bacteria. When the mesophilic bacterium Bacillus sphaerius was added to fresh culture medium containing the thermophilic spore-forming aerobic bacterium B. stearotherophilus the latter could

transfer its thermophily, allowing B. sphaerius to grow at 55°C. It is thought that the transferred material is nucleoprotein. Work on anaerobic cellulose fermenting bacteria (McBee, 1948) showed that the products of fermentation were carbon dioxide, hydrogen, ethanol, formic acid, acetic acid, lactic acid, succinic acid and glycerol. Some hydrolysis of cellulose to cellobiose and glucose was also noted. Russian workers (Loginova, Golovacheva and Shcherbakov, 1966) have found that anaerobic thermophilic bacteria will break down cellulose to a greater extent when in a symbiotic relationship with aerobic thermophilic bacteria. It is thought that this enhancement is due to the production of vitamins, complex nitrogenous compounds and the removal of the products of fermentation of cellulose.

The role of bacteria in the biodegradation of farm wastes is difficult to elucidate. Müller (1964) believes that self-ignition of hay is caused by B. subtilis and B. stearothermophilus. The odours from farm wastes (Stephens, 1971) are usually due to bacteria which are able to anaerobically reduce urea to ammonia and its amine derivatives, and proteins to hydrogen sulphide and thio alcohols. These bacteria are usually undesirable and can be inhibited by aerobiosis. Another group of bacteria present will be the potential pathogens such as those excreted in the faeces and those which find the waste a suitable environment. These are more difficult to control, although the use of antibiotics in feedstuffs may reduce their

numbers. The third group of bacteria which are found in farm wastes are those which are able to decompose the waste plant material which forms the bulk of the farm waste. These will be present in the environment and on straw and bedding. McCoy (1966) working with bovine manure from a lagoon found a significant number of pectolytic and cellulolytic bacteria, but a predominance of proteolytic, nitrate reducing and lactic acid producing bacteria. All her counts were based on the Most Probable Number technique carried out at 30°C aerobically and anaerobically.

The bacterium Cellulomonas isolated by Han and Srinivasan (1968) has been used in the production of protein from waste bagasse in the U.S.A. (Callihan and Dunlap, 1969). The process has reached plant scale and the authors claim that an economic evaluation of the process has shown it to be a viable proposition. Newman (1959) reported the isolation of a thermophilic cellulolytic bacterium from composting refuse; and Stutzenberger, Kaufman and Lossin (1970) determined the cellulolytic activity of the indigenous flora in municipal waste composting. They isolated Aspergillus fumigatus, Thermoactinomyces sp. and Bacillus sp. The actinomycete isolated was shown to be active on cellulose agar at 60°C.

The incidence of pathogens in animal wastes is of considerable importance when consideration is made of treatment and disposal methods. If the treatments lead

to the production of a feedstuff then pathogens must be removed. Composting has been found to effectively inhibit growth of pathogens (Jalal, 1969) but it is likely that the spore formers will be able to survive the maximum temperature recorded. It is fortunate, however, that spore forming bacteria are only found in the Bacillaceae. This group consists of two genera, Bacillus and Clostridium, both capable of a wide variety of diseases in man. When considering the precautions necessary for the production of a feedstuff it is important that control measures regarding the output of pathogens are restricted to prevention of normal enteric pathogens which may cause, not a fatal or near fatal reaction in the animal to which they are given, but will be transferred to humans via the meat they eat. Outbreaks of animal diseases which can be diagnosed are controllable at the animal level with total destruction of the wastes. The transference of enteric bacteria to humans from food has received much attention (Bower, 1970) since the introduction of antibiotics in animal feeds in 1953 (HMSO, 1969) for use as fattening enhancement agents (Braude, Townsend, Harrington and Powell, 1962) and disease controlling factors. Strains of antibiotic resistant organisms have developed in the animals, and these have been transferred to humans where they are able to transfer their resistance to susceptible bacteria (Williams Smith, 1969) thus making the use of normal doses of antibiotics ineffective. The Swann Report (HMSO, 1969) condemned the indiscriminate use of antibiotics for this

reason and suggested that control methods be instituted. The carry over of antibiotics in the waste may also occur which may also be important if the product is to be used as a recycled feedstuff. Although the persistence of antibiotics in animal wastes has received little attention, and only then in connection with their effects on the breakdown process, it seems likely that any residues in a feedstuff might effectively decrease the usefulness of normal doses of antibiotics used to combat disease. Research into the stability of any antibiotic in relation to any processing of the waste required would need to be done to safeguard against this. Unlike antibiotics research into the accumulation of insecticide residues in animal tissues has been investigated because of the possibility that the residues may accumulate in human tissue up to a toxic level when the meat is eaten. For example, low levels of DDT have been detected in heat sterilised poultry waste (Taylor, 1971). However, feeding sheep diets containing up to 75% waste for 80 days did not result in substantial residues in the fat or liver. The author did not state the safe levels but reported that levels were comparable to a control diet used. El Sabban et al. (1970) fed autoclaved, cooked and dried poultry waste to cattle. Although levels of chlorinated hydrocarbons and arsenic in the rations or the waste products of the poultry were not known, feeding the waste did not seem to increase the levels of the chlorinated hydrocarbons in the fat of the cattle. There were no detectable residues of lindane, aldrin, dieldrin or heptachlor found

in the fat of the cattle.

The role that the three main groups of thermophilic micro-organisms - fungi, bacteria and actinomycetes - play in the breakdown of a variety of substrates shows that the possibility of their controlled use in the treatment of animal wastes is desirable. Some species of the thermophilic fungi, in particular, have been shown to degrade cellulose very quickly. The advantages of a thermophilic stage are many; the removal of pathogens has been shown to occur between 50°C and 60°C and the number of species of micro-organisms able to grow is drastically reduced, making the process controllable from an ecological viewpoint. The need for a mesophilic and thermophilic stage which normally occurs in natural composts has not been elucidated, but it has certainly been found that composting is speeded up by using a continuous thermophilic stage. Certainly vant Hoff's law that the rate of a reaction approximately doubles or trebles for every 10°C rise up to its optimum may be important in considering the economics of a mesophilic and thermophilic stage. The populations of thermophilic organisms need to be considered if the breakdown is to be efficient. The presence of primary sugar fungi and cellulose decomposers is a necessity for a complete breakdown. The addition of inocula has been considered by Golueke, Card and McGauhey (1954). They tested the effects of the addition of fresh material, horse manure, bacteria and soil on the temperature, increase in ash and decrease in carbon in a

municipal refuse compost. The inocula additions made no difference to these parameters and it was suggested that an adequate bacterial population was already present in the compost.

The effect of antibiotics and copper compounds as fattening agents has been found to have not only an adverse effect on the resistance of pathogens but also on the organisms which naturally degrade the waste. Robinson, Draper and Gelman (1971) have found that 500 parts per million (ppm) of copper in aerated pig urine will inhibit all microbial breakdown of soluble nitrogen compounds. They have found that pig wastes can contain up to 750 ppm of copper. The effects of antibiotics will probably parallel this observation. It is thus important that for fast and efficient treatment of farm animal wastes antibiotic and copper contents should be kept to a non-toxic level.

It seems clear from the above review that much more needs to be done to elucidate the role of fungi, actinomycetes and bacteria in the biodegradation of farm wastes. Assumptions from work done on municipal composting and sewage treatment, as well as with just plant material composts carry very little validity when applied to farm wastes, particularly those of animal origin. Municipal refuse with its high paper content of up to 54% (Higginson, 1965) is of a different composition from farm waste. This is true also of straw and plant material composts of an

artificial composition. However, techniques and guidelines in research provided by these studies may be used to reveal colonisation patterns, biochemical activities, and processes which will combine the results from these studies to enable us to create conditions for the active biodegradation of animal wastes and remove an ever increasing pollution problem.

2.2 THE ISOLATION OF MESOPHILIC AND THERMOPHILIC FUNGI FROM SLURRY AND STRAW BEDDING

The above review has suggested that bacteria, fungi and actinomycetes are able to tolerate thermophilic temperatures. Certain species from all the groups have been isolated from composting plant material and municipal rubbish and have been shown to play an active part in the breakdown process. The desire that fungi should be used as the agents of breakdown which was suggested in the first chapter can be further substantiated from the review above. Workers studying composting material have noted fungi and actinomycetes to be the main agents in the breakdown of the cellulose constituent. Stutzenberger (1971) has suggested that actinomycetes may be important in the breakdown of cellulose. However, work done on the actinomycetes to date in natural substrates suggests that they are slow colonisers and can be out grown by the fungi. The biochemical studies by Chang (1967) have shown that fungi, and in particular

the thermophilic species, are very important as cellulose degraders in straw composts. It is thus important having chosen fungi as the agents of waste breakdown that their occurrence within the substrates to be used is investigated. This will provide information as to the extent to which cellulolytic species are found and also the frequency to which they occur. The results will also supply information to the process work, i.e. whether there is a need for inocula addition and the optimum pH ranges for maximum activity of cellulolytic and non-cellulolytic species alike.

Thus the isolation of fungi from pig slurry and deep litter bedding was undertaken to determine the range and frequency of thermophilic and mesophilic species present. The isolations were made at a range of pH values so that optimum occurrences could be noted for those fungi considered to be of importance in any process developed.

2.2.1 Materials and Methods

Two isolation media were used - Eggins and Pugh cellulose agar and Eggins and Pugh mineral salts agar with starch and glucose substituted for cellulose (see Appendix I for composition of agars). These agars have been found to be of considerable use in the isolation of cellulolytic and non-cellulolytic species of fungi by other workers at the Biodeterioration Information Centre. The salts provide a buffering effect which keeps the medium pH at between 5.6

and 5.8 and the inclusion of the dye rose bengal (di-sodium 4,5,6,7-tetrachloro-2,4,5,7, tetraiodofluorescein) suppresses the growth of bacteria (Ottow, 1972). The agars were buffered at pH 4.0, 6.6, 7.6 and 8.6 using McIlvaine's citrate-phosphate buffer (see Appendix II for preparation), using either 1N hydrochloric acid or 1N sodium hydroxide to bring the agars to their respective pH values. Agar at pH 5.6 was also prepared which needed no buffering. All pH values described are those after autoclaving as there were sometimes slight changes in the pH (see figure 5). Sterilisation was carried out at 10 lb/sq. in. for twenty minutes and the subsequent pH determination carried out whilst the agar was still hot using a small sample from each of the bottles. A Pye (model 78) pH meter was used with an Ingold pH electrode. The temperature was compensated for on the meter during the measurements. Three temperatures were used for the isolations - 25, 40 and 48°C. The incubators used for the temperature control were maintained at a high humidity by bubbling air through water baths placed on the floor of the incubators. Ten replicate plates were prepared for each temperature, pH and media.

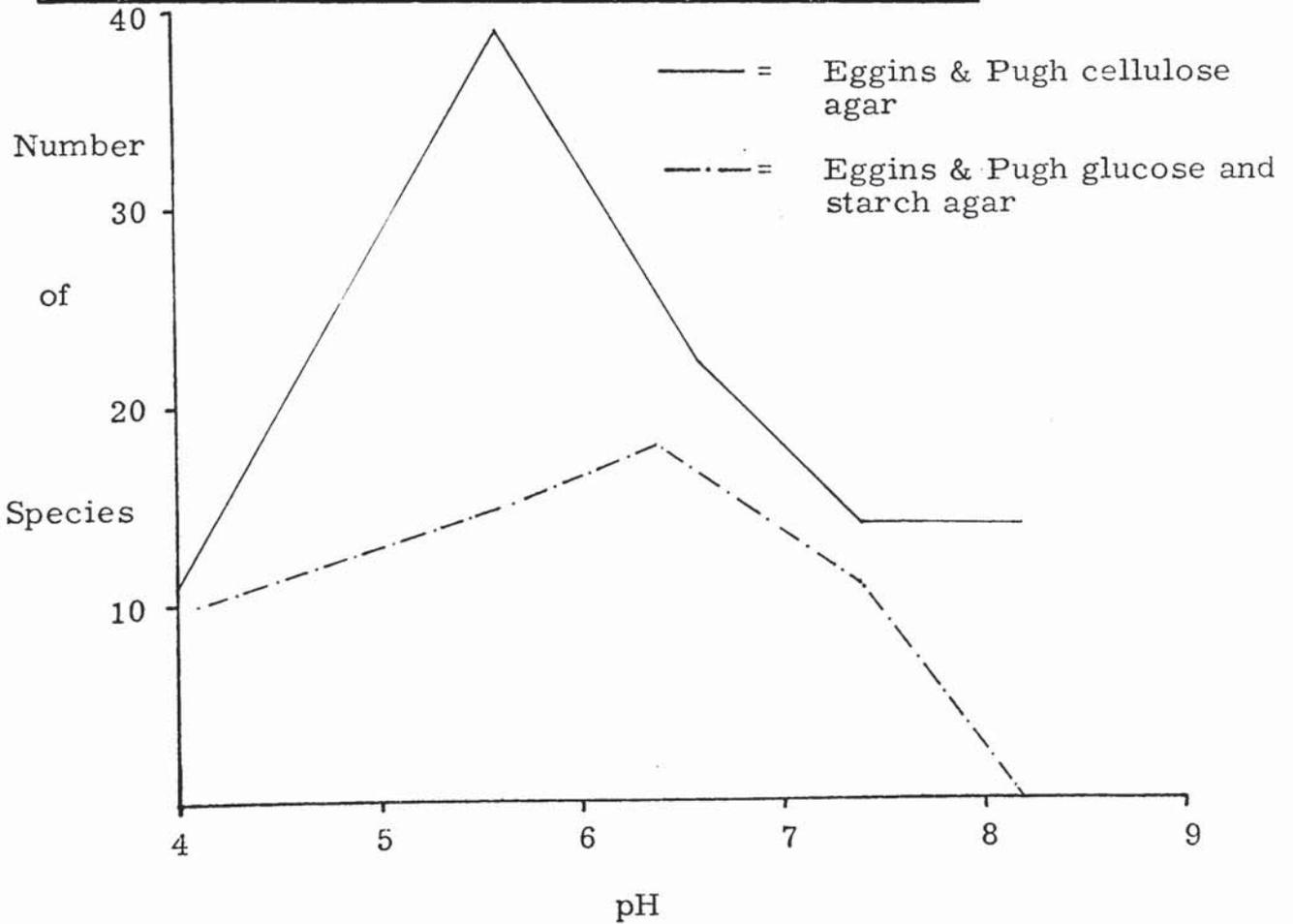
The samples for isolation were collected from either fresh pig slurry or from deep litter bedding and placed in sterile containers for transport to the laboratory. Dilutions of 10^{-1} were made using sterile distilled water containing glass balls for efficient separation of fungi from tissue and other debris. 1ml. samples were then pipetted into

FIGURE 5
pH OF AGAR AFTER AUTOCLAVING

Eggin's & Pugh	Glucose and Starch
4.0	4.1
5.6	5.6
6.6	6.4
7.4	7.4
8.2	8.2

FIGURE 6

NUMBER OF SPECIES ISOLATED AT VARIOUS pH VALUES



petri dishes, and then the agar was poured and mixed thoroughly. Identifications and frequencies were determined after twelve days incubation. Observation and subsequent identifications were made using the 'Sellotape' technique described by Lloyd (1965). This enabled whole structures to be observed which might otherwise have broken up using normal mounting procedures. Mounting and staining of the cell material was effected using lactophenol/cotton blue prepared according to Smith (1971).

Percentage frequency of isolation (PFI) was determined by noting on how many of the ten plates a particular fungus appeared. For example, a fungus isolated from five out of ten plates would have a PFI of 50%. The PFI's are presented in histogram form in figures 7 to 12.

2.2.2 Results and Discussion

a. Isolations from deep litter bedding

Figure 6 shows that the highest number of species (39) isolated from the cellulose agar was greater than that from the glucose-starch medium (18) and that these numbers occurred at pH's 5.6 and 6.6 respectively. This would seem to indicate a flora which preferred the cellulose substrate and was able to actively utilise it. The overall optimum pH was 5.6 showing the preference of fungi to the acid ranges, although it will be seen below that many of

TABLE 6

ISOLATES FROM STRAW BEDDING AT 25°C
CLASSIFIED ACCORDING TO SIU'S (1951) CLASSIFICATION
OF CELLULOLYTIC ACTIVITIES



* Shown to clear cellulose agar by Malik (1970)

the active cellulose degraders were isolated at significant frequencies in the alkaline ranges. The efficiency of the Eggins and Pugh agar as an isolation medium was further supported, its pH being 5.6 and there being the greatest numbers of fungal isolations from it. Glucose starch as a selective isolation medium could not be distinguished from the cellulose based agar on the basis of the species isolated in this investigation.

It is necessary next to discuss the activity of the species isolated, on a cellulose based substrate such as straw. The review by Siu (1951) gives lists of fungi which have been found growing on cotton textile, a material with a high cellulose content. Of the fungi isolated at 25°C on the cellulose and glucose-starch media 9 of the 22 species isolated had been isolated on cotton and the following species classified by Siu as being strong, moderate, definite or weak in their cellulolytic activities (see Table 6).

Malik (1970) using modified Rautela and Cowling tubes containing Eggins and Pugh cellulose agar, also found the fungi isolated here to have the ability to clear cellulose agar (see Table 6). This was in connection with prevention of biodeterioration of which such fungi were known to be causative agents. However such activities can also be related to biodegradation and thus used in this present investigation.

Thus 16 of the 22 species have been classified as cellulolytic. The classification of Siu seems to have no quantitative basis although it may be used as an indicator, in this investigation, to show the flora capable of degrading the cellulose fraction of the straw.

The pH ranges of the fungi at 25°C (figures 7 and 8) indicate where the optimum growth rates and activities occur. It can be seen that Humicola spp., Monilia sp., Paecilomyces sp. and Mucor spp. were isolated over a wide range of pH values although this was dependant upon the medium. Mucor spp. had a wider range on glucose-starch (4.1 - 7.4) than on cellulose agar (4.0 - 6.6). Humicola spp., Monilia sp. and Paecilomyces sp. occurred at pH 6.4 only on glucose-starch but from 4.0 - 8.2 on cellulose agar. This indicates a much wider tolerance for the cellulose and presumably a preference for this substrate. Other species such as Aspergillus fumigatus, Chaetomium spp. and Penicillium spp. appeared to have definite optimum pH ranges. Aspergillus fumigatus had a PFI of 90% on cellulose agar at pH 4.0 and 10% at 6.6. These optima are in agreement with Mills (1973) who showed that the cellulase from this fungus had two optima at pH 4.2 and 6.2. Mills, however, extracted his cellulase at thermophilic temperatures. However, Levinson and Reese (1950) obtained similar results for a mesophilic strain. On glucose-starch agar Aspergillus fumigatus had an optimum pH of 5.6 (PFI 90%) but had significant PFI's of 40% at pH 4.1 and 6.4. The Chaetomium spp. had maximum PFI's of

FIGURE 7
Eggin's-Pugh cellulose medium at 25°C

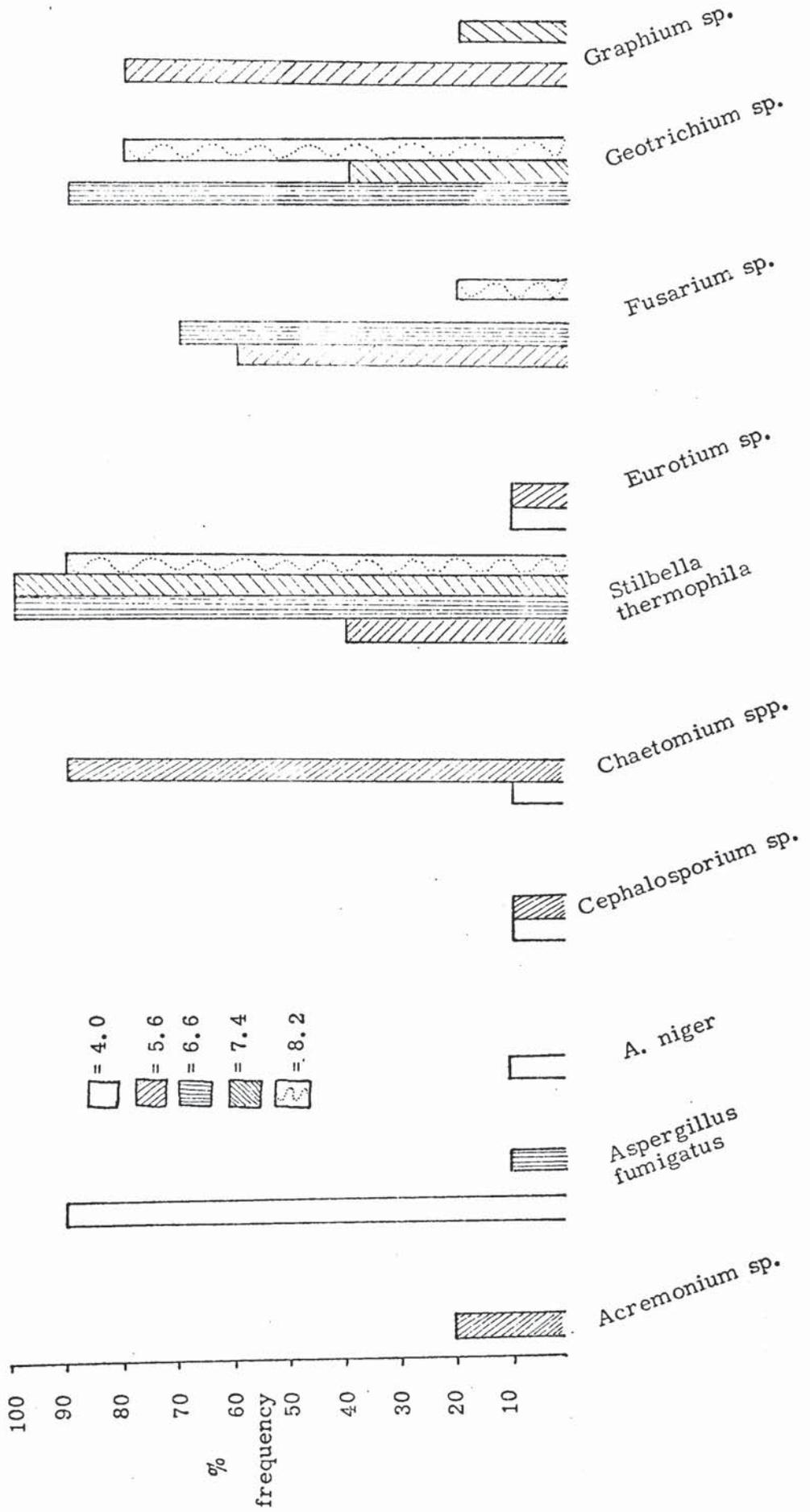


FIGURE 7

Eggs-Pugh cellulose medium at 25°C

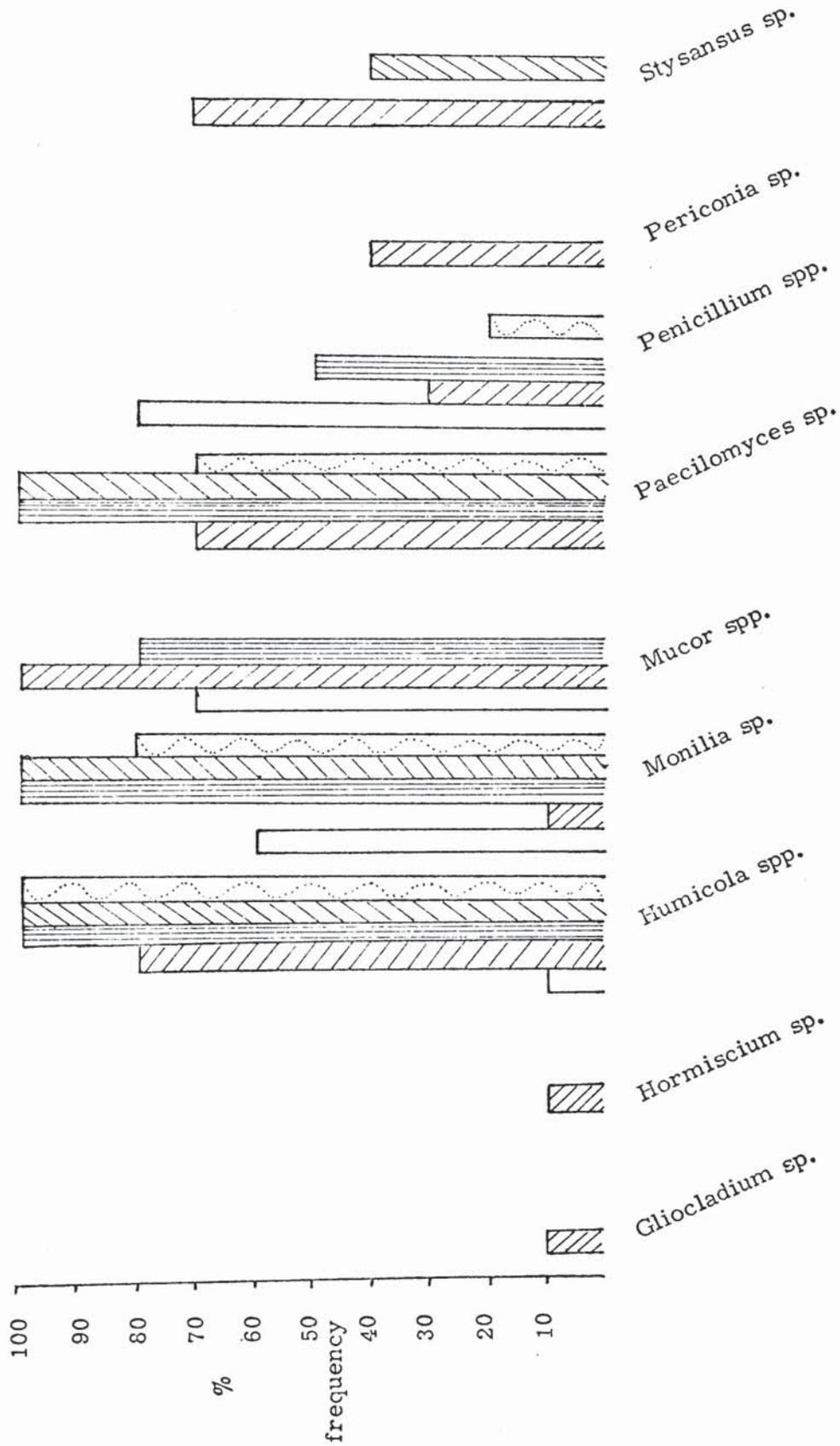


FIGURE 7
Eggin's-Pugh cellulose medium at 25°C

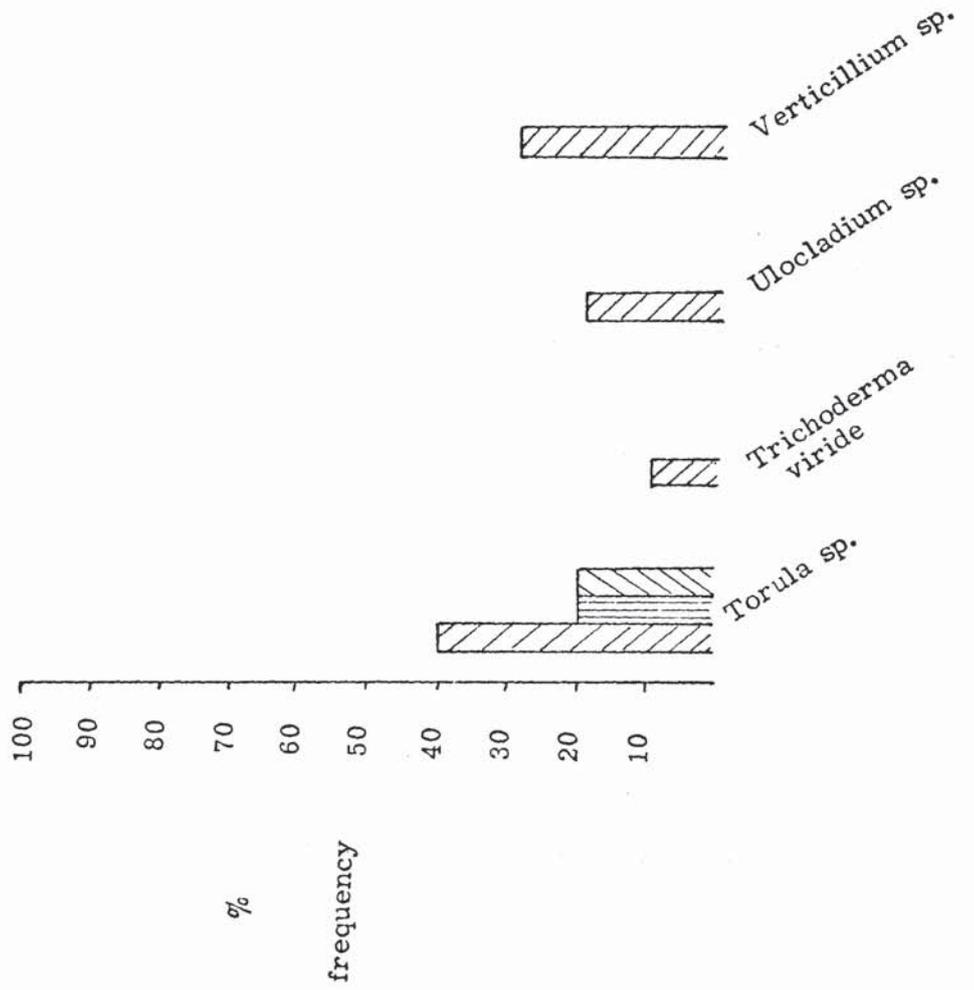


FIGURE 8

Glucose-Starch Medium at 25°C

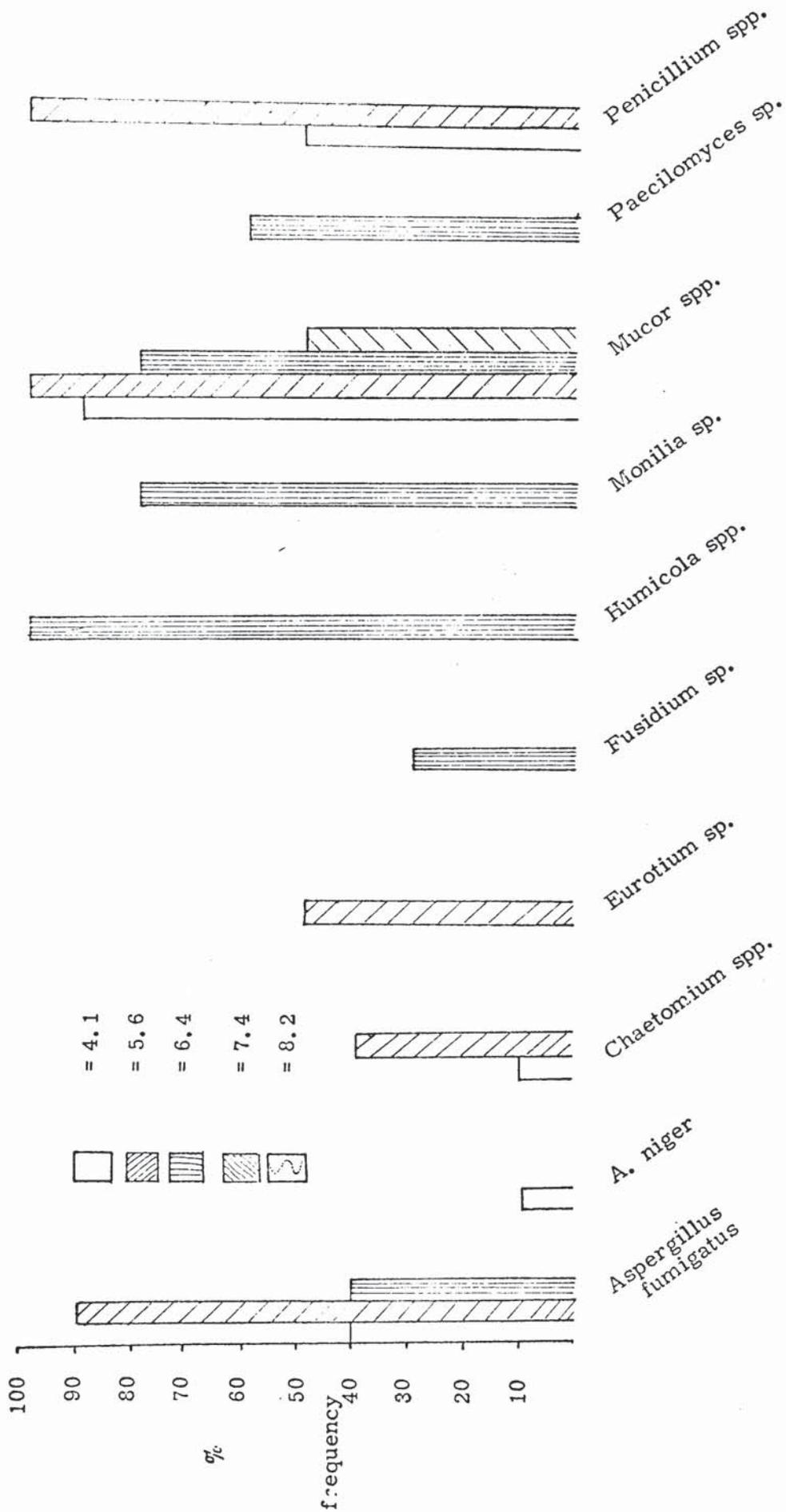
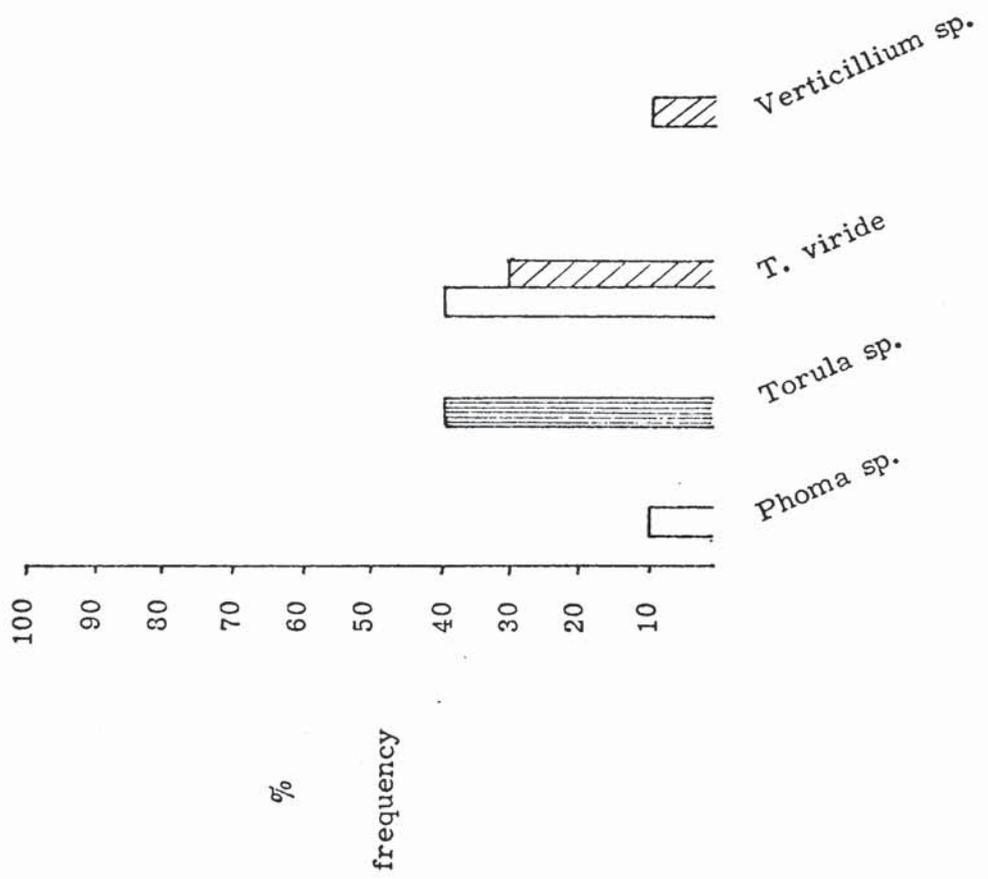


FIGURE 8

Glucose-Starch Medium at 25°C



90% and 40% on cellulose and glucose-starch agars respectively at pH 5.6. Penicillium spp. preferred pH 4.0 on cellulose agar (PFI 80%) and 5.6 on glucose-starch (PFI 90%).

Apart from those above species which exhibited acid pH preferences there were those species with high PFI's in both the alkaline and acid ranges. Stilbella thermophila was isolated only on the cellulose agar where it had a range from pH 6.6 - 8.2 with PFI's of 100% and 90%. Geotrichum sp. had two high PFI's of 90% at pH 6.6 and 80% at pH 8.2 on cellulose agar. Although Humicola spp., Monilia sp., and Paecilomyces sp. were isolated over the range of pH's investigated it can be seen that the highest PFI's tended to occur towards and in the alkaline ranges, notably between 6.6 and 7.4 on cellulose agar. It will be seen later that thermophilic Humicola spp. were isolated at alkaline pH values.

The information gained from these isolations at 25°C shows that an active cellulolytic flora was present in the straw bedding used for the investigations. Such a flora can only be judged as adequate when quoted in terms of the breakdown process and the product required, but given the correct pH ranges selected species may be able to pretreat the cellulose fraction during the filtration stage when the straw is still at ambient temperatures. The results from this isolation programme also indicate a simple control procedure for suppressing Aspergillus fumigatus. By

selecting a pH at or above 6.6 Humicola, Monilia and Paecilomyces will be encouraged. However, as will be seen later, an alkaline pH may adversely affect the nitrogen content and favour the growth of bacteria.

At 40°C no mesophilic species were isolated (see figures 9 and 10). The thermotolerant Aspergillus fumigatus increased its activity having a PFI of 100% on cellulose agar and 90% on glucose-starch agar at pH 5.6. Mills (1973) found that this fungus caused maximum strength losses in cellulose paper at 5.8. The activity of Aspergillus fumigatus above 5.6 decreased and the PFI's at 6.6, 7.4 and 8.2 were 60%, 40% and 10% respectively. Chaetomium thermophile had a narrow range for isolation (pH 5.6) on cellulose and on glucose-starch only occurred on one plate in ten at pH 5.6 and 6.4 - an indication of the preference for cellulose substrates. It should be noted here that for this and subsequent isolation work no attempt was made to differentiate between the two varieties coprophile and dissitum as proposed by Cooney and Emerson (1964). Mills (1973) and Malik (1970) have found there to be very little difference in the cellulolytic activities of the two varieties, both being good cellulose degraders. Thus, for the purpose of this investigation, differentiation was not deemed to be necessary. Mucor pusillus was only isolated on cellulose between pH 4.0 and 6.6, preferring pH 4.0 with a PFI of 60%. Mucor pusillus has been reported to be non-cellulolytic (Chang, 1967) and in this respect, isolations on the glucose-starch

FIGURE 9
Eggin-Pugh Cellulose Medium at 40°C

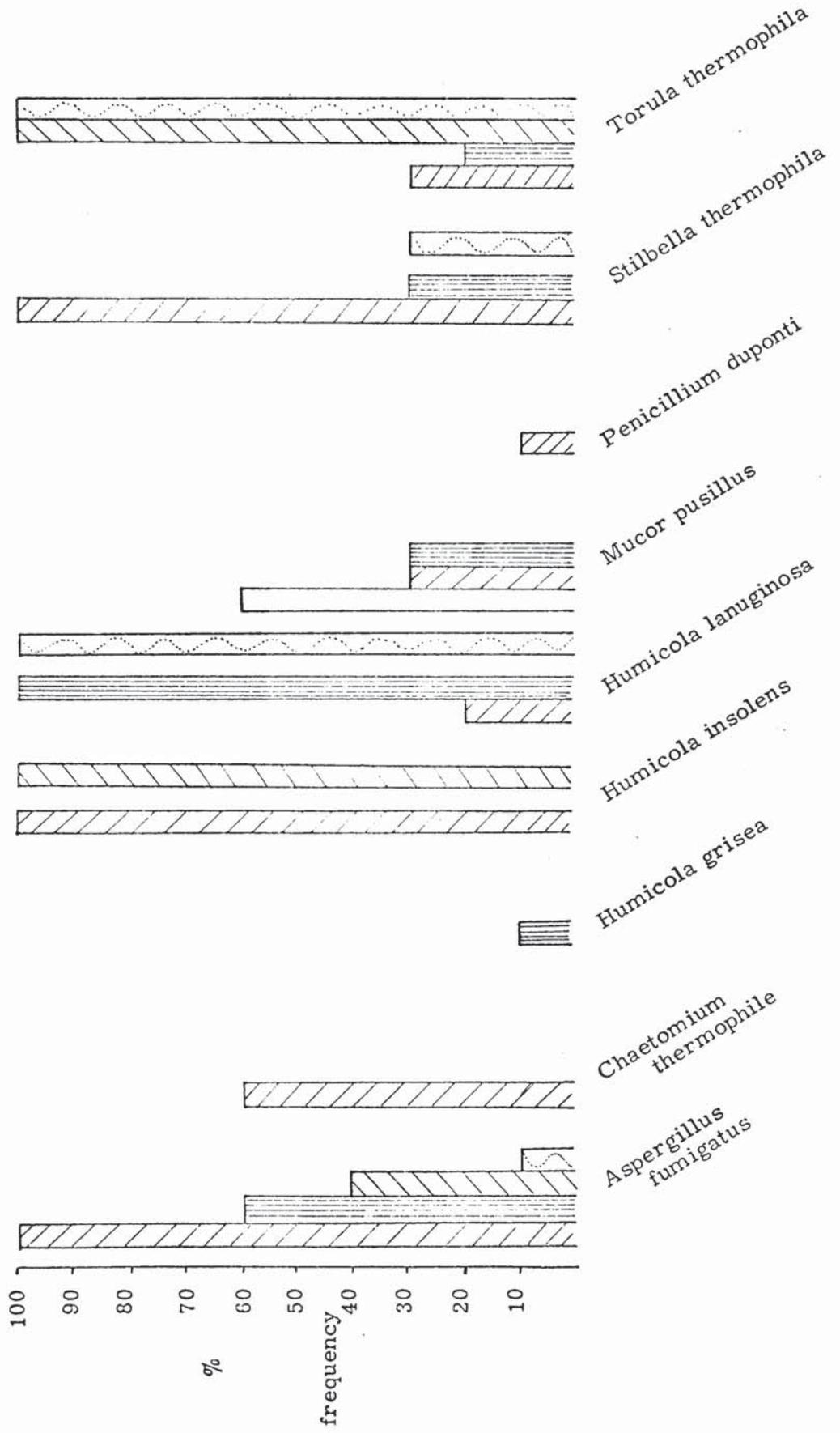
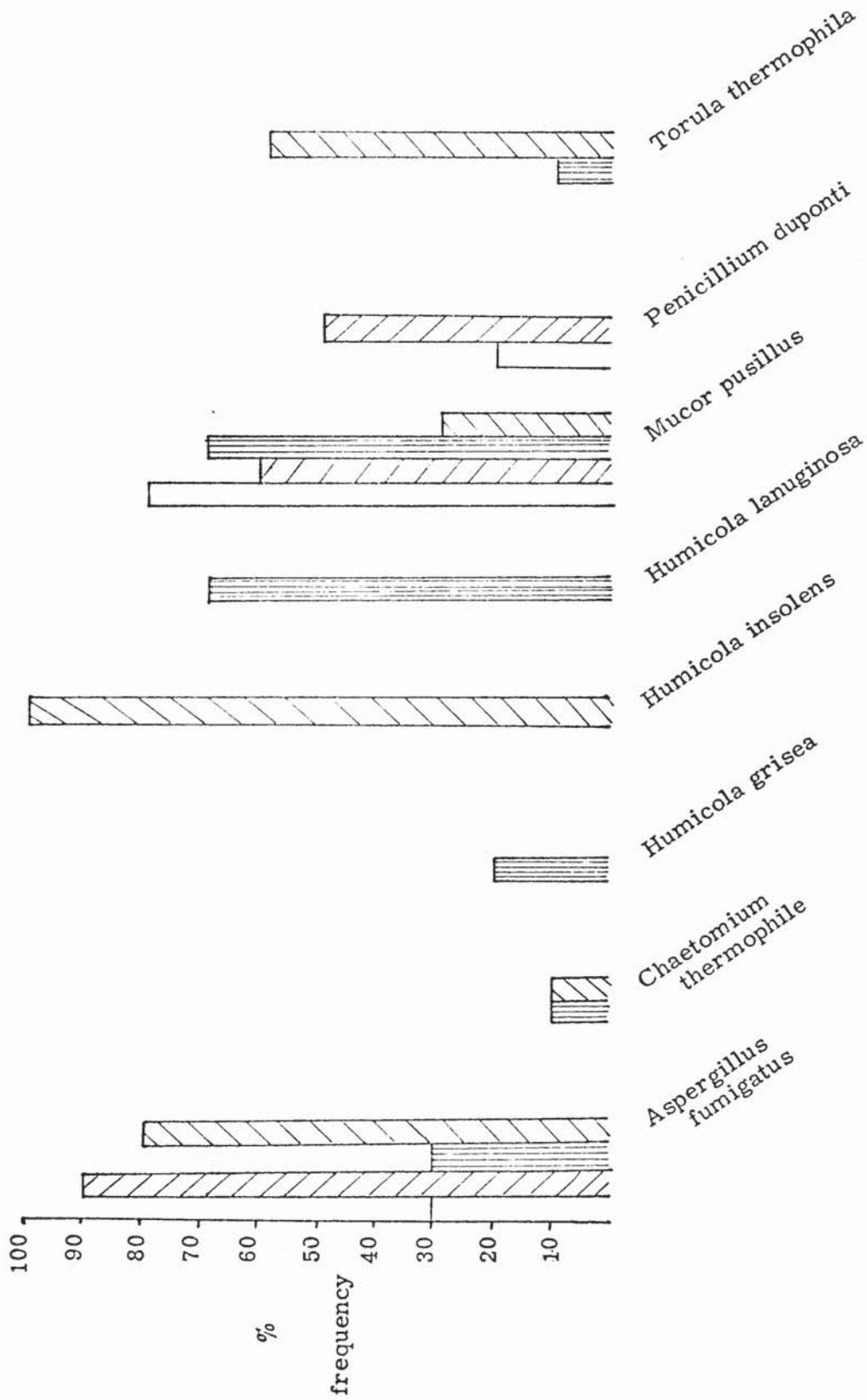


FIGURE 10

Glucose-Starch Medium at 40°C



medium were higher (maximum of 80% at pH 4.1) and covered a wider pH range from 4.1 to 7.4. Humicola insolens and Stilbella thermophila had optimum PFI's at 5.6 on cellulose agar, although Humicola insolens was also isolated (PFI 100%) at pH 7.4. Humicola lanuginosa and Torula thermophila preferred the alkaline ranges on cellulose agar but were isolated at pH 5.6 and 6.6 where Humicola lanuginosa had PFI's of 20% and 100% respectively. Torula thermophila, on the other hand, had PFI's of 30% and 20% respectively. However, the high number of isolations at pH 7.4 - 8.2 suggest a selective dominance at these values where competition from fast growers such as Aspergillus fumigatus, Mucor pusillus and Chaetomium thermophile is suppressed by adverse pH conditions. Humicola grisea and Penicillium duponti were only isolated at pH 6.6 and 5.6 on cellulose agar where they had PFI's of 10%. Narrow pH ranges were recorded for isolations on the glucose-starch medium. Humicola lanuginosa, although non-cellulolytic (Pugh, Blakeman and Morgan-Jones, 1964) was only isolated at pH 6.4 with a PFI of 70%. Humicola insolens and Torula thermophila only occurred between pH 4.1 and 5.6. As these two fungi are cellulolytic they probably preferred the cellulose agar and were able to colonise it at a wider range.

On cellulose agar at 48°C (figures 11 and 12) Aspergillus fumigatus was suppressed to occur only between pH 4.0 and 5.6 with a low PFI of 20%. However, on the glucose-starch substrate it still had PFI's of 80% and

FIGURE 11

Eggin's-Pugh Cellulose Medium at 48°C

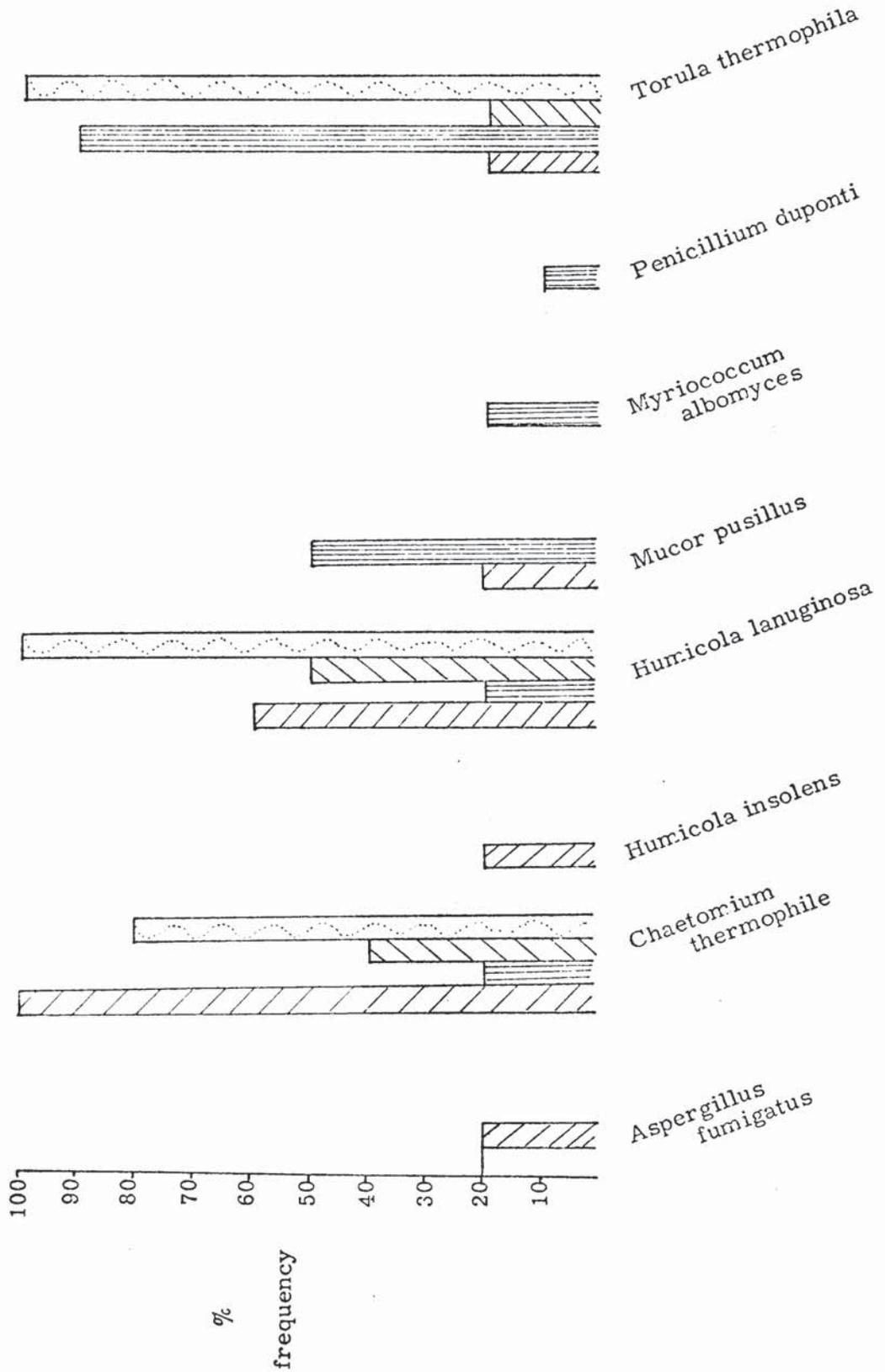
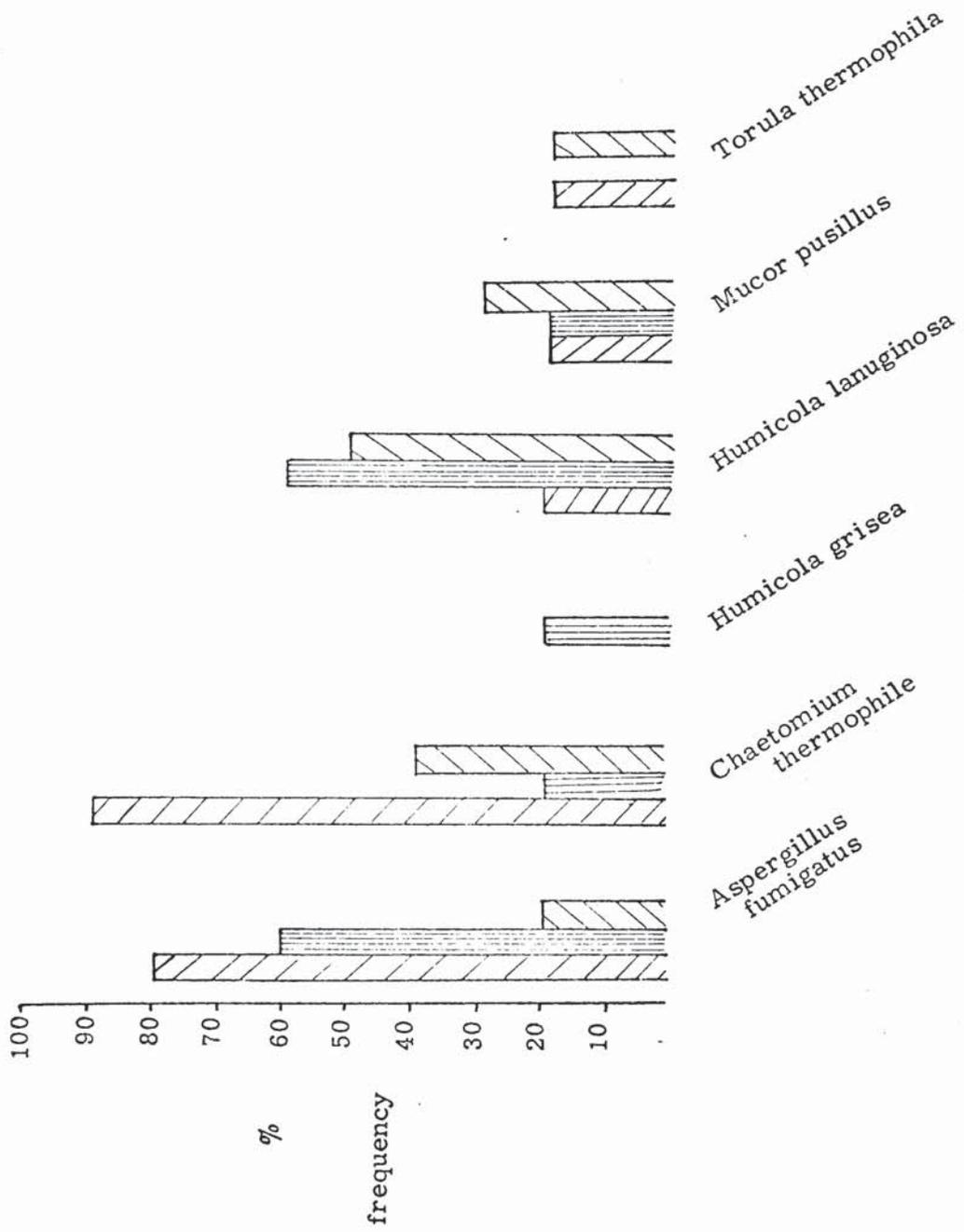


FIGURE 12

Glucose-Starch Medium at 48°C



60% at pH 5.6 and 6.4. Chaetomium thermophile was isolated between 5.6 and 8.2 at higher PFI's than at 40°C. The maximum PFI of 100% occurred at pH 5.6 although there was also one of 80% at pH 8.2. It is interesting to compare the isolation frequencies of Chaetomium thermophile with Humicola lanuginosa on cellulose agar as the latter has been reported to be able to utilise the soluble sugars produced by the former (Chang, 1967) and has been termed a 'secondary sugar' utiliser (Garrett, 1963). The optimum PFI's for both fungi occurred at pH 5.6 and 8.2 (PFI's of 100% and 60%, and 80% and 100%) and the lowest PFI's (20% for both) occurred at pH 6.6. At pH 7.4 the PFI's were 40% (Chaetomium thermophile) and 50% (Humicola lanuginosa). Thus it would appear that on a cellulose substrate Humicola lanuginosa may be able to utilise the product of cellulose hydrolysis from Chaetomium thermophile. Observations of the growth of the two fungi showed them to be growing together on the plates. Mucor pusillus was only isolated at pH 5.6 and 6.6 with PFI's of 20% and 50% respectively. Humicola insolens was only isolated at pH 5.6 (PFI 20%) and Humicola grisea was not isolated at any pH on cellulose agar. Torula thermophila was found to have an optimum pH of 8.2 (PFI 100%) but had a significant PFI (90%) at pH 6.6. Below this, however, the isolations were very low. On glucose-starch all the fungi had a narrower pH range of isolation (5.6 - 7.4). Humicola lanuginosa did not have similar isolation frequencies to Chaetomium thermophile, possibly because of the absence of cellulose. This would suppress

Humicola lanuginosa according to Chang (1967) who found that this fungus preferred the hydrolysis products from Chaetomium thermophile to the starter sugars investigated. Thus the glucose and starch supplied might not have been good substrates for Humicola lanuginosa although Chaetomium thermophile was able to use them to give a maximum PFI of 90% at pH 5.6. Torula thermophila and Humicola grisea had low PFI's of 20% and Mucor pusillus had a maximum PFI of only 30% at pH 7.4. However, it occurred from pH 5.6 - 7.4 whereas on cellulose agar it was only isolated at pH 5.6 and 6.6.

The isolation and selective colonisation of the cellulose substrate over a wider pH range has shown that at thermophilic temperatures there is an indigenous flora capable of cellulose breakdown. The fungi which are dominant each appear to be pH dependant although at pH 8.2 Chaetomium thermophile, Humicola lanuginosa and Torula thermophila were all at their optimum frequencies. At this pH Aspergillus fumigatus and Mucor pusillus were suppressed. However, even at acidic ranges on cellulose Aspergillus fumigatus and Mucor pusillus had low PFI's and thus factors other than pH may be important. At 40°C these fungi had higher PFI's and wider pH tolerances. Aspergillus fumigatus has a maximum growth rate at about 50°C and Mucor pusillus has been reported to have an optimum growth rate at around 40°C (Cooney and Emerson, 1964). Thus it might be expected that those thermophiles with optimum growth rates

at around 50°C would dominate the culture and suppress the slower growers. Both Aspergillus fumigatus and Mucor pusillus are known to cause animal and human mycoses (Cooney and Emerson, 1964; Emmons et al., 1963). It is thus important that the conditions for the suppression of these undesirable fungi be known so that the parameters for any process can be adjusted to exclude such species. Thus it would appear from this isolation programme that a temperature of around 50°C would be correct for the thermophilic stage and that the pH, although not strictly determined should be between 7.4 and 8.2. This will select out the cellulolytic species able to utilise the straw and aid rapid breakdown.

A second isolation programme was conducted to determine the contribution, in terms of fungal flora, which the slurry might make. 1 ml. samples of slurry (diluted 1 in 10 with sterile distilled water) were pipetted into petri dishes (ten replicates), and glucose starch and cellulose agars buffered to those values shown in Tables 7 and 8 were poured onto the plates. The incubation temperatures were 48, 39 and 25°C and the length of incubation was 14 days.

b. Isolations from pig slurry

The results (Tables 7 and 8) show that a very limited flora was present in the slurry. Actinomycetes had the highest PFI's at 25°C and 39°C on both media and had

TABLE 7

ISOLATIONS FROM CELLULOSE AGAR
(EXPRESSED AS PFI)

pH Organism	4.2	5.9	6.4	7.3	8.1
<u>a. At 25°C</u>					
Actinomycetes		90	80	70	60
Aspergillus fumigatus	70		10		
Aspergillus spp.	10			30	
Chaetomium sp.		10			
Penicillium spp.	20	40	40	30	40
Phoma sp.				10	10
<u>b. At 39°C</u>					
Actinomycetes		100	90	50	40
Aspergillus fumigatus			10		
Humicola lanuginosa					20
<u>c. At 50°C</u>					
Actinomycetes		20			10
Aspergillus fumigatus	10				
Humicola lanuginosa		10			

TABLE 8

ISOLATIONS FROM GLUCOSE-STARCH AGAR(EXPRESSED AS PFI)

pH Organism	4.5	5.5	6.2	7.0	7.9
<u>a. At 25°C</u>					
Actinomycetes		70	60	60	60
Aspergillus niger	40	20	40	10	
Aspergillus spp.	30	70	70		
Humicola spp.		20			
Mucor sp.			10		
Penicillium spp.	70	10	30		
Phoma sp.		10			
<u>b. At 39°C</u>					
Actinomycetes		80	70	50	30
Aspergillus fumigatus		10	10	10	10
Aspergillus niger	50	40	30	20	20
Humicola lanuginosa		30			
Mucor pusillus		10		10	
<u>c. At 50°C</u>					
Actinomycetes		60			
Humicola lanuginosa		10			

limited isolations at 50°C. More isolations occurred on glucose-starch (35) than on cellulose agar (26) and the Aspergillus spp. were more frequently isolated in the acid than alkaline ranges. At 39 and 48°C the range of thermophilic species was restricted to Mucor pusillus and Humicola lanuginosa. It would appear that the slurry does not contribute much to the flora in the solid waste. However, the use of the slurry as a source of carbon, nitrogen and moisture will become evident in subsequent chapters.

This isolation programme has shown that the majority of the flora capable of breakdown of the straw were present in the straw bedding fraction of the waste. The slurry did not contain a very wide range, particularly at thermophilic temperatures where only non-cellulolytic species (Humicola lanuginosa and Mucor pusillus) were isolated. It is thus likely that the slurry does not contribute to the active flora in the process. However, nutrients and moisture will be the main contributions as well as the purification which the slurry will undergo. These properties will be investigated later so that a more complete picture can be arrived at.

CHAPTER 3

C H A P T E R 3

THE BIODEGRADATION OF SEMI-SOLID PIG WASTE
UNDER NORMAL CONDITIONS ON THE FARM

- 3.1 Introduction
- 3.2 Materials and methods
- 3.3 Results and discussion

3.1 INTRODUCTION

The process of composting has received much attention as a possible solution to domestic waste treatment. However, at the present time, composting plants do not seem to be very successful due to the quality of the product and the associated high application rates as fertilisers (Fulbrook et al., 1973). The microbiological aspects of the process are little known at the present time. However, the knowledge of the parameters of composting town waste is great in relation to that known about animal wastes, and observations made on town waste are not necessarily the same for animal wastes. The substrates contained are different. Town wastes contain variable amounts of paper, metals, textiles, meat and vegetable wastes and ashes. Animal wastes are more easily defined and their composition has been described previously. The substrates present will affect the dominant micro-organisms and thus the conditions in the composting process. It will be interesting to compare the conditions in the town waste composting and that of the semi-solid pig waste used in the studies for this thesis.

Studies in town waste composting have been done by Stutzenberger, Kaufman and Lossin (1970) and Mills (1973). Stutzenberger et al. found that it took one week for the centre of a windrow (a low pile of municipal waste) to reach 60°C. The highest temperature recorded was 74°C. After

three weeks the pile cooled to between 40 and 55°C (measurements were taken at various depths in the waste) and remained so for eight weeks when the investigation ceased. Mills (1973), also using town waste which was windrowed, obtained a rapid increase in temperature. Within 48 hours the outside of the heap had reached 62°C and the centre 58°C. The temperature then slowly decreased until the heap was at 42°C after 25 days. The temperature further decreased to between 35 and 40°C where it remained until day 54. Ambient temperature was reached after 76 days. Chang and Hudson (1967) studied the ecology of wheat straw composts which reached temperatures of between 67 and 72°C after 5 days, remaining so for 2 days. Temperatures of between 40 and 50°C then prevailed for another 3 weeks. This was followed by a fall to ambient. These three examples of the temperature relations in composts show a variation in the maximum temperatures reached, the times taken for the attainment of these maxima and the length of time which the thermophilic phase of above 40°C lasted. It has been suggested that variations are due to the amount of insulation which the material provides, the substrate itself, nutrients available, aeration (Gray, Sherman and Biddlestone, 1971), the age of the material (Festenstein, et al., 1965), and climatic conditions. It is thus important that these factors be considered when studying composting processes, so that it may be possible to arrive at a set of optimum conditions for natural composting.

The pH parameter is important in determining the microflora which are active in the process. Town waste has been found to have a starting pH of between 5.7 (Stutzenberger et al., 1970) and 5.5 (Mills, 1973) and an increase to a final pH of between 7 and 8 with no intermediate rises higher than 8.0. The Chang and Hudson straw compost started at pH 6.5 and showed an initial rise to about 7.5 when they noted ammonia production. The pH then returned to neutrality where it remained for the remainder of the investigation. Gray, Sherman and Biddlestone (1971) have stated that animal manures have a starting pH below neutrality, which falls in the first mesophilic stage due to the production of simple organic acids. The increase in temperature then causes the pH to rise above 7.0 with the liberation of ammonia. Finally, the pH evens out at just above neutrality. It would appear from the work done on the pH changes that the compost starts in the acidic region, increases to between pH 8 and 8.5 with the liberation of ammonia, depending upon the concentration of nitrogen containing compounds, and then falls to the alkaline side of neutrality for stabilisation. pH has been used as a criterion for the completion of the composting process (Jann, Howard and Salle, 1959). It was found that a fully stabilised compost would maintain an alkaline pH at 55°C for 24 hours under anaerobic conditions, i.e. there was no further anaerobic fermentation on storage.

The moisture content of the material will determine

the microflora which is active. In composts of high moistures greater than 85% where there is no forced aeration it is likely that bacteria of the anaerobic group will be dominant. Below this moisture content different groups of bacteria, fungi and actinomycetes will be able to colonise the waste due to increased aerobicity. The compost of Chang and Hudson started at 75%, increased to 80% in the centre in the early stages and then fell to 70%. The town waste compost of Mills (1973) began at 60%, gave a slight increase to 62.5% after 7 days, but then evened out at between 55 and 53% after 76 days. Mills noted that where there was compaction of the waste in the centre of the windrow, waterlogging occurred and this caused a localised acidic pH which kept the temperature lower than at the sides of the windrow.

The effects of aeration on the temperature relations of waste composting have been studied by Schulze (1962) and Bell (1969). Schulze designed an automatic temperature control for keeping composts at an optimum temperature (45°C). This was based upon the concept that aeration is necessary for respiration which in turn produces heat. When there is no air respiration is drastically reduced and the temperature decreases. Schulze found that he could control composts throughout their thermophilic phase at $45 \pm 2^{\circ}\text{C}$ by using controlled aeration. Bell found, using poultry manure, that until aeration was introduced, the compost did not heat up. It is likely, however, that if aeration is too

great heat will be lost in the exhaust gases. Schulze used between 17 and 36 cubic feet per pound of the initial material per day, and Bell introduced 200 ml per minute for 1.5 kg. of material.

A summary of the conditions in a selection of composts is shown in Table 9 for comparison.

The fungal flora naturally occurring in animal wastes, causing their breakdown, are known as the coprophilous fungi. Such a group are taxonomically unrelated but have become commonly adapted to their habitat (Hudson, 1972). Like other breakdown systems involving micro-organisms a coprophilous succession has been determined beginning with those fungi able to utilise the simple sugars and ending with the lignolytic fungi. Thus the species involved in a particular succession will depend upon the substrates present. However, it is generally recognised that the Mucorales utilise the simple sugars, then the Ascomycetes and Fungi Imperfecti degrade the cellulose, and finally the Basidiomycetes which utilise both cellulose and lignin. In this way animal wastes are broken down naturally and the rate at which this breakdown occurs will depend upon the parameters of pH, temperature, aeration and substrates discussed above. By controlling the parameters, the coprophilous succession, once determined, can be optimised to bring about breakdown as quickly and as efficiently as possible.

TABLE 9

KEY

- (1) Stutzenberger et al (1970)
- (2) Mills (1973)
- (3) Chang and Hudson (1967)
- (4) Willson (1971)
- (5) Bell (1969)
- * Investigation ceased
- ** Compost was considered stable when temperature dropped below 40°C and would not return above it.

TABLE 9
A COMPARISON OF FIVE COMPOSTS FROM DIFFERENT SOURCES

Type of Compost Parameters	Town Refuse (1)	Town Refuse (2)	Wheat Straw (3)	Dairy Cow Manure (4)	Poultry Manure (5)
Maximum temperature (°C)	74	62	68	63	50
Time taken to reach maximum temperature (days)	7	2	5	12	4
pH RANGE	initial	5.5	6.5	7.5 - 11	-
	maximum	5.3	8.5	7.5 - 11	-
	final	7.5	7.3	7.0	-
MOISTURE RANGE (%)	initial	60	75	75	51.9
	final	-	54	70	-
Time compost remained above 40°C (days)	56*	25	28	56 (with turning)	7
C:N RATIO	initial	50:1	-	-	28.2:1
	final	-	-	-	20.7:1
Aeration rate	Natural	Natural	Natural	Natural	200ml/min/1.5kg material
Length of composting time (days)	56	76	60	56**	14
Quality of product	60% cellulose degraded	71% cellulose degraded	71% cellulose degraded	No odour. No flies attracted	May be used as soil conditioner
Method of composting	Windrow	Windrow, covered with plastic sheet	Chopped straw placed into wooden bins	Placed in wooden bin	Bench-scale fermenter (5litre:)

A study was undertaken to determine the composting process of the semi-solid pig waste. The purpose of this investigation was two-fold. No literature could be found on the composting of pig wastes and so it was necessary to determine the parameters and coprophilous succession which have been discussed above. Such parameters would provide guidelines as to the natural breakdown processes which occur in pig wastes and the fungi involved. The other purpose was to determine whether normal composting of the pig waste was an adequate system for rapidly and efficiently biodegrading the wastes. The results would also act as a control to be compared to any results obtained in improving the biodegradation process.

3.2 MATERIALS AND METHODS

Approximately half a ton of semi-solid pig waste from a deep-litter pen eight weeks old was formed into two heaps with the approximate dimensions: 1.7m x 1.0m x 0.5m high and 1.7m x 1.0m x 0.2m high. The larger heap was about twice as high as the other heap but had the same length and breadth measurements (Plate 4). This gave respective volumes for the large and small heaps of 0.85 cu. m. and 0.34 cu. m. The purpose of this was to determine the minimum volume needed for the attainment and maintenance of thermophilic temperatures. This would then provide information for process work regarding the thermophilic stage. Screened substrate tubes described



PLATE 4

THE LARGER HEAP AFTER 10 DAYS COMPOSTING

The polythene sheeting has been pulled back to show the fruiting bodies of Coprinus sp. on the top right hand side of the heap

by Eggins and Lloyd (1968) and subsequently used to demonstrate the selective colonisation of cotton textile by fungi (Allsop and Eggins, 1972) were buried at two levels in the larger heap. These levels were described as the outer layer (OL) and the inner layer (IL). The OL was between 0 and 4 inches from the surface and the IL from 4 inches in to the centre of the heap. Initial temperatures, carbon, nitrogen, moisture and pH measurements were made taking an average of four samples from each level. The air temperature was also taken and subsequent air temperatures were taken using a maximum and minimum thermometer. Carbon determinations were carried out according to the 'New Zealand' method ($\%C = (100 - \%ash)/1.8$) described by Mills (1973) as being of adequate accuracy. The micro-Kjeldahl method of nitrogen determination was used for nitrogen measurements and temperatures were taken at six points within each layer using a long reach mercury thermometer. The moistures were determined by drying 5g. samples in an oven at 80°C for 48 hours. Screened substrate tubes were removed at intervals and plated out onto Eggins and Pugh cellulose and glucose-starch agars. This was to obtain a successional pattern of the flora present throughout the process. Climatic conditions were also noted.

3.3 RESULTS AND DISCUSSION

The temperature increases in the large and small heaps (Figures 13 and 15)

FIGURE 13

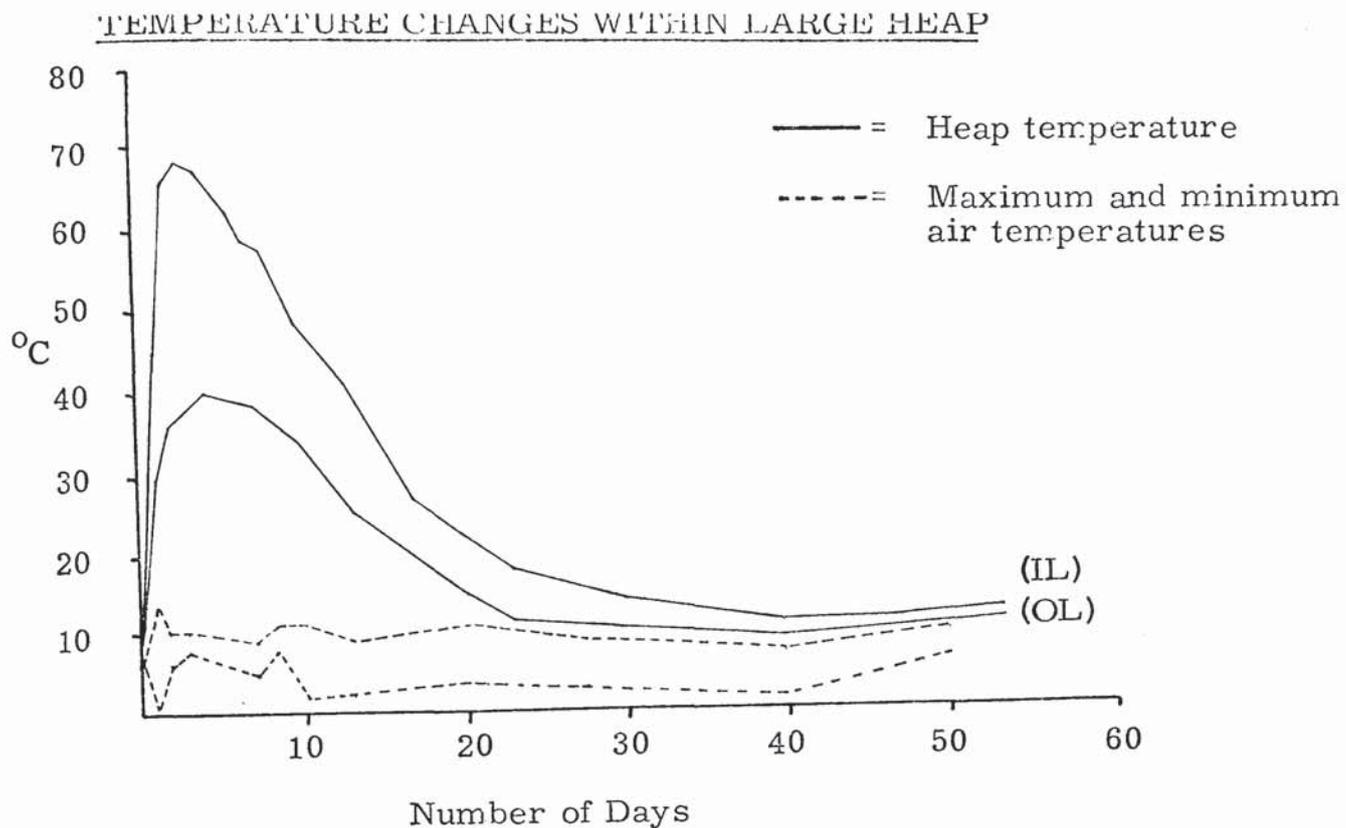
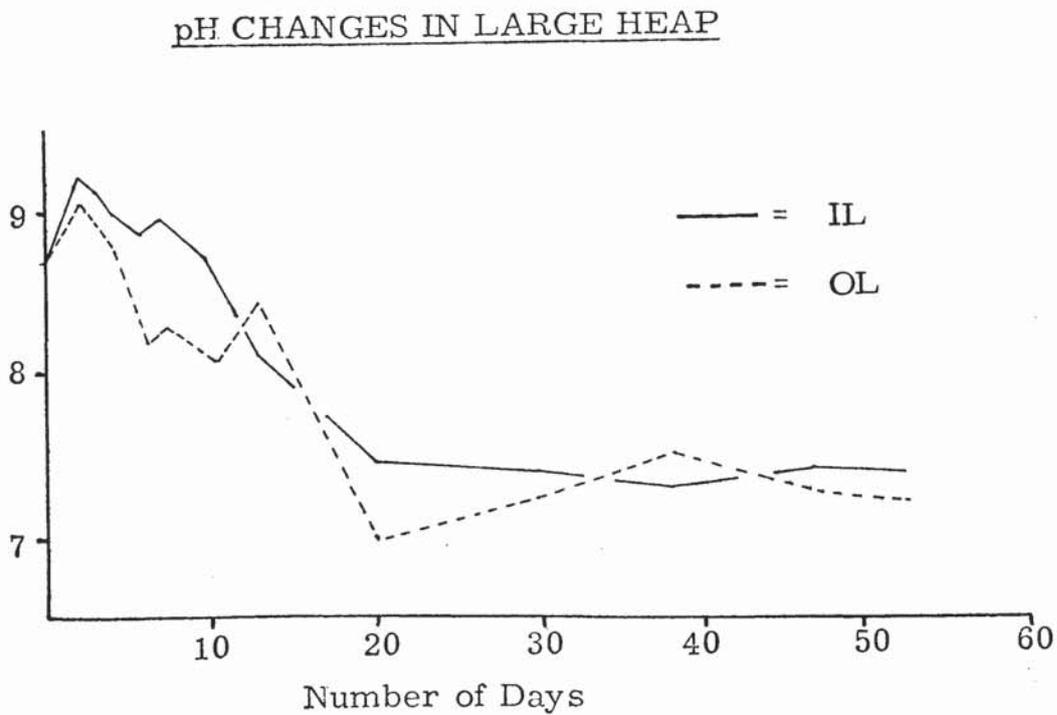


FIGURE 14



showed a distinct difference. The small heap did not rise above 31.0°C and thus did not reach a thermophilic phase. Within the larger heap there was, as could be expected, a differential between the inner and outer layers. The inner layer reached a maximum of 68°C after 3 days and then decreased over the next 17 days to 20°C without a plateau as described by Mills (1973) for town refuse and Chang and Hudson (1967) for wheat straw. The temperature levelled out at just above ambient after 30 days. The outer layer reached a maximum temperature of 40°C after 4 days, decreasing to 20°C after 15 days and then following the ambient temperature very close to the temperature of the inner layer. Although the air temperature underwent changes between 2°C and 13°C during the thermophilic phase it did not cause rises and falls within the compost to correspond to the air temperature changes. After the thermophilic phase, however, the temperature of the heap was probably subject to the influence of the surroundings. The compost of Mills (1973) was set up during the winter but remained above 40°C for 56 days. It is likely that the available oxygen plays an important part. Schulze (1962) has found that a thermophilic phase is favoured by the controlled introduction of air. Moisture level measurements (see Table 10) on the heap showed that the levels varied between 79% and 85%. The centre of the heap appeared waterlogged and it is likely that these high moisture levels prevented air circulation and thus restricted the thermophilic phase above 40°C to about 14 days in the inner layer. Turning

the heap may have improved aeration. However, this would have involved mechanical means which were not available. Also the results would not have represented a normal farm compost heap which would be impractical and uneconomic to turn regularly.

The pH of the small heap (figure 15) increased from 8.65 to 8.75 and then decreased to 8.2 after 11 days indicating activity but not enough to give a stable compost characterised by a stable pH. The larger heap (figure 14) rose from 8.70 to 9.05 (OL) and 9.20 (IL) and then after 20 days had decreased to 7.0 (OL) and 7.45 (IL). The IL then stabilised at about 7.4 whilst the OL stabilised at 7.3 after 40 days.

Ammonia evolution was noted for the first 4 days and this was followed by an earthy smell characteristic of actinomycete establishment. The final compost after 55 days had a pleasant earthy smell characteristic of a gardening compost.

The analysis of carbon (Table 10) showed an overall decrease from 49.1% to 46.5% (OL) and 38.5% (IL), a decrease of 5.1% and 21.6% respectively of the total. The nitrogen, surprisingly, did not initially decrease due to ammonia release. In contrast it increased and showed an overall increase from 1.22% to 1.97% (OL) and 2.33% (IL). The moisture contents did not decrease to a level which would

favour good aerobic conditions. This was probably due to the compaction of the heap and condensation from the cooling down process. The inner layer seemed to undergo the greater biodegradation judged by the carbon reduction, and this probably caused the larger apparent nitrogen increase.

TABLE 10

ANALYSIS OF LARGE HEAP

No. Days		0	7	15	21	27	42
Carbon (%)	OL	49.10	47.90	45.80	45.50	48.90	46.50
	IL		43.90	44.00	43.00	44.20	38.50
Nitrogen (%)	OL	1.22	1.62	1.36	1.56	1.16	1.98
	IL		1.42	2.38	2.16	1.61	2.33
Moisture (%)	OL	-	-	84.90	83.30	85.00	82.50
	IL		-	79.00	81.80	85.20	82.30

The flora successional patterns showed a dependence upon pH (figure 16). Bacteria were initially dominant at above pH 9 as well as Chaetomium thermophile, Aspergillus

fumigatus and actinomycetes which were isolated in the first day as the pH was rising to 9. At this high pH these species were not further isolated until the pH came down below 9 when after 3 days Aspergillus fumigatus was isolated for one further day and the actinomycetes appeared for the remainder of the isolation programme. Humicola lanuginosa was isolated after 6 days and the fructifications of Coprinus sp. appeared on the surface of the heap after 10 days. Humicola insolens was found after 16 days and Stilbella thermophila was isolated after 17 days when the temperature was at about 22°C and the pH was at 7.3. The isolations from both layers were similar and so differentiation has not been discussed between the layers. When the temperature dropped below 20°C no further isolations were made as the investigations were limited to the thermophiles.

Although not an exhaustive analysis of the heap it can be seen that information as to the parameters of temperature, pH, moisture and carbon reduction have been obtained. If these parameters are compared where possible to those in Table 9 the maximum temperature of 68°C can be compared to that of wheat straw compost and the pH maximum is in the range of the cow manure compost. The compost reached its maximum after 3 days which was comparable to the poultry manure compost and the final pH was 7.3 - 7.4 in agreement with the range found in the table. The moisture was higher than those shown and, coupled with this, the time the compost remained above 40°C was shortened although similar

to the poultry manure. However, aeration is probably an important factor in the length of the thermophilic phase. Thus the composting characteristics of pig waste can be compared to other animal wastes already investigated and can be used as a control to compare with the biodegradation in accelerated processes. The flora present can also be compared to see whether altering the natural process affects the dominant fungi and whether certain desirable fungi can be encouraged.

Thus the extent of biodegradation under natural conditions has been studied. The next two chapters will discuss the optimisation of parameters to increase the rate of biodegradation and encourage desirable fungi.

C H A P T E R 4

CHAPTER 4

THE ACCELERATED BIODEGRADATION OF PIG WASTE - AN EVALUATION OF THE PARAMETERS

4.1 Introduction

4.2 Materials and methods

4.2.1 The effects of temperature on
biodegradation

4.2.2 The effects of inocula additions

4.2.3 The effect of pH on nitrogen loss

4.3 Results and discussion

4.3.1 The effects of temperature on
biodegradation

4.3.2 The effects of inocula additions

4.3.3 The effects of pH on nitrogen loss

4.1 INTRODUCTION

Before trying to develop a thermophilic breakdown process involving fungi it is necessary to discuss and investigate in detail the parameters which will determine the rate at which the process proceeds. The parameters of temperature, pH, aeration and nutrients may affect the microflora present and its biodegradative activities.

The fungi have been divided into two main groups according to their temperature relations - the mesophiles and thermophiles. The temperature limits of growth of each group overlap but certain upper and lower limits distinguish the two groups. Thermophilic fungi have been defined by Cooney and Emerson (1964) as those fungi with a minimum temperature for growth of 20°C and an optimum growth rate at or around 50°C. Mesophiles in contrast are able to grow at temperatures below 20°C but their upper limit is usually between 38 and 40°C. There is, however, a certain group of thermotolerant fungi which are debatable thermophiles. Thus, Aspergillus fumigatus will grow at 50°C (maximum temperature) but is able to exist below 20°C (Cooney and Emerson, 1964). This fungus would not be termed a thermophile by the definition of Cooney and Emerson. This is also true of certain synemmatous fungi which have similar ranges to Aspergillus fumigatus and have been termed thermotolerant (Mirza, 1970). In considering a process which will operate at 50°C or above it is unlikely that thermotolerant

fungi will become dominant and contribute to the overall biodegradation process. However, below 50°C their activities may need to be considered. The effect of temperature on the decomposition of plant materials has been discussed by Waksman and Cordon (1939). They found that mixed cultures of thermophilic fungi gave a more rapid breakdown of straw and hay at 50°C than did mesophilic cultures at 28°C. Waksman, Cordon and Hulpoi (1939) also found that decomposition was most rapid at 50°C. Cooney and Emerson (1964) have listed the temperature limits of growth for 13 thermophilic fungi. They have found that the optimum growth rates are between 40 and 50°C whilst the limits ranged from 20°C (Mucor pusillus) up to 60°C (Humicola lanuginosa). The analyses of Yung Chang (1967) showed that as the temperature increased so did the rate of decomposition. She found in particular that Chaetomium thermophile could decompose 40% of the total weight of straw at 45°C over 3 weeks. This was equivalent to the total activity of the microbial population in the compost. It was also found in pure culture that at 45°C and 35°C utilisation of pure cellulose was greater than at 25°C. However, information regarding the cellulolytic activity of the flora at a range of temperatures is still scarce and preliminary investigations must be determined for farm waste substrates.

Once an optimum temperature for growth has been established it must be correlated with the enzyme activities

of the fungi. Mills (1973) has measured cellulolytic activities of the thermophilic fungi over a range of pH values at 50°C and observed the nature of growth of the thermophiles. He has found that cellulolytic activity is not dependant upon growth. For example Sporotrichum thermophile gave a very heavy growth and sporulation between pH 3.0 and 6.0, whilst at 7.0 and 8.0 growth was poor. However, cellulolytic activity was good over the whole range. In contrast the thermotolerant Aspergillus fumigatus only grew well and had cellulolytic activity at pH 3.0 and 5.8. If it is wished to upgrade the waste to produce microbial protein then maximum growth conditions to give maximum biomass must be instituted. However, this decision may not arise if analyses show an adequate nutritional level without further control.

The length of the biodegradation process will influence the end product in terms of quality, quantity and value. There will be an optimum length for the process qualified by defined parameters above and below which it is uneconomic to run. It has been shown in the previous chapter that the length of composting determines the amount of breakdown of cellulose. It is necessary to determine how the rate of breakdown proceeds, that is, whether there is a direct linear relationship between biodegradation and time or whether the relationship follows the normal growth kinetics and has a lag, log and stationary phase. From this analysis it will be possible to predict an optimum process time.

The measurement of biodegradation may have many parameters and these will be defined by the characteristics of the product required. Thus, if it is wished to remove just odour by the action of micro-organisms, then methods would have to be devised to determine odour either by chemical analysis or using an odour panel (Sobel, 1972). The concluding section of Chapter 1 listed the problems which untreated farm wastes may cause, and to tackle these problems it is necessary to devise analyses methods which can be related to them. The reduction in bulk, reduction in handling problems and a possible increase in the value of the product may be measured by analysis of the breakdown of the constituents of the waste. This will reduce the bulk and make handling easier. A nutritional and fertiliser analysis of the product will also determine the value. Odour production is a very subjective problem and unfortunately the olfactory senses still remain as the final analysis. Disease hazards can be measured by monitoring the pathogenic organisms present, and water pollution by analysis of the waste for inorganic nitrates, phosphates and the 5 day BOD test. The resident microflora in the process will determine how the problems above are solved and this microflora will in turn be determined by the parameters of pH, temperature, aeration, C:N ratio and moisture content. Thus, an appreciation of the needs of certain groups of micro-organisms in terms of the above parameters will determine the extent to which the problems are solved in terms of the analysis techniques.

The fungi need oxygen for growth and their respiration produces carbon dioxide and water. This is also true for the aerobic bacteria, but there are groups, including important pathogens, which can exist only under anaerobic conditions. As there is no oxygen available the final hydrogen ion acceptor in the electron transfer chain may be carbon, sulphur or more complex sulphhydryl compounds to produce respectively methane, hydrogen sulphide, thiols and skatols. These compounds are very volatile producing the obnoxious odours associated with untreated farm and domestic wastes. Further, the anaerobicity of the waste will favour establishment of the anaerobic intestinal bacterial pathogens which may cause a whole range of diseases in animals and man.

By selecting out the thermophilic fungi for the process it is further possible to free the material of the non spore-forming intestinal pathogens by raising the temperature to between 50°C and 56°C (Jalal, 1969). Also, the group has been well defined in its temperature relations and enzyme activities by a variety of authors (Fergus, 1967, 1969, 1971; Cooney and Emerson, 1964; Celerin and Fergus, 1971; Tansey, 1972; Mills, 1973; Barnett and Fergus, 1971; Broad and Shepherd, 1970; Somkuti and Babel, 1967; Somkuti and Babel, 1968) and their results can be used as guidance. The thermophilic fungi then occupy a unique ecological niche in which they can be the dominant species with very little interference to their activities providing the

conditions for optimum growth prevail. It is first necessary to determine how the biodegradation of the farm waste proceeds at thermophilic temperatures in terms of the analysis techniques outlined above, and the degree of control needed to enhance the thermophiles in the process.

4.2 MATERIALS AND METHODS

It was first necessary to construct some laboratory-scale apparatus to simulate the proposed two-stage filtration and thermophilic breakdown process outlined in Chapter 1. The thermophilic stage involved the design of a laboratory fermenter into which raw waste could be placed and the whole subjected to thermophilic temperatures. With this in mind containers for the waste were constructed, as shown in figure 17, from aluminium petri dish sterilisers (28.5 cm x 12.0 cm). Each container (termed fermenter) could take about 1 kg. of solid waste. It should be noted that Bell (1969) constructed similar containers (30 cm x 15 cm) and he channelled aeration through the top of the container which was sparged from the bottom. It was found that this presented many practical problems and a direct air line to the bottom of each fermenter was used. An air pump was used in conjunction with each fermenter (see Plate 5), air being first drawn through sterilised glass-wool. An air flow meter could be attached to control and monitor the air flow into the fermenter. A hole drilled in the side could accommodate

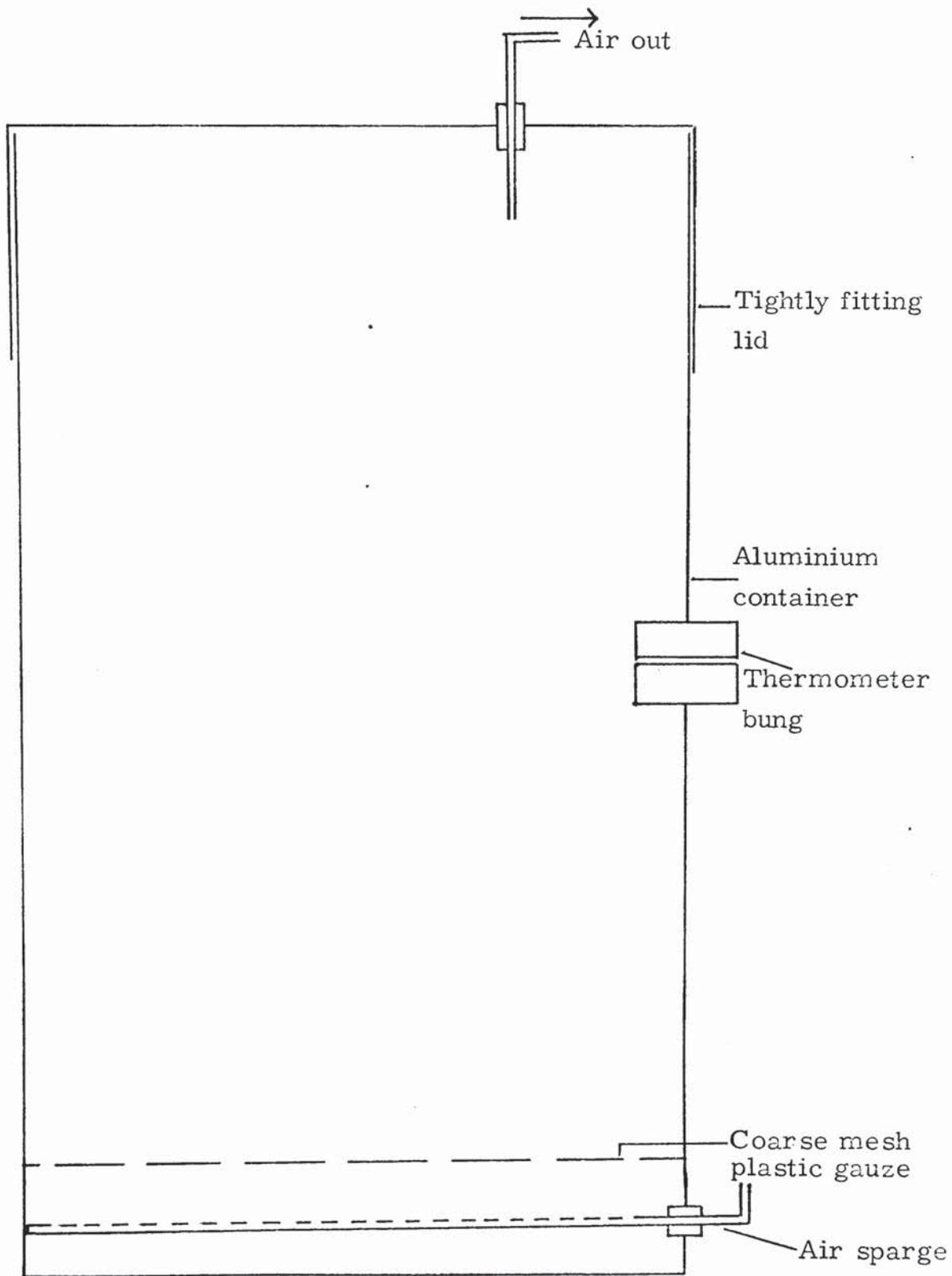


FIGURE 17

A LABORATORY-SCALE FERMENTER FOR DETERMINATION
OF THERMOPHILIC PROCESS PARAMETERS



PLATE 5

LABORATORY-SCALE FERMENTER PAINTED
MATT-BLACK AND AIR PUMP WITH FILTER

a thermometer and the whole of the outside was painted matt black to aid heat absorption. The complete apparatus, excluding the air pump, could be placed in a high humidity incubator for the maintenance of a constant temperature. It was hoped that this fermenter could provide information for the design of a pilot-scale plant.

The pig waste used for all the investigations was collected from a deep litter pen which had just been cleared. Wheat straw was used as the bedding and the time it had been in the pen ranged from 1 to 8 weeks. Temperatures of between 35°C and 40°C were recorded in the centre of this bedding before it was cleared. The material was bagged in polythene containers, transported to the laboratory and used as quickly as possible. Slurry was also collected from beneath the slatted floor pens and utilised within one day of collection. In order to simulate the first stage of the process outlined in Chapter 1 where it was proposed to filter the slurry to remove cellulose fibre, plastic drainpipe tubes (4'6" x 3") were charged with 2 kg. of the solid waste, and the liquid slurry added at hourly intervals during the day for 3 days. This was to enable large additions of cellulose fibre and extra nitrogenous compounds. It was found that the small cross-section area of the tube caused clogging by the slurry, and subsequently the straw was spread on a grid approximately 18" square to a depth of 6". This system would more approximate the large scale beneath-pen system which will be explained in Chapter 8. About 1 litre of slurry was added per 2 kg.

solid waste. This gave an even covering of the solid with filtered fibre and did not cause clogging. It should be noted at this point that the methods used in handling the wastes were as the farmer might do under normal circumstances. The reason for this was that any process developed would have to appeal to the farmer for its simplicity and ease of handling. Thus sterility procedures were only used when necessary for microbiological work, and the weighing of proportions was done using a large spring balance and bucket.

The effect of three parameters on the biodegradation process was measured in this Chapter.

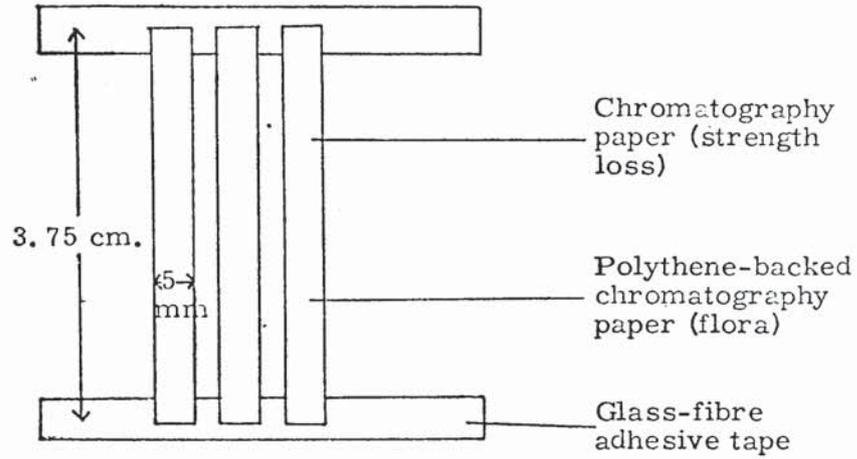
4.2.1 The effects of temperature on biodegradation

It was decided to measure the cellulolytic activity of the microflora at 18, 25, 40 and 50°C. This was done to see whether the flora present at thermophilic temperatures did give a greater cellulolytic activity and hence greater breakdown of cellulose in the substrates used for this investigation. To do this the screened substrate tube method of Eggins and Lloyd (1968) was modified so that it could be used in the fermenters. Figure 18 shows how these were constructed. Three strips of Whatman chromatography paper cut to exactly the dimensions shown by hand, so that the alignment of the fibres ran along the length of each strip, were stuck to the adhesive tape. This was wound around each tube and covered

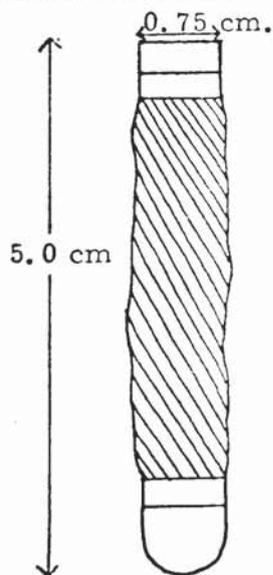
FIGURE 18

THE MODIFIED SCREENED SUBSTRATE TUBE
 USED IN THE FERMENTERS TO DETERMINE FUNGAL
 SUCCESSIONS

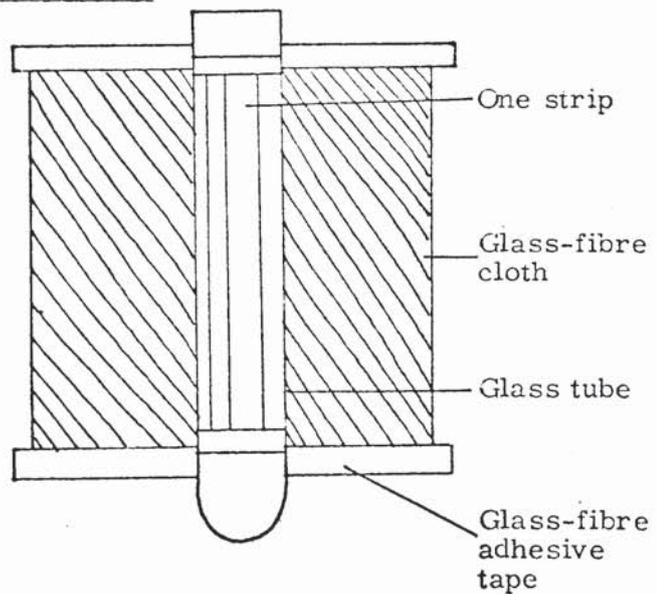
Arrangement of strips around tube



Completed tube



Arrangement of cloth and strips around tube



by glass-fibre cloth (Malik, 1970). The tubes were then autoclaved at 10 lb/sq. in for 20 minutes and placed randomly throughout the fermenter. A strength testing machine as used by Mills (1973) determined the strength loss of the strips, six of which were tested at any one time. Carbon, nitrogen, ethanol soluble fraction, pH and ash determinations were determined on samples throughout the process. The ethanol soluble fraction determinations were carried out according to Mills (1973) and the material was ashed in a crucible (about 1g. accurately weighed) in a muffle furnace at 550°C for 4 hours. The fermenters were placed in incubators containing, at 40°C and 50°C, aerated water baths to maintain a high humidity and prevent drying out. Filtered air was introduced at the rate of 0.25 litres/kg. waste/minute.

4.2.2 The effect of inocula additions

The work of Golueke, Card and McGauhey (1954) suggested that inocula additions made no difference to the biodegradation of municipal refuse and concluded that an adequate bacterial population was present. The isolation work in Chapter 2 also showed that the thermophilic fungi had high percentage frequencies of isolation, but whether these were in fact adequate could only be determined by adding other sources of thermophiles. Mills (1973), Barnes (1972) and Malik (1970) have isolated thermophilic fungi from, respectively, composted town refuse, composted sewage

sludge/town refuse add-mixture and soil. Such wastes might be incorporated into farm wastes as an add-mixture providing an outlet and method of disposal if transport costs permitted. It was decided to make 10% additions (wet weight) of the above materials to fresh waste and to see whether any increases in biodegradation could be obtained. The value of end-product recycling was also tested. The recycling of a proportion of the end product as a seed and accelerator is well known in domestic waste systems and for oxidation ditch operation where fresh incoming waste is mixed with a percentage of the outgoing treated material. Thus a fermenter containing waste was run for 17 days and then 10, 25 and 40% additions were made (wet weight) to fresh waste. The analyses of biodegradation were determined throughout the incubation period.

4.2.3 The effects of pH on nitrogen loss

It has been seen that the initial pH determines how the pH throughout the composting process changes. Those composts with a starting pH in the acidic ranges tended to move to the alkaline side of neutrality and those wastes which were alkaline to begin with tended to decrease to neutrality. Mills (1973) has shown that maximum growth and enzyme activity of the thermophilic fungi occurs in the acidic region although some species were able to produce cellulase in alkaline conditions. The previous results have shown that the normal pH changes in the biodegradation of the pig waste do

not include acidic ranges and that the process in terms of odour, nitrogen removal and dominant flora was undesirable. By changing the initial pH of the material it may be possible to produce a pH range which is more favourable to the thermophiles. This would increase their activities, favouring biodegradation of the cellulose and nitrogen constituents and reducing the final bulk and polluting capability. Thus the waste, after having been trickled with the liquid slurry for 3 days, was brought to 3 pH values of 4.0, 5.0 and 6.0 using citric acid (150, 100 and 50g respectively). The fermenters were placed in a high humidity incubator at 50°C and aeration introduced (0.25 l/kg). Measurements of pH were made daily and nitrogen determinations were made to see whether there was any loss due to ammonia production.

4.3 RESULTS AND DISCUSSION

4.3.1 The effects of temperature on biodegradation

The results are shown graphically in figures 19 to 23. It can be seen that for all three fermentations there was an initial rise in pH up to between 8.2 (at 40°C) and 8.5 (at 25°C) within 2 days which then levelled out for a further 2 days and subsequently began to fall. However, at 40°C the pH fell to 7.5 after 3 days and then began to rise over the remainder of the incubation period until it reached 8.0 after 12 days and remained there until the 17th day. The 25°C

FIGURE 19
EFFECT OF TEMPERATURE ON pH

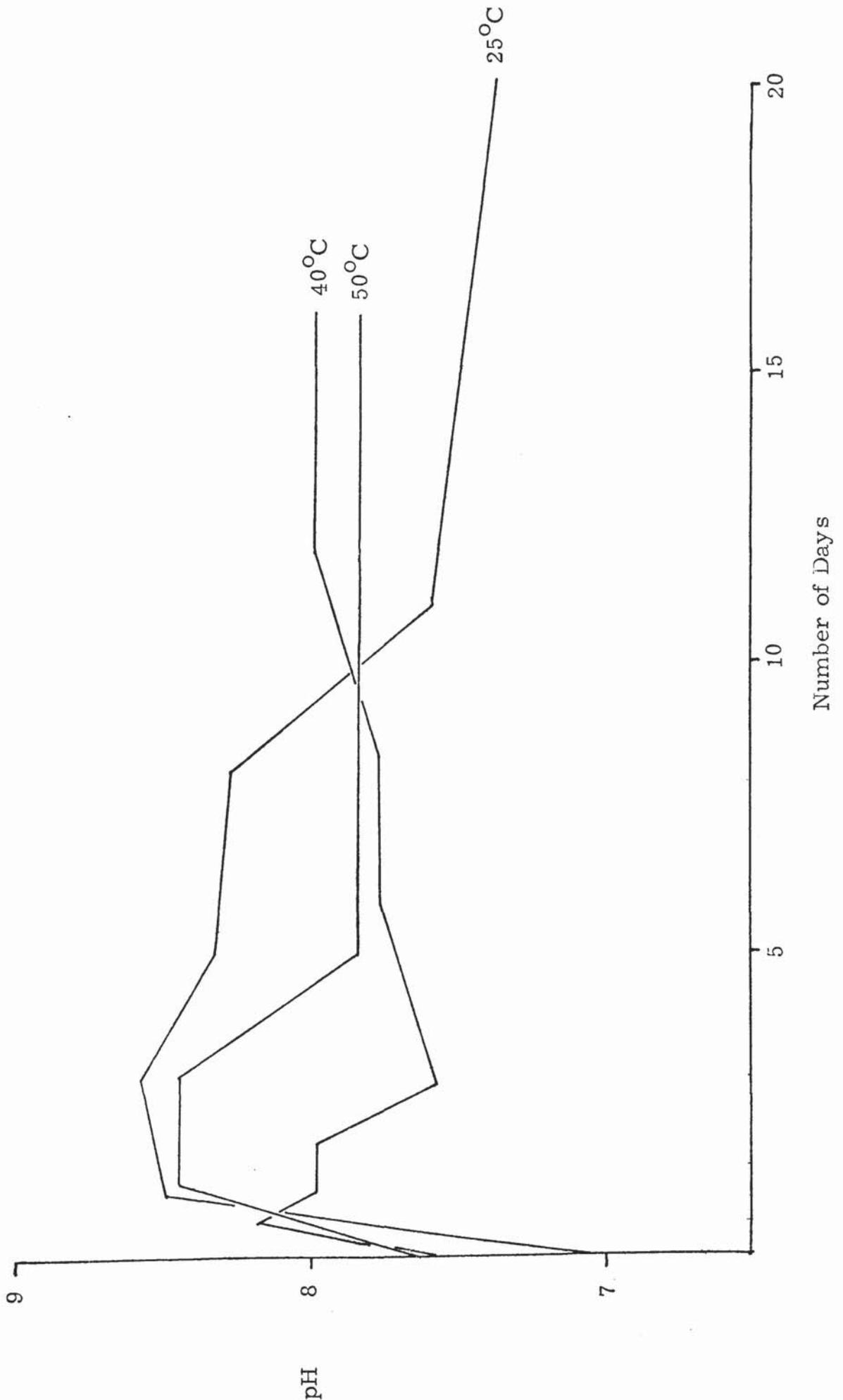
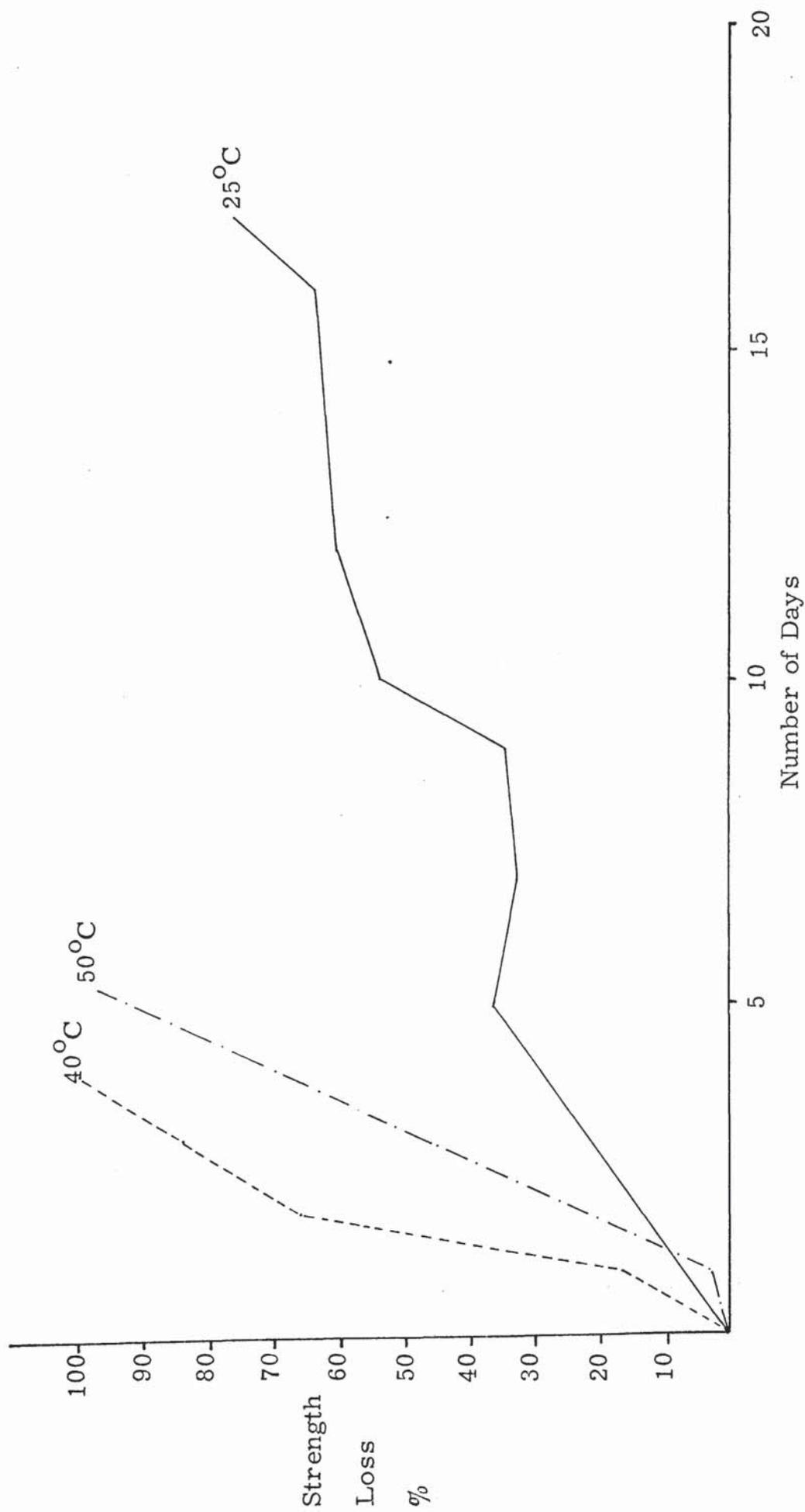


FIGURE 20

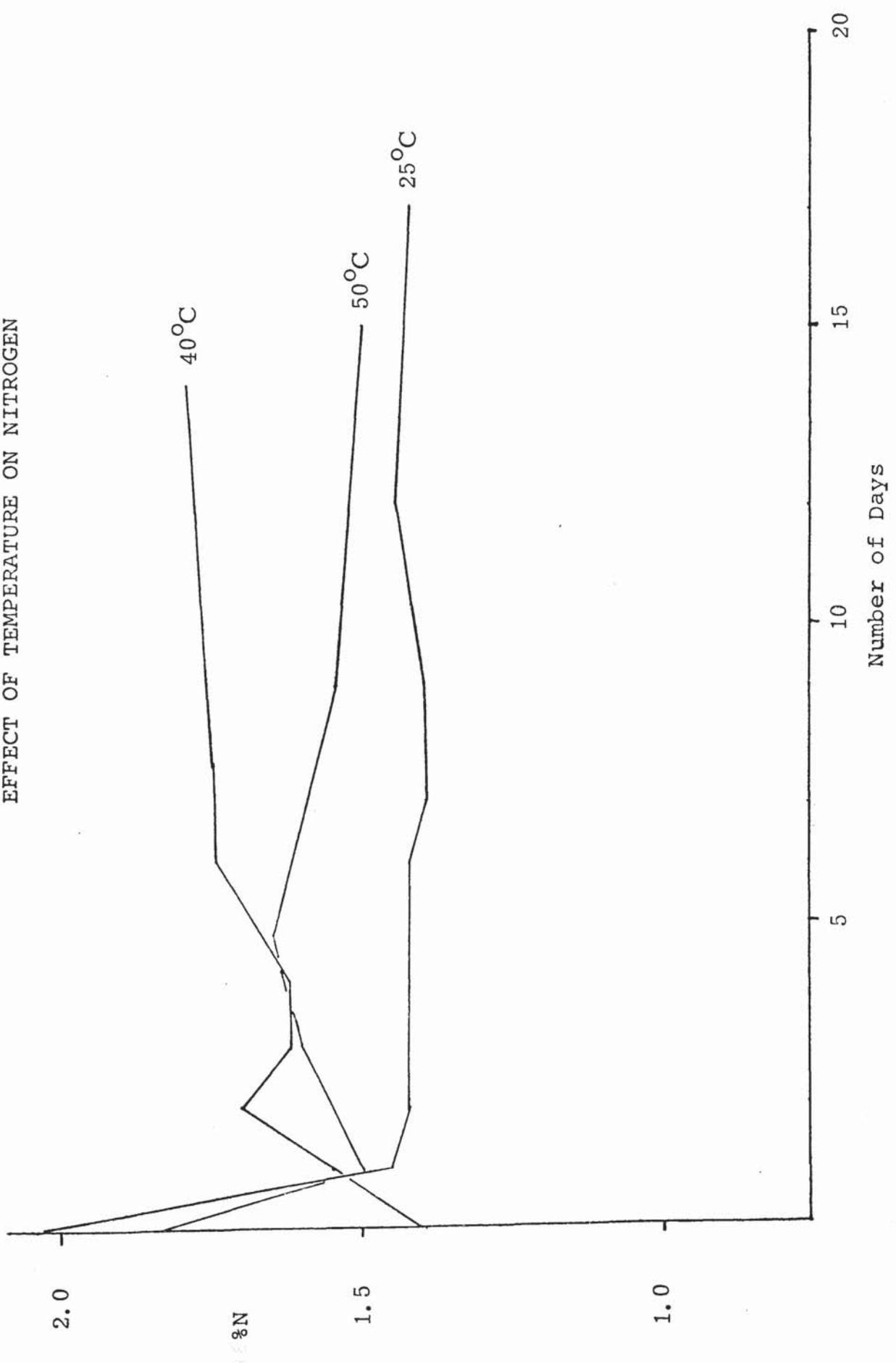
CELLULOLYTIC ACTIVITY AT VARIOUS TEMPERATURES



and 50°C fermentations both fell slowly to give final pH values of 7.5 and 7.8 respectively. If stabilisation of the pH is taken as a criterion for completion of the composting process (Jann, Howard and Salle, 1959) then the 50°C process which stabilised after 4 days could be taken as the best temperature. Stabilisation of the pH at 25° and 40°C was not complete until after 11 - 12 days. However, as will be seen from later results, this criterion need not necessarily be true when considering the biodegradation of the waste.

The results of the measurement of cellulolytic activity by strength loss of uniform strips of cellulose paper are shown in figure 20. At 25°C strength loss of the strips reached 75% after 17 days whereas at 40° and 50°C the strength losses reached 100% after respectively 4 and 5 days. The only reason for this difference is shown on the graph to be a lag of one day at 50°C after which the slopes of the two lines were almost the same, indicating similar rates of cellulose utilisation. It should be noted that although measurements of strength loss were taken the relationship of these results to the biodegradation of other cellulose based materials is limited. This is because the availability of the cellulose in these materials will differ from that in cellulose paper. Thus these results have only a monitoring job and can only quote the relative cellulolytic activities of the flora at various temperatures. It was found to be impossible to find representative and uniform strips of straw which could be used in place of the cellulose paper to give more meaningful

FIGURE 21
EFFECT OF TEMPERATURE ON NITROGEN



results. Later in this thesis will be seen the results of an analysis of the cellulose contents of the initial and final samples of each fermentation carried out. This will show directly and quantitatively the extent of biodegradation.

The results of the nitrogen determination show a considerable drop in the first day of fermentation followed by either a stabilisation (25°C) or a slight increase and decrease with a levelling off after 9 days (50°C). However, at 40°C there was an increase in the nitrogen content and a levelling off after 6 days. The changes in nitrogen content observed in this investigation and subsequent investigations can be explained in two ways. The micro-kjeldahl method measures organic nitrogen (Humphries, 1956) and thus if there is any change in the organic forms of nitrogen such as proteins, free amino acids or amines, there will be a change in the micro-kjeldahl percentage. The first explanation based upon this premise is as follows: The transport of the nitrogen can occur by two routes. It can be reduced by micro-organisms to give ammonium ions and these will produce gaseous ammonia, depending upon the alkalinity of the substrate (Culp and Culp, 1971), or the micro-organisms can oxidise the nitrogen to give inorganic nitrates and nitrites. Both nitrogen reducing and oxidising microflora will probably be present in the waste. Thus initial loss of micro-kjeldahl nitrogen may be due either to gaseous ammonia production, the oxidation of reduced organic nitrogen to inorganic nitrates and nitrites, or a combination

of both. The subsequent increase is due to the reduction of the inorganic forms of nitrogen to build cell tissue which produces protein and increases the micro-kjeldahl nitrogen. The second explanation lies in the observation that there may be large decreases in bulk of the waste so that the organic nitrogen, even if it does not increase by conversion of the inorganic form, will still increase as a percentage of the whole sample unless nitrogen is lost to the atmosphere or fixed from the atmosphere by the nitrogen fixing bacteria. Thus the initial decrease in nitrogen is explained by release of ammonia and a subsequent increase explained by the reduction in carbon and cellulose bulk. Any levelling out of the nitrogen content shows that there is an equilibrium between the organic and inorganic forms of nitrogen, showing either no reduction in carbon or no build up of protein. It will be seen in subsequent runs that where the nitrogen figures increase the carbon figures decrease. However, it is likely that, as the micro-kjeldahl method measures organic nitrogen, the increases will be due to microbial conversion to organic nitrogen which would mean an upgrading of the waste in terms of crude protein.

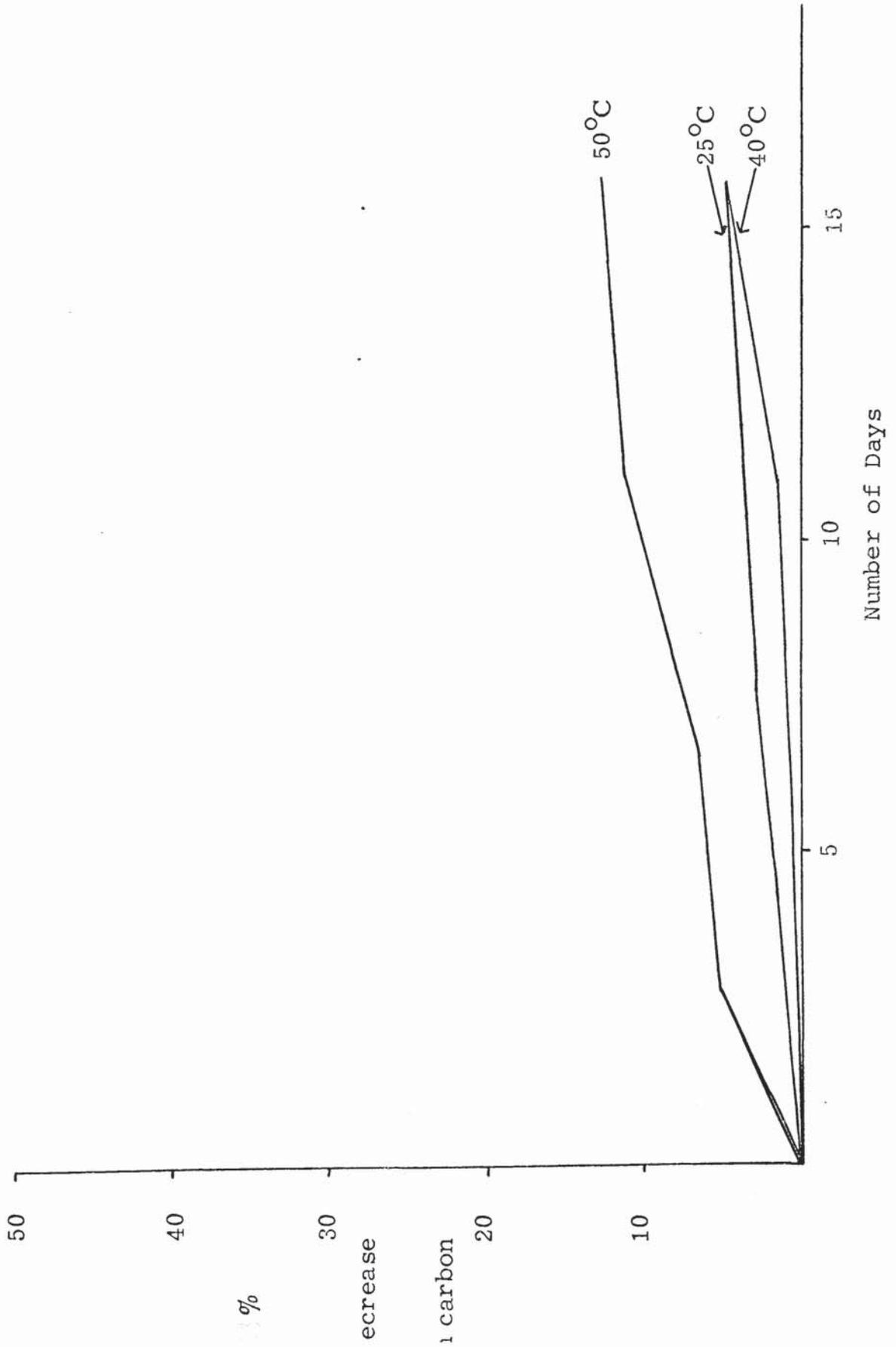
It is very important that nitrogen loss as ammonia be minimised so that it can be used to produce protein. It will be seen later that by altering the initial pH loss of nitrogen can be minimised. In these results the greatest loss of nitrogen (30%) occurred at 25°C where the pH reached 8.7. At 50°C where the pH increased to 8.2 the

loss was 16%. The anomalous result at 40°C may be explained in terms of the loss of other materials as explained above. However, examination of the decreases in carbon at various temperatures will show that there was very little carbon utilisation at 40°C. It thus seems likely that the conversion of inorganic nitrogen to protein was greatest at this temperature and that this may have been due to only a small increase in pH, compared to the other runs, which gave rise to no loss of nitrogen through ammonia release.

It will be seen later that carbon and cellulose breakdown provide more useful information as to the extent of the biodegradation process, whereas the nitrogen content is only important in that it should be monitored to see whether certain parameters affect it and thus alter the amount of available nitrogen which can be converted to protein.

It was found that the best way to present the carbon results was to show their relative decreases so that conclusions could be made as to the extent of biodegradation in these terms. The results for the three temperatures are shown in figure 22. The greatest carbon decrease was given by the 50°C process. This reached 13% after 17 days. At 25°C and 40°C there was a 5% decrease after 17 days. These figures, although not very high, do indicate that 50°C would seem to be the best temperature for the process. A more full discussion

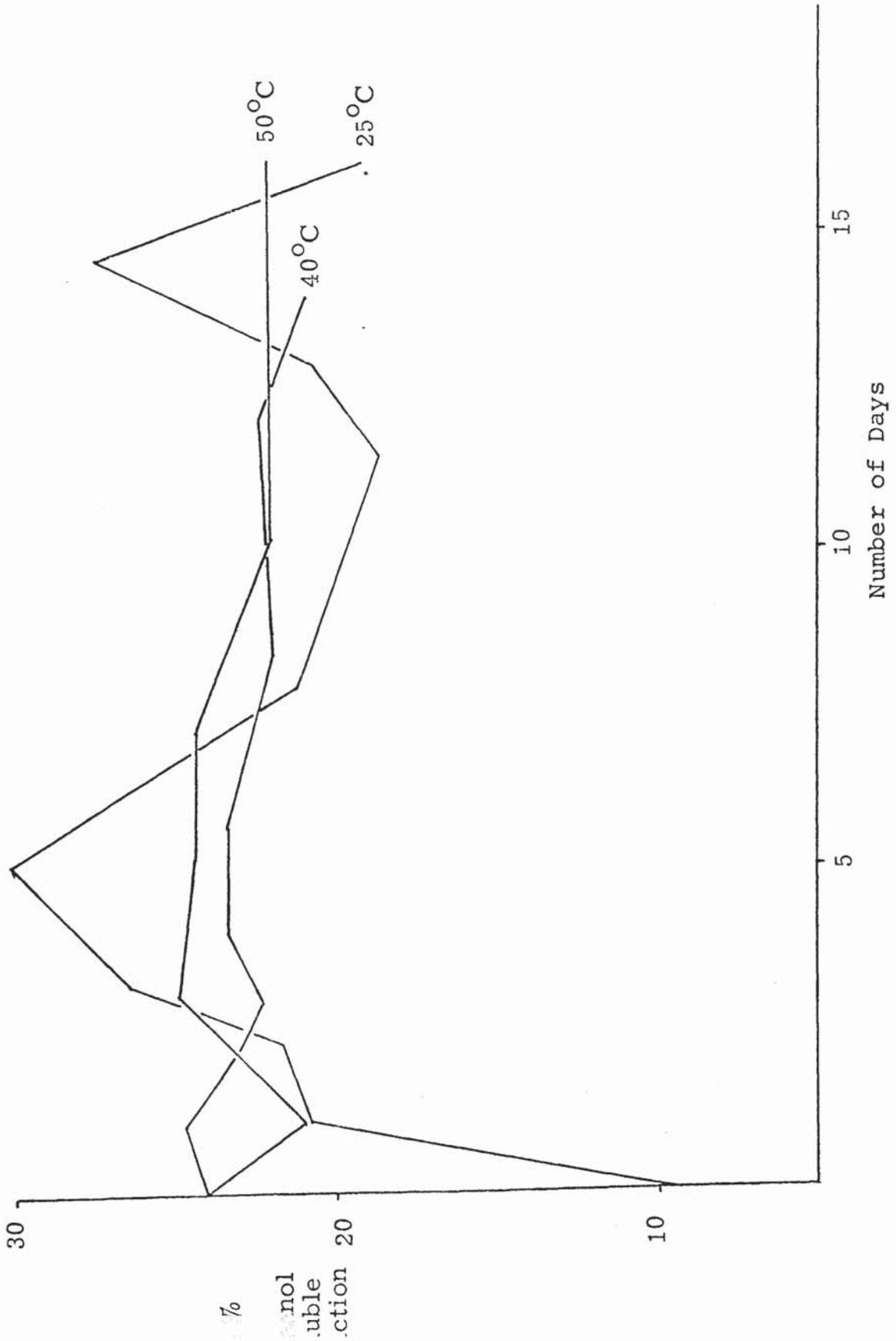
FIGURE 22
EFFECT OF TEMPERATURE ON CARBON REDUCTION



of these results can be made when the cellulose analysis results are discussed to see if there are correlations between the two.

The determination of the ethanol soluble fraction (see figure 23) was introduced to see if soluble sugars were produced initially in the process by the cellulolytic fungi and subsequently utilised by the secondary sugar fungi (Garrett 1963). Although not very pronounced the fractions do show that for the 50°C and 25°C processes there is an initial increase in the fractions followed by decreases. At 40°C there was an initial decrease suggesting preferential utilisation of any soluble sugars present but this was then followed by an increase, a plateau lasting about 4 days, and then a decrease. The plateau of increased ethanol soluble fraction at 50°C only spanned 2 days suggesting a shorter cellulolytic dominance before the secondary sugar fungi stage. It is interesting to note that where the initial ethanol fraction was low (9%) as for the 25°C run the solubilisation of higher saccharides was great producing more substrate for subsequent utilisation. The second peak in the 25°C run, although not as large, indicates a repeat of the first cycle. The value of these determinations is limited in making such statements and must be strengthened by isolation work to elucidate the presence of the different groups of fungi at the various stages in the process. Chang (1967) gives no discussion of this fraction stating only that it contained sugars, glucosides, essential oils, colouring matter and

FIGURE 23
EFFECT OF TEMPERATURE ON ETHANOL SOLUBLE FRACTION



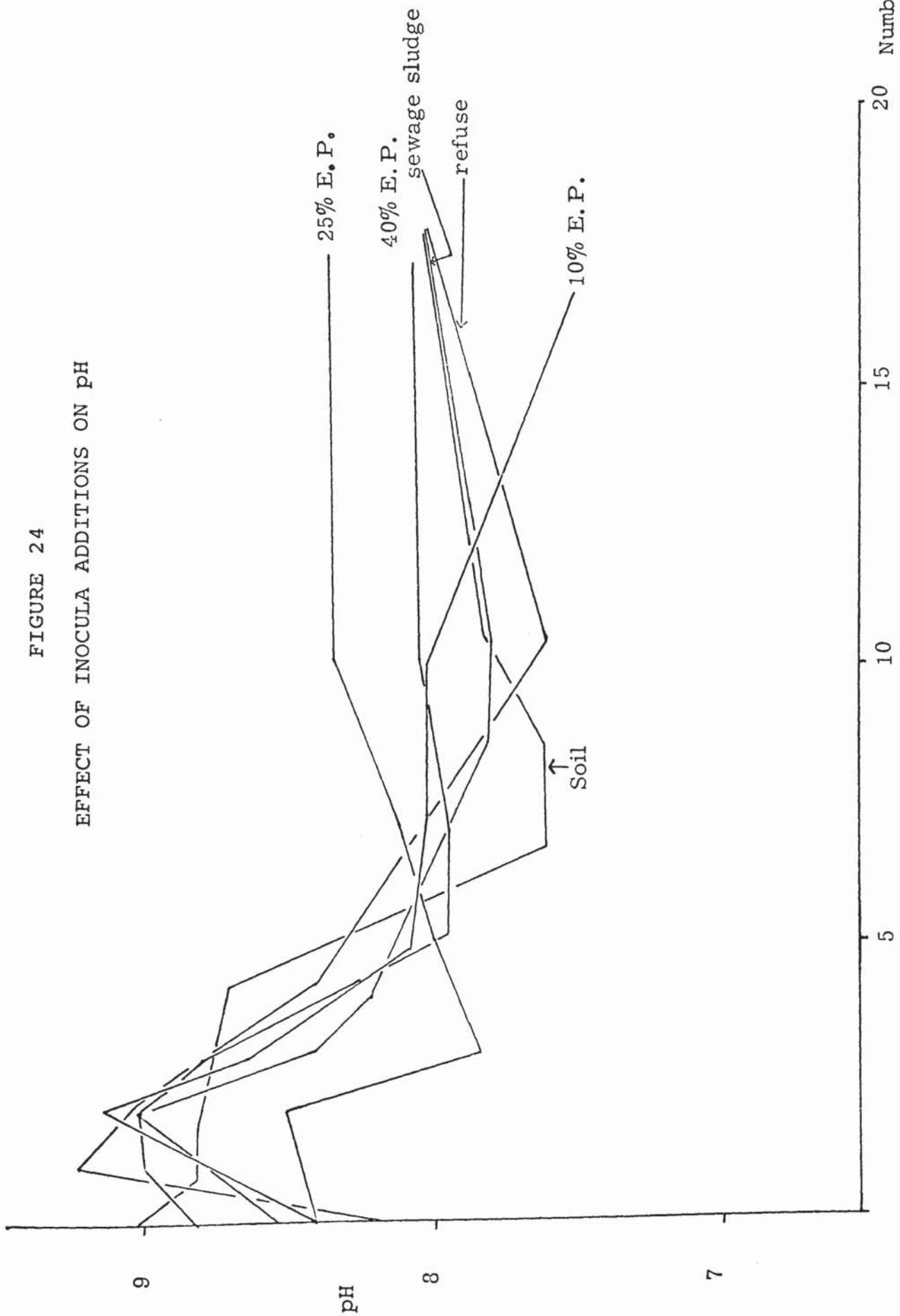
resinous substances. The ethanol soluble fraction determination is a precursor to cellulose determinations and thus it will continue to be quoted in future work to see if further results can be correlated with these.

4.3.2 The effects of inocula additions

The results from the addition of sewage sludge, refuse, soil and end-product are shown in figures 24 - 27. On studying the pH curves it can be seen that in all but two cases there was an initial appreciable increase in pH (9.3 being the highest recorded for the refuse inoculum). This was recorded after 2 days and was followed by a decrease to below the initial pH, and then a terminal pH of between 8.3 and 7.5. For the soil and 25% end-product inocula there was respectively a fall and very little initial increase. However, the fall may have been due to a high initial pH of 9.05. The final pH of 8.0 was the same as for the sewage sludge and refuse inocula, but there was an intermediate fall in pH to 7.6 followed by a rise to 8.0.

The 25% end-product inoculum had a pH rise of 0.5 in the first 2 days which may be negligible, and a fall to 7.8 followed by a rise to a plateau of 8.4 where it remained until the 17th day. It is necessary to relate the nitrogen and carbon decreases to the pH before conclusions can be

FIGURE 24
EFFECT OF INOCULA ADDITIONS ON pH



Number of Days

drawn as to the best inoculum investigated. There were organic nitrogen losses for the sewage sludge, refuse and soil inocula of respectively, 0.25%, 0.1% and 0.28% which meant overall losses as a percentage of the original contents of respectively: 17.2%, 9.8% and 21.8%. The refuse inoculum seemed to regain all of its lost organic nitrogen, being converted from the inorganic forms with no apparent loss to the atmosphere. Having previously stated that when the pH increases there may be nitrogen loss to the atmosphere the soil fermentation pH fell also giving nitrogen loss. This may have been due to the starting pH of 9.0 which fell to 8.8 after the first day followed by a gradual fall of about 0.5 units over the next 3 days. This meant that the material still remained alkaline and may have lost ammonia nitrogen. However, as explained above, the apparent loss of organic nitrogen may have been due to the conversion to inorganic forms of nitrogen. The pH of the other two fermentations (sewage sludge and refuse) quickly declined to below 8 which seemed to arrest the release of ammonia and nitrogen loss. Thus it is not only important to consider the increase in pH but also the initial pH and how quickly it decreases to below a value which keeps the ammonium ion in solution or bound up with other ions.

The addition of end-product inocula gave an increase in organic nitrogen - the 10% gave an increase of 0.55%, the 25% gave an increase of 0.26% and the 40% inoculum gave no increase. This would indicate products in the case of the 10% and 25% inocula additions which were enhanced in their

FIGURE 25 - EFFECT OF INOCULA ON NITROGEN

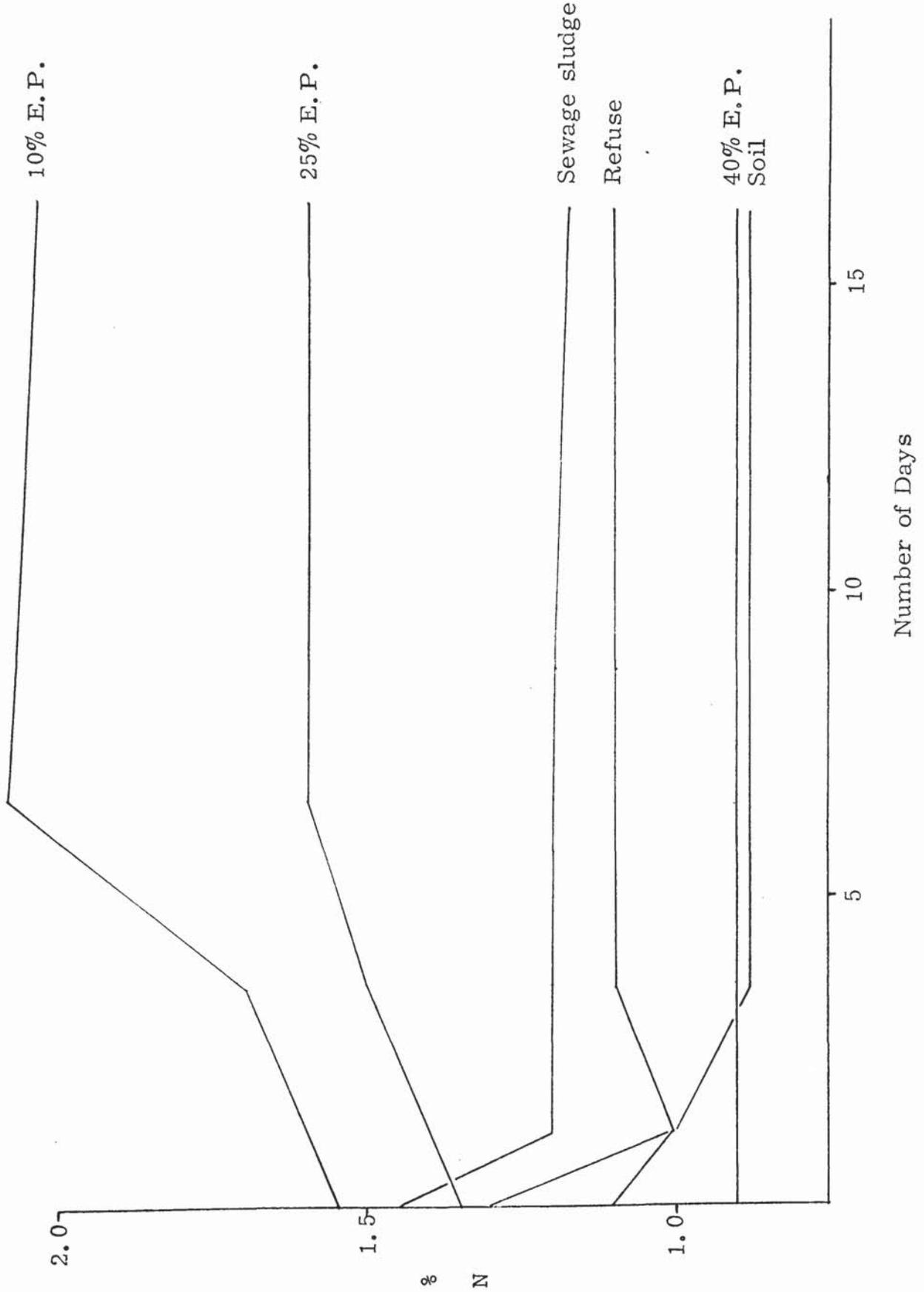
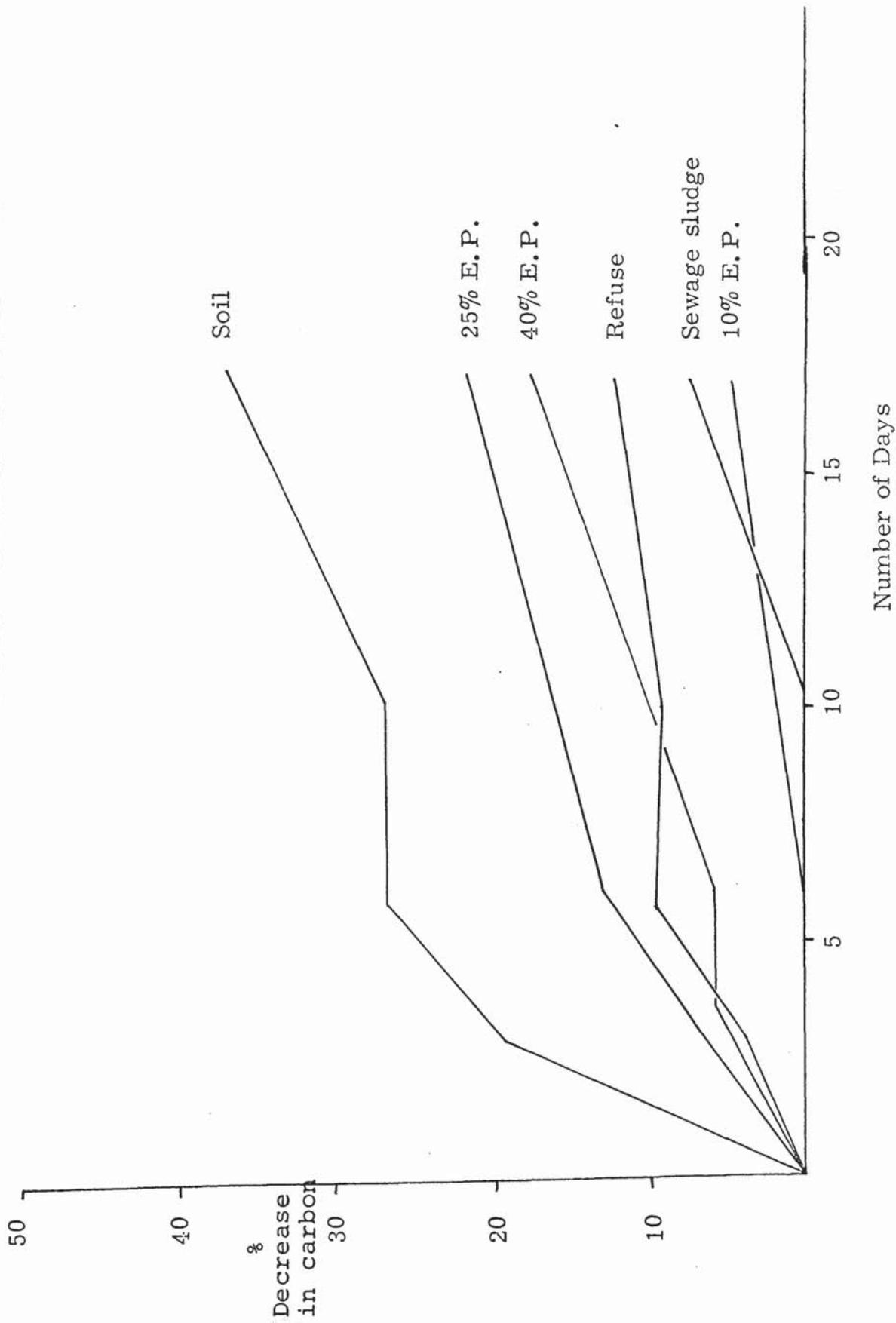


FIGURE 26
EFFECT OF INOCULA ADDITIONS ON CARBON REDUCTION

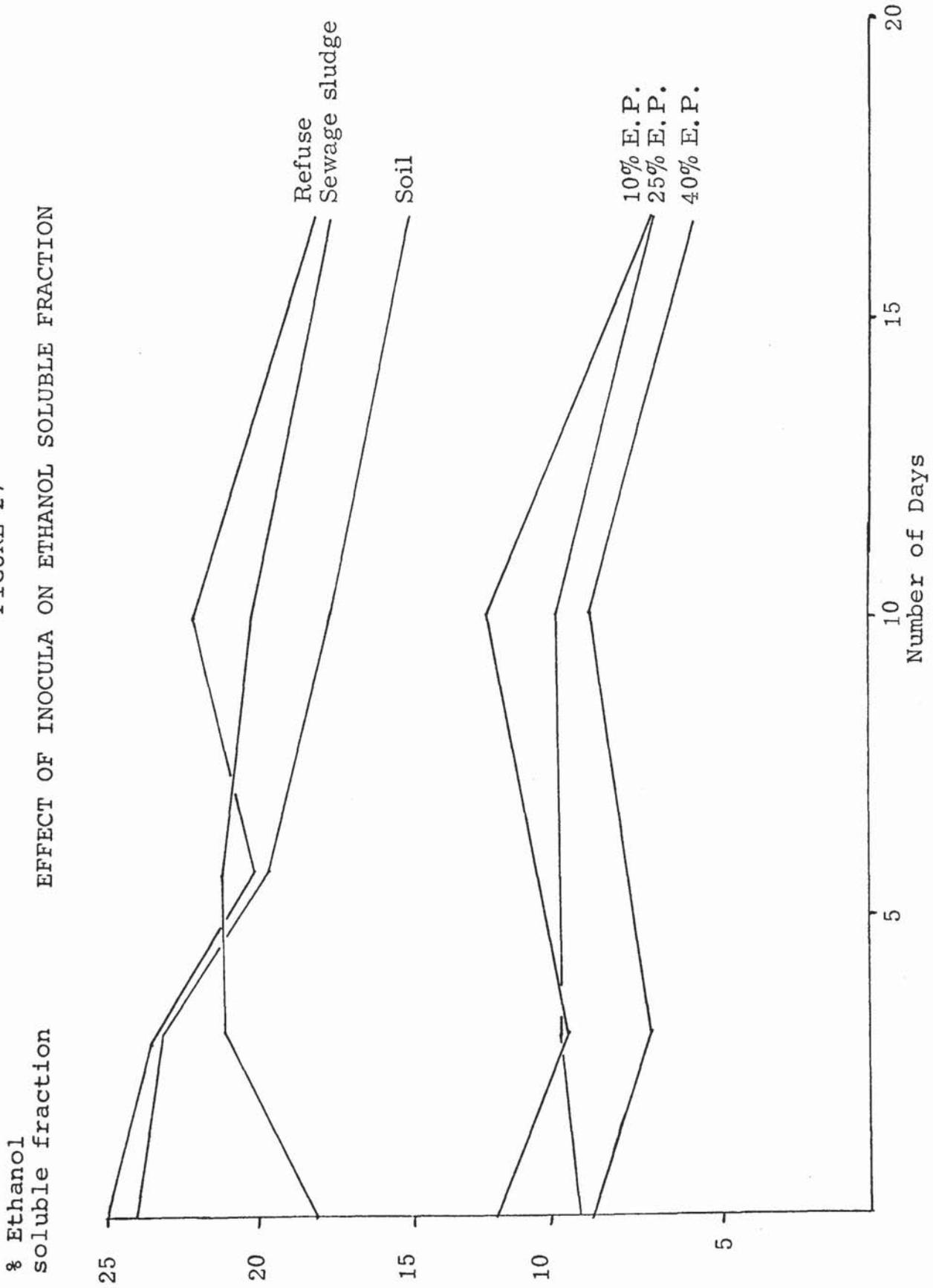


protein content. These increases cannot be correlated with decreases in carbon (see below) and this suggests it is more likely that the nitrogen increases did not result from a reduction in the bulk of the materials.

The carbon decreases show the difference in the biodegradation processes of the six inocula. The soil was found to give the greatest decrease (38%). The 25% end-product gave 22% and the 40% end-product gave 18%. The 10% end-product gave the lowest decrease of 5% with refuse and sewage sludge giving 12 and 7% respectively. Thus it can be seen that a 10% end-product inoculum, sewage sludge and refuse are probably unsuitable additions. Later the effect of pathogens in these inocula will further substantiate this view. The addition of 40% end-product might mean adding very large amounts which would give mixing problems on the practical side. The incorporation of 10% soil into the starter fermentation and then adding 25% of the product to a new batch containing soil might provide the best breakdown.

The ethanol soluble fractions were monitored for this investigation and it was found that for all cases excepting the sewage sludge and 25% end-product inoculum, there was a decrease in solubles followed by an increase and then a further decrease to a value lower than the initial one. This again would indicate the primary utilisation of soluble sugars, perhaps in preference to cellulose, followed by the exhaustion of these sugars and the selective ability of cellulose decomposers to solubilise the cellulose and produce an increase in the fraction. The secondary sugar utilisers

FIGURE 27
EFFECT OF INOCULA ON ETHANOL SOLUBLE FRACTION



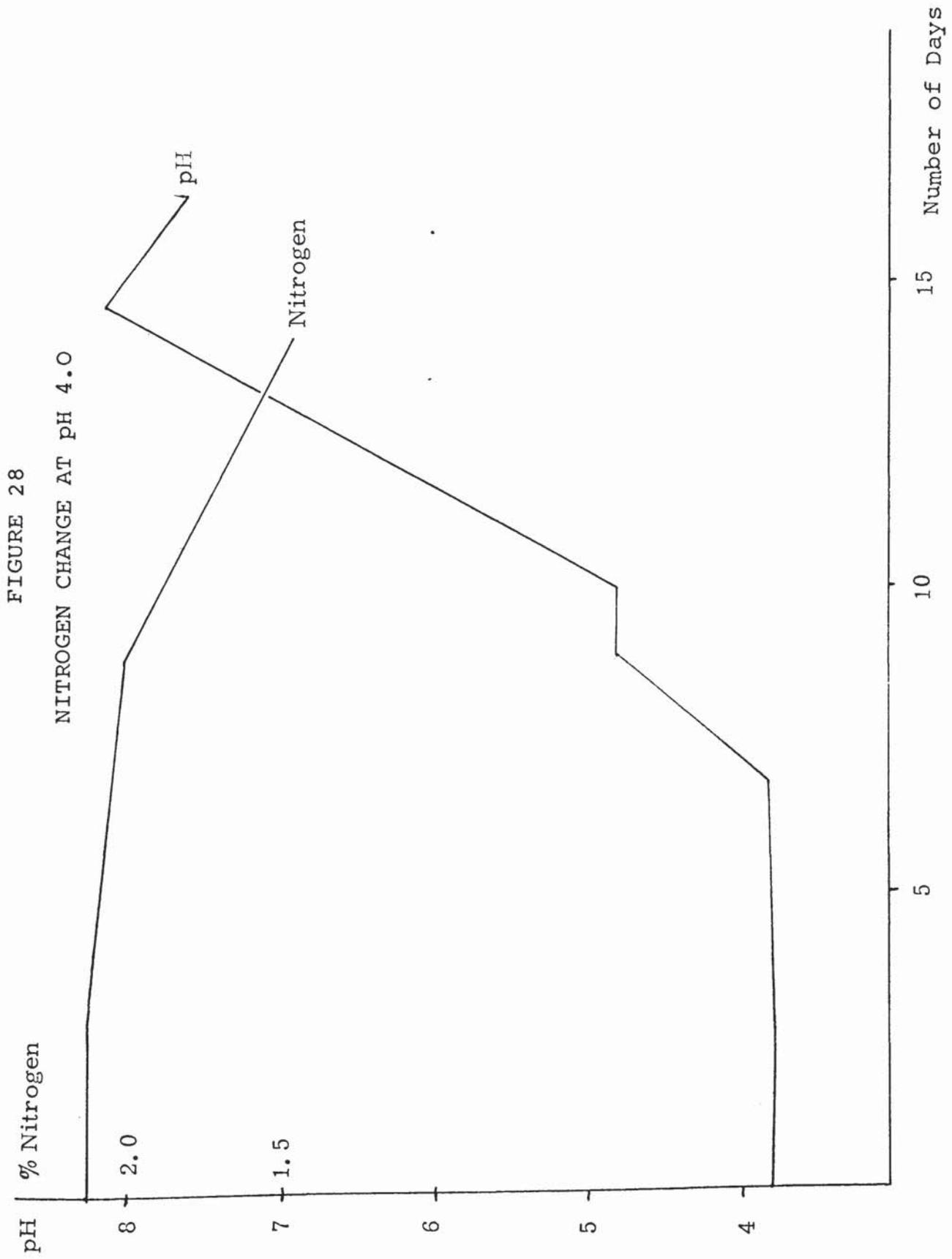
25
20
15
10
5

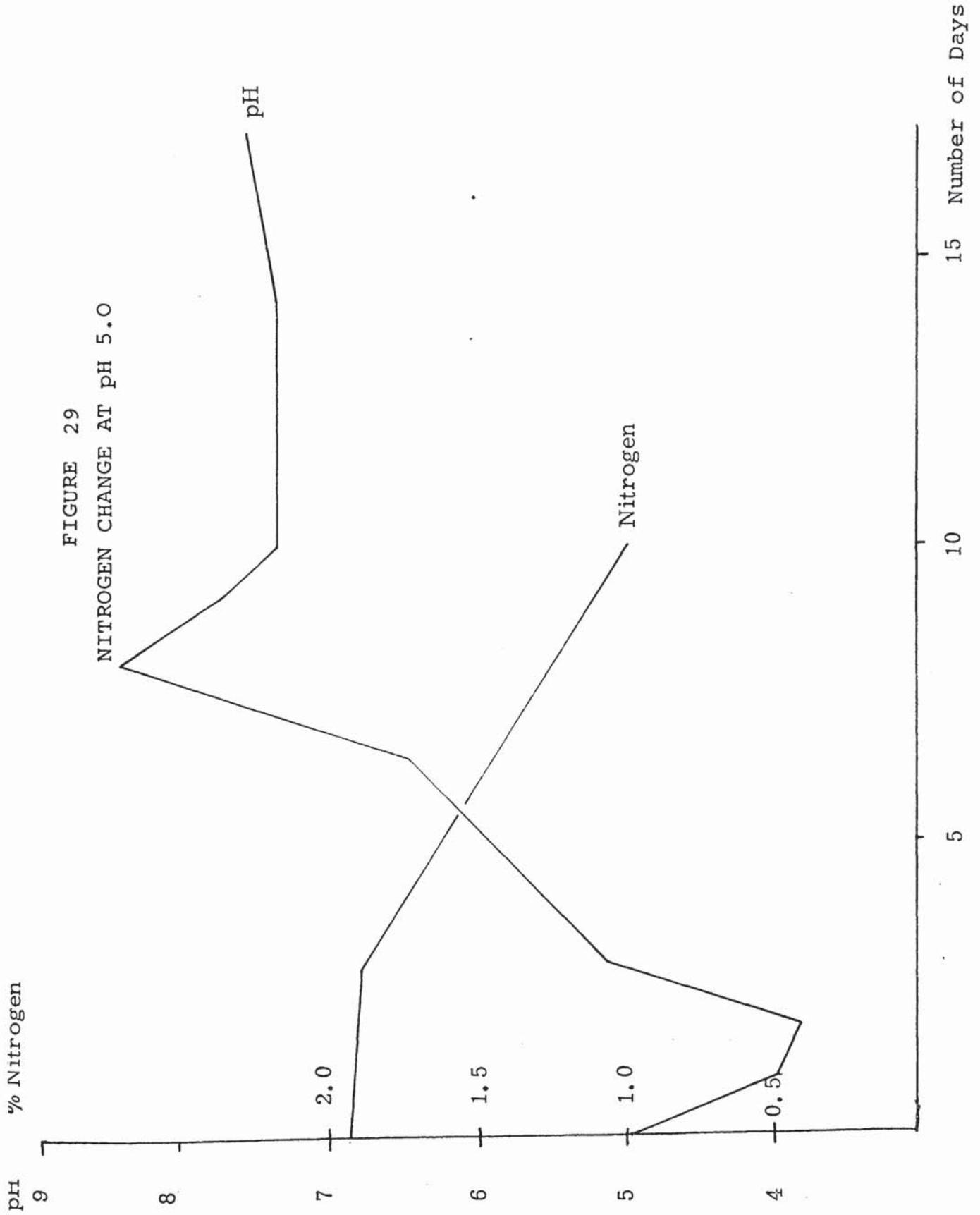
5
10
15
20

are then able to use the soluble sugars produced and cause a further decrease in the fraction. The other two fermentations had no initial drop in the fraction which may have indicated that conditions favoured the cellulose decomposers although, in the case of the sewage sludge inoculum, carbon decreases in the first 10 days were nil.

4.3.3 The effects of pH on loss of nitrogen

The results from this investigation are shown in figures 28 - 30. It can be seen that the lower the pH the longer was the time that was spent in the acid range. At pH 4.0 the fermentation remained at this value for 7 days in which no microbial activity was observed, after which there was an increase over a further 7 days to 8.2 followed by a decrease to 7.8. At an initial pH of 5.0 there was a decrease for the first 3 days to pH 3.8 and then a gradual increase over 5 days when the pH reached 8.4. This decreased and stabilised at between 7.2 and 7.3 after 10 days. After 3 days the process with an initial pH of 6.0 had increased to 8.8, and this was followed by a decrease over the remaining 14 days to reach 7.2. The nitrogen determinations show that the longer the pH remained acidic the longer the organic nitrogen loss was contained. It can be seen that at pH 4.0 and 5.0 the appreciable falls in nitrogen content (0.6 and 0.9% respectively) occurred when the pH increased into the alkaline range. For pH 6.0 the decrease in nitrogen (from



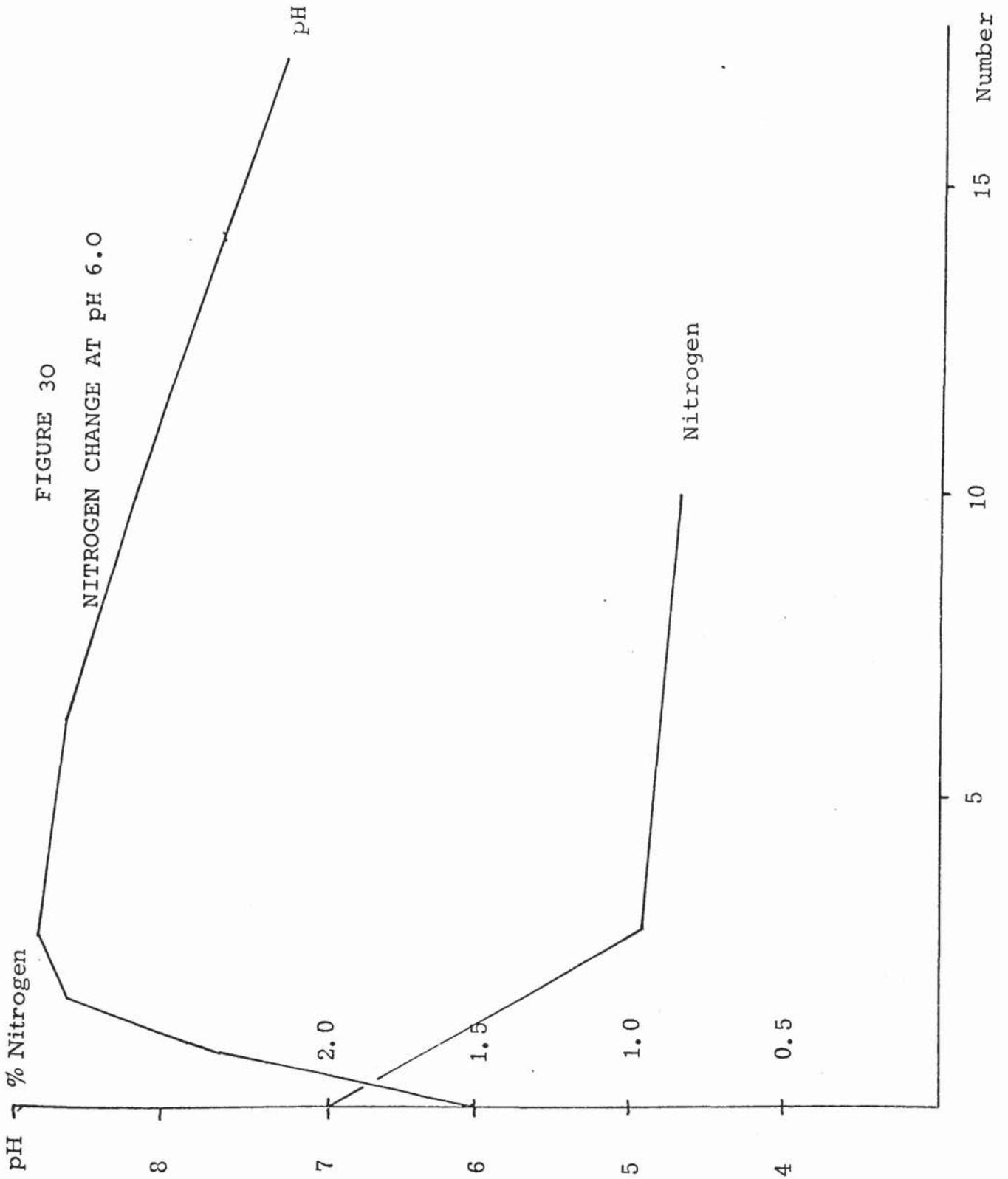


1.90 to 0.93%) occurred in the first 3 days and, when the pH began to decrease, the loss in nitrogen was reduced. The losses of 0.6, 0.9 and 1.0% nitrogen represent overall percentage losses in terms of the total initial nitrogen content of 28, 45 and 50%. Thus losses in organic nitrogen can be reduced by altering the initial pH of the material. During the acidic stage of the process at pH 4.0 and 5.0 there was negligible drop in nitrogen. It may be desirable to include some cheap chemical with buffering properties into the thermophilic stage to encourage this pH range and also, as a consequence, to encourage the establishment of a highly active thermophilic fungal flora to utilise the nitrogen in preference to oxidation by bacteria or conversion to gaseous ammonia which may be irretrievably lost. The optimum starting pH may be pH 5.0 as 4.0 is too low for the optimum activity of most thermophilic fungi. If these fungi can convert the nitrogen before the pH increases then there may be no loss of nitrogen as ammonia. This problem also has applications in odour control. Ammonia and the other more offensive gases are released in alkaline wastes due to bacterial dominance. By keeping the pH low these odours can be minimised.

The control of pH may also be used to control certain unwanted organisms. The reduction of undesirable bacteria at acidic pH's has been discussed already, but certain fungi such as Aspergillus fumigatus and Mucor pusillus, both animal pathogens (Emmons et al., 1963; Cooney and Emerson, 1964) can be controlled using adverse pH ranges. As was seen in the isolation work, these fungi had very low frequencies above 5.6.

FIGURE 30

NITROGEN CHANGE AT pH 6.0



Mills (1973) has investigated the pH optima for the cellulolytic activity of Aspergillus fumigatus and has found that there are two pH optima of 4.2 - 4.4 and 6.2 - 6.4. It can now be seen that to prevent the activity of these fungi the pH should be kept in the neutral region range whereas for minimal nitrogen loss and bacteria control the pH should be in the acid ranges. The effect of temperature, however, is able to affect the fungi in that at 48°C and above Aspergillus fumigatus and Mucor pusillus are not at their optimum activity and may be suppressed by other more active fungi. The parameters of inocula additions with pathogen removal at 48°C and 50°C, the effect of 56°C on pathogen removal, the length of the process and fungal successions will be discussed in the next chapter together with analysis for cellulose and a discussion of the optimum conditions for a large-scale process in the light of the results obtained from these two chapters.

CHAPTER 5

CHAPTER 5

THE ACCELERATED BIODEGRADATION OF PIG WASTE - AN EVALUATION OF THE PARAMETERS

- 5.1 Introduction

- 5.2 Methods
 - 5.2.1 The effects of temperature and inocula on pathogen removal
 - 5.2.2 The effect of 56°C on the biodegradation process
 - 5.2.3 The effect of the length of the process on biodegradation of the waste
 - 5.2.4 Determination of the flora successions in the process

- 5.3 Results and discussion
 - 5.3.1 Effects of temperature and inocula on pathogen removal
 - 5.3.2 Effect on biodegradation at 56°C
 - 5.3.3 The effect of the length of the process on biodegradation of the waste
 - 5.3.4 Flora successional patterns

5.4 Analysis of samples for starch, hemicelluloses
and cellulose

5.4.1 Methods

- a. Starch, dextrins and glycogen
- b. Hemicelluloses
- c. Celluloses

5.4.2 Results and discussion

5.1 INTRODUCTION

In this Chapter investigation will be made of the effects of temperature on intestinal bacterial pathogen removal and the rate of breakdown, and the effect of the length of the process on the rate of breakdown. The removal of the intestinal bacterial pathogens is important in controlling the transfer of such bacteria to animals if the waste is to be used as a feedstuff. This could cause diarrhoea or 'scours' to the recipients and affect the quality of the animal. Also, the transfer of bacteria to humans, via incompletely cooked meat, is greatly increased. The limits of this investigation mean that it is not possible to demonstrate whether other animal pathogens and parasites are reduced or inhibited by heat. It seems likely, however, that those pathogens with resistant resting stages such as the tapeworm cyst will be able to tolerate the thermophilic temperatures. Those non-resting stages voided in the faeces will probably be destroyed. A further investigation would be needed to determine the extent of treatment necessary for safe removal of all pathogens.

An analysis of the products to determine the extent of breakdown of cellulose and hemicelluloses will also be carried out to support the results obtained regarding the optimum parameters.

5.2 METHODS

5.2.1 The effects of temperature and inocula on pathogen removal

In Chapter 2 a discussion of pathogens revealed that at temperatures of 50°C and above the non spore-forming intestinal bacterial pathogens could be killed off. In considering a complete treatment process which may be linked with recycling it is necessary that pathogens be removed. The danger in handling the treated waste is also considerably lessened. The effect of temperature on pathogen removal was investigated by studying the removal of coliform salmonellae and non-lactose fermenting bacteria from the waste. Two agars both made by Oxoid Ltd., London, were used as selective media for these groups. McConkey's agar No. 3 (MCA) was used for coliforms and non-lactose fermenting bacteria. Desoxycholate citrate agar (DCA) (Hynes) was used for the salmonellae.

Three temperatures were investigated: 40°, 50° and 56°C. Jalal (1969) has found that the non-spore formers can be removed at 56°C and this temperature was included as still being within the thermophilic fungi growth ranges. Fermenters were set up with a 10% soil inoculum as for previous experiments and daily samples (lg. wet weight) were plated out (10 replicates) onto the two agars. Dilutions were made of the samples before plating out as follows:-

10^{-1} , 10^{-3} and 10^{-5} . 1 ml aliquots of the dilutions were added to each plate and the agar poured at about 45°C. Incubation was at 37°C for 18 hours. The colonies were counted at a suitable dilution using an illuminated background and detected by their development on the diagnostic media described above. The effect of the sewage sludge, refuse and soil inocula on pathogen removal was also investigated using samples from those investigations at 48°C and 56°C. This was to demonstrate whether the inocula introduced large amounts of pathogenic bacteria from other sources and whether these were viable under the different conditions existing in pig waste.

5.2.2 The effects of 56°C on the biodegradation process

As a fermentation at 56°C was set up to test for pathogen removal it was decided to carry out a process run at this temperature to determine whether biodegradation was further increased above the 48°C process run. The results from a 56°C fermentation coupled with the pathogen removal results would indicate whether the process was more feasible at this temperature. Three fermenters were set up containing 10% sewage sludge, 10% refuse or 10% soil respectively. The material mixed with straw bedding had previously been treated with liquid slurry as for the other investigations. An air line was introduced into each fermenter with an aeration rate

of 0.25 litres/minute. It was found necessary at 56°C to humidify the air before passing through the waste as the latter tended to dry out. Analyses for carbon, nitrogen, pH and ethanol soluble fraction were carried out at intervals throughout the 17 day incubation period.

5.2.3 The effect of the length of process on biodegradation of the waste

It has been stated previously that from an economic viewpoint there will be an optimum length for the process in regard to the quality of the product. In all the previous investigations 17 days was chosen because the pH stabilised out after this period. However, it may be that when the process reaches this pH there is further fungal activity which causes large amounts of biodegradation. Thus, the process was allowed to proceed for 24, 31 and 38 days to determine whether the extra time produced appreciable biodegradation. The investigation was carried out at 50°C and 56°C as pathogen removal at this latter temperature had been shown by other authors to be a better temperature to work at. Three fermenters were set up at each temperature with a 10% soil addition (shown to give greatest biodegradation at both temperatures) and samples were taken initially and after 24, 31 and 38 days. The extent of biodegradation was determined for each sample.

5.2.4 Determination of the flora succession in the process

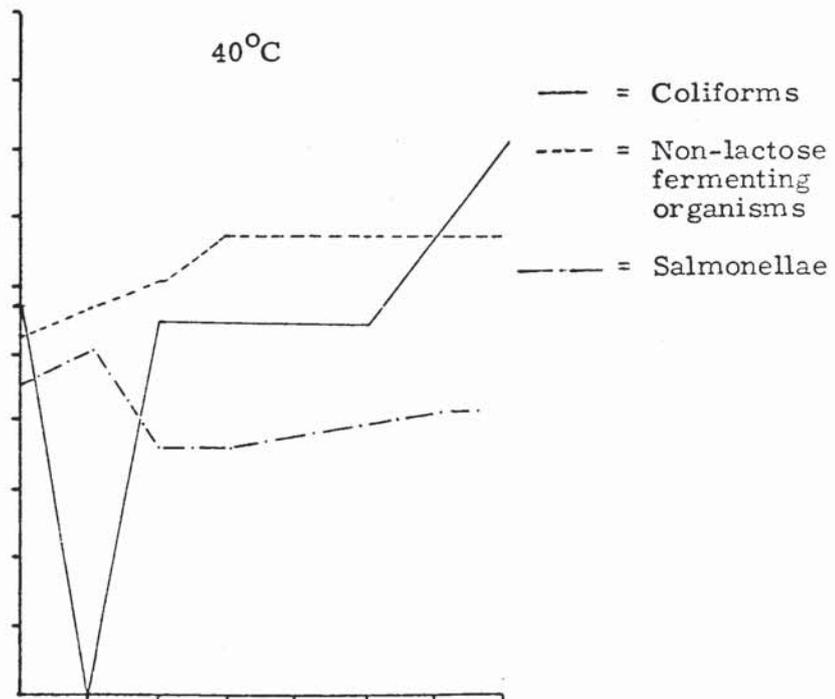
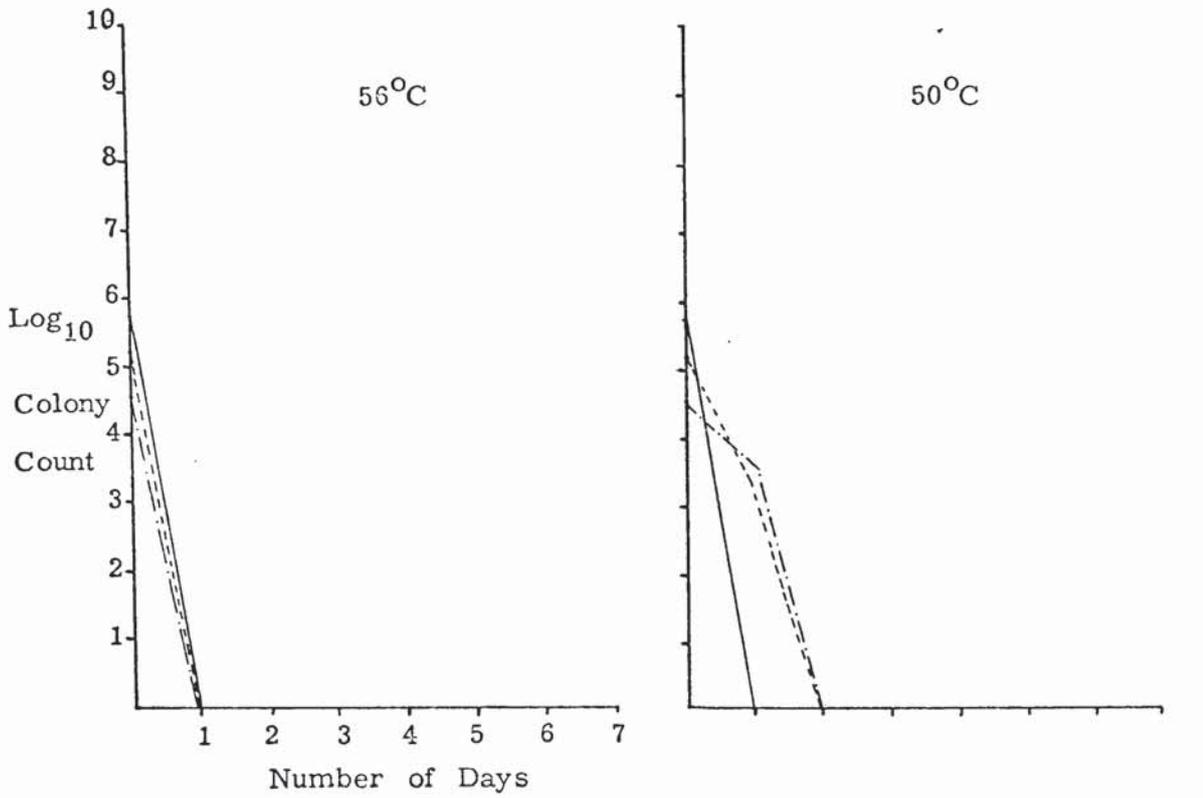
In order to determine which species were present under varying conditions throughout the fermentations, and thus create conditions favourable to those species which caused the greatest breakdown, a floral succession was determined. Using the modified screened substrate tubes and polythene-backed cellulose paper ('Benchcote', made by Whatman) to prevent the strips from falling apart on handling, strips were sacrificed from the 50°C run and from the sewage sludge, refuse and soil runs to determine the dominant flora present throughout the run. Two tubes were removed, each strip cut into four pieces and plated onto cellulose and glucose-starch agars for incubation at 48°C.

5.3 RESULTS AND DISCUSSION

5.3.1 The effect of temperature and inocula on pathogen removal

The effect of temperature is shown in figure 31. At 56°C all groups of pathogens monitored disappeared within one day. At 50°C the coliforms were killed off within one day whilst the salmonellae and non-lactose fermenting bacteria took 2 days. When the material was incubated at 40°C the coliforms showed a decrease in which none were recorded after

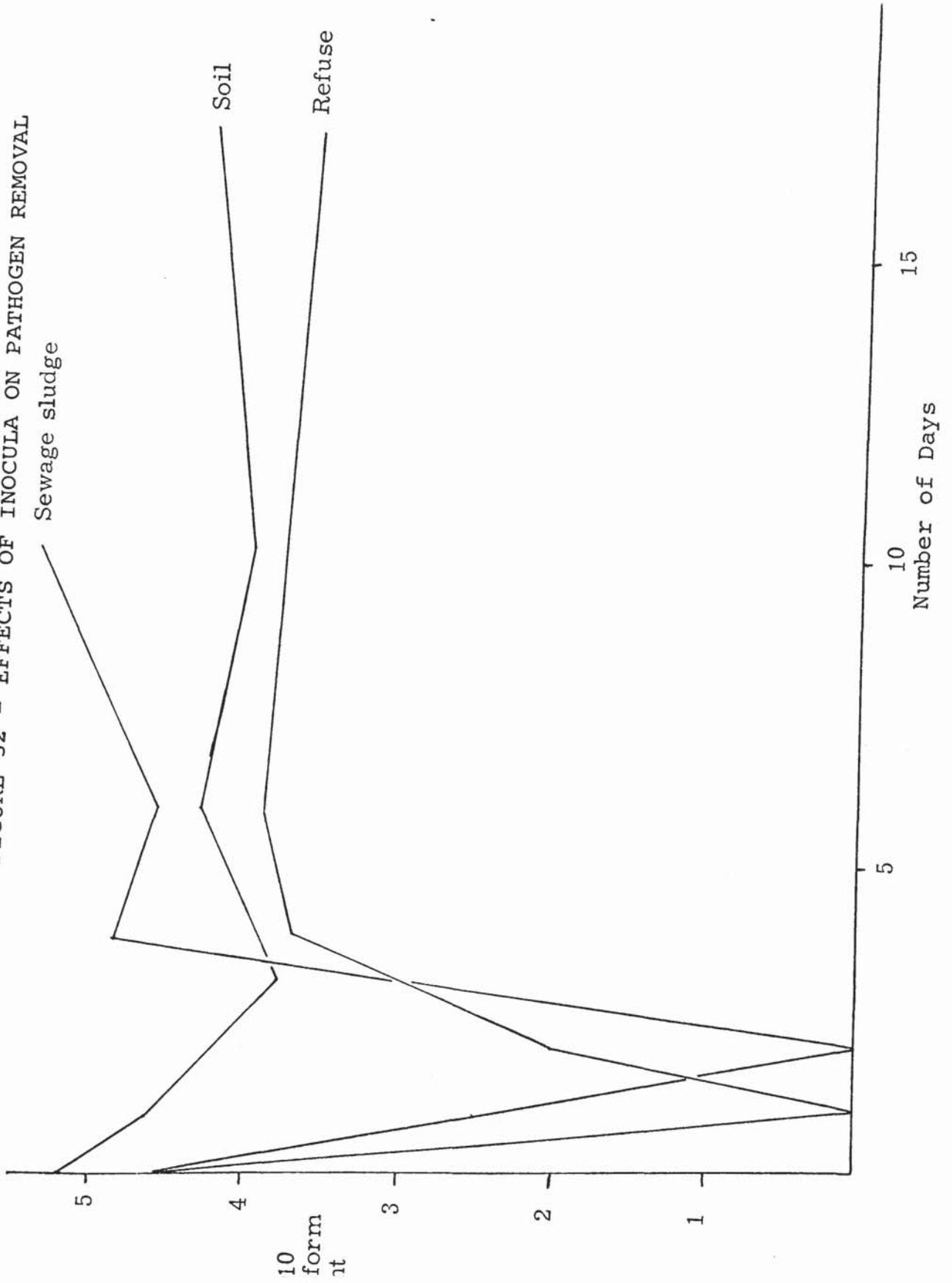
FIGURE 31
PATHOGEN REMOVAL



1 day, but subsequently the numbers increased reaching 10^8 /ml. after 7 days. The salmonellae showed a slight increase after 1 day followed by a decrease and then a slow increase reaching 10^4 /ml. in number after 7 days. The non-lactose fermenting bacteria showed an increase up to 1.5×10^6 organisms/ml. after 3 days and a plateau at this number for a further 4 days. The initial decrease and subsequent increase of coliforms may have been due to the death of non-resistant forms which was then followed by the establishment of the heat resistant strains which were able to gain dominance. Both the salmonellae and non-lactose fermenting bacteria did not increase at the same rate even though their initial concentrations were similar. They may have been at their top temperature limit of growth or they may have been partially suppressed by the coliforms.

In figure 32 are shown the results from coliform counts done for the sewage sludge, refuse and soil additions. Here can be seen a different situation at 2°C below the 50°C experiment described above. For the sewage sludge and refuse there is the characteristic fall to zero numbers in respectively the 2nd and 1st days of incubation followed by an increase as quick as the decrease. The sewage sludge continued to increase whilst the refuse coliforms reached a plateau of about 10^4 organisms/ml. The soil did not appear to give a large fall in numbers. However, this may have been due to the fact that no sample was taken for day 2 when there could have been this drastic decrease in viable numbers. The number of coliforms in the soil inoculum fermentation reached a plateau at about 1.3×10^4 /ml. after 5 days and remained

FIGURE 32 - EFFECTS OF INOCULA ON PATHOGEN REMOVAL



there until the 17th day.

The effect of the inocula seems to be to introduce a greater number of heat resistant strains into the material. The past history of the refuse and sewage sludge might support this as they were subjected to composting before their use as an inoculum. However, the soil was not and its effect may have been to increase the number of viable bacteria present. In comparing the three inocula the sewage sludge was the most unsatisfactory as the numbers of coliforms increased above the initial inoculum level. The soil and refuse gave plateaux which were below inoculum level and there were no subsequent increases over 14 days. However, it can be seen that for complete coliform removal this temperature was not high enough and the inocula additions were undesirable. The inocula fermentations were conducted at 48°C whilst the temperature investigations were carried out at 40, 50, and 56°C. The 2°C difference may have had some effect when comparing the 48°C with the 50°C experiment. The only real conclusions to be made from this preliminary investigation is that temperatures at and below 50°C with inocula additions would seem to be unsatisfactory in removing certain groups of pathogenic organisms. However, as will be seen later, temperatures above 50°C may not increase the biodegradation process and it is thus important to see whether other means of sterilisation can be used at very little extra cost to the overall running costs.

5.3.2 Effect on biodegradation at 56°C

The results for this investigation are shown in figures 33 to 36. The starting pH of the waste was 9.0 and there was only a slight increase to 9.1 before the pH dropped to level out at between 8.6 and 8.8. The final pH's for the sludge, refuse and soil additions were:- 8.55, 8.40 and 8.50 respectively. If this is compared to the 48°C process with the same inocula it can be seen that these final values were at about pH 8.0 which was tending towards more stable conditions. Further, the high initial pH with only a small increase and then a large drop has been observed for other runs where the initial pH was at or above 9. The soil addition at 48°C showed no increase initially and the 40% end-product addition rose from pH 8.8 to 9.0 before dropping quickly. Based on the final pH values it would appear that a more stable product is formed at 48°C than at 56°C.

As for the 48°C run the soil addition gave the highest decrease in carbon (33%), whilst refuse and sewage sludge additions gave 20% and 17% losses respectively. However, the soil addition decrease of 38% at 48°C was higher than that at 56°C. The refuse and sewage sludge additions had final decreases higher than those at 48°C but even so they were much less than the soil additions at both temperatures. Thus the decreases in carbon content would further support the process at 48°C.

FIGURE 33
EFFECT OF 56°C ON pH

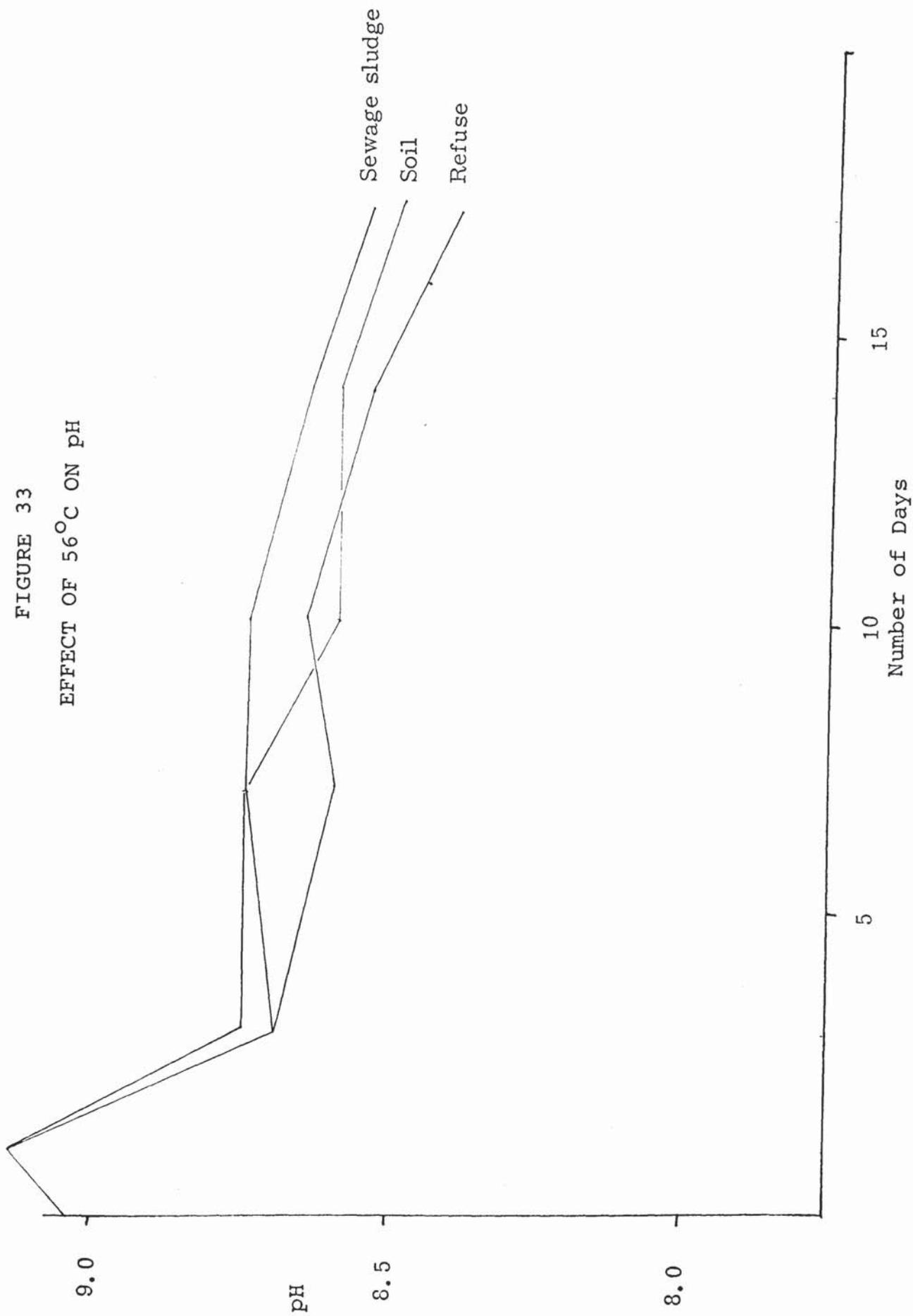


FIGURE 34
EFFECT OF 56°C ON CARBON REDUCTION

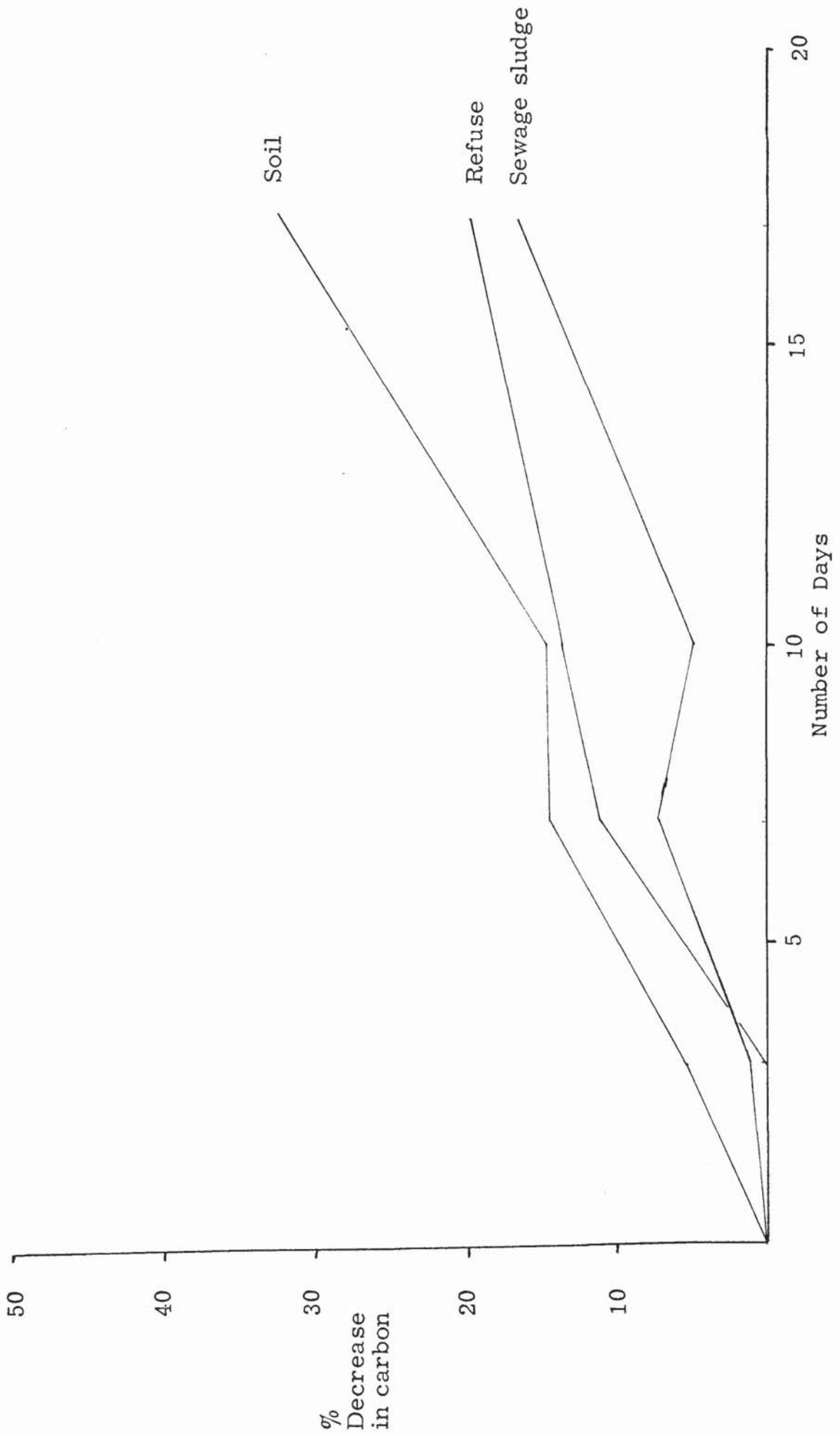


FIGURE 35
EFFECT OF 56°C ON NITROGEN

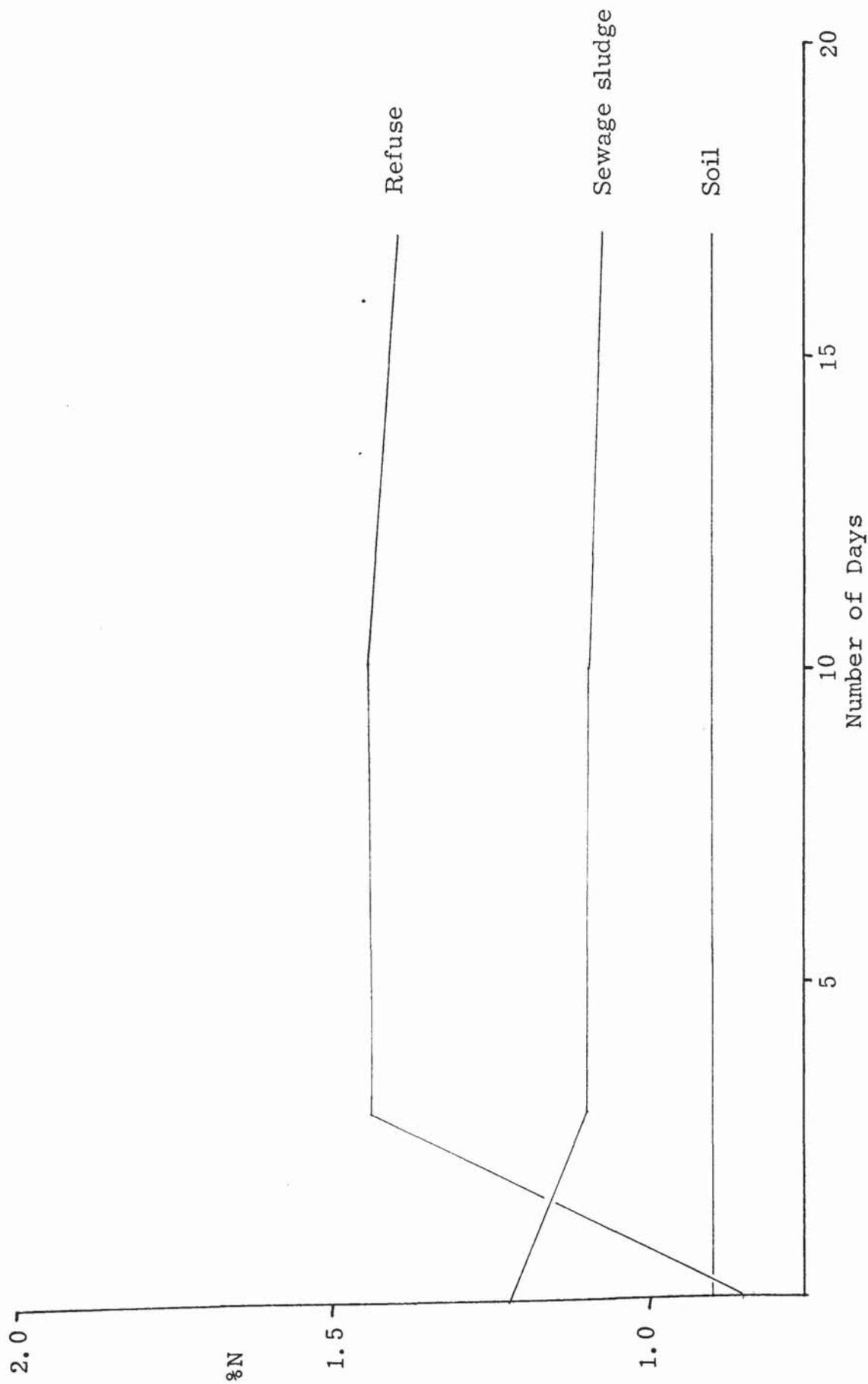
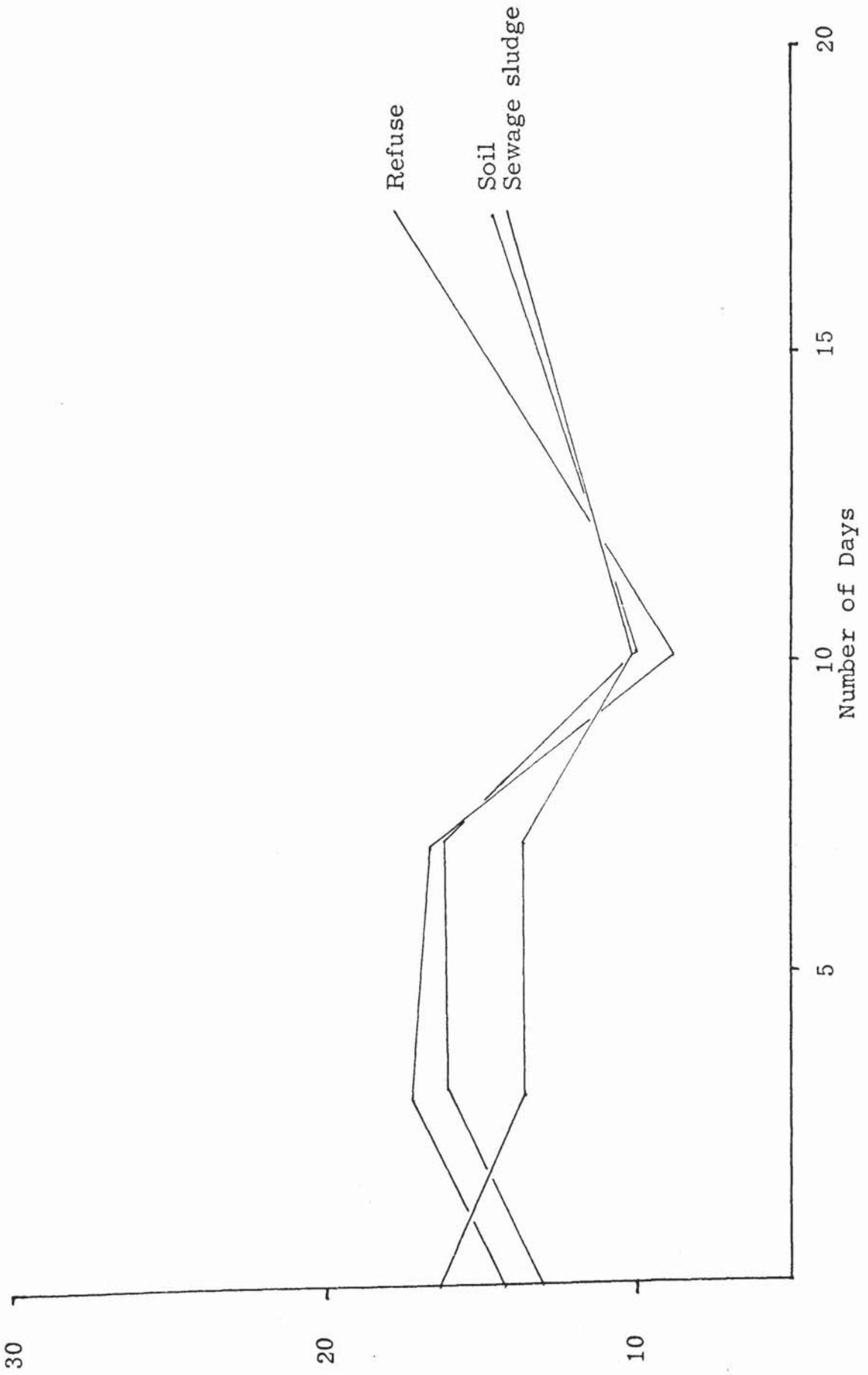


FIGURE 36
EFFECT OF 56°C ON ETHANOL SOLUBLE FRACTION

% Ethanol
soluble fraction



The nitrogen values gave very little change, excepting the refuse, where there was an increase. This may have been due to conversion to organic nitrogen and not reduction in carbon as increases in nitrogen were not observed for the soil and sewage sludge additions. The high initial pH of the refuse addition does not agree with the soil addition at 48°C where there was a decrease from 1.1 to 0.9% nitrogen over 3 days which would be more in agreement with the literature (Culp and Culp, 1971) which states that the more alkaline is the waste the more the equilibrium of dissolved ammonia with gaseous ammonia tends to the latter state. However, because of other factors such as the production of odours and the dominance of unfavourable bacteria an initial pH of 9.0 would be undesirable: this will be discussed at the end of this Chapter when all the information is to hand.

The ethanol soluble fraction showed an initial rise for refuse and soil, and a fall for sewage sludge. This was followed by a drop to below 10% for all three after 10 days and then there was a rise. There is no correlation here between the 48°C run (figure 27) and this run. At 48°C there was an initial fall for the refuse and soil followed by an increase for refuse, but a further fall for soil. The sewage sludge rose initially and then dropped slowly.

Based upon the above results it would appear that biodegradation was higher at 48°C and that 56°C was above the optimum temperature for fungal growth. However, the 33%

carbon decrease at 56°C could be termed as fairly reasonable compared with 38% at the optimum temperature of 48°C and might be acceptable in return for the pathogen removal which was effected at this temperature.

5.3.3 The effect of the length of the process on biodegradation

The results are shown in figures 37 and 38. It can be seen that a more stable product was formed after 38 days with regard to the pH. In this run pH values of 7.5 and 7.2 at 56°C and 48°C respectively were obtained. After 17 days at 56°C and 48°C the values were 8.5 and 8.0 respectively. In each case it can be seen that at 48°C the final pH was lower. At 56°C the pH stabilised itself after 24 days (7.3) whereas at 48°C it was still at 8.2.

There was an increase in the organic nitrogen for the 48°C process. This may have been due to both conversion from the inorganic forms of nitrogen and the accompanying loss in carbon which would have given the nitrogen a larger percentage of the dry weight. At 56°C the nitrogen value rose and then returned to just above its original value. This was also true for the carbon and thus sampling error may have been the cause here.

At 48°C the decrease in carbon was 78% after 38 days and at 56°C there was no decrease. Even after 24 days the decrease was 63%. This showed that at 48°C the biodegradation process

FIGURE 37 - EFFECT OF TIME AT 48°C

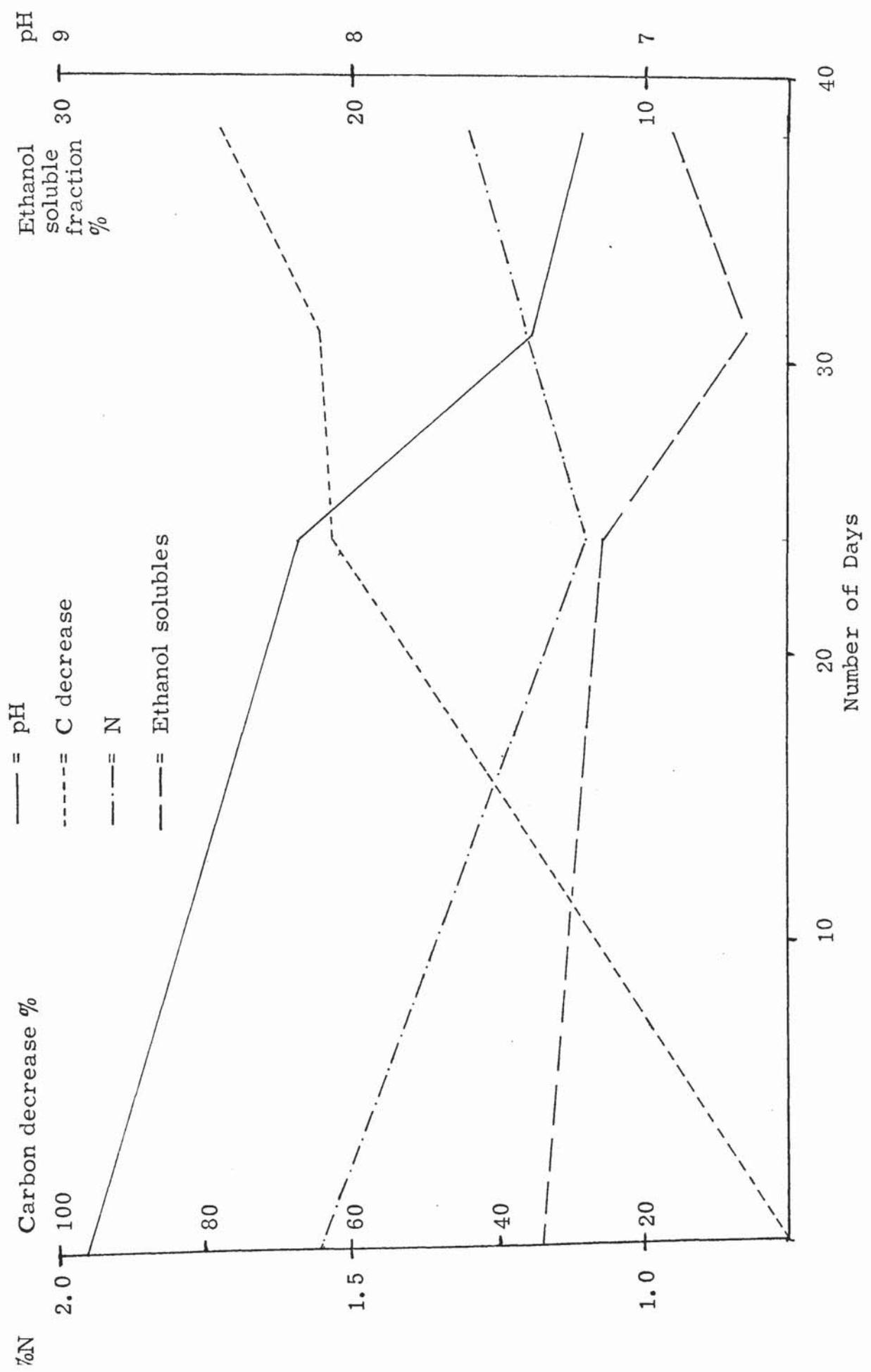
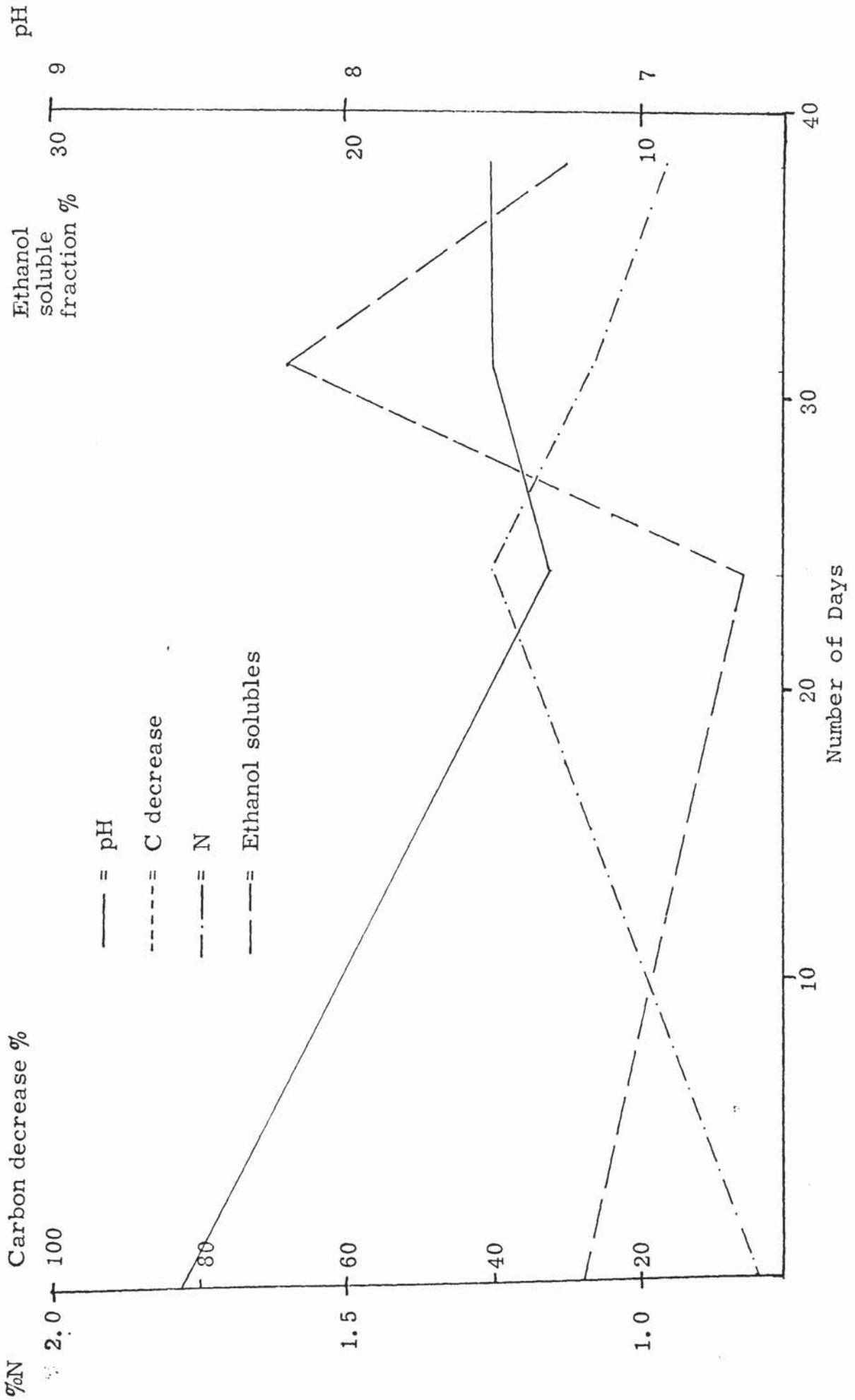


FIGURE 38 - EFFECT OF TIME AT 56°C



was still a linear process even after 38 days. Although the 56°C gave no decreases it was thought that perhaps the samples were faulty and so samples were taken for cellulose analysis (see later). This would determine the amount of cellulose hydrolysed by the organisms present.

From these results it would appear that a process run at 48°C with a soil inoculum gives linear biodegradation based upon carbon decreases. It is thus necessary to decide at which point the process is terminated and this will be determined by the product required. Amino acid analyses in Chapter 7 will determine the nutritional value of the product after 17, 24, 31 and 38 days to see if there is sufficient enhancement for use as a feed additive.

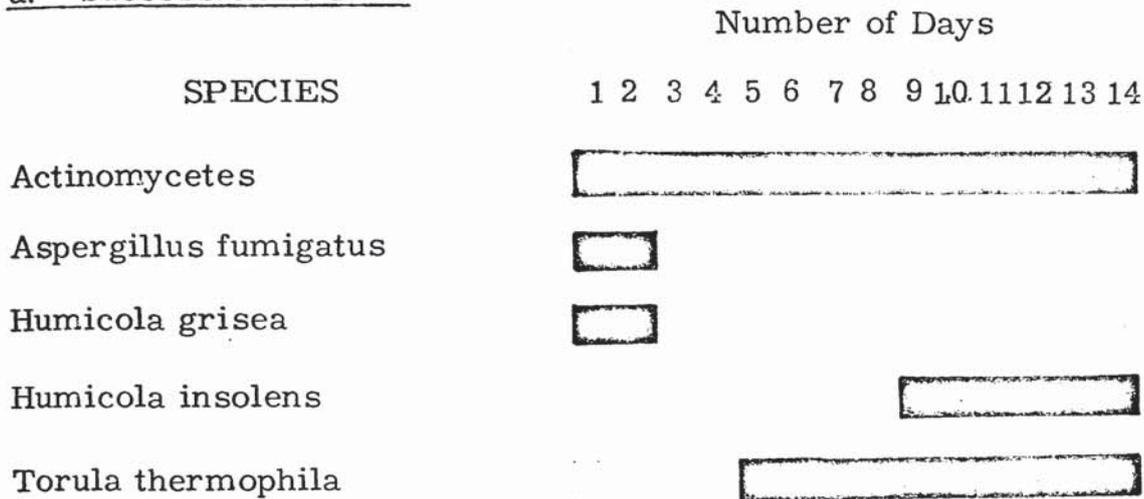
5.3.4 Flora successional patterns

The results from these are shown as histograms in figures 39 and 40. At 40°C actinomycetes, Aspergillus fumigatus and Humicola grisea were isolated initially for the first two days. The latter two fungi were then replaced by Torula thermophila after 4 days and then Humicola insolens after 8 days. Both of these fungi, together with the actinomycetes persisted until the end of the run. These isolations can be correlated with the pH values monitored during the runs (see figure 19). The pH rose from 7.5 to

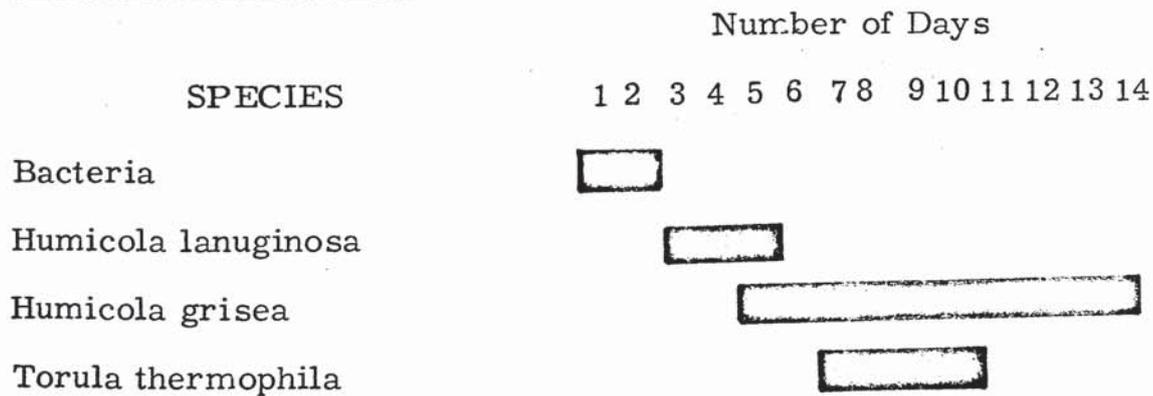
FIGURE 39

TO SHOW THE SUCCESSION OF THERMOPHILIC
MICRO-ORGANISMS AT 40°C and 48°C

a. Succession at 40°C



b. Succession at 50°C



8.2 in the first day favouring actinomycetes and Humicola spp. The occurrence of Aspergillus fumigatus, although unusual at such alkaline pH values (see isolation programme), could perhaps be explained by its dominance over other species initially due to the optimum temperature. However, it did not persist after 2 days and did not recur for the remainder of the run. The appearance of Torula thermophila after 4 days and Humicola insolens after 8 days was probably due to their optimum pH values being reached in the waste. Torula thermophila appeared at pH 7.5 and Mills (1973) has reported this fungus to have a broad optimum for cellulolytic activity with its top limit of 7.8. Humicola insolens appeared when the pH was at about 7.7. Mills (1973) has reported this to produce good strength loss of cellulose paper at pH 7.8. The persistence of the actinomycetes was probably due to the pH remaining well into the alkaline region. After the drop to pH 7.5 in the 4th day it rose to 8 after 12 days and remained there until the end of the experiment. The alkaline pH range in the run was probably the reason why only a selected group of thermophiles grew. Mills (1973) believes that selected species of thermophilic fungi are isolated in town refuse because the pH of the waste is optimum for their activity. These results would appear to agree with this in that only those fungi which were isolated predominantly in the neutral to alkaline pH's (Chapter 2) were found to be actively growing in this waste at alkaline pH's.

At 50°C (figure 39) bacteria were isolated in the first

two days, then Humicola lanuginosa was isolated from the 3rd to the 5th days. Humicola grisea persisted from day 5 until the end of the 14 days and Torula thermophila appeared from the 7th to the 10th day. The isolation of bacteria in the first 2 days was probably due to the increase in pH to 8.5 for 3 days. Then Humicola lanuginosa appeared and the pH fell to 7.8. As for the 40°C Humicola grisea preferred the more alkaline conditions and was first isolated at pH 7.8. The pH remained at 7.8 and Torula thermophila appeared 2 days afterwards. However, it was not isolated after 10 days. Humicola grisea may have suppressed its growth at this pH. As for the 40°C a limited number of fungi were isolated. However, no actinomycetes were recorded as would be expected. Instead, because of the higher pH initially reached, bacteria became dominant until there was a fall below pH 8.0.

The addition of inocula increased the number of species isolated (figure 40) to include Chaetomium thermophile, similar successional patterns being observed in all three runs. In the sewage sludge run bacteria were initially isolated and this may have been due to the past history of the inoculum coupled with the high pH of 9.0 recorded within the first 2 days. Chaetomium thermophile was isolated for the 1st day when the pH rose from 8.5 to 8.7, but did not appear for the next 2 days until the pH came down to 8.4 after 4 days. The actinomycetes were favoured by the high pH and they appeared at pH 8.8, persisted until the pH reached

9.0, and fell to 8.8 again and were not isolated until the 9th day when the pH was at 7.8. They then remained for the rest of the run. Humicola lanuginosa appeared after the bacteria and also after Chaetomium thermophile was first isolated. It may have been acting in its capacity as a secondary sugar utiliser. However, it was not re-isolated after the second appearance of Chaetomium thermophile. Humicola grisea was isolated after 6 days at pH 7.9 and persisted for a further 3 days until the pH reached 7.8. Torula thermophila appeared for 1 day at the same time as Humicola grisea but remained for longer when isolated after the 9th day at pH 7.8. Humicola insolens was isolated after 7 days also at pH 7.8 and persisted until the end of the run when the pH rose to 8.0.

For the refuse inoculum there is a similar pattern except that even though the pH increased to 9.3 no bacteria were isolated and the actinomycetes persisted throughout the run, perhaps because the refuse provided more diverse species able to tolerate the high alkaline conditions. Chaetomium thermophile was isolated for the 1st day when the pH rose from 8.2 to 8.8. It was then probably suppressed by the adverse pH and appeared 2 days later when the pH was again at 8.5, but did not persist. Humicola lanuginosa appeared the second time that Chaetomium thermophile was isolated and only for one day. Humicola grisea appeared on the 6th day at pH 8.2 and was isolated for 3 days. Humicola insolens also appeared at this pH but persisted for the rest of the

run. Torula thermophila was isolated at pH 8.2 for one day only and then reappeared after 10 days until the end of the run when the pH rose from 7.6 to 8.0.

The pH curve for the soil inoculum did not show any increase initially and this is reflected in the isolation pattern. The actinomycetes were isolated for 3 days until the pH dropped to 8.7. They did not reappear until the 10th day as for the sewage sludge inoculum when the pH was 7.7. Chaetomium thermophile appeared after 1 day and then again after the 2nd. This was followed by Torula thermophila which appeared after 3 days (much earlier than in the above two inocula) when the pH was 8.7, and persisted for the length of the run. Humicola insolens was isolated at pH 7.7 after 6 days and Humicola grisea for one day at pH 7.6 in the 8th day.

These patterns have shown that the colonisation of the thermophilic fungi is pH dependant and that the process pH should be controlled to encourage certain desirable fungi and discourage bacteria. The following pattern of colonisation can be stated from these isolations. As the pH rises Chaetomium thermophile will establish itself if present. Above pH 9 the bacteria are dominant and actinomycetes will also be present. As the pH falls Chaetomium thermophile re-colonises the waste and Humicola lanuginosa may appear. The actinomycetes disappear below pH 8.8 - 8.7 only reappearing at about 7.6. This may be due to competition

by the species which become established at pH 8.8. The order in which these appear is: Humicola grisea, then Humicola insolens and finally Torula thermophila although in the soil run Humicola grisea appeared last. These results may be compared with those in Chapter 3 where Chaetomium thermophile, bacteria and actinomycetes were also the first isolated followed by a similar pattern to that above.

5.4 ANALYSIS OF SAMPLES FOR STARCH, HEMICELLULOSES AND CELLULOSE

It has been seen that although carbon decreases showed a differential rate of biodegradation under varying conditions they could not give information as to the breakdown of the components within the waste, and may have given a false picture in regard to bulk reduction which is due to cellulose breakdown in this particular waste. Thus samples used for the ethanol solubles determination were subjected to analyses for starch, hemicelluloses and cellulose. The initial and final samples were used in each case two replicates being done for each sample. The analyses were carried out in the following manner:-

5.4.1 Methods

a. Analysis for starch, dextrans and glycogen

The method used was that described by Weinmann (1947).

The total extract from each ethanol soluble analysis was placed in a 100 ml. conical flask and 10 ml. distilled water were added. The flask contents were titrated with a glass rod to mix the material, and the flasks were placed on a boiling water bath to steam for 30 minutes. Filter funnels were placed in each flask to prevent evaporation. This process was to coagulate the starch. The flasks were then allowed to cool and 10 ml. acetate buffer pH 4.45 were added to each flask. The buffer was made up as follows:- 3 volumes of acetic acid (12.5 ml. glacial acetic acid per litre) were added to 2 volumes of sodium acetate solution (27.2 g. sodium acetate per litre). To 1 litre of the buffer was added 1 g. powdered thymol as a preservative. 10 ml. of 0.5% diastase solution was then added, the flasks shaken and stoppered, and then placed in a 37°C incubator for 44 hours. The material was filtered through, dried and previously weighed, 9 cm. filter papers and the whole then dried in an oven at 80°C overnight. The extract was determined by the difference in weight.

b. Analysis for hemicelluloses

This was done using that method described by Mills (1973) and Chang (1967). The whole of the extract from the starch analysis was placed in a 100 ml. flask and to this were added 5 ml. 24% (w/v) potassium hydroxide. The hemicelluloses were extracted by shaking (on a shaker) the flasks for 4 hours at room temperature. The material was then filtered through

weighed filter papers and washed with IN HCl until acid, followed by water. The filter papers were dried and weighed.

c. Cellulose analysis

This was done by hydrolysis with 72% sulphuric acid (Chang, 1967). The method was speeded by using Canavescini's modification (Canavescini, 1970). 5 ml. 72% H₂SO₄ were added to the whole of the extract from the hemicellulose analysis and the flasks allowed to stand at room temperature for one hour. The acid was then diluted to 4% and autoclaved for 1 hour at 121°C. The acid was then decanted off and the residue filtered through weighed filter papers and washed well with water. The papers were dried and weighed. The residue was regarded as a mixture of lignin and ash.

5.4.2 Results and discussion

The results are shown in Table 11. Temperature did not seem to have much effect on cellulose breakdown (experiments 1, 2 and 3) although, as seen earlier, cellulolytic activity was much greater on cellulose strips at 48°C than at 25°C. The soil inoculum gave the greatest cellulose decrease of 29.7% in experiment 5 whilst refuse gave an increase of 3.6 (see Table 12). This may have been due to either the hemicellulose decrease which was larger than for sewage

TABLE 12

KEY TO TABLE 12

Sample No.

1	=	25°C
2	=	40°C
3	=	48°C
5SL	=	sewage sludge
5R	=	refuse
5S	=	soil
7SL	=	sewage sludge
7R	=	refuse
7S	=	soil
8A	=	10% end product
8B	=	25% end product
8C	=	40% end product
10D24	=	56°C
10D31	=	56°C
10D38	=	56°C
10D24	=	56°C
11D24	=	48°C
11D31	=	48°C
11D38	=	48°C

180 -
TABLE 11

Sample No.	Ethanol Solubles	Starch Etc.	Hemicelluloses	Cellulose	Lignin and Ash
1O	5.1	15.7	43.2	16.6	19.4
1F	9.3	17.3	37.2	15.5	20.7
2O	24.0	14.4	43.5	5.6	12.5
2F	21.4	14.8	35.5	6.6	21.7
3O	24.0	12.9	35.5	11.6	16.0
3F	22.5	15.5	27.8	11.9	22.3
5SLO	18.0	11.3	26.7	10.7	33.3
5SLF	17.6	11.2	22.5	8.8	39.9
5RO	25.0	10.2	31.5	7.8	25.8
5RF	17.9	13.9	26.2	11.6	30.4
5SO	24.0	11.8	20.4	11.1	32.7
5SF	15.0	9.8	19.0	7.8	48.9
7SLO	16.3	8.5	20.8	32.0	22.4
7SLF	14.0	7.5	18.8	35.2	24.5
7RO	14.0	6.4	22.8	36.0	20.8
7RF	17.5	11.7	16.9	35.5	19.4
7SO	13.2	8.8	23.0	35.5	19.3
7SF	14.6	12.5	17.9	34.6	20.4
8AO	12.1	13.0	28.7	36.0	10.2
8AF	7.2	17.3	34.2	30.1	11.2
8BO	8.6	13.1	24.3	29.0	25.0
8BF	6.7	13.5	28.5	23.4	27.9
8CO	9.0	11.6	20.8	24.1	34.5
8CF	5.5	9.5	19.7	21.8	43.5
10DO	12.0	1.5	19.4	27.1	39.9
10D24	6.2	12.4	23.6	30.2	27.6
10D31	22.1	21.0	3.0	23.6	30.1
10D38	12.7	1.3	17.9	33.6	34.5
11DO	13.4	5.4	-	35.8	45.4
11D24	11.3	3.9	9.7	18.8	56.3
11D31	6.5	6.0	11.0	20.8	55.7
11D38	9.1	5.7	19.3	13.3	52.6

ANALYSES OF SAMPLES FROM PROCESS RUNS

sludge and soil or to the non-homogeneity of the material. At 56°C the cellulolytic activity in the soil inoculum, although still the highest of the 3 inocula was cut to a 2.4% decrease. There was very little activity in the sewage sludge where the cellulose content increased by 10% relative to the dry weight of the material. The refuse inocula was favoured at 56°C where there was a cellulose decrease of 1.5%. This is in agreement with the carbon decreases which were greater at 56°C than at 48°C for the refuse inoculum.

The effect of end-product re-cycling with no extra soil added was to cause the greatest cellulose breakdown of 19.3% for the 25% addition. 10% end-product addition also gave good breakdown (16.4%) but neither of the results improved upon the soil addition. However, it might be improved if soil were added as well as some of the product. The 40% addition was not very satisfactory in comparison to the other two results and was considered to be impractical for a large-scale operation where it might involve difficult mixing and handling problems and would also take up a large amount of space in the process which could be used for fresh waste, thus decreasing the efficiency of the operation.

The cellulose reductions for experiments 10 and 11 show that 48°C favours greater breakdown of cellulose. The negative decreases in cellulose breakdown at 56°C support the carbon increases obtained at this temperature and it thus seems likely that 56°C may be too high an optimum for the

EXPLANATION OF TABLE

1. All figures are expressed as percentages of the original sample dry weight.

2. The sample notation is as follows:
 - a. Numbers 1, 2, 3, 5, 7, 8, 10 and 11 refer to the following experiments:-
 - 1 = run at 25°C
 - 2 = run at 40°C
 - 3 = run at 48°C
 - 5 = inocula additions at 48°C
 - 7 = inocula additions at 56°C
 - 8 = end product additions
 - 10 = effect of length on process at 56°C
 - 11 = effect of length on process at 48°C

 - b. O and F refer to initial and final samples respectively.

 - c. SL = sewage sludge inoculum
R = refuse inoculum
S = soil inoculum
A = 10% re-cycled end-product added to raw waste
B = 25% re-cycled end-product added to raw waste
C = 40% re-cycled end-product added to raw waste

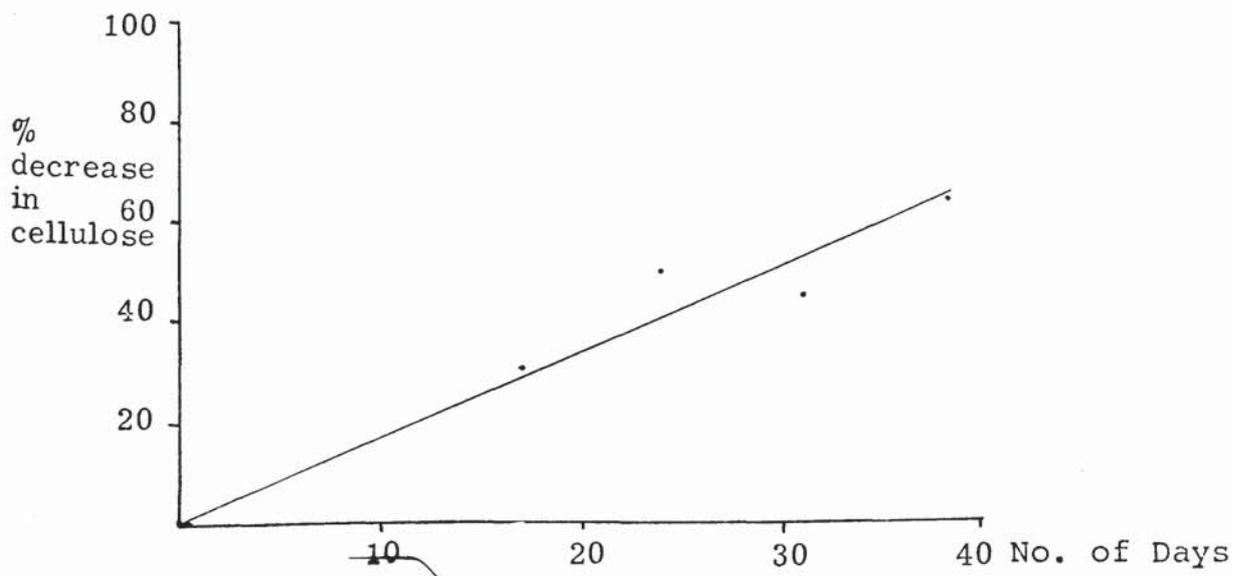
 - d. DO, 24, 31 and 38 refer to the number of days incubation for experiments 10 and 11.

TABLE 12

TO SHOW DECREASE IN CELLULOSE OF
SAMPLES FROM FERMENTERS

Sample No.	Decrease in cellulose %
1	6.6
2	+ 1.8
3	+ 2.6
5SL	1.8
5R	+ 3.6
5S	29.7
7SL	+10.0
7R	1.5
7S	2.4
8A	16.4
8B	19.3
8C	9.6
10D24	+11.0
10D31	11.1
10D38	+12.4
11D24	47.6
11D31	41.8
11D38	62.9

FIGURE 41
TO SHOW LINEAR RELATIONSHIP BETWEEN CELLULOSE
DECREASE AND TIME AT 48°C WITH SOIL INOCULUM



thermophilic flora which is active in breaking down the cellulose constituent of the waste.

A graph (figure 41) shows the linear relationship between the percentage cellulose decrease and time at 48°C with a soil inoculum. It can be seen from this that between 17 and 38 days the extent of cellulose breakdown is directly proportional to time. Below 17 days although the straight line has been extrapolated to zero it is unlikely that it will be straight in the first 2 or 3 days, Chang (1967) has found that the greatest activity is in the first 5 days. However, there is probably a short lag period. Thus, from this graph the extent of cellulose breakdown can be determined for any period between 17 and 38 days and that the extent of the breakdown depends upon the length of the process; Chang (1967) found that the greatest cellulose breakdown occurred in the first 34 days for straw. It now remains to relate this to the nutritional analysis to see whether an optimum length can be obtained within the limits stated above.

A scan through the ethanol solubles column shows a trend towards a decrease in the fraction at the end of the process, showing that the soluble sugars were utilised fairly efficiently by specific groups of micro-organisms. The starch fraction on the other hand sometimes increased but this could not be correlated with increases or decreases in the other fractions. The only explanation may be that given by Chang (1967) who states that the starch may be converted to glycogen,

a fungal reserve product. Where this happened there may have been a relative increase due to non-utilisation and where there was no storage the starch fraction decreased, being utilised as an energy source. The hemicelluloses were utilised, in many of the samples, to a greater extent than cellulose. This may have been due to their easier availability for breakdown and the relative ease with which they could be hydrolysed.

The results from the analysis for cellulose have shown to be mostly in agreement with the carbon reduction and pH results presented previously. They have shown that a soil inoculum, a temperature of 48°C, end-product re-cycling and the length of the process all enhance the cellulose biodegradation. The analysis has also shown that 56°C, inocula such as sewage sludge, refuse and 40% of the product do not produce in relative terms a highly biodegraded and stable product. It is not possible to show on paper the aesthetic quality of some of the successful products, but they may be described as odourless, friable and easily handled after grinding to a powder (see Plate 6).

A review of the results from the above investigations in this and the preceding chapter can now cast light on the parameters for a thermophilic process.

The optimum temperature would appear to be 48°C because of the greater biodegradation, although at 56°C pathogen

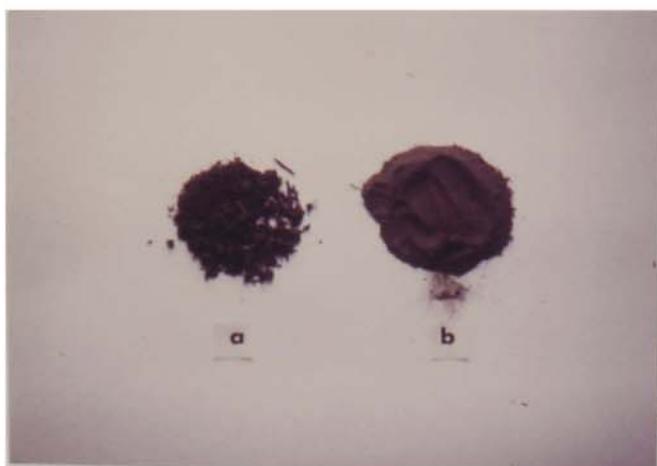


PLATE 6

DRIED PRODUCT FROM EXPERIMENT 5S AFTER
17 DAYS

- a. before grinding
- b. after grinding

removal is more complete. However, it might be more efficient if sterilisation was done after the process and incorporated with drying to make sure that the procedure was complete. Flash driers and other high temperature driers could be used to both dry and sterilise. However, this might destroy the protein, and thus the use of microwaves (Anon, 1971) and air drying might be better. The acid pH ranges would seem to be preferable from an odour and nitrogen loss point of view. Also the thermophilic fungi prefer neutral to acid pH value for optimum activity. High alkaline pH's produce ammonia and encourage undesirable bacteria and have been found to be toxic to some species of fungi (Cochrane, 1963; Leal, Lilly and Gallegly, 1970). It was found that aerobic conditions were necessary, and that odours could be easily controlled (with the exception of ammonia) under such conditions. Low rates were necessary but exact information as to optimum rates was not obtained due to the insufficient monitoring equipment and inaccurate pumps. It is thought, though, that only low rates are necessary. This has been borne out by Schulze (1962) and Bell (1969) who have used low aeration rates in their experiments. It is likely that passive air movement through the waste caused by hot air upward movement may be sufficient. Of course the loading of waste into the process is important to avoid anaerobic pockets but the nature of the straw bedding lends itself to large spaces being formed within the waste. The use of inocula did enhance the process to varying degrees and this result does not agree with Golueke, Card and McGauhey (1954) who found no enhancement. However, their

analyses were not as complete as these and did not include a direct measurement of cellulose. Soil and a re-cycling of 25% of the end-product gave the best biodegradation in regard to the production of an innocuous material with a much decreased bulk. However, the inocula did introduce more resistant pathogens, but it is envisaged that pathogen removal could be done by high temperature drying. Of the 3 inocula used sewage sludge was the least useful producing a product which was unstable, sour and had a high pathogen count. The refuse contained non-biodegradable plastics and would produce an aesthetically unpleasing product apart from its low standard of performance as an inoculum. The use of soil does not involve transportation cost and there is no short supply of it in the country. The soil could be roughly sieved and sprinkled in with the waste as it is placed in the large-scale process.

At 48°C the extent of biodegradation appeared to be dependant upon time and there was a linear relationship meaning that economic factors could be more easily calculated and that the quality of the product determined the process length.

The flora present was limited and seemed to follow a pattern which was pH dependant. In general it was found that at pH 9 and above bacteria were dominant and that not until the pH reached below 8.0 did the thermophilic fungi establish themselves. The pH range within each run also limited the

species present, although with the exception of Humicola lanuginosa all the species were highly cellulolytic. Aspergillus fumigatus was only isolated briefly at 40°C and Mucor pusillus was not isolated in any of the runs investigated.

It is thus likely that any large-scale thermophilic process utilising thermophilic fungi as the dominant agents of biodegradation would incorporate the following parameters:-

1. Optimum temperature 48 - 50°C
2. pH controlled between 5.5 and 7.5
3. Addition of soil and recycling of a proportion of the end product as an inoculum
4. Low but sufficient aeration rate
5. Process length to be between 17 and 40 days

It is now necessary to discuss the effect of the slurry on the growth of thermophiles and to determine the nutritional value of the product. These will be discussed in the next two chapters.

CHAPTER 6

CHAPTER 6

THE GROWTH OF THERMOPHILIC FUNGI ON PIG SLURRY

- 6.1 Introduction

- 6.2 Respirometric measurements
 - 6.2.1 Materials and methods
 - 6.2.2 Results and discussion

- 6.3 The growth of thermophilic fungi on pig slurry in pure culture
 - 6.3.1 Materials and methods
 - 6.3.2 Results and discussion
 - a. Effects of pH
 - b. Effect of carbon:nitrogen ratio

6.1 INTRODUCTION

The growth and utilisation of the solid fraction of the waste has been studied so far and an indication of the extent of biodegradation of the waste under varying conditions has been given. However, even though slurry was used in an ad-mixture it has been impossible to determine how the thermophiles are affected by its presence and whether they are able to utilise it. The biodegradation of the cellulose fraction of the slurry may be included in the analysis for cellulose in the solid waste (Chapter 5). It remains to investigate the effects of the liquid fraction on the growth of the thermophiles. A convenient and accurate method of measuring growth is by using the Warburg constant volume respirometer to measure the respiration rate of the organisms under different conditions. The apparatus can be maintained at a constant temperature using a water bath and the measurement of oxygen uptake can be read direct from a manometer. The Warburg respirometer has been used to study the metabolism of cells and how they are affected by metabolic inhibitors or activators (Umbreit, Burris and Stauffer, 1951) and in this respect can be used to test nutrient sources. Lee and Oswald (1954) have applied the technique to the measurement of biochemical oxygen demand (BOD), comparing it with the dilution method. They found that the latter method was cheaper and more samples could be handled at any one time. However, for information regarding the intermediate stages such as lags, first and second stage

oxidation, and nitrification the Warburg respirometer could be of great value. An extension to the measurement of BOD has been investigated by Schulze and Hoogerhyde (1967) who have attempted to characterise waste waters according to their biodegradability in terms of the oxygen uptake by the micro-organisms. In this way specific respiration rates can be determined for each type of waste water and this may be related to the extent of biodegradation. They used a Warburg respirometer and a differential manometer which operates at constant pressure and temperature and measures a change in volume. The differential manometer gave similar results to the Warburg apparatus and was a more simple apparatus. Thus the Warburg respirometer may be used to measure the respiration rates of the organisms found in waste waters. It may be possible, therefore, to test the effects of pH, temperature and inocula additions to the liquid fraction of the slurry under controlled and defined conditions. In this way predictions can be made as to whether the slurry has any adverse or promoting effects on the biodegradation process.

6.2 RESPIROMETRIC MEASUREMENTS

6.2.1. Materials and methods

The slurry used was allowed to settle to remove the fibrous material. A description of the Warburg respirometry apparatus used and the technique has been given by Gareth

Morris (1968) and his method has been followed in these experiments. Before the experiments were started a flask was assigned to each manometer and the manometer constant was calculated according to the equation below:-

$$K = \frac{V_g \frac{273}{T} + V_f \alpha}{P_o}$$

where V_g = volume of available gas in μl
 T = experimental temperature in degrees absolute
 V_f = volume of fluid in manometer flask in μl
 α = absorption coefficient of the exchanged gas
 in the liquid contents of the manometer flask
 at temperature T
 P_o = normal atmospheric pressure, expressed not in
 mm mercury but in mm of manometer fluid
 (usually = 10,000 mm manometer fluid)

It can be seen that the manometer constant will change with temperature and thus was calculated for each temperature investigated. This constant is used in the calculation of the volume of gas absorbed according to the equation:-

$$V = hK$$

where V = volume of gas exchanged in μl at STP
 h = distance in mm by which the meniscus moves
 in the left-hand column of the manometer
 K = manometer constant

A correction factor must also be applied to h before calculating V . This factor is supplied by the thermobarometer which is, in effect, a blank and takes into account the changes in atmospheric pressure. Thus, any value for h on the thermobarometer must be added on or subtracted from the values of h for the samples and the measurement of the thermobarometer be done at the same time as the samples.

To prevent any contamination of material which might affect the respiration rate for each experiment the flasks were washed in chromic acid overnight and rinsed with distilled water. When necessary the flasks were sterilised at 15 lb/sq. in. for 15 minutes. The liquid used for the manometer tubes was Brodie's solution and had a low oxygen absorption coefficient. Its composition was as follows:-
23 g. sodium chloride made up to 500 ml. with distilled water and crystal violet added as a colouring agent.

The respirometer used in these investigations was made by Braun with a circular arrangement of 14 manometers the flasks of which were immersed in a thermostatically controlled water bath accurate to $\pm 0.5^{\circ}\text{C}$. The flasks were agitated at a rate of 120 revolutions per minute.

It was decided to test the effects of pH and temperature on the unsterilised slurry for the first investigation. The oxygen uptake was measured and thus it was necessary to

prepare a 20% (w/v) solution of potassium hydroxide 0.5 ml. of which was pipetted into the centre well of each flask which contained a filter paper wick cut so that it did not protrude above the well. The wick was used to increase the surface area of the KOH and so increase the absorption of carbon dioxide, the liberation of which would have affected the results. If the wick had protruded above the well there might have been the danger of seepage into the sample which would change the pH. 2.5 ml. of the buffered slurry (using McIlvaines' citrate/phosphate buffer) were placed in the flask, duplicate samples being set up. The thermobarometer in each case contained 2.5 ml. water and 0.5 ml. KOH. The slurry was buffered to pH 2, 4, 7, 8 and 10 at 30°C (pH 7.0 was the original pH of the material) and pH 2, 4, 6, 7, 7.5, 8 and 10 at 48°C (pH 7.5 was the original pH here). The results are graphically shown in figures 42 to 45.

In order to test the effects of inocula a 10% (w/v) mixture of slurry and soil was prepared. Two mixtures were set up, one containing sterilised slurry (10 lb/sq. in. for 20 minutes) and the other unsterilised slurry. The pH was buffered to pH 7.5, the optimum pH determined in the first experiments (see results). For comparison unsterilised slurry alone was used and the endogenous soil respiration was monitored. The experiments were conducted at 30°C and 48°C and the results are presented in figures 42 to 45. In an extension to the above experiment an attempt was made to test the growth of pure cultures of thermophiles on sterilised

slurry. However, after several attempts to obtain growth this method had to be abandoned in preference to the method described later in this Chapter.

6.2.2 Results and discussion

The highest oxygen uptake at 30°C was greater than at 48°C (900 µl. and 400 µl. respectively after 10 hours) (Figs. 42 & 43). However, at pH 2 and 4 the rates were very similar. In both cases the natural pH of each slurry had the highest oxygen uptake and the alkaline pH values had greater respiration rates than the acid ranges. This may be indicative of two things. Firstly, the microflora at these pH's was mostly bacterial which had a higher respiration rate than the fungi which grew in the acid regions. The different rates need not necessarily indicate less activity at pH 6, 4, and 2 as it is known that fungi are able to tolerate and remain active at low oxygen tensions. Secondly, it has been shown earlier and by other authors (Mills, 1973; Malik, 1970) that fungi are active at pH 7 - 8. Thus, the optimum respiration rates at these pH's may have been due to both the adaption of the microflora and the synergistic effects of bacteria and fungi. At 48°C the slurry buffered at pH 10 had a lag of one hour which may have indicated that at this temperature the bacteria able to grow at this pH were greatly reduced and needed to adapt themselves. These experiments have shown that although the respiration rate is

FIGURE 42

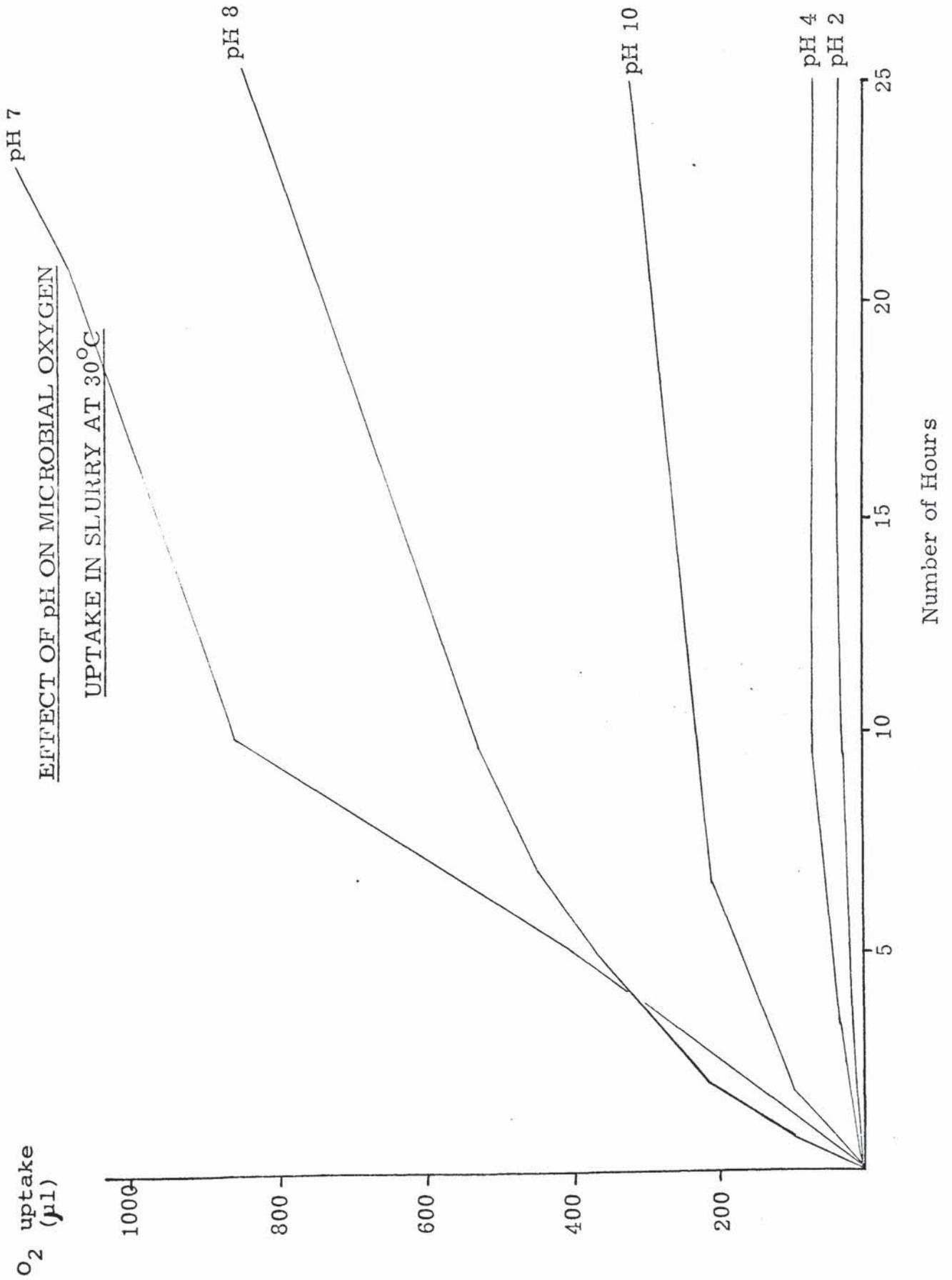


FIGURE 43

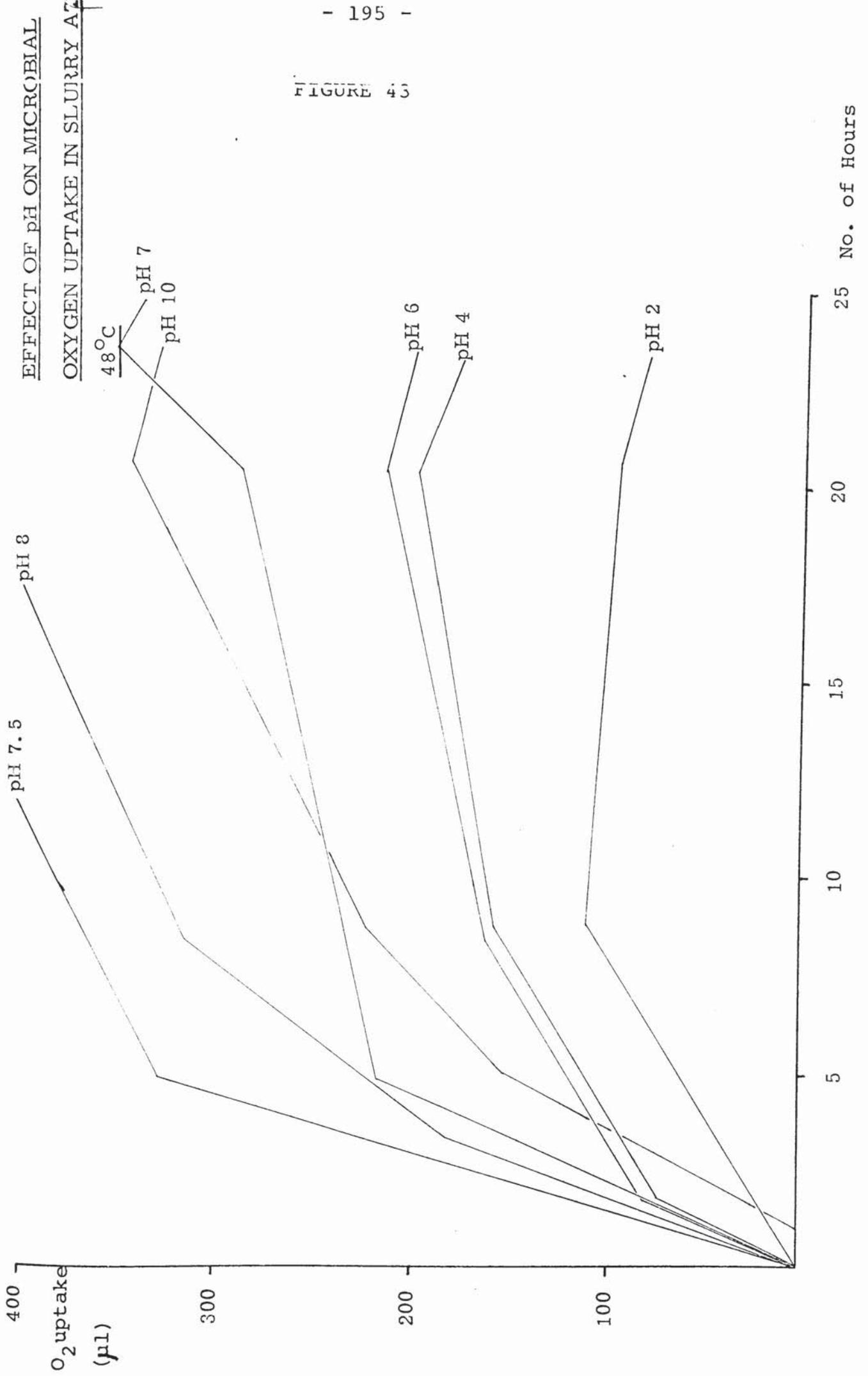


FIGURE 44

EFFECT OF SOIL ADDITIONS ON OXYGEN UPTAKE AT 30°C

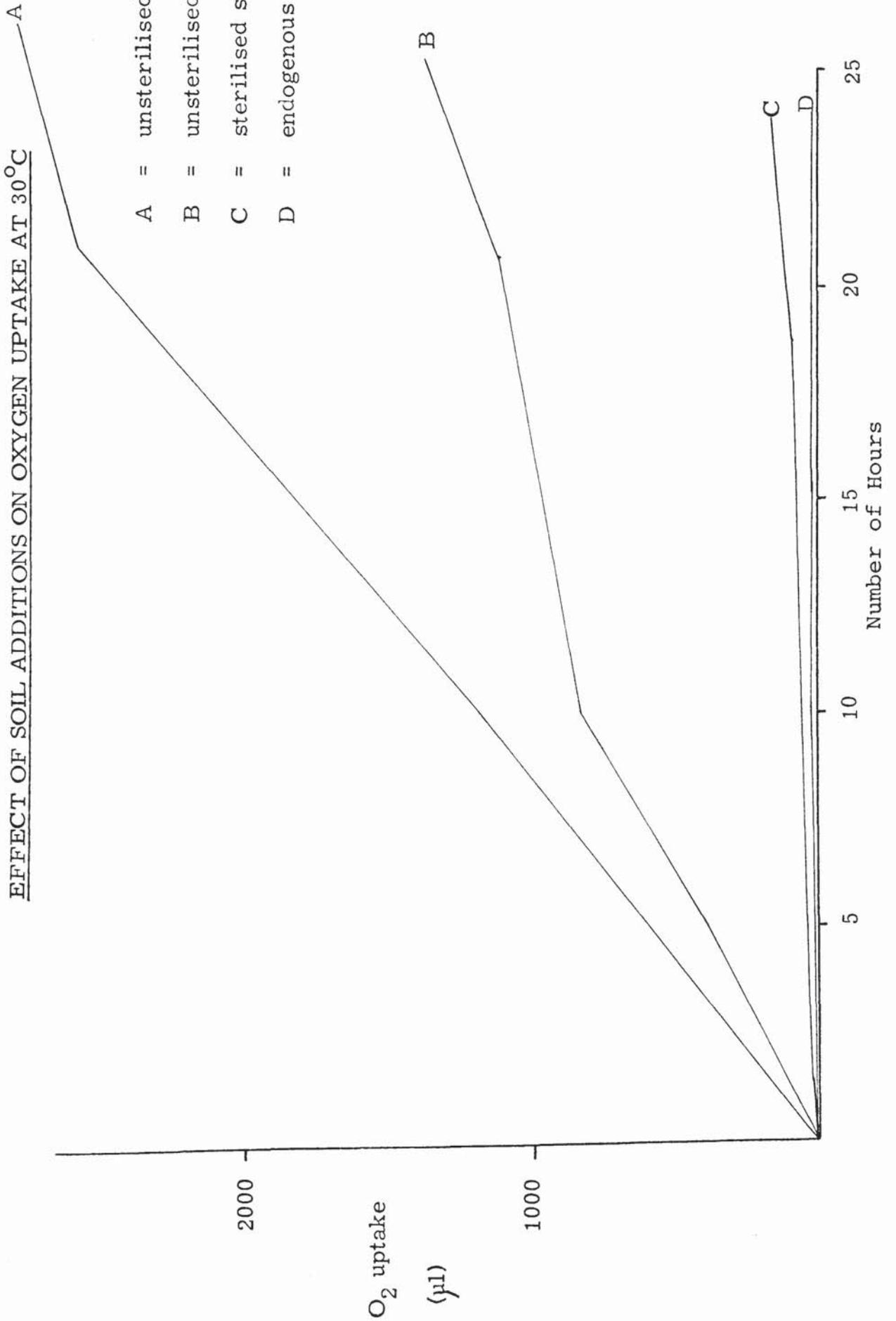
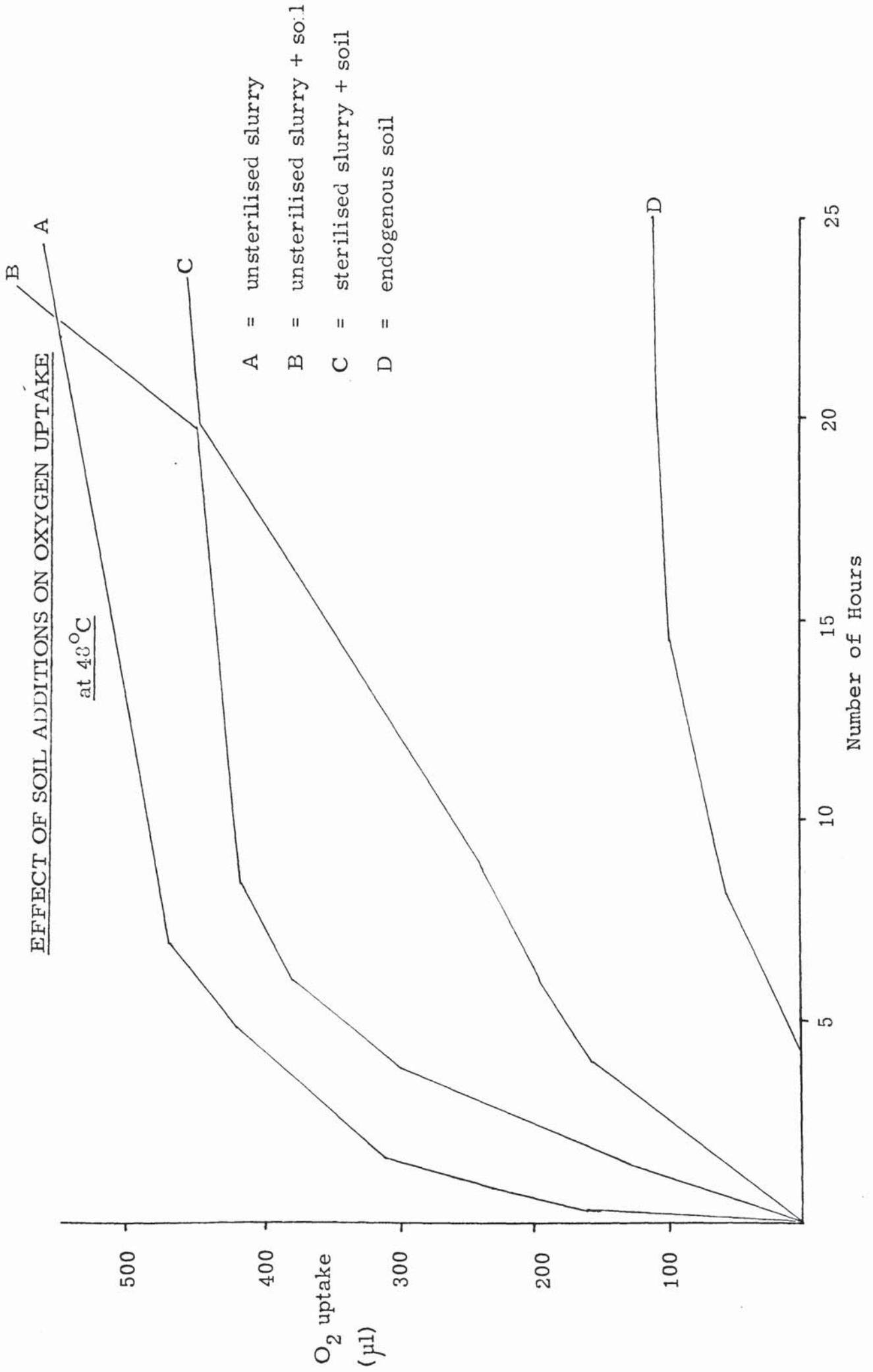


FIGURE 45



reduced at 48°C there is still activity and that, where fungi predominate (pH 4 and 6), the temperature does not appear to affect the respiration rate. At pH 2 activity is very low possibly due to chemical oxidation.

The effects of soil inocula (figures 44 and 45) again showed a greater respiration rate at 30°C than at 48°C due probably to the higher viable flora present. The effect of the unsterilised slurry and soil at both temperatures was to initially depress the respiration over the unsterilised slurry in the first 20 hours. After this, however, at 48°C the rate increased above the unsterilised slurry and at 30°C there were the beginnings of a similar trend although not so pronounced. This may have been due to antagonisms and the time taken for the soil micro-organisms to adapt themselves. The antagonistic effects may have been caused by antibiotic production from the actinomycetes, and toxins produced by bacteria and fungi. It can be seen that the organisms in the slurry do have a part to play in the respiration rate as the sterilised slurry and soil gave a lower rate at 30°C but a surprisingly higher initial rate at 48°C. However, this tailed off after 7 hours and became the same as the endogenous rate for soil at a higher oxygen uptake level. The effect of sterilised slurry on the endogenous soil rate shows that it provided utilisable substrates for the micro-organisms present and was thus biodegraded.

Mills (1973) used a soil inoculum for determining at

thermophilic temperatures the biodegradation of plasticisers. However, for pure culture work to see if selected thermophilic fungi would utilise the same plasticisers he used agar plates containing the plasticiser as the sole carbon source. In order to test pure cultures for their growth on pig slurry it was decided to use a pig slurry agar which will be described below. Pure culture work will also be attempted using a Warburg respirometer but the results of Mills' work seem to indicate that there may have been problems of contamination or growth using the respirometer. Agar plates can also provide information on the morphology and type of growth which the pig slurry as a substrate encourages.

6.3 THE GROWTH OF THERMOPHILIC FUNGI ON PIG SLURRY IN PURE CULTURE

Respirometry was able to show the utilisation of pig slurry with a mixed flora at varying temperatures and pH values. However, as the thermophilic fungi have been postulated as the main agents of biodegradation, and conditions created to favour their growth, it is necessary to determine their selected growth on pig slurry. For pig slurry it was found that respirometry could not be used to maintain sterile conditions with pure cultures. An agar containing pig slurry and adjusted to varying pH values and carbon to nitrogen ratios was prepared to test the growth of the thermophiles on such a medium. This would give more

specific information as to those fungi which favoured the substrate and the optimum pH ranges. The results could also be compared to those from the solid waste isolations to see whether similar trends were present.

6.3.1 Methods and materials

The growth of the thermophilic fungi isolated from the bedding and slurry was determined at a range of pH values and carbon to nitrogen ratios. The basic agar was prepared by steam sterilising the raw slurry and ball-milling it for 3 days to break up the fibrous material. The slurry was then made into an agar using 1.5% Oxoid agar No. 3 and autoclaved at 10 lb/sq. in. for 20 minutes. Plates were poured and plugs (0.8 cm diameter) of 10 day old cultures were inoculated onto the surface. Five replicates were set up for each variable and colony diameters were measured as an indicator of growth (Brancato and Golding, 1953). The following pH values were investigated 4.0, 5.6, 7.0, 8.0 and 9.0 (values after autoclaving) using McIlvaines' phosphate/citrate buffer and IN HCl or NaOH. By adding 3.3, 6.6 and 9.9 g. urea it was possible to adjust the C:N from 20:1 to 10:1, 7.5:1 and 5:1 respectively. This second investigation was carried out at pH 5.6 as the pH results showed this to be the optimum value for growth.

6.3.2 Results and discussion

a. Effects of pH

The results are shown in figure 46. The optimum pH for all the fungi was 5.6 and there was no recorded growth at pH 4.0. Torula thermophila, Chaetomium thermophile, Humicola grisea and Humicola insolens had high growth rates at this pH after initial lag periods of one day in which adaption to the new substrate was probably taking place. Humicola lanuginosa and Malbranchea pulchella were less active but still grew well producing a dense fluffy mycelium and yellow conidia respectively. Mucor pusillus had an initial high growth rate at pH 5.6 equal to Torula thermophila for 2 days but this levelled off into a stationary phase for the remainder of the investigation. At pH 7, 8 and 9 growth was less, notably so in Mucor pusillus where there was little growth at pH 8 and none at 7 and 9. Humicola insolens had lag periods of 2 and 5 days respectively for pH 7 and 9; there was no growth at pH 8 in this case. Humicola lanuginosa and Torula thermophila had the best growth in the alkaline ranges. This observation may be substantiated by the isolation programme which found these fungi to be prevalent at between pH 7 and 9. Chaetomium thermophile and Humicola grisea grew well at pH 7 and 8, Humicola grisea having a higher growth rate at pH 8 than Chaetomium thermophile.

It should be noted that to state from these results

FIGURE 46

GROWTH OF THERMOPHILIC FUNGI

ON BUFFERED PIG SLURRY

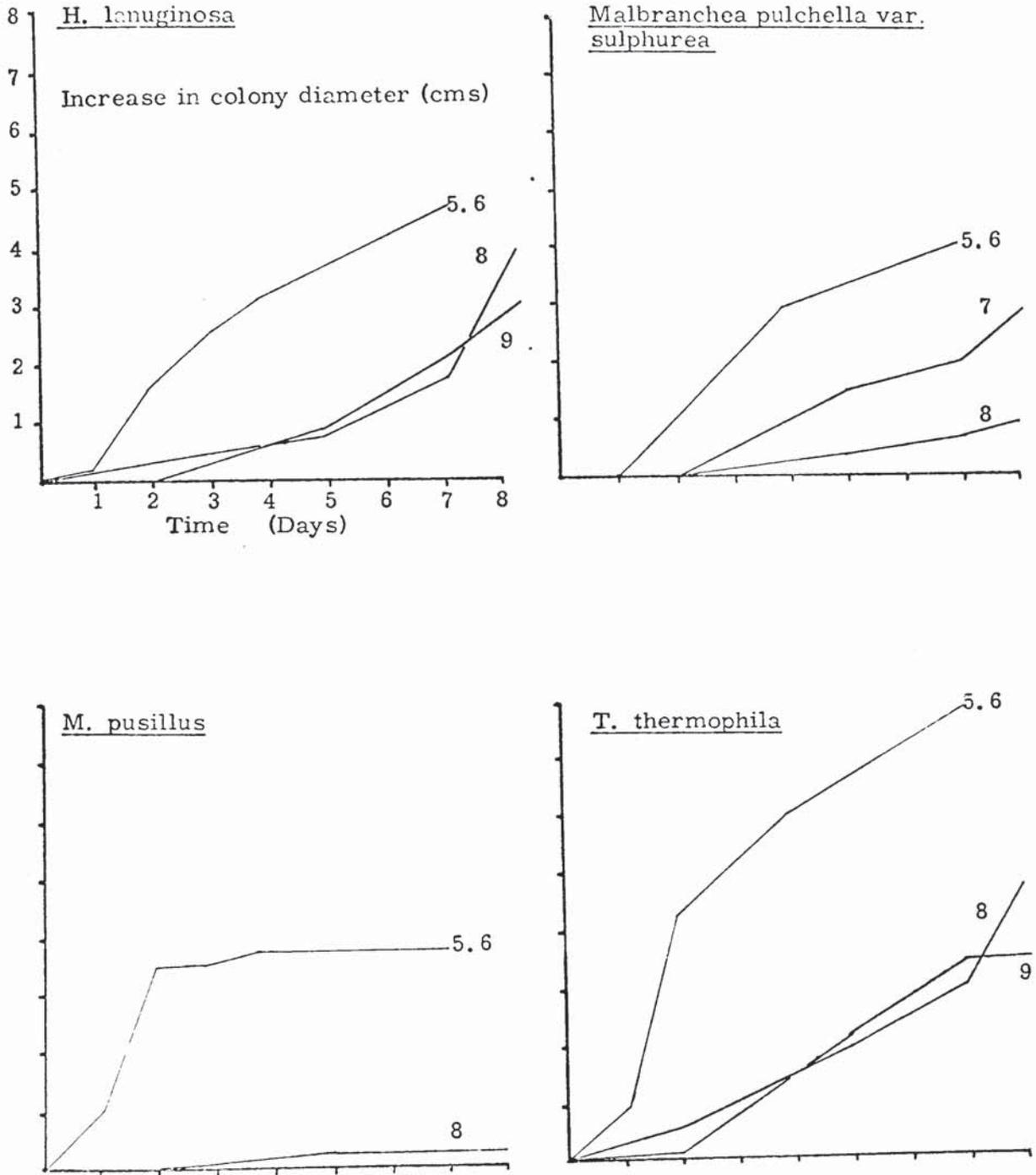
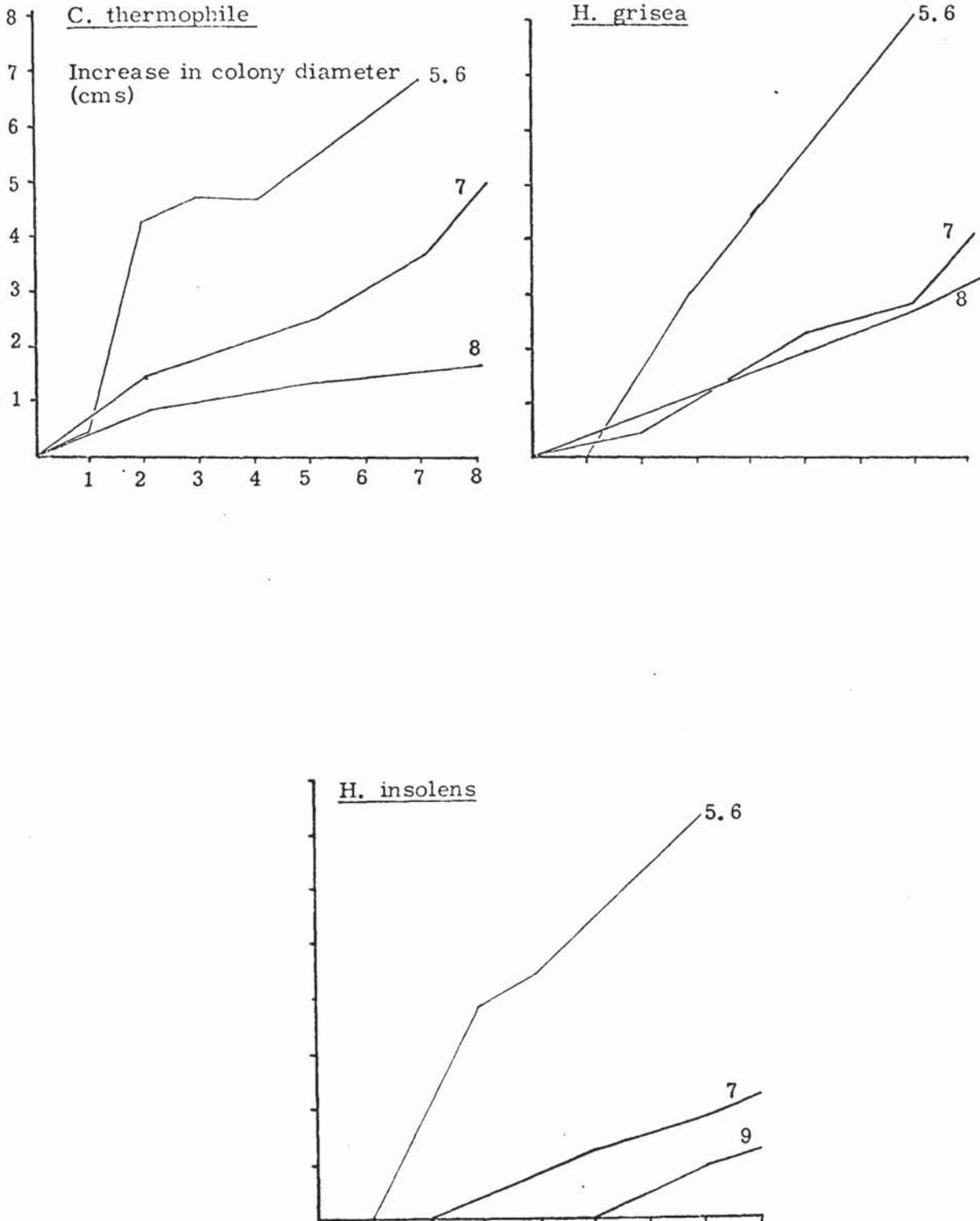


FIGURE 46 (continued)

GROWTH OF THERMOPHILIC FUNGI

ON BUFFERED PIG SLURRY



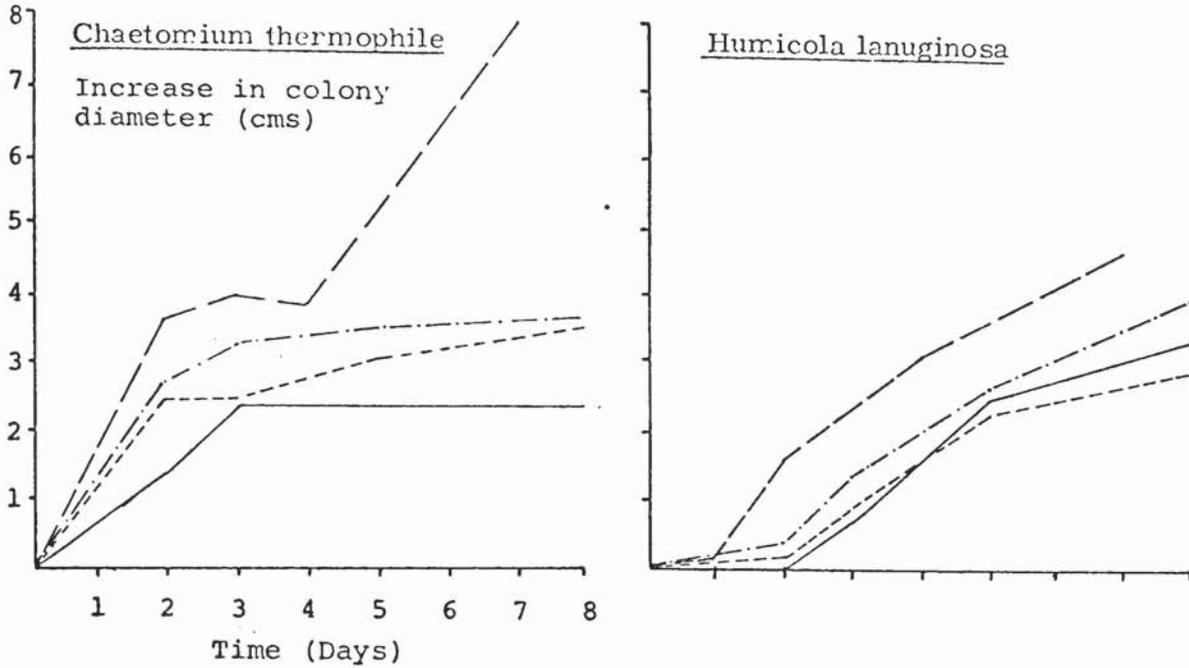
that, for instance, Torula thermophila has a higher growth rate than Malbranchea pulchella, would be premature. Before such a statement could be made every parameter would have to be investigated including the inherent growth rates. However, it is possible from this investigation to say which fungi are more suited to growth on pig slurry agar and at which pH they are favoured. In this way comparisons can be made with work done on other substrates, either natural or artificial, to determine similarities and differences in growth parameters. Thus a more complete picture may be obtained as to the usefulness of the thermophilic fungi in biodegradation processes and the control of those processes.

b. Effect of carbon:nitrogen ratio

The results of this investigation appear in figure 47 and plate 7 shows the growth of selected fungi on the agar. The general effect of lowering the C:N ratio was to depress the growth rate. The 3 C:N ratios investigated are shown compared with the results from the last investigation at pH 5.6. It can also be seen that for all the fungi, with the exception of Humicola lanuginosa and Aspergillus fumigatus, the lower the C:N ratio the lower was the growth rate. Both varieties of Chaetomium thermophile grew very slowly at 10:1 and 7.5:1 after 3 days whilst at 5:1 growth after 3 days ceased, and a stationary phase took over. A similar observation was noted for Humicola grisea but here the C:N ratios of 10:1 and 7.5:1 continued at a higher rate than

FIGURE 47

EFFECT OF C:N RATIO ON GROWTH OF
THERMOPHILIC FUNGI ON PIG SLURRY



see continuation for key

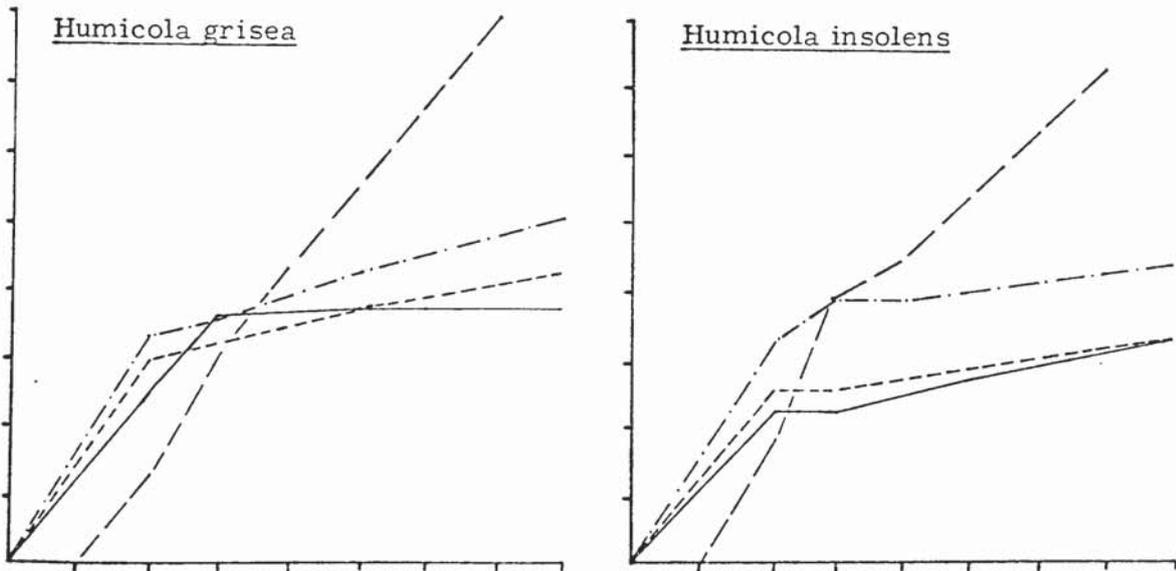
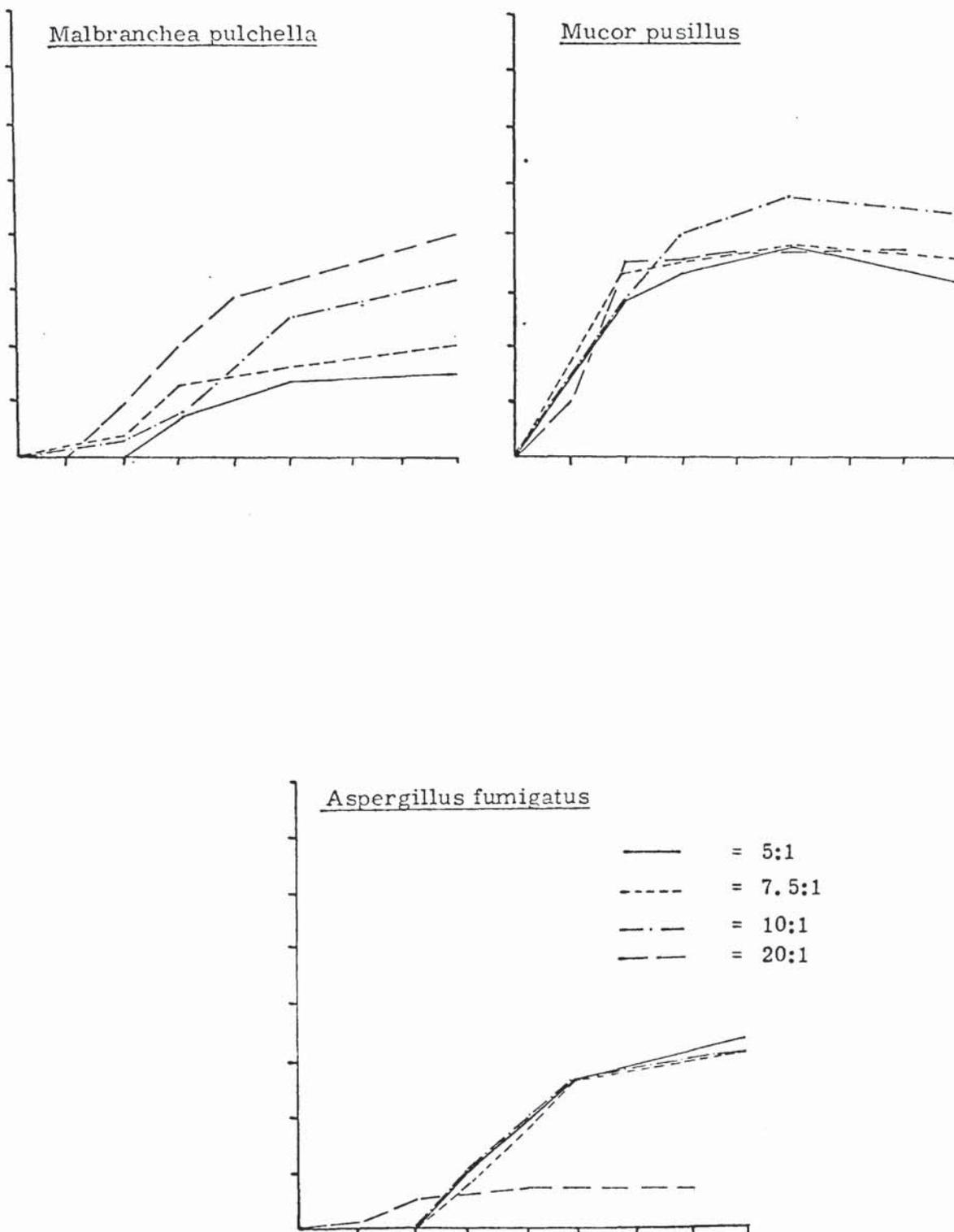


FIGURE 47 (continued)



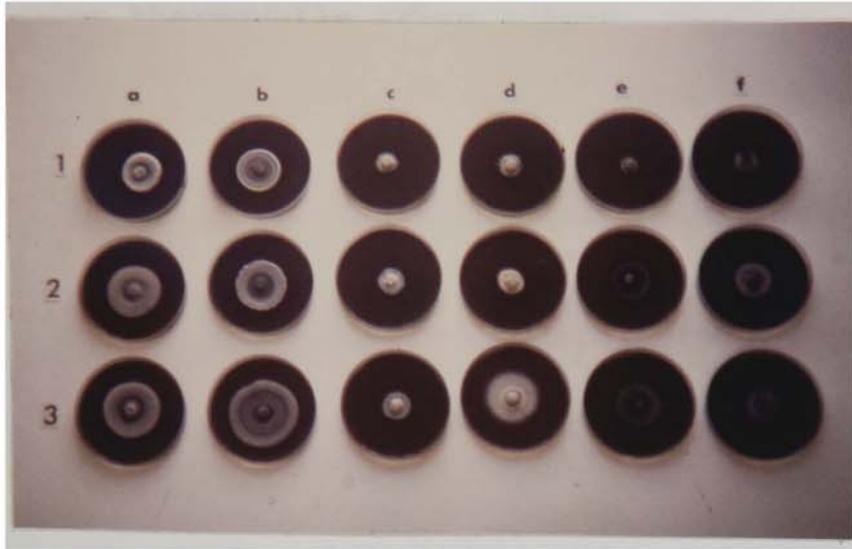


PLATE 7

GROWTH OF THERMOPHILIC FUNGI ON PIG SLURRY AGAR
 AT DIFFERENT C:N RATIOS AFTER 8 DAY INCUBATION
 AT 48°C

1	=	5:1)	
)	
2	=	7.5:1)	C:N ratio
)	
3	=	10:1)	

a	=	<i>Aspergillus fumigatus</i>
b	=	<i>Humicola lanuginosa</i>
c	=	<i>Sporotrichum thermophile</i>
d	=	<i>Malbranchea pulchella</i> var. <i>sulphurea</i>
e	=	<i>Chaetomium thermophila</i> var. <i>coprophile</i>
f	=	<i>Chaetomium thermophila</i> var. <i>dissitum</i>

Chaetomium thermophile after 3 days. Malbranchea pulchella, although having an initial growth rate which was higher at C:N ratio of 20:1 in the first 4 days then decreased to a similar rate to the other ratios. This showed that in this case the first 3 to 4 days were important for differentiating the growth rates. Mucor pusillus and Aspergillus fumigatus, which have already been described as undesirable because of their pathogenicity, were found to have higher growth rates at the three lowest C:N ratios. This was most pronounced for Aspergillus fumigatus where at 20:1 the growth rate became stationary after 4 days. Mucor pusillus, although less pronounced in its differences, showed a death phase or a decrease in the growth rate after 4 days, presumably due to autolysis.

The respirometric and growth experiments have shown that the thermophiles, and in particular the thermophilic fungi, are able to colonise and grow on pig slurry under aerobic conditions. The optimum pH for the growth of mixed cultures in the respirometry investigations was found to be between 7.0 and 7.5 and in pure culture work 5.6 to 6.6. The difference in optima was probably due to the influence of bacteria which tended to favour conditions above neutrality and were very active in mixed cultures. As found in the last Chapter Humicola lanuginosa, Humicola grisea, Torula thermophila Chaetomium thermophile and, to a lesser extent, Humicola insolens were able to tolerate alkaline pH values between 7 and 9.

The addition of extra nitrogen to the slurry depressed the growth rate either initially or after several days. It was found that the natural C:N ratio of the slurry used in the investigations gave optimum growth conditions in pure culture. It can be seen that addition of straw to slurry which would increase the C:N ratio would not have detrimental effects on the growth of thermophilic fungi as a ratio below 20:1 has been shown to adversely affect the growth rates of the thermophiles. Thus a 20:1 ratio could be considered as the threshold value above which the addition of straw to increase the C:N ratio would aid the biodegradation process in terms of providing an energy source for utilisation of the nitrogen source.

Thus, it is likely that the slurry will have little effect on the process and may, when filtered, be able to concentrate carbon and nitrogen substrates for fungal growth on the semi-solid waste.

CHAPTER 7

CHAPTER 7

THE NUTRITIONAL VALUE OF THE PRODUCT

- 7.1 Discussion of nutritional values of animal wastes

- 7.2 Determination of amino acid spectra
 - 7.2.1 Materials and methods
 - 7.2.2 Results and discussion

7.1 DISCUSSION OF NUTRITIONAL VALUES OF ANIMAL WASTES

It has long been recognised by farmers that pigs kept on straw bedding seem to thrive better than those allowed to roam free. It is further known that the pigs will eat the straw bedding and that the nutritive value of the straw is increased by fungal and bacterial up-grading; the micro-organisms being able to provide certain vitamins and proteins which the pigs find useful. The upgrading of the straw is aided by the animal waste which provides a nitrogen source and moisture. The depth of bedding may also provide insulation for thermophilic conditions. Thus, there is a partial recycling system of the waste materials via an intermediate organism which is able to utilise the waste and upgrade its nutritive value. Many other animals re-ingest their own faeces, the condition being known as coprophagy (Durham et al., 1966). There are examples from the wood lice up to the cow, the main purposes being either to increase the efficiency of the nutrient removal, or to absorb vitamins produced by the micro-flora in the gut and passed out through the faeces. These vitamins such as vitamin A for the rabbit, and the B-complex from cattle can be absorbed on re-eating the faeces or may provide a supplement to vitamin deficient feedstuffs (Bohstedt, et al., 1943). Also, pigs have been used to salvage grain from the faeces of cattle fed on high grain rations (Anthony and Nix, 1962). It is thus not unusual for animals to indulge in coprophagy in their natural habitat and the acceptance of the recycling

of wastes is only novel to man who seems to have no need to re-ingest his wastes possibly a reflection of his efficient digestive system.

However, there is an increasing realisation amongst farmers and research workers alike that certain farm animal wastes are of nutritive value either when recycled to the same animal or to another species. Because of the fear of the spread of disease amongst the animals strict regulations regarding the feeding of wastes - including waste foods - to animals are in existence. In Great Britain The Diseases of Animals (Waste Foods) Order 1957 makes it an offence to feed 'waste foods' to animals unless those foods have been boiled. The American Food and Drug Administration has stated (Taylor, 1971) that it has not sanctioned the use of poultry manure as a feedstuff because of the possibility of pathogens and toxic metabolites being present. When it receives enough information as to the safety and quality of a particular waste it will review the situation. However, at the present time in the U.S.A. legislation prevents the use of animal wastes as feedstuff supplements. Alexander et al. (1968) on studying the bacteria isolated from poultry litter have found that 60% of the samples contained Clostridium spp. Salmonella spp., although isolated less frequently, were found in those wastes being fed to livestock and thus creating conditions for the spread of salmonellosis. Species were also isolated capable of causing disease in cattle. Another important hazard has been mentioned by El-Sabban et al. (1970). They investigated the build-up of arsenic and chlorinated

hydrocarbons in the tissues of animals fed waste material and found them to be in quantities of less than 1 ppm. However, the build-up of accumulative toxic residues in the tissues of animals needs to be carefully monitored to make sure it does not reach a dangerous level. It is not uncommon practice though for dried poultry manure to be included in the rations of cows or pigs, and of course it is very difficult to prevent the animals from indulging in coprophagy which seems to do them little harm. Thus, arguments against the feeding of waste materials which are based upon the introduction of diseases can be counteracted by providing conditions where the disease-producing organisms cannot grow or by some form of sterilisation procedure. The main argument for and against the use of animal wastes should be based upon the nutritive value, the toxins present and the comparative costs of producing the upgraded material relative to the costs of feed additives bought from external sources.

The nutritive value of poultry manure has been investigated by several authors both in Great Britain and the U.S.A. (El-Sabban et al., 1970; Evans et al., 1968; Long et al., 1969) where it has been used for trials involving ruminants. Evans et al. (1968) have found that dried poultry waste (DPW) can constitute up to 50% of a feed to calves provided the ration was not introduced too abruptly. Sheep also fed the diet were slaughtered and were awarded a top grade for quality. In the U.S.A. similar work (Long, Bratzler and

Frear, 1969) has been done with beef cattle and sheep to test the effects of hydrolysed and cooked poultry waste which was subsequently dried before feeding. The researchers found no ill-effects to the quality or rate of gain of the ruminants when the levels of hydrolysed and cooked DPW were at 28% and 25% respectively. Apart from the comparative costs of DPW with other feed additives the arguments for using this waste are based upon the nitrogen source which is uric acid. Uric acid is 10,000 times less soluble than urea (Evans et al., 1968) and is thought to be less toxic because the more soluble urea will release ammonia at certain levels. Thus DPW can be used in higher concentrations. However, uric acid is unstable on storage and will break down to urea and ammonium salts. Drying out the waste prevents this. Evans et al. (1968) have also shown that there is a wide range of amino acids present in DPW but methionine and lysine were at too low a level (0.88 and 1.89%) to provide the whole amount of amino acid required.

Anthony (1966) has fed mixtures of cow manure and grain (40:60) to beef cattle and experienced no difficulty in acceptance and palatability by the animals. He found further that the manure was a source of thiamine, niacin, pantothenic acid, vitamin B₁₂ and provided an adequate amount of the essential amino acids. It is thought that these compounds are synthesised in the rumen and then excreted in the faeces and urine. In a later paper Anthony (1969) describes his system of 'wastelage' which involves the

mixing of fresh cow manure with ground grass hay in the ratio of 57:43 and then storing the mixture in a silo until fed. It is then fed direct or mixed with concentrates. An amino acid analysis of the wastelage in comparison with a control diet has shown it to contain about 20% more than the total amino acid content of the control diet, making it, together with DPW, a valuable source of protein. The value of cow manure as a supplement in poultry rations has been investigated by Palafox and Rosenberg (1951). They found that air and oven dried manure did not suppress egg production, body weight, hatchability and feed consumption when it was included in the feedstuff at 5 and 10% levels. At 15% some depression of the above factors was noted but on adding enough herring meal to bring the protein level to 16% the depression was removed. This suggested that the manure provided inadequate protein at the higher levels when it replaced other supplements in the feedstuff. However, a 10% addition might mean a saving of money where supplements need to be imported.

The nutritive value of pig waste has received less attention in the past, possibly because of its high strength and handling problems. Recently, however, Harmon, Day, Jensen and Baker (1972) have studied the nutritive value of the solid residue from an oxidation ditch on rats. They found that this solid residue mixed with corn could replace between one half and one third of the protein of the soybean control meal and that the feed intake was not affected showing that

TABLE 13

A comparison of the amino acid composition of various wastes

Type of waste Amino acid	Poultry ^a	Cow manure ^b 'Wastelage'	Pig oxidation ^c ditch residue	Control ^b Feed
Aspartic acid	3.29	6.31	1.28	2.53
Threonine	1.92	3.27	0.67	1.42
Serine	1.85	2.50	0.57	1.52
Glutamic acid	4.18	7.63	1.59	4.50
Proline	0.63	4.02	0.61	4.20
Glycine	2.68	4.15	0.86	1.09
Alanine	2.34	5.34	0.98	3.43
Valine	4.04	3.74	0.75	2.04
Methionine	0.88	0.59	0.22	0.45
Isoleucine	2.72	2.22	0.51	1.37
Leucine	2.99	3.22	0.92	3.45
Tyrosine	1.36	Trace	0.56	Trace
Phenylalanine	1.79	Trace	0.72	Trace
Lysine	1.89	4.10	0.72	1.39
Histidine	0.72	1.06	0.28	0.92
Arginine	1.56	1.83	0.74	1.91
Ammonia	-	0.13	-	0.27
TOTAL AMINO ACIDS	34.84	50.13	11.98	30.49

a = Evans et al. (1968)

b = Anthony (1969)

c = Harmon et al. (1972)

1. All figures are expressed as percentages of total protein.
2. Loss of protein during acid hydrolysis occurs.

the material was palatable. The addition of 1% lysine and 0.1% tryptophan together in the experimental feed significantly increased the rate of gain of the animals showing that these amino acids were limiting in the waste. If a comparison is made of the amino acid composition of the three types of waste described above it is possible to demonstrate their relative nutritional values. This is shown in table 13 where a comparison is made of DPW, 'wastelage' and pig oxidation ditch residue (ODR). The last column shows a normal diet fed to cattle and it can be seen that all the wastes, with the exception of the pig ODR, have a greater percentage of amino acids than the normal diet. The 'wastelage' and pig ODR had undergone a fermentation and physical separation respectively which may have increased the amino acid composition either by microbial synthesis or concentration. However, the analyses show that the wastes are a potential source of nutrients either for direct recycling or on a rotation basis and it is hoped that the low figure recorded for pig ODR can be increased by selective fungal upgrading.

The total amino acid content, although indicative of the up-grading of a waste material, does not predict the nutritive value to the animal. Not all the amino acid content will be digestible and not all of the digestible acid will be available. The digestible fraction is that which is absorbed from the gut and the available part of this is actually used by the animal to produce protein

(Gadd, 1972). Thus, in comparing the value of protein concentrates and upgraded waste materials it is more valuable to calculate the available amino acid contents and this may be done by nutritional trials from which an economic value can be determined. The amino acid value of pig foods is generally quoted as the percentage of available lysine, as this has been found to be the first limiting amino acid in pig growth (Gadd, 1972).

Thus it has been seen that animal wastes can provide a source of cheap amino acids and vitamins. The technology of the processing of such wastes is still in its infancy but recycling of wastes could become an economic proposition. It is, therefore, necessary that an analysis of the products from experiments in Chapters 4 and 5 be done to determine the nutritive value of the products to show whether recycling is a possibility. In the following investigation amino acid spectra will be determined for the biodegraded samples to give a preliminary indication of upgrading. This will provide information for pilot-scale plant operation which will produce enough material for feeding trials and hence determination of amino acid availability.

7.2 DETERMINATION OF AMINO ACID SPECTRA

7.2.1 Materials and methods

In order to determine the nutritional value of the

samples obtained from the fermentation experiments conducted in Chapters 4 and 5 an amino acid analysis was carried out for the initial sample and this was compared with the amino acid spectrum of the final sample to see whether any upgrading had occurred. The analysis procedure was carried out according to Welcher (1963). It was first necessary to remove soluble carbohydrates and fats in order to prevent any interference which might decrease the amounts of amino acid recorded. It should be noted at this stage that all equipment used was kept scrupulously clean using chromic acid to prevent contamination and a blank was carried out to see whether the reagents contained amino acids. The carbohydrates were removed using the method originally described by Weinmann (1947) and subsequently used by Chang (1967). This method has been described for the determination of starches and dextrans earlier and it need only be stated that 1g. sample (weighed accurately) of the dried material was used without previous Soxhlet extractions being done. After this first extraction the insoluble material was removed by centrifugation and washed with hot water, acetone, hot benzene:ethanol 95:5 (v/v), ethanol and ether. All the washings were carried out in a fume cupboard the washing being done through a No. 1 porosity sintered glass funnel with a Buchner flask and a vacuum pump to draw away the solvents. It is claimed (Welcher, 1963) that this procedure will increase the nitrogen three to five fold. The dried material was weighed and determined as a percentage of the original dried sample so that final amino acid figures could

be quoted in terms of a percentage of the original sample and not the extract.

The next step is the hydrolysis of the proteins. This was carried out in sealed pyrex test-tubes drawn out in the middle in a flame. The sample was placed in each tube after the tubes were partially drawn-out leaving a hole large enough for sample introduction and to this was added 3 ml. of 6N HCl. Welcher has suggested that when small amounts of protein are present the HCl should be present in quantities of between 1,000 and 5,000 times the weight of the protein. The 3 ml. quantity was approximately 1,000 times the average amount of protein calculated to be present in all the samples. The tubes were then drawn-out completely the ends being sealed in the flame. The hydrolysis was then carried out in an oven at $110 \pm 1^{\circ}\text{C}$ (Eaker, 1968) for 24 hours (Welcher, 1963). The tubes were then broken open and the hydrolysate washed into tubes to undergo filtration. The filtration was in two stages. The straw material was first removed using Whatman No. 1 filter papers, and then the hydrolysate was decolourised using a small amount of activated charcoal ('Norit', Sigma Chemical Co.). The filtrate was then corrected to pH 2.2 using 5 N sodium hydroxide and buffered by adding 1 ml. of a sodium citrate buffer made up as follows to pH 2.2:-

Sodium citrate (N)	0.20
Citric acid (g)	21.00
NaOH, 97% (g)	8.40
HCl conc. (ml.)	16.00
Phenol (g)	0.40

Made up to 1 litre with distilled water

The final volume of the hydrolysate was measured so that the dilution could be taken into account in the calculation. Most of the samples after washing increased in volume from 3 ml. to 18 ml. The samples were used immediately or stored in the refrigerator at 4°C. A blank was prepared with the samples to determine whether any amino acids were in the reagents. The standards used were in a concentration of 0.1 μ moles/ml. excepting proline which was at 0.2 μ moles/ml.

The amino acid analyser used was based on that originally described by Spackman, Stein and Moore (1958) and was an Evans Electro-selenium Amin-al 194 with a pen chart print-out. The column lengths were, for the basic amino acids, 10 cm., and for the neutral and acidic amino acids, 80 cm. The material in the column was a bonded polymer resin and the time to do one analysis of the basic, neutral and amino acids was 4 hours 25 minutes. The colour reaction reagent was made up as follows:-

Ninhydrin	40g.
4M sodium acetate buffer	500 ml.
Methyl oxyethanol	1.5 litres
Stannous chloride	0.8 g.

The absorption of the colour was done at 440 μ and 570 μ . The latter wavelength was necessary to scan for proline. This amino acid analyser was not able to detect cystine and thus this amino acid will not appear in the results. 1 ml. aliquots of each sample buffered to pH 2.2 were introduced onto the top of the columns. An example of the chart print-out is shown in figure 48.

7.2.2 Results and discussion

The results for the amino acids were calculated in the following way, using printed results sheets, examples of which appear in figures 49 to 50. The calculation is best illustrated using the peak in the chart for aspartic acid:-

Peak reading	=	0.162
Base line	=	0.040
Total height	=	Peak-base = 0.122
Half height	=	0.061

The width is now measured by firstly drawing a line across the peak from A to B 0.061 units above the base line and

FIGURE 48

ACIDIC AND NEUTRAL AMINO ACID CHART PRINTOUT

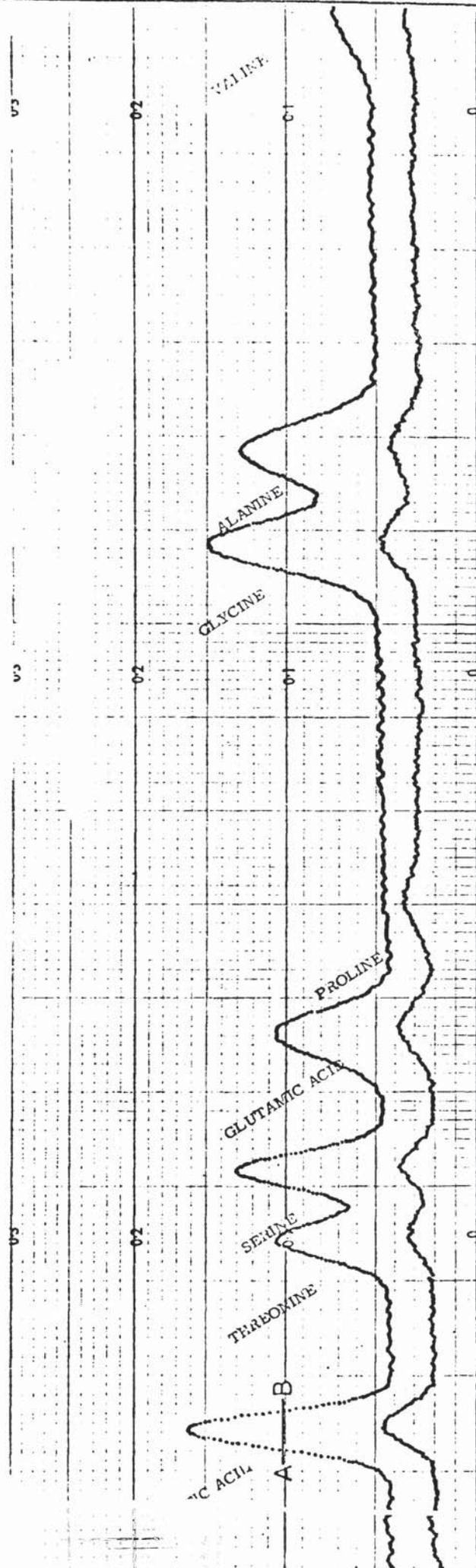


FIGURE 49
AMINO ACID ANALYSIS

Analysis No. 90/KJS/IV.....

Date. Nov 15th 1972.....

Column No. 1 Basic analysis (10 cm).....

Flow rate.....

Sample.....

Buffer Pressure. 50 lbs/in².....

Ninhydrin Pressure. 30-40 lbs/in².....

Channel 1. 359.....

Channel 2. 66.....

Amino Acid	Peak Reading	Base Line	Half Height	Total height (H)	Width (W)	H x W	$\frac{H \times W}{C}$
Tryptophane							
Lysine							
Histidine							
Ammonia							
Arginine							

Notes:

AMINO ACID ANALYSIS

Analysis No. 40/KTS/41..... Date... Nov. 15th 1972.....

Column No. 2..... Neutral + Acidic..... Flow Rate.....

Sample.....

Buffer Pressure... 140-190 lb/in²..... Ninhydrin Pressure.....

Channel 1... 321..... Channel 2... 62.....

Amino Acid	Peak Reading	Base Line	Half Height	Total Height (H)	Width (W)	H x W	$\frac{H \times W}{C}$
Aspartic							
Threonine							
Serine							
Glutamic							
Proline							
Glycine							
Alanine							
Cystine							
Valine							
Methionine							
Isoleucine							
Leucine							
Tyrosine							
Phenylalanine							

then counting the dots above that line on the curve. To facilitate this procedure green dots are placed at every sixth black dot so that these can be counted and the result multiplied by six.

Thus,

$$\begin{aligned} \text{Width} &= 38 \\ \text{Height x Width} &= 0.122 \times 38 = 4.64 \end{aligned}$$

C. is a constant which is calculated from the standards which are run before the samples

$$\begin{aligned} C &= H \times W \text{ for the standards} \\ C \text{ for aspartic acid} &= 19.360 \mu \text{ moles/ml.} \end{aligned}$$

Thus, the amount of aspartic acid in 1 ml. sample

$$= \frac{4.64}{19.360} = 0.24 \mu \text{ moles}$$

As the samples were diluted during washing and filtering it was necessary to make a correction factor for this figure so that the answer was now in terms of the extracted sample. In order to convert the units of μ moles/ml. to percent of the original dry weight sample an equation was devised:-

$$\begin{array}{l} \% \text{ amino acid in} \\ \text{original sample} \end{array} = A - A \left[1 - \left(\frac{B}{C} \right) \right]$$

where

$$A = \% \text{ amino acid in extract}$$

$$= \frac{3 \times \text{correct amino acid result in } \mu \text{ moles/ml.} \times \text{mol. wt.}}{\text{weight sample for hydrolysis} \times 10^4}$$

$$B = \text{Wt. of sample after extraction}$$

$$C = \text{Original sample weight}$$

A computer programme using the Fortran language was constructed to do the above calculation and an example of the printout sheet together with the programme is shown in figure 51. The programme was punched on cards and the data cards for each sample could be inserted in the programme for the calculation.

The results are tabulated in tables 14 and 15.

To relate the results to other waste feedstuffs and normal feed supplements so that comparisons could be made, the final amino acid fractions were expressed as a percentage of the total crude protein (Kjeldahl nitrogen x 6.25) present. The results are shown in tables 14 to 15. From these results those samples with the best biodegraded products may first

EX

EXAMPLE OF PROGRAMME AND RESULTS SHEET

PRINTING OF :SBP0620.KEN(1/81B) PRODUCED ON 15FEB73 AT 03.08.07

#OUTPUT BY LISTFILE IN :SBP0620.KEN ON 15FEB73 AT 03.08.07

DOCUMENT :SBP0620.KEN(1/81B)

STARTED :SBP0620.KEN(15FEB73) 11.47.28
 03.04.10 0.01 USED URGENCY M
 03.04.13 JOB IS NOW FULLY STARTED
 03.05.22 0.01 COKE GIVEN 18176
 FORTRAN COMPILATION BY EXXAT NY 4C DATE 15/02/73 TIME 03/04/20

```

0001 LIST (LPI)
0002 PROGRAM (FXXX)
0003 INPUT 1 = CRU
0004 INPUT 2 = TRU
0005 INPUT 3 = CR1
0006 OUTPUT 2 = LPU
0007 OUTPUT 6 = LP1
0008 COMPRESS INTEGER AND LOGICAL
0009 COMPACT
0010 TRACE 2
0011 END

```

```

0012 TRACE 1
0013 READ FROM (CR)
03.04.27 FREE +TRU, 13 TRANSFERS
0001 C SAMPLE 13
0002 C A=PERCENT AMINO ACID
0003 C B=CORRECT VALUE
0004 C D=MOLECULAR WEIGHT
0005 C E=REFLUX EXTRACT WEIGHT
0006 C F=HOLE EXTRACT WEIGHT
0007 C G=ORIGINAL WEIGHT
0008 C H=(3*B*D)/(E*10**4)
0009 C P=1-(F/G)
0010 C A=H-H*P
0011 MASTER KENNETH
0012 READ(1,36)N
0013 36 FORMAT(12)
0014 T=0
0015 WRITE(2,40)
0016 40 FORMAT(12X,19H PERCENT AMINO ACID)
0017 DO 59 I=1,N
0018 E=0.0517
0019 F=0.8118
0020 G=1.000
0021 READ(1,42)B,D
0022 42 FORMAT(2F0.3)
0023 H=(3*B*D)/(E*10**4)
0024 P=1-(F/G)
0025 A=H-H*P
0026 WRITE(2,46)A,B,D,E,F,G
0027 46 FORMAT(//10X,4HA = ,F3.2,5X,4HB = ,F7.3,5X,4HD = ,F6.2,
45X,4HE = ,F6.4,5X,4HF = ,F6.4,5X,4HG = ,F6.4)

```

KEY TO TABLES

1. All figures are expressed as percentages of the total crude protein

2.	Try	=	Tryptophan
	Lys	=	Lysine
	His	=	Histidine
	Amm	=	Ammonia
	Arg	=	Arginine
	Asp	=	Aspartic acid
	Thr	=	Threonine
	Ser	=	Serine
	Glu	=	Glutamic acid
	Pro	=	Proline
	Gly	=	Glycine
	Ala	=	Alamine
	Val	=	Valine
	Met	=	Methionine
	Iso	=	Isoleucine
	Leu	=	Leucine
	Tyr	=	Tyrosine
	Oala	=	Phenylalanine

3.

Experiment Number	Description of Experiment
1	Breakdown at 25°C
2	Breakdown at 40°C
3	Breakdown at 48°C
5SL, R and S	Run at 48°C with 10% additions of sewage sludge, refuse and soil respectively
7SL, R and S	Run at 56°C with same additions as in experiment number 5
8A, B and C	Run at 48°C with 10%, 25% and 40% end-product additions respectively
11DO, 24, 31 and 38	Run at 48°C. Analysis for amino acids after 0, 24, 31 and 38 days

O = initial sample at day 0

F = final sample at day 17

4. N/D = not detected

5. T = trace present but not measurable

TABLE 14

Quantitative analysis of basic amino acids

Amino acid Sample	Try	Lys	His	Amm	Arg
1O	0.07	0.24	T	0.31	T
1F	0.10	0.70	T	0.23	T
2O	0.11	0.09	T	0.02	T
2F	0.33	0.48	T	0.04	0.19
3O	0.06	0.35	0.05	0.34	0.08
3F	0.18	0.21	T	0.27	T
5SLO	0.72	0.13	T	0.88	T
5SLF	0.11	0.18	T	0.16	T
5RO	N/D	N/D	T	N/D	T
5RF	0.13	0.14	T	0.15	T
5SO	0.14	0.21	T	0.15	T
5SF	0.11	0.12	T	0.10	T
7SLO	0.12	0.12	T	0.13	T
7SLF	N/D	N/D	N/D	N/D	N/D
7RO	0.01	0.14	T	0.12	N/D
7RF	0.18	T	T	0.04	N/D
7SO	N/D	N/D	N/D	N/D	N/D
7SF	0.13	0.09	T	0.11	N/D
8AO	0.57	0.16	T	0.24	N/D
8AF	0.97	0.55	T	0.42	N/D
8BO	0.45	0.29	T	0.17	N/D
8BF	0.63	0.34	T	0.28	T
8CO	0.14	0.05	T	0.09	T
8CF	0.24	0.11	T	0.16	N/D
11DO	N/D	0.19	N/D	0.15	N/D
11D24	T	0.17	T	0.18	T
11D31	T	0.29	T	0.24	T
			T	0.24	T

TABLE 15
Quantitative analysis of neutral and acidic amino acids

	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Iso	Leu	Tyr	ϕ ala	Total amino acid
10	0.43	0.24	0.34	0.22	0.19	0.19	0.17	0.51	N/D	N/D	0.03	0.20	0.32	3.46
1F	0.39	0.19	0.19	0.42	0.11	0.18	T	0.15	T	T	T	0.18	0.21	3.05
20	0.25	0.13	0.12	0.27	0.12	0.15	0.09	0.33	0.02	0.13	0.16	T	T	1.99
2F	0.64	0.51	0.37	0.74	0.04	0.39	0.27	0.68	0.05	0.26	0.45	1.07	1.09	7.60
30	0.54	0.31	0.32	0.72	0.34	0.25	0.14	0.64	T	0.23	0.39	T	T	4.76
3F	0.36	0.22	0.42	0.16	0.14	0.16	0.11	0.74	0.03	0.19	0.22	N/D	T	3.41
5SLO	0.20	0.13	0.14	0.25	0.10	0.11	0.08	0.17	T	0.04	0.09	T	T	3.04
5SLF	0.29	0.16	0.19	0.31	0.12	0.19	0.13	0.53	T	0.06	0.09	T	T	2.54
5RO	0.20	0.78	0.10	0.17	0.08	0.10	0.06	0.15	T	T	T	T	T	1.64
5RF	0.21	0.15	0.15	0.30	0.17	0.16	0.11	0.15	T	0.08	0.13	T	T	2.38
5SO	0.33	0.18	0.19	0.36	0.16	0.20	0.13	0.15	T	0.09	0.15	T	T	2.80
5SF	0.18	0.10	0.14	0.24	0.09	0.11	0.08	0.38	T	0.05	0.09	T	T	1.82
7SLO	0.30	0.14	0.07	0.31	0.18	0.18	0.22	T	T	0.09	0.13	T	T	2.09
7SLF	N/D	0												
7RO	N/D	0.27												
7RF	N/D	0.22												
7SO	N/D	0												
7SF	0.25	0.13	0.10	0.26	0.13	0.13	0.03	N/D	T	T	0.11	T	T	1.47
8AO	0.36	0.15	0.16	0.28	T	0.21	0.22	N/D	T	0.07	T	T	T	2.42
8AF	0.55	0.23	0.34	0.48	T	0.42	0.35	N/D	T	T	T	T	T	4.31
8EO	0.35	0.15	0.16	0.38	0.15	0.22	0.24	N/D	T	T	0.13	T	T	2.69
8EF	0.59	0.30	0.33	0.57	0.27	0.40	0.51	N/D	T	T	0.14	T	T	4.36
8CO	0.14	T	T	T	T	T	T	T	T	T	T	T	T	0.42
8CF	0.19	0.09	0.11	0.21	T	0.12	0.13	N/D	T	T	T	T	T	1.36
11DO	0.19	0.09	0.08	0.13	T	T	T	0.14	T	0.03	0.05	T	T	1.05
11D24	0.15	0.06	0.06	0.19	T	0.12	0.12	0.16	T	0.04	0.09	T	T	1.34
11D31	0.36	0.13	0.13	0.42	T	0.26	0.27	0.35	0.03	0.10	0.10	T	T	2.77
11D38	0.39	0.19	0.14	0.44	T	0.29	0.30	0.30	0.06	0.11	0.08	T	T	2.54

be discussed to see whether the amino acid contents are equivalent to other wastes. It would appear that the wastes in table 16 have appreciably higher amino acid contents than any of the samples investigated. Even the pig oxidation ditch residue had a higher total amino acid content (11.98%) than any of the samples. It is likely that because the straw content of the samples was high the nitrogen, and hence protein, content would be proportionally lower. The poultry, cow manure and pig wastes in table 13 were of low straw content and probably high bacterial concentration which might have given higher amino acid contents. Temperature may also have played an important part, in that the numbers of species of bacteria and fungi were limited, and this reduced the amino acid percentages. In more detail a comparison has been made between the levels of amino acids regarded by the Agricultural Research Council (1967) to be essential and the levels of the final products from the 48°C run (3F), the 48°C run with the addition of 10% soil (5SF), the addition of 25% end-product (8BF) and the 48°C run to test the effect of time on the biodegradation process (11D24, 31 and 38). It can be seen that with the exception of methionine there are sufficient levels to meet the essential amino acid levels. The 48°C run after 38 days had sufficient methionine (0.78%) but was deficient in lysine and tryptophan. The only other samples to contain methionine were the final product of the 48°C run (3F) and 31 days after the beginning of the 48°C run to test the effect of time on biodegradation (11D31) where, although not containing the

TABLE 16

Levels of essential amino acids in samples
compared with suggested levels

Amino Acid	Amino acid as percent of total dried matter						
	3F	5SF	8BF	11D24	11D31	11D38	Suggested level(1)
Lysine	1.83	2.26	3.40	2.47	3.82	T	0.7-0.75
Methionine	0.28	T	T	T	0.44	0.78	0.6-0.7 ⁽²⁾
Tryptophan	1.56	1.83	6.26	T	T	T	0.15-0.2
Threonine	1.98	1.73	3.04	0.86	1.68	2.28	0.5-0.6
Isoleucine	1.66	0.95	T	0.63	1.24	1.37	0.75
Crude protein	10.00	9.00	9.00	6.90	7.55	8.10	15-16.5

(1) ARC (1967)

(2) Sum of methionine + cystine

T = trace of amino acid recorded

3F = Product from 48°C run (no soil added) after 17 days

5SF = Product from 48°C run with 10% soil added

8BF = Product from 48°C run with 25% end-product added

11D24 = Sample after 24 days (10% soil added) at 48°C

11D31 = Sample after 31 days (10% soil added) at 48°C

11D38 = Sample after 38 days (10% soil added) at 48°C

recommended levels, did have more complete essential amino acid spectra, 11D31 being deficient only in tryptophan and 3F having the complete range. It is interesting to note that, with the exception of lysine, there was an increase in essential amino acids in experiment 11. The absence of tryptophan was probably due to its breakdown during acid hydrolysis as has been reported from several sources (Welcher, 1963; Roger et al., 1972). The figures for total crude protein (7 - 10%) in the samples did not satisfy the A.R.C. requirement of 15 - 16.5%. Taking the total amino acid contents as a criterion for a good feed additive the final product from the 40°C run with no soil added (2F) had the highest percentage of 7.6 whilst the final products from the 48°C with 10% (8AF) and 25% (8BF) end-product added had 4.31 and 4.36% respectively. The final products of experiments 3 and 5S and the 24, 31 and 38 day samples from experiment 11 had contents of 3.41, 1.82, 1.34, 2.77 and 2.54% respectively. This would indicate that good biodegradation does not necessarily mean that the product will make a complete feedstuff. However, these preliminary investigations have shown that the amino acid content of the waste can be increased and thus upgraded. Until nutritional trials have been done on large samples to show how much of the amino acids are available it is impossible to gauge their value. As was discussed in the introduction, the total amino acid percentage does not indicate its nutritive value. It is necessary to determine the availability to each animal to which it is fed. Thus,

the high lysine percentages for example (Table 16) obtained might have a low availability because of the form in which the lysine is present.

The possibility of recycling of the product must not be pre-judged from these investigations as there is much more nutritional work to be carried out. Before this can be done, however, appreciable quantities of a fairly constant composition of waste product must be obtained. This may be ad-mixed with other ingredients to arrive at an acceptable level. The high price of certain feed additives such as fish meal and ground-up grass might stimulate the incorporation of a suitable treated waste product into a compound feed for either ruminants or non-ruminants.

CHAPTER 8

CHAPTER 8

LARGE-SCALE PROCESS OPERATION

- 8.1 Introduction

- 8.2 Development of the mesophilic filtration stage
 - 8.2.1 Introduction
 - 8.2.2 Design and construction of equipment
 - 8.2.3 Methods
 - 8.2.4 Results and discussion

- 8.3 Development of thermophilic stage
 - 8.3.1 Introduction
 - 8.3.2 Design and construction of plant
 - 8.3.3 Methods and materials
 - 8.3.4 Results and discussion

- 8.4 A discussion of the economics of the process

- 8.5 An examination of a further treatment process for the liquid run-off

8.1 INTRODUCTION

The laboratory work done so far has indicated that the accelerated upgrading of intensively produced pig waste is possible on a small scale. The parameters have been determined and it has been shown that a thermophilic stage is necessary in the treatment process. The natural extension to the laboratory work is large scale development of the thermophilic stage incorporating into its design the parameters determined in Chapters 4 and 5.

However, before commencing the up-scaling of the thermophilic stage it is necessary that the mechanical separation or mesophilic filtration stage be investigated. Results from laboratory studies would be meaningless because of the lower limit of size at which informative tests could be carried out. It is thus necessary that an on-farm site be set up to test separation of solids from liquid 'in situ'. In this way more useful results, which are directly applicable to a large-scale separation stage, may be obtained.

The two stages outlined in Chapter 1 are thus investigated below. It is hoped that these two stages may be run together in the following manner. The filtered solids will be placed in the thermophilic stage where they will undergo biodegradation. The filtered liquid fraction will be pumped through the solids in order to prevent drying out and to perhaps further reduce the polluting ability. It is likely that the liquid will still contain inorganic nitrates and phosphates, and further treatment will be necessary. Section 8.4 outlines possible solutions for the final disposal of the liquid.

8.2 DEVELOPMENT OF THE MESOPHILIC FILTRATION STAGE

8.2.1 Introduction

An investigation of the parameters for the thermophilic stage has elucidated the optimum conditions which might be tested on a large scale (see 8.3). However, the filtration of the slurry was conducted in an arbitrary fashion in order to retain the fibrous matter and some of the moisture. It was considered that to get more realistic results as to the filtration efficiency of the straw material on-farm experiments should be conducted with representative samples of solid and liquid waste.

The importance of the separation of the solids from slurry has only recently been recognised. Gowan (1972) reviews various pieces of equipment by which this can be done either in the animal house or outside it. He states that this equipment is expensive and the separated liquid still has a high BOD (about 20,000 mg/l) needing much treatment. Glerum, Klomp and Poelma (1971) tested various centrifuges, filters, sedimentation tanks and vibrating screens as separators of pig slurry and found that the centrifuge and sedimentation procedures gave the best results. If the solids are not separated then any subsequent treatment or handling method will be more costly because of the need to periodically remove the build-up of solids and the need for more powerful pumps to move the slurry.

Hepherd and Charlock (1971) have advocated the separation of the slurry prior to biological treatment using a high-rate biological filtration tower to lower the BOD. They believe that an effluent with a lowered solids content could be channelled through smaller bore pipes, cause less run-off, and be applied in greater quantities before causing adverse conditions. Their main problem, however, was the treatment of the separated solid and they suggested that it be stored in straw bale enclosures and periodically removed to the land.

The use of straw as a separating medium has many advantages. It is cheap because there is a surplus in the grain producing countries of the world. A survey of the yields of wheat, barley and oat straws (Fulbrook, 1972) has shown that from 1959 to 1970 an average of 4,534,000 tons of straw were produced per year with a retail price which ranged from £3.50 to £5.00 per ton depending upon season, type and locality. Between 9 and 12 million tons of straw are produced in total and thus between one half and one third may be considered to be a waste product with no retail value. In practice many farmers with a harvester, baler and some labour can remove straw free of charge and it is probable that the above charges relate to labour and transport costs. Straw is also a good substrate for cellulolytic activity and attracts a flora able to decompose it in the presence of moisture: thus it is biodegradable. The mechanical and physical properties of straw make it a

good moisture absorber and able to retain air spaces for filtration purposes. Other filter media in comparison do not conform to all of the above parameters and, probably most important of all, are not naturally found and handled by farmers in their daily routine. Straw is used as a bedding and for insulation on the farm and the farmer is acquainted with techniques used to handle it. Thus any new process instituted would be better recognised by him because of the use of straw.

Having decided that straw should be the filtration medium it is next necessary to decide how it should be employed. In order to make any system acceptable to a farmer, very little change in house or equipment design is desirable in order to cut costs. Thus it was decided that a straw pad could be placed beneath the dunging slats in the channel where the slurry would collect. In this way realistic results could be obtained as to the filtering capacities of the straw and the pigs themselves would act as continuous dosing devices. Mechanical dosing devices are expensive and need extra attention. The placing of straw beneath the slats would not involve extra work for the farmer. Hephherd and Charlock (1971) suggested that straw bales might be used to absorb slurry, but they dismissed the idea because application rates (probably done periodically from a tank) were not high enough to treat the wastes from more than about 500 pigs. It will be seen later that by putting straw beneath the slats the number of pigs need not be the limiting factor.

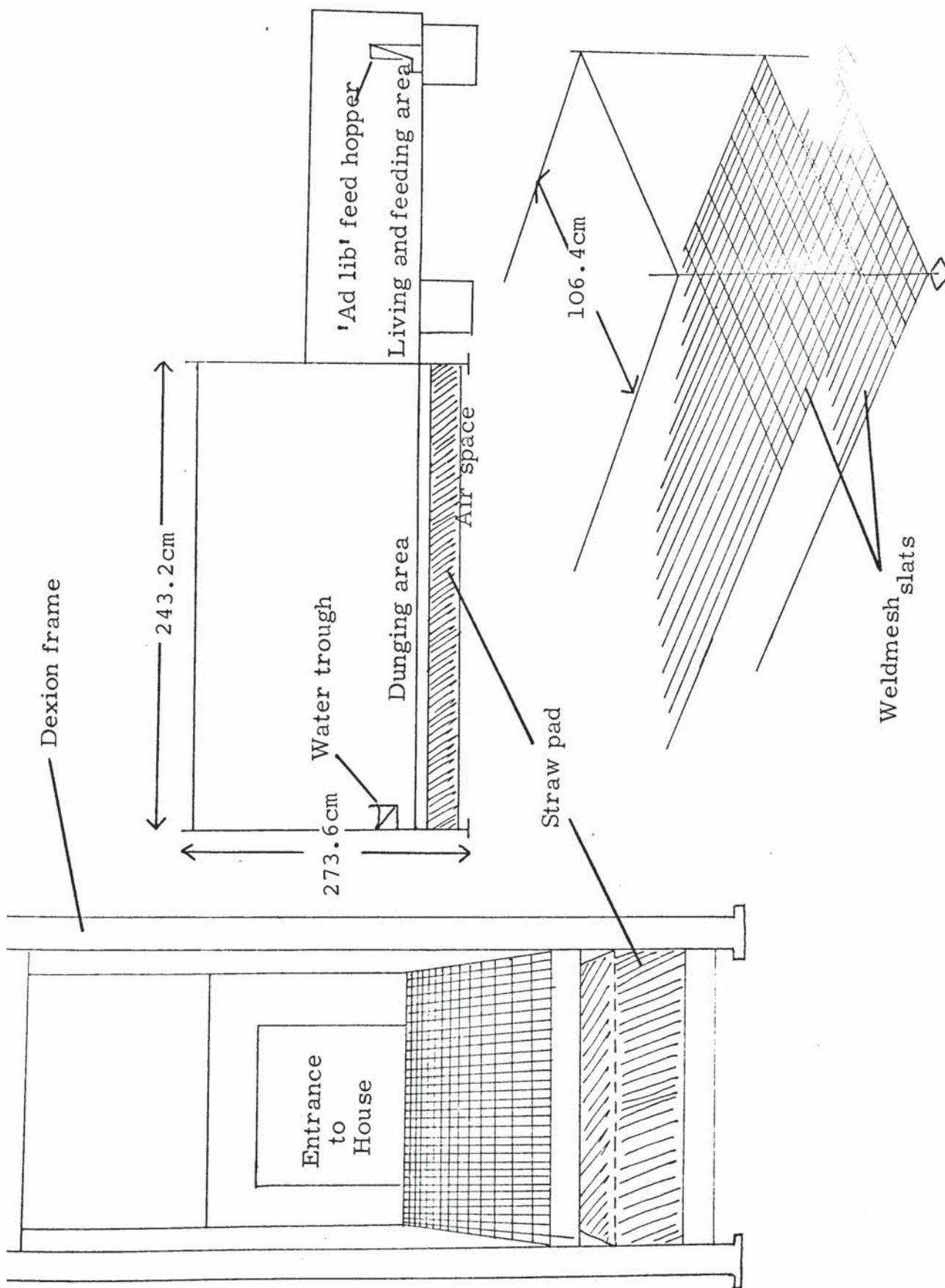
Also, the pigs do not compress the straw into an anaerobic pad of sour smelling waste which would lower the filtering capacity and encourage anaerobic bacteria. In deep litter bedding systems this often happens producing an anaerobic and unstable product often waterlogged. Thus the use of slats will prevent compression of the straw, keep the pig clean and probably reduce disease as the waste does not remain in close contact with the pig. The uncompressed straw can remain aerobic, reducing any odours and keeping its filtering properties throughout the process. A space beneath the straw pad was also incorporated to aid passive aeration of the pad and to aerate the drops falling from beneath the pad.

8.2.2 Design and construction of equipment

Two experimental pens were constructed on the farm to accommodate 15 pigs each from weaning at about 8 weeks until ready for slaughter, usually 6 - 7 weeks later. These pens replaced the fattening stage when the pigs were usually kept on slats with a slurry system. The pens were set up as shown in figure 52 and plates 8 to 11. The framework was constructed of Dexion 'Speedlock' beams which were quick to construct, could hold the weight required, and could be adjusted with ease to vary the slat heights. The ease with which the framework could be put up was shown in that two people took 40 minutes to construct both frames. Box beams

FIGURE 52

DESIGN OF EXPERIMENTAL PIG PEN



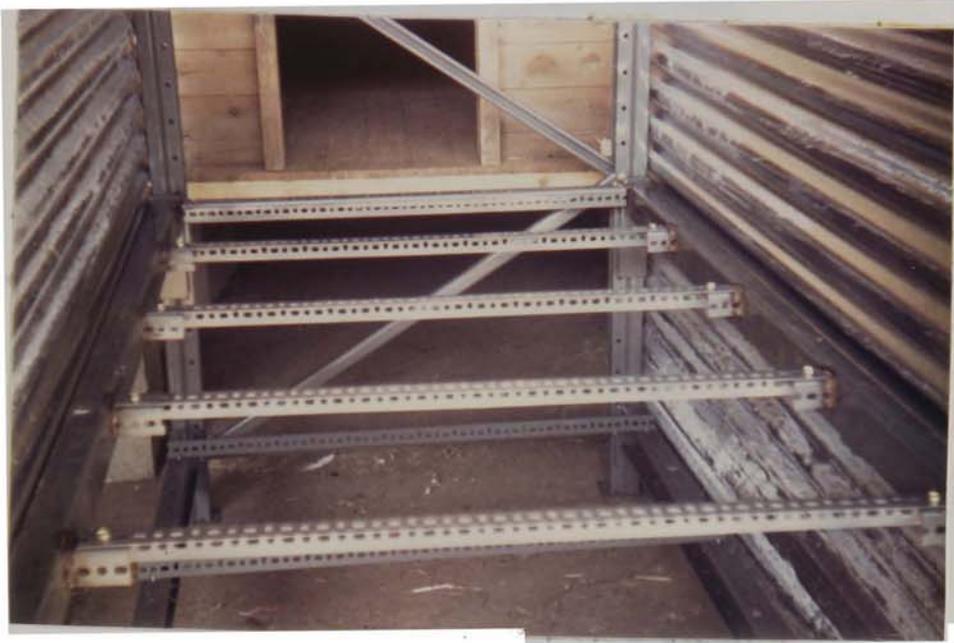


PLATE 8

ARRANGEMENT OF CROSS-MEMBERS ON FRAMEWORK TO
SUPPORT 'WELDMESH' SLATS



PLATE 9

DEXION FRAMEWORK WITH REMOVABLE SIDES FOR
ACCESS TO STRAW FILTER

Living quarters are in wooden pen to the rear

(3½") were used to support the upper slats and 3" open beams to support the straw-holding lower slats. The slats used were made from 'Weldmesh' pig flooring. It was made from 5 s.w.g. drawn steel wire welded to give a mesh size of 3" x ¾". This was designed to support pigs of weight 85lb to 190lb with Dexion angle iron cross supports at 15" centres (see Plate 10). The angle iron was bolted to supports welded onto the sides of the beams. The slats were wired to the cross supports and the side beams. Weldmesh was chosen because it was used extensively as a pig flooring material, being acceptable to pigs, because it could conveniently be cut to size and because it had a large voidage (over 50% according to manufacturer) to prevent clogging.

The living areas were made from wood and placed on blocks to bring them to the level of the top slat. The sides of the framework were filled in with corrugated sheeting, the bottom sheets of each pen attached with wire so that access to the straw could be gained by lifting the sheet during the experiments without disturbing the pigs (see Plate 9). Drinking troughs were placed on a sheet of 'Weldmesh' cut to form the front of the pen (see Plate 11). 'Ad-lib' feeding troughs were placed in the back of the living area. The feeding and drinking facilities were purposely placed apart so that the pig would have to go to the drinking trough across the dunging area and would preferentially dung there because of the lower temperature. Pigs will, in general, dung in the cooler parts of the pen. It is usually



PLATE 10

TOP SLAT IN PLACE WITH STRAW FILTER BENEATH



PLATE 11

COMPLETED PEN WITH DRINKER INSTALLED AND
STRAW FILTER BENEATH

necessary to first encourage them to do this by placing the water trough in this area and by placing dung on the slats. The pigs will not foul their living area if these precautions are taken and all the wastes will pass onto the straw. The pens were situated in a barn and thus the dunging areas were left uncovered. This protection from rain meant that no extra water was present to affect the analysis of the waste. A 6" gap was left beneath the bottom slat for passive aeration and polythene sheeting placed to collect the filtered liquid. Trays were placed beneath each gap to collect samples for analysis.

Two preliminary experiments were conducted to see if this system was feasible. They both showed that if straw was placed above the top slat and no gap was left between the straw and the top slats the waste built up above the slat and pigs, although not affected physically, became dirty and the pen was not very clean (see Plates 12 and 13). Thus, in all the investigations a gap was left to prevent build up above the slats.

8.2.3 Methods

For each experiment conducted 15 weaned pigs at about 8 weeks old were used for each pen. They were fed from an 'ad-lib' hopper on a dry feed (see appendix 4 for composition) and water was provided from a self-filling trough. The



PLATE 12

FIRST PRELIMINARY EXPERIMENT WHERE STRAW WAS
PLACED ON THE TOP SLAT

An exposed portion of the slat can be seen in
the far left-hand corner



PLATE 13

SECOND PRELIMINARY EXPERIMENT TO SHOW
BUILD-UP OF WASTE ABOVE SLAT WHERE NO GAP
BENEATH SLAT WAS LEFT

Feeding trough is situated in top left-hand corner

experiments were conducted during the winter months and it was thus necessary to insulate the living area with straw to prevent hypothermia and encourage dunging on the slats. The pigs themselves also provided insulation by their numbers. When the pigs were first placed in the pens they had to be encouraged to go to the water trough over the slats and a check needed to be made for the first few days on whether there were any signs of dehydration, indicated by dizziness and irregular movement in the pigs. After a few days the pigs went to the water of their own free will and dunging was confined exclusively to the slats.

Experiments were designed to determine the following parameters:-

- a. The optimum depth of straw for filtration.
- b. The length of time the straw could remain as a filter before replacement.
- c. The best loading procedure in preparing the filter.
- d. The acceptance of the system by the pigs and the farmer.
- e. The quality of the liquid filtrate.
- f. The amount of labour required.

Straw was laid on the bottom slat to depths of 12, 15 and 18" using bale sections placed on top of each other to avoid the trickling of liquid through the gaps between the sections. A layer of loose straw was then spread on top to provide a rough initial filter. A 4" layer of straw was also laid in a similar fashion to the above depths but in this experiment fresh straw was spread over the solid waste at weekly intervals to give separate layers of straw and dung. Sample trays were placed beneath the bottom slat, away from the water troughs to avoid dilution from spillage. The following analyses were conducted on the two fractions:-

Analysis	Solid	Liquid
Moisture	4, 12	-
pH	4, 12	4, 12, 15, 18
Temperature	4, 12	-
Carbon	4, 12	-
Nitrogen	4, 12	-
Solids content		4, 12, 15, 18
BOD ₅		4, 12, 15, 18
Phosphate		4, 12
Nitrate		4, 12
Copper		4

Numbers refer to depths of straw on which analyses were carried out

Flora successions were also determined for the 4" and 12" investigations using the screened substrate tubes of Eggins and Lloyd (1968). The strips were plated out onto cellulose and glucose-starch agars at 25°C.

For the analysis of the solid material four samples were taken from each pen. The liquid was collected for nitrate and phosphate analysis (see Appendices 5 and 6) from shallow trays which were emptied after each collection. Samples of pre-filtered material were also collected for solids content and BOD₅ analysis (Society for Chemical Analysts, 1958). Trays were placed on top of the straw to collect a representative sample of urine and faeces which were then mixed into a slurry. Observations were made on the condition of the pigs, the slats and the filter throughout the investigations. As it was not possible to attend the pens continually (weekly visits were made) the farmer agreed to look after the daily running, and he was asked whether any extra or less labour was required in comparison to the other systems he employed. He was also asked whether he thought the system to be of use in present and future intensive pig rearing management.

8.2.4 Results and discussion

Figures 53 and 54 show the carbon, nitrogen, pH and moisture changes for the 4" and 12" straw filters. For

FIGURE 53

ANALYSIS RESULTS FOR 4" STRAW FILTER

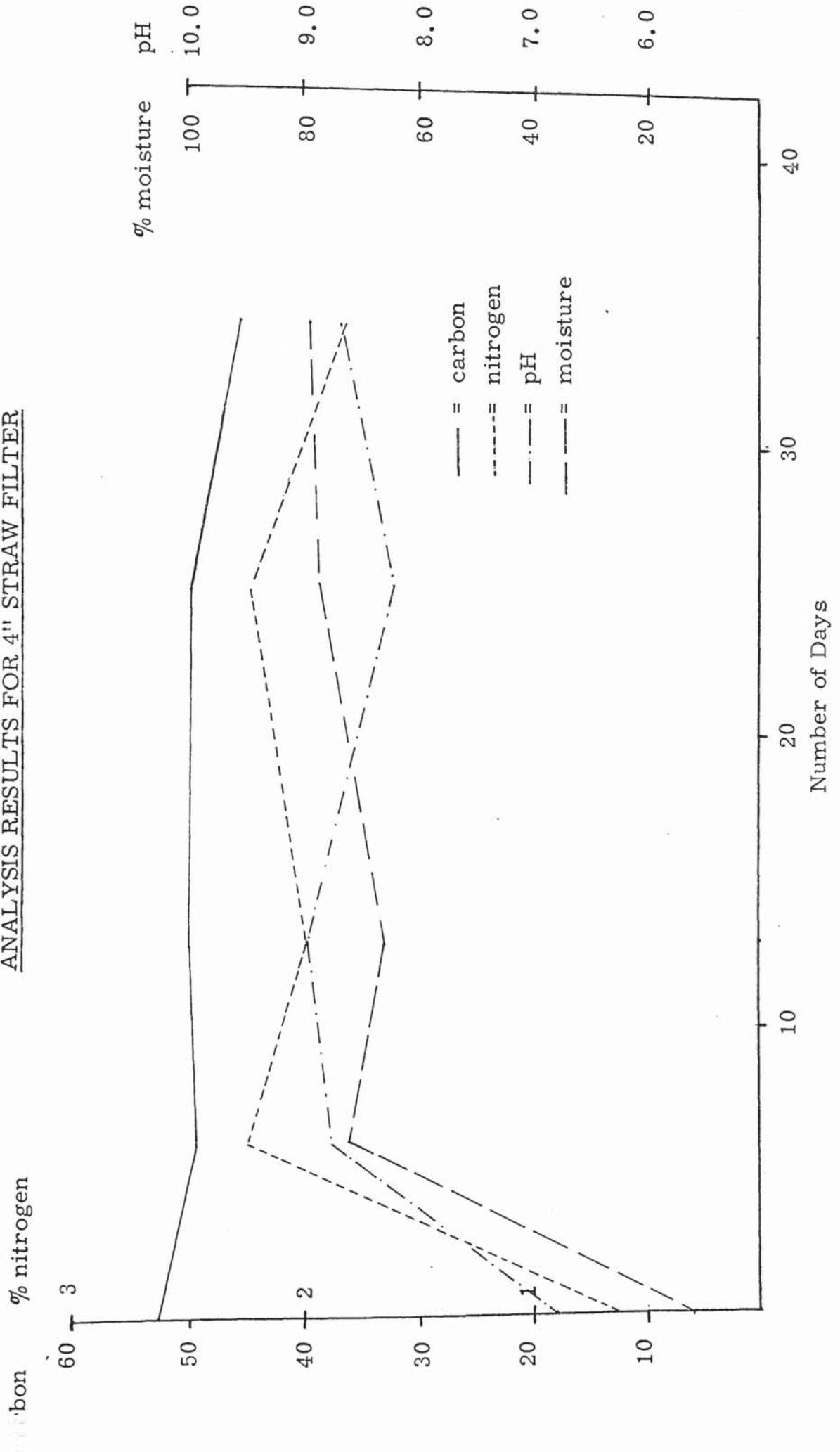
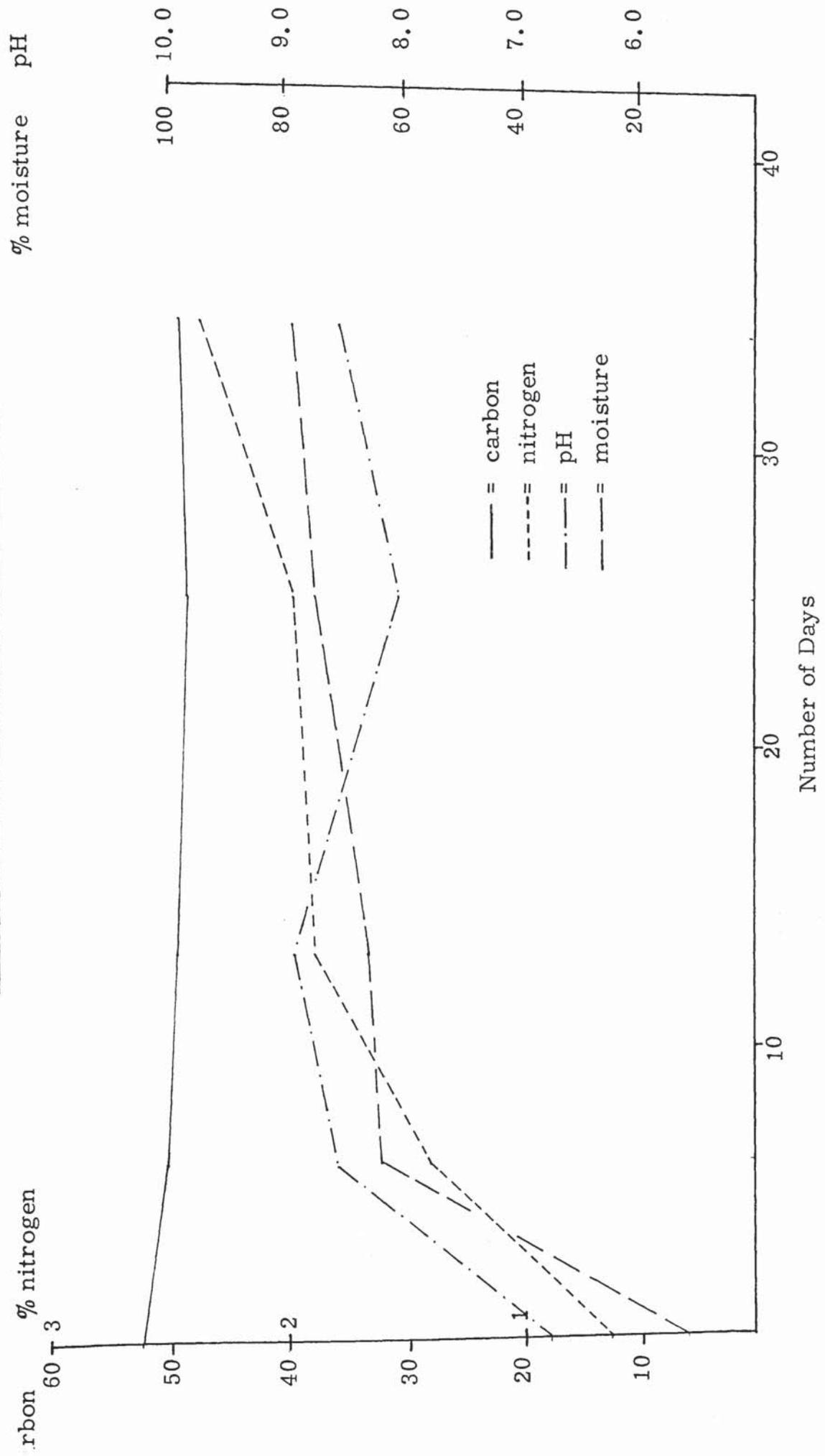


FIGURE 54
ANALYSIS RESULTS FOR 12" STRAW FILTER



both cases the decrease in carbon was from about 52% to between 48 and 49%, a negligible decrease in relation to that at 50°C. However, this small decrease may be due to the increase in carbon from the solid waste which off-sets the biodegradation of the carbon compounds. As would be expected the nitrogen increased due to the deposition of the waste. The 12" straw filter increased its nitrogen content from 0.63% up to 2.40% whilst the 4" filter increased from 0.63% to 1.82% after 34 days. The larger filter was thus able to retain more nitrogen although between 6 and 25 days the nitrogen of the 4" filter fluctuated between 2.27% and 2.24%. The increase in moisture contents up to just below 80% in both cases gave a product which retained its structure and was not waterlogged. The pH remained alkaline at about 8.6 with a peak of 8.9 for both filters and was thus probably a little too high for dominant fungal growth.

The inorganic nitrate and phosphorus contents, the total solids and the BOD of the liquid filtrate are shown in Table 17. The nitrate concentration in both filtrates decreased over the 5 weeks, the 4" one giving a lower content (3 ppm) than the 12" filter (5 ppm). It would appear from this that the nitrate was either physically retained in the straw filter or was being biologically reduced by nitrate reducing micro-organisms. In contrast the phosphate concentration in the filtrate increased throughout the experimental period. There was a 40-fold increase in the 12" filter from 180 ppm to 8,800 ppm after 5 weeks. The 4" filter increased from

TABLE 17

ANALYSIS OF LIQUID FILTRATE FROM
4" AND 12" STRAW FILTERS

4" STRAW FILTER

Day	Nitrate (ppm)	Phosphate (ppm)	pH	Total solids content (%)	BOD ₅ mg/l
6	7	160	9.0	4.20	10,700
13	5	1,720	8.2	4.05	4,500
25	12	2,130	8.8	5.80	11,800
34	3	1,870	8.9	2.32	2,040

12" STRAW FILTER

Day	Nitrate (ppm)	Phosphate (ppm)	pH	Total solids content (%)	BOD ₅ mg/l
6	10	180	8.7	5.70	9,400
13	4	2,050	8.3	4.20	4,600
25	12	3,730	8.5	1.43	3,000
34	5	8,800	8.7	2.49	7,900

BOD₅ of Pre-filtered waste = 8,000 mg/l

160 ppm to 1,870 ppm a much lower increase showing that phosphate could be retained either physically or biologically. The BOD for both filters decreased over the weeks although a high result for the 12" filter (7,900 mg/litre) was obtained after 34 days.

The pre-filtered waste had a BOD of 8,000 mg/litre and thus there was an initial increase after 6 days. However, the 12" filter then decreased to 3,000 mg/litre after 25 days whilst the 4" filter reached 2,040 mg/litre after 34 days. The increase in efficiency of the filters can be shown in the BOD and nitrate figures, and also an examination of the solids content further supported this. The total solids content of both filters had similar decreases - the 12" filter decreasing from 5.7% to 2.49%, and the 4" filter from 4.2% to 2.32%. A correlation between the solids content and BOD can also be seen in table 17. In the 12" filter the total solids increased from 1.43% to 2.49% after 34 days, and this was coupled with a BOD increase from 3,000 to 7,900 mg/litre. Similarly in the 4" filter an increase of total solids content from 4.05% to 5.8% gave a BOD increase from 4,500 to 11,800 mg/litre. The subsequent decrease in solids content gave a corresponding decrease in BOD. The organic carbon content is probably in the solid fraction and this would directly affect the BOD. Robbins et al. (1972) have found that under certain conditions the organic carbon content may be correlated with the BOD. However, high nitrogen, metal and physical factors do play an important

TABLE 18

ANALYSIS OF LIQUID FILTRATE
FROM 15" and 18" STRAW FILTERS

15" STRAW FILTER

DAY	BOD ₅	SOLIDS CONTENT %
7	6,662	2.33
14	9,300	1.66
21	6,540	1.79
28	5,100	1.16
35	2,900	1.08
42	2,860	1.29

18" STRAW FILTER

DAY	BOD ₅	SOLIDS CONTENT %
7	2,812	1.70
14	15,600	4.62
21	10,280	2.97
28	8,324	1.37
35	3,080	1.46
42	1,600	0.84

BOD₅ of pre-filtered waste = 11,900 mg/l (7 days)
18,950 mg/l (35 days)

Solids content of pre-filtered waste = 6.42%

part in BOD determinations and the authors state that a strong correlation only exists in diluted animal wastes where the effects of other chemicals, in lower concentrations, are minimised.

The copper content of the filtrate remained fairly constant at about 9 ppm over the 6 weeks. The amount of copper in the feedstuff was 180 ppm and thus the pigs probably retained much of it.

The results for the 15" and 18" straw filters are shown in table 18. This investigation was conducted for 6 weeks and it can be seen that there was a further BOD decrease in the 6th week. After 5 weeks the BOD contents for the 15" and 18" filters (2,900 mg/litre and 3,080 mg/litre) were similar to those for the 4" and 12" filters (3,000 and 2,040 mg/litre) after 5 and 4 weeks respectively. The solids contents, however, were lower in comparison to the 4" and 12" filters, the final figures being 1.29% (15" filter) and 0.84% (18" filter). The pre-filtered solids content was determined as 6.42%. Two pre-filtered BOD determinations showed BOD's of between 11,900 mg/litre after 7 days and 18,950 mg/litre after 35 days. This showed reductions of between 80 and 85% after 7, 35 and 42 days but, as for the 4" and 12" filters there was an increase in which the physical and biological activity was not very efficient. The low figure after 7 days may have been due to the amount of waste produced which was probably less when the pigs were just weaned. This increase and subsequent decrease in BOD suggests that the build-up of solid material may aid the filtration activity retaining more solids and encouraging biological utilisation of carbon and nitrogen, thus reducing the BOD.

It was originally envisaged that a build-up of liquid would occur on top of the filter. Such 'ponding' did not in practice occur, the solid waste building up above the straw and the liquid trickling through (see Plate 14). In practice although the 4" filter did not give improved analysis results it was found that a thin layer of straw placed on the solid fraction each week gave a product of a more loose consistency which might be better for retaining aerobic conditions in the thermophilic stage. The build-up of solid above the filter did not reach the top slats after 6 weeks when there was a 24" space between the two slats. The space beneath the bottom slat certainly improved the odour of the solid waste. As opposed to the grey evil smelling untreated slurry the liquid fraction had a brown colour, was almost odourless (see Plate 15) probably being aerated as it dripped from the bottom slat to the floor. The normal pig odour, a not unpleasant and lingering odour, could not be removed, but this would not cause any nuisance problems as dispersal in the air was complete.

The labour required for the maintenance of the filter was nil. It was only necessary to feed the pigs and encourage them to walk on the slats. Although the farmer had some reservations about the pen design he thought the straw filter, if it could be treated, would provide a cheap and passive method of slurry treatment.

The isolation programme carried out on the 4" and 12"



PLATE 14

SOLID WASTE COLLECTION ON TOP OF 4" FILTER

Note screened substrate tubes buried in straw
and detected by long metal loops



PLATE 15

A COMPARISON OF UNFILTERED (left) AND FILTERED
(right) WASTE FROM 4" FILTER

Note foam present on left but not right

PERCENTAGE FREQUENCY OF ISOLATION OF FUNGI

FROM 4" STRAW FILTER

a. Glucose starch at 25°C					
Species \ No. weeks	0	1	2	3.5	5
Cladosporium sp.	50	-	-	-	-
Penicillium spp.	10	-	40	60	100
Cephalosporium sp.	30	-	-	-	-
Trichoderma viride	-	20	10	-	-
Mucor sp.	-	-	40	100	100
Aspergillus fumigatus	-	-	40	100	100
Fusarium sp.	-	-	-	100	-
Aspergillus sp.	-	-	-	60	-
Aspergillus niger	-	-	-	20	-
b. Cellulose agar at 25°C					
Species \ No. weeks	0	1	2	3.5	5
Humicola sp.	50	40	80	60	20
Cladosporium sp.	40	-	-	-	-
Chaetomium sp.	10	-	20	-	-
Graphium sp.	10	-	-	-	-
Torula sp.	10	-	20	-	-
Cephalosporium sp.	50	-	-	-	-
Trichoderma viride	-	10	-	60	20
Penicillium sp.	-	10	40	60	40
Monilia sp.	-	-	40	60	60
Paecilomyces sp.	-	-	10	40	-
Fusarium sp.	-	-	40	20	-
Aspergillus fumigatus	-	-	20	40	40
Mucor sp.	-	-	80	60	80
Phoma sp.	-	-	-	-	40

TABLE 20

PERCENTAGE FREQUENCY OF ISOLATION OF FUNGIFROM 12" STRAW FILTER

a. Glucose starch at 25°C					
Species \ No. weeks	0	1	2	3.5	5
Cladosporium sp.	50	-	-	-	-
Penicillium spp.	10	-	-	100	80
Cephalosporium sp.	30	-	-	-	-
Mucor sp.	-	-	30	100	100
Aspergillus fumigatus	-	-	-	100	100
Aspergillus spp.	-	-	-	100	-
Fusarium sp.	-	-	-	100	-
Trichoderma viride	-	-	-	100	-
Aspergillus niger	-	-	-	20	-
b. Cellulose agar at 25°C					
Species \ No. weeks	0	1	2	3.5	5
Humicola sp.	50	60	100	70	70
Cladosporium sp.	40	-	-	-	-
Chaetomium sp.	10	-	20	-	-
Graphium sp.	10	-	-	-	-
Torula sp.	10	-	20	-	-
Cephalosporium sp.	50	40	-	-	-
Mucor sp.	-	-	20	20	90
Penicillium spp.	-	-	20	-	20
Fusarium sp.	-	-	-	60	-
Aspergillus fumigatus	-	-	-	40	30
Monilia sp.	-	-	-	60	100
Trichoderma viride	-	-	-	70	40
Phoma sp.	-	-	-	-	80

filters (Tables 19 & 20) showed that the fungi isolated from dilution plates constituted a range of cellulolytic and non-cellulolytic soil inhabiting flora, capable of colonising the filter under the right condition of growth. The isolation of new species not present initially showed that fungal spores from the air or the waste were being introduced; Aspergillus fumigatus illustrates this, not being isolated until the second week from the 4" filter and not until the third week for the 12" filter. The establishment of a flora with a high percentage frequency of isolation took between 2 and 3 weeks to appear, although the fungi present in the straw initially, with the exception of Humicola sp. and Penicillium spp., were not isolated after the second week.

The screened substrate tubes, which indicated those fungi actively growing, showed (Tables 21 and 22) a more narrow number growing on the filter. Both Mucor sp. and Aspergillus fumigatus were isolated throughout the investigation whilst Chaetomium sp. and Monilia sp. did not appear until the third and fifth weeks respectively.

For both the dilution plates and screened substrate tube isolations similar fungal floras for the 4" and 12" filters were revealed. The range of fungi shows that species were present which were able to colonise a range of substrates under varying pH conditions. However, the biodegradation analyses indicated that substrate utilisation

ISOLATION OF FUNGI FROM SCREENED SUBSTRATE
TUBES BURIED IN 4" STRAW FILTER

a. From glucose starch at 25°C				
Species \ No. weeks	1	2	3.5	5
Aspergillus fumigatus	+	-	+	-
Penicillium sp.	+	-	+	+
Trichoderma viride	+	-	-	-
Mucor sp.	-	+	+	+
Chaetomium sp	-	-	-	+
Humicola sp.	-	-	-	+
Monilia	-	-	-	+
b. From cellulose at 25°C				
Species \ No. weeks	1	2	3.5	5
Humicola sp.	+	+	-	+
Penicillium spp.	+	-	-	+
Botrytis sp.	-	+	-	-
Gliocladium sp.	-	+	-	-
Mucor sp.	-	+	+	+
Monilia	-	-	-	+

+ = species isolated on medium
- = species absent from medium

TABLE 22

ISOLATION OF FUNGI FROM SCREENED SUBSTRATE

TUBES BURIED IN 12" STRAW FILTER

a. <u>From glucose starch at 25°C</u>				
Species \ No. weeks	1	2	3.5	5
Mucor sp.	+	+	+	+
Aspergillus fumigatus	+	+	-	+
Penicillium sp.	+	-	-	-
Humicola sp.	-	-	+	+
Chaetomium sp.	-	-	+	+
Monilia	-	-	-	+

b. <u>From cellulose at 25°C</u>				
Species \ No. weeks	1	2	3.5	5
Humicola sp.	+	-	+	+
Mucor sp.	-	-	+	+
Chaetomium sp.	-	-	+	+
Penicillium sp.	-	-	-	+
Monilia sp.	-	-	-	+

was restricted due to the daily loading of fresh substrate. It seems likely that bacteria, because of the alkaline pH, were responsible for the reductions in BOD and nitrate uptake, if biological action made a contribution. As no adverse odours associated with anaerobicity occurred, the bacteria were probably aerobic and thus more desirable than the anaerobes in such a system.

This investigation, by no means complete at this stage, has shown that raw pig waste can be passively and cheaply filtered 'in situ' to reduce the polluting ability and solids contents of the waste, and increase its handling characteristics. The depth of the filter seemed to have little effect on the quality of the end product. However, important factors such as the effects of the solid fraction as a filter aid and the best way to load fresh straw onto the filter at intervals need to be investigated.

8.3 DEVELOPMENT OF THE THERMOPHILIC STAGE

8.3.1 Introduction

In the previous Chapters an investigation of the parameters for biodegradation has suggested that at temperatures at or above 40°C the rate of breakdown and the stabilisation of pig waste can be increased over both mesophilic and natural composting conditions. The parameters of temperature,

pH, aeration, inocula addition and the length of the process have all provided important information which can be used in the design and operation of a pilot-scale plant to biodegrade pig waste produced from the pigs on the straw-pad filtration system thus linking the mesophilic and thermophilic stages described at the end of Chapter 1. A further link is the use of the thermophilic stage to trickle the liquid run-off from the pads in order to further purify the liquid. The investigation below will elucidate the design and the efficiency of operation of the tower.

8.3.2 Design and construction of plant

At the end of Chapter 1 a thermophilic stage was briefly outlined, and in Chapters 4 and 5 laboratory scale investigations determined the optimum conditions necessary for a quick efficient breakdown of the waste at thermophilic temperatures. From this data it was possible to design a pilot-scale 'on farm' plant to be linked (see figure 55A) with the pens described previously in this Chapter. The final design adopted is shown in figure 55. It consists of a tower made of concrete blocks (46 cm x 23 cm x 15 cm) with four 'Weldmesh' platforms (122 cm x 122 cm) at intervals of 60 cm. Beneath the bottom platform a sloping drain was placed which led to a storage tank. Holes were drilled into the walls beneath the bottom platform to allow air to be drawn in passively. The front was covered with an insulated

FIGURE 55

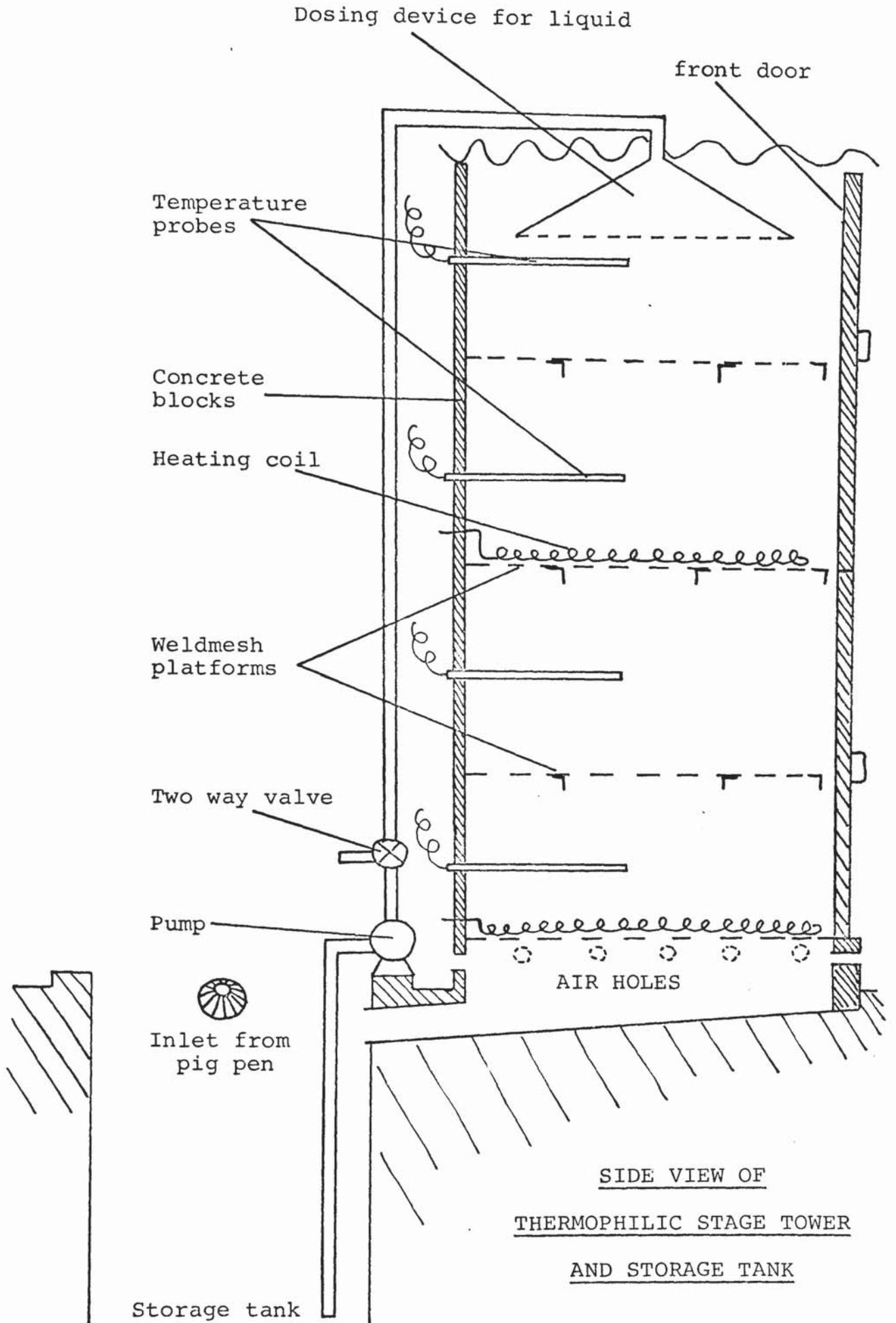
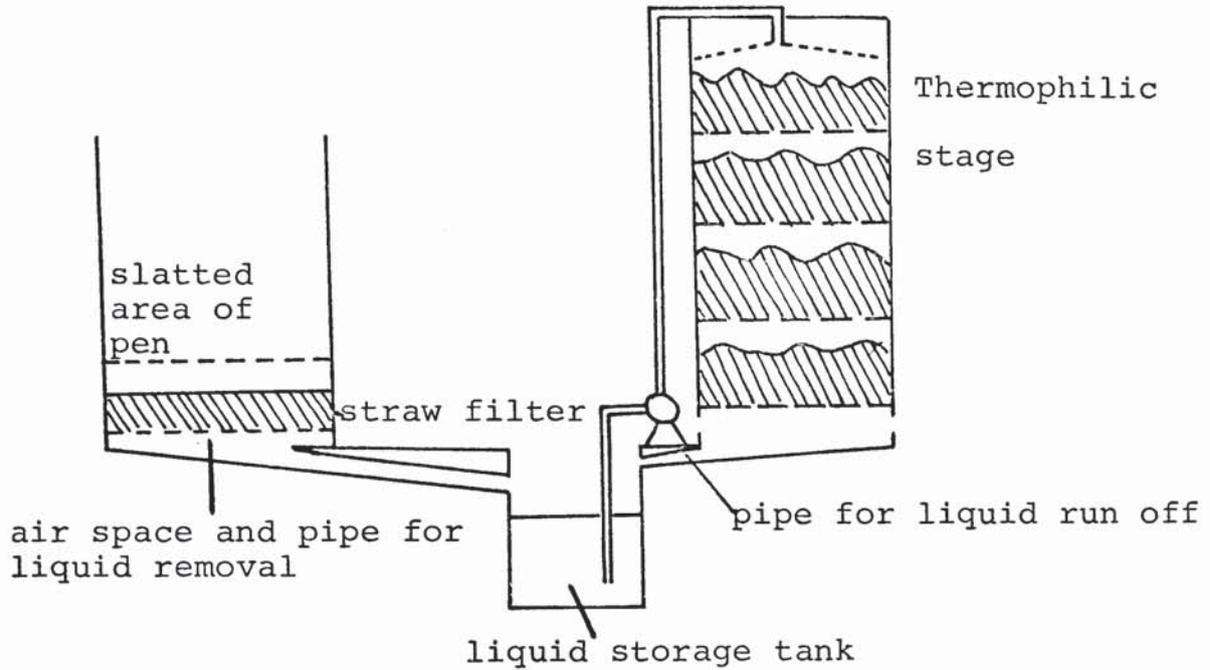


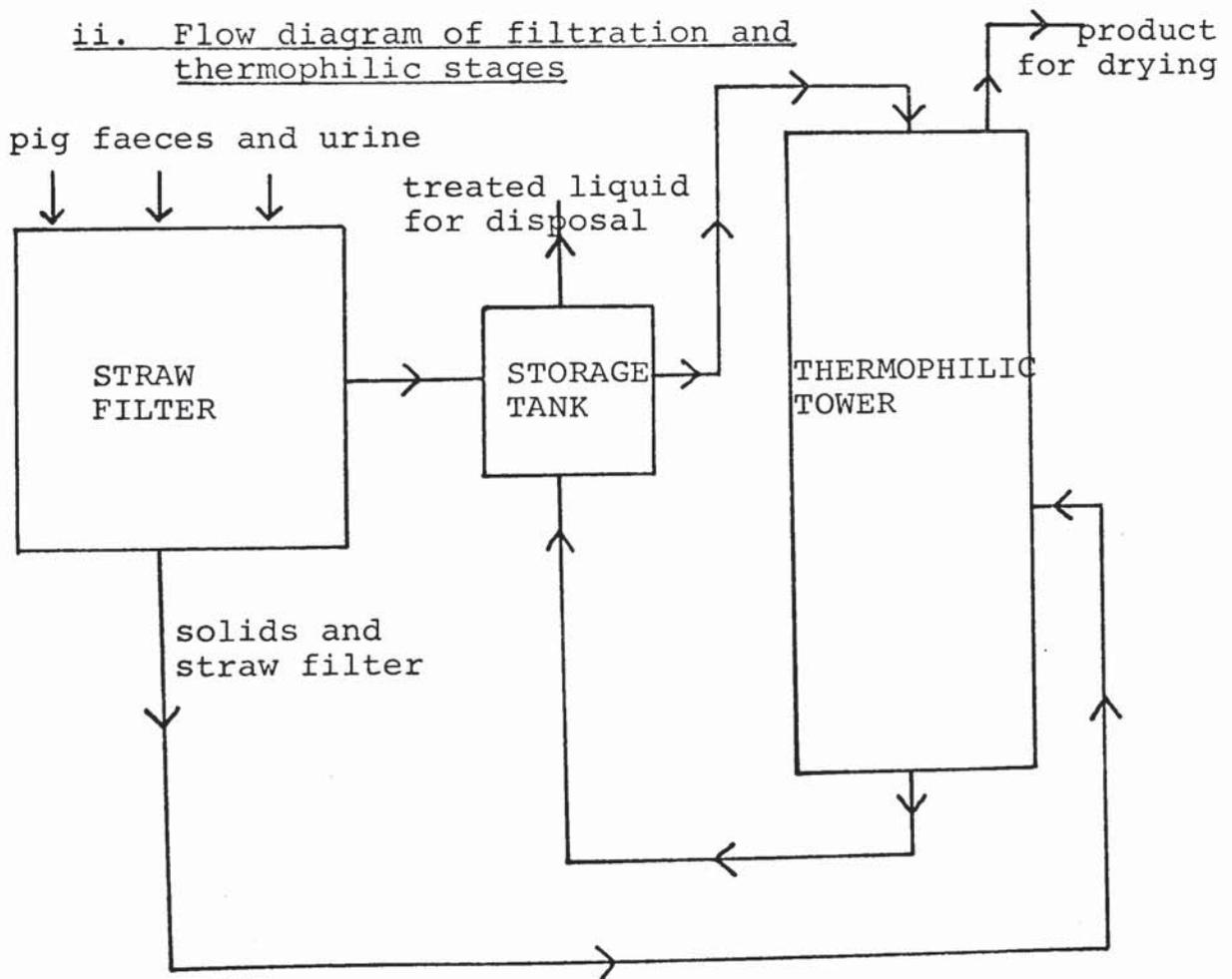
FIGURE 55A

ARRANGEMENT OF PIG PEN WITH TOWER

i. Side view of one pen with thermophilic tower



ii. Flow diagram of filtration and thermophilic stages



door in two sections and the roof made up of corrugated iron sheets so that air could pass out through the corrugations. Temperature probes were inserted at each level in the form of thermistors enclosed in aluminium tubing. Silicon rubber tubing placed beside the thermistors was used to remove air for the determination of oxygen to carbon dioxide ratios. Provision was made to enable heating cable to be placed on the platforms together with the necessary thermostats. From data obtained from the compost heap experiments in Chapter 3 it was decided that a volume of waste (122 cm x 122 cm x 61 cm) would heat up to thermophilic temperatures and thus each level in the tower occupied such a volume. Four platforms were used (Plate 16) because, for practical reasons, this was the maximum height (240 cm) at which the waste could be loaded manually. A discussion of the aeration requirements in Chapter 5 showed that, although only a low rate of aeration was needed, it was necessary to keep the waste aerobic. Thus, the holes at the bottom of the tower were to encourage slow but definite passive flow of air which would be stimulated by an increase in the temperature of the tower contents. A space of about 7.6 cm between each level would also aid aeration of the level above and also transfer heat. In this way it was hoped that heated air would aid the establishment and maintenance of a thermophilic phase. It was envisaged that the liquid run-off from the straw pad beneath the pens might be further purified by passing it through the tower contents. In this way any additives to control pH or encourage growth could be



PLATE 16

PILOT SCALE TOWER FOR THERMOPHILIC STAGE
WITH FOUR PLATFORMS

inserted. The drain at the bottom of the tower was designed to channel this run-off back to a storage tank. The front of the tower was made as a removable door both for sampling purposes and to enable the platforms to be front loaded.

It was further proposed that the tower act as a multi-operation plant, that is, more than one stage of the treatment process be carried out within the same plant. This would simplify the process from engineering and practical aspects, reduce the area required for treatment and perhaps the time required for the treatment. Thus it was proposed that the tower might be used to dry and sterilise the product after the thermophilic stage and whilst the product was still 'in situ'.

Apart from designing the tower according to the parameters investigated on a laboratory scale, the ease of operation by the farmer was also incorporated. Access by a tractor with a front loading mechanism to introduce the fresh waste was allowed for and the farm staff were encouraged to help run the tower and pens.

The waste to be placed on each platform was to come from the accompanying straw pads which were to be completely removed and placed on the platforms. In this way the solid fraction consisting of the filtered solids and the straw could undergo thermophilic biodegradation. However, before this was possible, and to test the operation of the tower and

the sampling procedures, a preliminary experiment was set up using deep litter waste - also a mixture of dung and straw, but usually more compacted. It was hoped that this would provide a 25% starter inoculum for mixing with the straw pad in subsequent runs. It will be seen, however, that time allowed only for the completion of the preliminary run although it is proposed to continue the operation of the tower.

8.3.3 Methods and materials

Each of the four platforms (numbered 1 to 4 from the top) was loaded with deep litter bedding to a depth of about 54 cm. A temperature probe was inserted into the middle of the waste and six screened substrate tubes, each containing five strips of polythene-backed cellulose paper for fungal isolations, were placed throughout the waste. Initial samples were taken from the four levels and the door put in place. At the same time two pens were set up containing 17 weaned pigs each and a straw pad 4" deep (Plate 17). The pigs were encouraged to dung on the slatted area and to go to the water by barring them entry to the covered section of the pen. The following analyses were made on the solid fraction from the tower and the liquid fractions from the straw pad:-



PLATE 17

ARRANGEMENT OF PEN AND TOWER ON FARM

TEST	SOLID	LIQUID
Temperature °C	+	-
Moisture content %	+	-
pH	+	-
Carbon content %	+	-
Nitrogen content %	+	-
Starch	+	-
Hemicellulose	+	-
Cellulose	+	-
BOD ₅	-	+
Fungal succession	+	-
Pathogen count	+	-

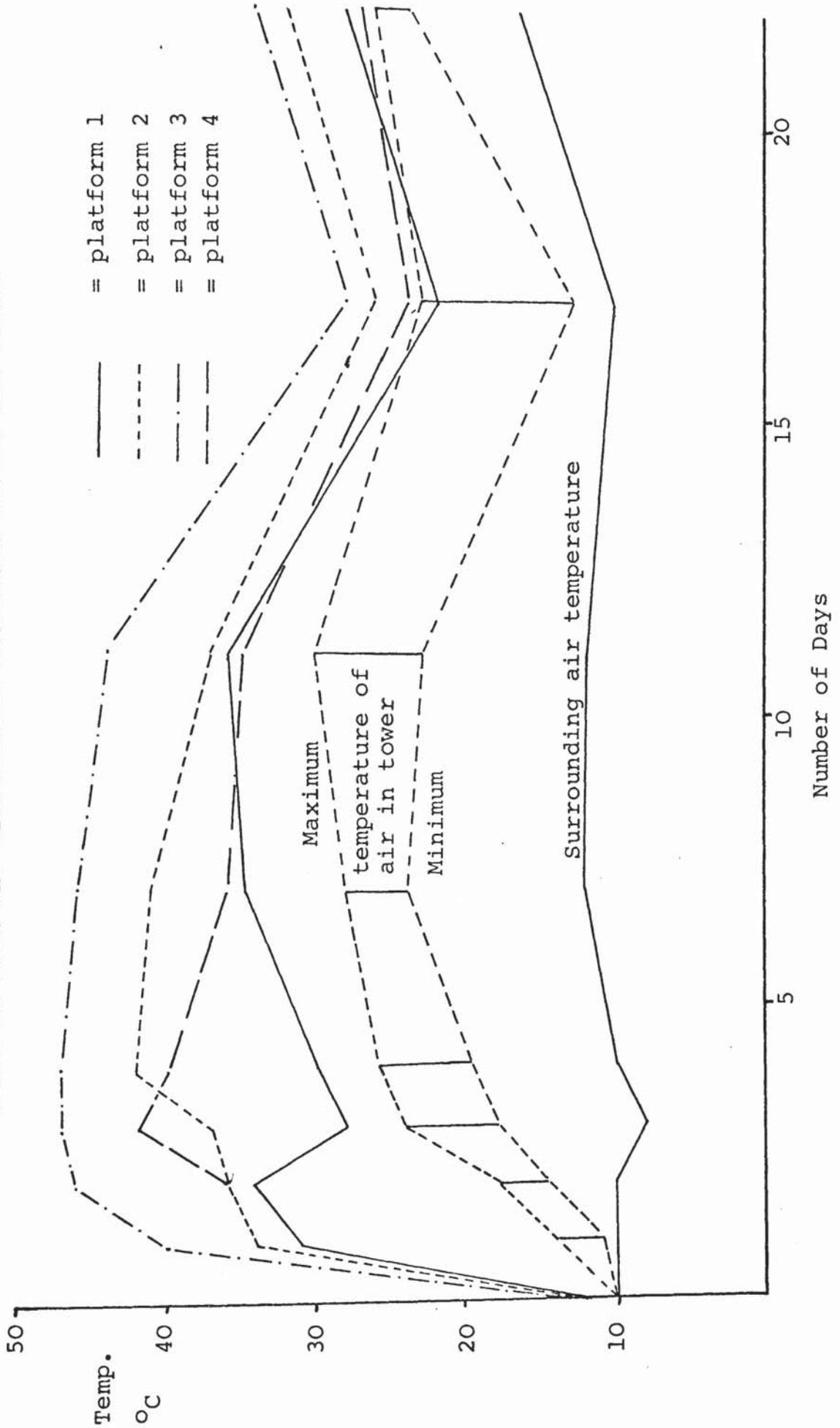
A sample of the raw faeces plus urine was also collected and a determination of solids and BOD₅ made. A note was made of the amount of liquid collected in the tank per week so that an estimate of the amount produced per pig per day could be made.

8.3.4 Results and discussion

The temperature changes within the tower are shown in figure 56. It can be seen that throughout the run there was a temperature gradient between the platforms. Platform 3 maintained the highest temperatures whilst platform 1 maintained the lowest, and platforms 2 and 4 lay between these two limits. In particular the highest temperatures recorded were 47°C (platform 3 after 3 days), 42°C (platform 4 after 3 days), 42°C (platform 2 after 4 days), and 36°C (platform 1 after 11 days). The temperature for platform 3 remained above 40°C between the 1st and 11th day, whereas platforms 2 and 4 were only above 40°C between the 3rd and 7th day, and platform 1 never reached 40°C. In all cases the initial rate of temperature increase was greatest in the first day after which there was a slow decrease in temperature back to mesophilic temperatures which all the platforms had reached after 17 days. However, it can be seen that there was an increase between the 17th and 22nd day. This may have been due to two factors. The first was the sudden increase in air temperature from between 10° and 12°C up to 16.5°C which affected the air temperature in the tower minimising the difference in maximum and minimum temperatures. The second factor may have been the addition which was made on day 17 of liquid filtrate from the pig pens which introduced new substrate and bacteria, causing renewed activity and an increase in temperature. It is likely that the effect of both the air temperature and

FIGURE 56

GRAPH TO SHOW THE TEMPERATURE INCREASES WITHIN THE TOWER



substrate addition was important in affecting the temperature of the waste. It is interesting to note that in the 17th day when the tower waste was at its lowest temperature the gap between the maximum and minimum air temperatures was also greatest ($23^{\circ} - 13^{\circ}\text{C}$) and it may be that a low night temperature caused the drop in temperature. As only the day temperature of the air was recorded it was not possible to show the extent to which the surrounding temperature dropped but it is probable that it did.

The temperature results in this preliminary experiment have shown that it is possible to attain thermophilic temperatures on a large scale without the input of heating. The gradient obtained in this experiment has shown that insulation is important in maintaining this phase and that the use of heating coils to maintain a defined temperature might be necessary. The laboratory scale work indicated that 48°C was the optimum temperature for maximum biodegradation. In this run platform 3, which was well insulated by the other platforms, reached 47°C and remained at 46°C for 5 days. If insulation could be provided around the tower very little heat input would probably be necessary to attain and maintain 48°C for the length of the thermophilic stage.

Table 23 shows the analysis results for the solid waste from the tower. The overall pH change was small - from 9.0 to 8.50 - although the product after 22 days had an earthy smell and was easy to handle and crumble. It was probable

TABLE 23
ANALYSES FROM FIRST TOWER RUN

No. of Days analysis	0	1	2	3	4	11	17	22
pH	9.0	9.0	8.8	8.6	8.7	8.3	8.7	8.5
Moisture %	77.83	76.99	-	71.97	76.50	71.10	74.00	72.00
Nitrogen %	2.15	2.08	1.92	1.88	1.79	1.98	2.28	2.39
Ash %	19.52	-	-	20.00	24.64	29.60	22.20	23.70
Carbon %	44.71	-	-	44.44	41.85	39.10	43.50	42.60
Cellulose%	29.5	-	-	-	-	-	-	11.00

*Average of 8 samples (P < 0.05)

that actinomycetes were important in the process although fungi were isolated (see below). There was no initial increase in pH suggesting no large scale ammonia loss. However, the organic nitrogen dropped due either to ammonia loss or conversion to inorganic nitrates, but this then rose to 2.39% which was greater than the original content (2.15%). This showed conversion of the inorganic nitrates back to organic nitrogen, probably as protein. The crude protein value rose from 13.5% to 15% over the 22 days and thus biodegradation had taken place. The carbon level fell from 44.7% to 39.1% after 11 days but then rose to 42.6% after 22 days. This may have been due to sampling error but did show that biodegradation of the carbon sources had taken place. An average reduction of 8.6% of the total organic carbon content compared with the carbon reduction in the 50°C laboratory run (see figure 23) shows that the results are very similar, the 50°C run having an average decrease of about 10% of the total carbon. This difference was probably due to the lower temperatures attained in the tower run. However, it is hoped that control of the temperature and the addition of a proportion of the end product may well increase the biodegradation to equal the figures obtained for similar runs in the laboratory.

The cellulose decrease from 29.5% to 11.0% represented an overall decrease of 63% cellulose. This was a large improvement on the laboratory-scale results after 17 days of 38% at 48°C. However, it would be necessary to do more

TABLE 24

ANALYSIS OF LIQUID FILTRATE FROM PIG PENS

Sample Day	Liquid from filter (mg/litre)	Sample from storage tank (mg/litre)	Solids content (Average of two pens) (%)
0	-	-	-
1	12,340	-	-
4	7,810	-	3.1
7	8,640	-	3.0
17	1,540	1,040	-
22	1,700	1,200	1.29

1. BOD_5 of prefiltered waste = 13,280 mg/litre

2. Solids content of prefiltered waste = 23.0%

3. Figures are expressed as averages of the two pens

large-scale work to support these results.

An analysis of phosphate from the liquid run-off from the two pig pens with the 4 inch filter showed a small decrease from 925 ppm to 836 ppm over 17 days and the nitrate contents dropped from an average of 10 ppm to 6 ppm. These levels are both very low for spraying purposes where they would be easily dispersed in the soil. The BOD₅ (see table 24) of the liquid decreased from 12,340 mg/litre on day one to 1,700 mg/litre at day 22 a reduction of about 85%. The pre-filtered waste had a BOD₅ of 13,280 mg/litre so that only a small reduction occurred after one day. However, after 4 days this reduction had increased to about 50%. The solids content of the waste was reduced from 23.0% to 1.29% after 22 days. The high initial figure may have been due to evaporation in the hot weather. However, a low solids content of just over 1% did indicate that a reduction had taken place removing solids which might have caused handling problems. The emptying of the storage tank presented no difficulties and there was only about 5 mm of sludge on the bottom which could be washed out easily.

The isolations from the tower (Tables 25 & 26) showed a good range of mesophilic and thermophilic cellulolytic fungi together with actinomycetes which were persistent throughout the process. Most of the mesophiles appeared only at the beginning and end of the run when the temperature was within their range for growth. The isolation frequencies of the thermophiles

were fairly constant, Torula thermophila although only being isolated after the thermophilic stage when the temperature fell below 40°C. Aspergillus fumigatus was very persistent at all the isolations, having overall higher percentage frequencies of isolation at 40°C where it was at its optimum growth rate. It is interesting to note that more thermophiles (4) were isolated at 40°C on cellulose agar than at 48°C (2). This may have been due to the average thermophilic temperature which was between 40 and 45°C. The thermophiles may have become adapted to this range and preferred it in the isolation work. Although not isolated in the laboratory, Coprinus sp. fruiting bodies were observed growing on the waste when it had cooled to below 40°C. These remained abundant until the end of the investigation probably contributing to the biodegradation process.

Although the suppression of Mucor pusillus was complete at 48°C it was still in evidence at 40°C with isolation frequencies of 20% and 60%. However, the presence of large quantities of cellulose, which Mucor pusillus could not utilise, probably acted as a control mechanism. By monitoring the amounts of liquid produced from the pens it was possible to determine the quantity of filtrate produced per pig per day. In the first eleven days the average was 0.4 litres/pig/day. As the pigs grew the average increased to 1.2 litres/pig/day and remained at this level. The averages of 5 litres per day which were mentioned as a working average were above this figure probably because they included rain water which was excluded from these pens and the storage tank.

TABLE 25

PERCENTAGE FREQUENCIES OF ACTINOMYCETES AND FUNGI ISOLATED FROM THE TOWER ON EGGINS AND PUGH CELLULOSE AGAR

Species	No. of Days			
	2	4	11	22
<u>Isolations at 25°C</u>				
Actinomycetes	10	60	40	20
Aspergillus fumigatus	40	40	40	60
Aspergillus sp.	10	-	-	-
Cephalosporium sp.	-	-	-	20
Eurotium sp.	10	-	-	-
Graphium sp.	20	-	-	-
Humicola sp.	60	40	40	20
Monilia sp.	-	-	-	20
Mucor sp.	10	-	80	60
Paecilomyces sp.	-	-	-	20
Phoma sp.	-	10	40	60
Trichoderma viride	60	-	-	-
<u>Isolations at 40°C</u>				
Actinomycetes	20	20	-	-
Aspergillus fumigatus	60	20	100	80
Chaetomium thermophile	10	-	-	-
Humicola lanuginosa	10	-	-	-
Mucor pusillus	-	-	-	60
Torula thermophila	-	-	-	40
<u>Isolations at 48°C</u>				
Actinomycetes	30	20	20	20
Aspergillus fumigatus	40	40	80	60
Humicola lanuginosa	30	15	40	20
Torula thermophila	-	-	-	100

TABLE 26

PERCENTAGE FREQUENCIES OF ACTINOMYCETES AND FUNGI ISOLATED
FROM THE TOWER ON GLUCOSE STARCH AGAR

No. of Days Species	2	4	11	22
<u>Isolations at 25°C</u>				
Actinomycetes	60	40	20	20
Aspergillus fumigatus	70	60	40	60
Aspergillus sp.	10	-	-	-
Eurotium sp.	40	-	-	-
Mucor sp.	20	40	40	20
Penicillium sp.	60	20	-	-
<u>Isolations at 40°C</u>				
Actinomycetes	50	30	-	20
Aspergillus fumigatus	40	40	100	30
Mucor pusillus	20	-	-	20
Torula thermophila	-	-	-	20
<u>Isolations at 48°C</u>				
Actinomycetes	80	30	20	100
Aspergillus fumigatus	30	20	80	-
Humicola lanuginosa	20	-	20	-

The results so far obtained from the large-scale thermophilic stage have supported the laboratory-scale work in that the thermophilic fungi were dominant and that they could cause large amounts of breakdown and stabilisation of the waste. The results obtained do not indicate that all the problems have been solved and that full-scale commercial operation could begin but they do offer some direction to any future research.

8.4 A DISCUSSION OF THE ECONOMICS OF THE PROCESS

A very rough economic comparison of the two stage process with other complete systems will demonstrate firstly how inexpensive this system is for what it achieves, and secondly that the fate of all the products has been postulated in a more positive fashion than the other systems, usually involving economic returns for the capital outlay. The comparison is best made in a table form (Table 26a). The other two systems are the "Halmarl" system and the "Porcomat" system, the latter coming from Switzerland and the former from England. The Halmarl system is very similar to human sewage treatment but involves a more vigorous aeration and biological treatment unit, and incorporates an electrolytic method of solids separation. The Porcomat claims to use chemical methods of stabilisation which are not sensitive to varying environmental conditions. It can be seen that although the other systems do a more complete

TABLE 26a
COMPARISON OF TWO COMPLETE PIG WASTE TREATMENT SYSTEMS WITH THE BENEATH PEN SYSTEM

	Beneath Pen System	Halmarl System	Porcomat System
Type of treatment	Mechanical and biological	Mechanical and biological	Mechanical and chemical
Extent of treatment	Produces upgraded, odourless solid product and reduces BOD ₅ of liquid to about 1,000 mg/litre	Produces fibrous solid and liquid with BOD ₅ to Royal Commission standards	Produces fibrous solid and water for washing down purposes
Specialised equipment used	Heating coils	Aeration equipment electrolytic tanks	Homogeniser, rotating drum, catalyst
Maintenance	Low to maintain as stages are few	Low when working	Low when working
Types of waste treated	Semi-solid and liquid slurry	Liquid slurry	Liquid slurry
Capital cost per 1,000 pigs	About £1,000	£5,000	Not known
Running costs/1,000 pigs	Equivalent to cost of heating the waste	£800 per year	About £200 per year
Economic returns	Feed additive from solid	None	Solid as fertiliser or feed additive
Fate of products	Recycling of solid and spraying of liquid	Solid - not known liquid - discharged to stream	Solid - fertiliser or feedstuff liquid - washwater
Space required	Separation stage in pig house and thermophilic stage in tower form	12' x 12' for 2,000 pigs	4 x 4 x 2 metres for 1,000 pigs
Type of process and number of stages	Batch with two stages	Continuous with 6 stages	Continuous with 4 stages

treatment process they need a slurry for operation, the fates of the products are less defined, the number of stages are greater and this involves more maintenance. Also, as far as operation is concerned, more specialised equipment is needed and trained men would be needed for repairs. The capital cost of the beneath pen system has been roughly estimated at £1,000. This is a fifth of the cost of the Halmarl system which probably has a similar capital cost to the Porcomat system. The running costs of the beneath-pen system would depend upon the requirement of heating and it is likely that because it is a batch process operating for 3 weeks in about 6 weeks the yearly costs would be very low, in comparison to a continuous process. A detailed cost analysis is impossible on the pilot scale process at present but when more work has been done it seems likely that the system may be able to compete with other complete treatment systems costing much more.

The results from the liquid run-off have shown that there are still nitrates and phosphates remaining, and that the BOD₅ is still about 1,000 mg/litre. This would indicate that further treatment may be possible or that disposal to fields would be of more benefit to crop fertility. Further treatment methods for the liquid are discussed in the next section.

8.5 AN EXAMINATION OF A FURTHER TREATMENT AND DISPOSAL
PROCESS FOR THE LIQUID RUN-OFF

It was expressed earlier that the liquid fraction from the straw filter, although considerably decreased in its polluting ability, would still need further secondary treatment if it were to be released into sewage systems or streams. Various areas of research have been carried out to try to treat such liquids with low solids contents in an attempt to remove inorganic nitrates and phosphates which have been shown to be little affected by the straw filter.

Sir Albert Howard (1946) first noted the use to which certain water plants could be used to take up the inorganic nitrate, phosphate and potassium. He was particularly interested in the water hyacinth (Eichhornia crassipes) and found that it could absorb large quantities of nitrogen, phosphorus and potassium from polluted water and could then be composted, the plant supplying enough water for watering to be unnecessary.

Since Howard's preliminary observations very little has been done until the series of papers by Boyd and his co-workers who have investigated the possibility of using fresh-water plants as protein sources after cleaning up polluted water. Boyd (1968) found that, on analysing a range of fresh-water plants for crude protein, the range was from 8.5% up to 31.3%. Of the 40 species studied 9 had

levels above 20% and 20 had levels above 15%. He suggests that use of these plants as food could alleviate world shortages but more work would need to be done to obtain a constant crop. Boyd then studied the nutritive value (Boyd, 1969) of 3 of the most serious water plant pests: Eichhornia crassipes, Pistia stratiotes (water lettuce) and Hydrilla sp. He found that protein and amino acid levels were comparable to other types of conventional high quality forage. However, this was after drying whereas in the fresh plant the protein level was below 2%. This would indicate dehydration prior to use as a feedstuff.

The possibility of mineral nutrient removal by water plants has been discussed by Boyd (1970). He studied the uptake of, in particular, nitrogen, phosphorus and potassium (see Table 27) by Eichhornia crassipes, Alternanthera philoxeroides, Justicia americana and Typha latifolia. One hectare of Eichhornia crassipes for example could remove 1,980 kg. nitrogen, 322 kg. phosphorus and 3,188 kg. potassium per year by continual culture. The possibility for use as a human protein source was also postulated (see Table 28) and it was found that from Justicia americana, one hectare could supply 301 persons with 70 g. of leaf protein concentrate (an accepted form and level of protein) for one year.

Thus it seems clear that, although the economics of such a system need to be determined, certain water weeds, under controlled conditions, could be encouraged to grow in artificial

TABLE 27

QUANTITIES OF ELEMENTS (kg/ha) THAT COULD BE
REMOVED PER YEAR BY CONTINUAL CULTURE OF SOME
AQUATIC PLANTS

Element	<i>Eichhornia crassipes</i>	<i>Justicia americana</i>	<i>Alternanthera philoxeroides</i>	<i>Typha latifolia</i>
Nitrogen	1,980	2,293	1,779	2,630
Phosphorus	322	136	198	403
Sulphur	248	204	180	250
Calcium	750	1,022	322	1,709
Magnesium	788	465	322	307
Potassium	3,188	3,723	3,224	4,570
Sodium	255	193	229	730
Iron	19	123	45	23
Manganese	296	13	27	79
Zinc	4	30	6	6
Copper	1	3	1	7

TABLE 28

METRIC TONNES OF CRUDE PROTEIN IN FODDER OR
PROTEIN IN LEAF PROTEIN CONCENTRATE THAT
COULD BE OBTAINED ANNUALLY FOR CONTINUAL
CULTURE OF ONE HECTARE OF SOME AQUATIC
VASCULAR PLANTS

Species	Crude protein in fodder	Crude protein in leaf protein concentrate
<i>Eichhornia crassipes</i>	12.4	-
<i>Justicia americana</i>	14.3	7.7
<i>Alternanthera philoxeroides</i>	11.1	4.7
<i>Typha latifolia</i>	16.4	-

ponds containing liquid effluent and it may be that the liquid from the thermophilic tower would be a good source of minerals. The work mentioned above gave the results from work done where cultivation is possible through the year. In Great Britain species would have to be selected which were either able to survive the winter or could be artificially heated. The use of water plants as sources of protein for pigs may provide a cheap alternative to imported additives by using the liquid effluent from the piggeries. A simple calculation below will indicate the number of pigs which could be supplied with this form of protein.

From the literature, in one year one pig will produce 9.5 kg. nitrogen which is available for plants. 1 ha. of Justicia americana is able to absorb 2,293 kg. nitrogen per year (see Table 28). Thus this amount of Justicia americana could remove the nitrogen from:-

$$\frac{2,293}{9.5} = 230 \text{ pigs}$$

10 ha. would be needed to remove the nitrogen from 2,500 pigs.

Fattening pigs are fed 290 g. protein per day. Justicia americana produced 7.7 metric tonnes of leaf protein concentrate per year. Thus about 700 pigs could obtain all their protein requirements from 1 ha. of continuously cultivated Justicia americana. This means that 28% of the 2,500 pigs from which the waste was obtained could have their nitrogen recycled.

By substituting for half the total protein double the number of pigs could be served.

Much work would need to be done on the design and flow rate into such reservoirs of plants, and the best method of harvesting and protein extraction, but it could provide another cheap feed additive whilst helping to prevent pollution and eutrophication by inorganic nitrates and phosphates. Work done (Cable, 1969) using grass-soil filters over large areas have shown that human waste waters can be treated from the secondary filtration stage to remove phosphates, nitrates, bacteria and viruses, and to reduce the BOD from 200 - 0.2 ppm. Bermuda grass has been shown to give the best mineral uptake figures although common salt build-up has been a problem in the reclaimed water. However, it is thought that desalinisation can be instituted.

The possible use of the liquid slurry as a fertiliser has been discussed and evaluated by several authors (Jones and Riley, 1970; Gowan, 1972). The Ministry of Agriculture, Fisheries and Foods has issued figures for the relative NKP values of fresh manure (see Table 29). However, such values will vary according to whether the manure is a slurry or has straw added (Gowan, 1972). The concentration of nitrogen, potassium and phosphorus from animal wastes either by the absorption by plants, the soil (in the case of phosphorus) (Taylor, 1967), or by chemical means (Salutskey et al., 1972) will serve to increase the value of the

product whilst extracting potential pollutants which would otherwise be lost from the system.

TABLE 29

The fertiliser values (per ton) of fresh manure
in 1970

(Jones and Riley, 1970)



The use of expensive tertiary filtration equipment as used in human sewage treatment would have to be ruled out because of the high cost. However, secondary filtration through biological filter beds is a possibility if space is available. This might yield a liquid which could be used as a wash-water.

The additional treatment methods could be added on to the basic two stage process, the extent of secondary treatment depending upon the use to which the farmer requires his liquid to be put.

CHAPTER 9

C H A P T E R 9

CONCLUSIONS

- 9.1 Discussion and conclusions
- 9.2 Proposals for future work

9.1 DISCUSSION AND CONCLUSIONS

Animal waste management has, in the past, been easy to cope with and this has been due to the open grazing habit where the animal acted as its own removal system and the soil acted as a treatment and disposal system. Such practices are still in common use today, but there is an increasing number of what may be termed intensive husbandry units. These are a result of the increased cost of land and labour, and the realisation that these can be overcome by close confinement housing and more efficient controlled feeding and breeding regimes. The Ministry of Agriculture, Fisheries and Foods has defined an intensive unit as one which contains more than 1 cow, 4 pigs or 100 chickens per acre of land. From this definition it can be calculated, using the Ministry's statistics, that 5% dairy cow and 23% pig holdings were intensive in 1969. The wastes from such intensive holdings are many times increased in volume per unit area of land because of the increased numbers. It has been shown in Chapter 1 that 5,000 pigs housed on 10 acres would need up to 150 acres for safe disposal of their wastes. This assumes that the land is available continuously which is rarely the case if overloading is to be avoided. The physical nature of the wastes is such that they are stronger than humans - the waste from one pig for example being equivalent to the waste of 3 humans. Calculations of the land available in the United Kingdom have shown that only a quarter is needed for safe disposal of the total wastes

produced per year. However, because of the uneven distribution of livestock and arable farms intensive farms usually need to transport their wastes beyond neighbouring fields which is costly and can be the limiting factor in intensive management. Because of this much of the waste is stored either in tanks, for slurries, or piled into heaps if it is in the form of a deep litter straw-based bedding. These wastes represent a vast store of carbon, nitrogen and phosphorus based compounds which are available for microbial utilisation. In Chapter 1 it was postulated that a microbial upgrading system be used to reclaim these nutrients which would otherwise be termed pollutants, and this would be directed to help solve the problems of waste management on intensive units. It was further decided that the thermophilic fungi would provide the optimum conditions for upgrading the waste. Together with a thermophilic reaction it was deemed necessary to separate out and reclaim for microbial upgrading as much solid from the slurry as possible so that the liquid fraction would be reduced in solids content and polluting ability.

The first investigation was to determine the extent and range of fungi, especially the thermophiles, present in intensively produced pig waste. Isolations carried out using deep litter bedding at a range of temperatures and pH values showed that a large number of cellulolytic species were present and that the thermophiles were well represented. Sixteen of the twenty-two mesophilic species

isolated were considered cellulolytic whilst at 40°C and 48°C 6 of the 10 species isolated were cellulolytic. The optimum pH range in which the greatest number of isolations were made was between 5.6 and 6.6. However, thermophiles such as Torula thermophila, Hemicola lanuginosa and Chaetomium thermophile also had high percentage frequencies at pH 7.4 - 8.2. An examination of the frequencies of Aspergillus fumigatus and Mucor pusillus, which are known to cause animal mycoses, revealed that they were most abundant at 40°C, having a wider pH tolerance. At 48°C their frequencies were much decreased and limited to the acid pH range whilst the other thermophiles had high frequencies and wider pH tolerances.

The isolations from the liquid slurry showed that a very limited flora was present. The number of species was reduced to 13 and of these 6 were cellulolytic. It is likely that the slurry does not contribute to the active flora in the breakdown process.

The isolation programme showed that a wide range of cellulolytic mesophilic and thermophilic fungi were isolated and that, within the pH range 7 - 8, these could be encouraged to grow at their optimum, being able to biodegrade the straw and other cellulosic fibres present.

Before attempting to investigate a new process involving thermophilic fungal action it was thought necessary

to study the natural breakdown process involving a thermophilic flora. This would act as a control to see whether more defined conditions could improve on the natural process. Also, it was an opportunity to investigate the composting process of pig waste to see whether any characteristic observations could be made to compare it to, or distinguish it from, composts made from other animal wastes. Compost heaps of two volumes were prepared - 0.85 cu. m. and 0.24 cu. m. The smaller heap did not heat up above 31°C whereas the larger heap reached 68°C after 3 days. This information was of value in deciding the size of the mass for a thermophilic phase so that as little heat as possible^{was} needed to be put in. A successional pattern of micro-organisms was observed beginning with the bacteria where the pH was at about 9. The actinomycetes, Chaetomium thermophile and Aspergillus fumigatus were also present. As the pH dropped to 8 Humicola lanuginosa was noted. This was probably acting as a secondary sugar fungus (Garrett, 1963) utilising the soluble products of cellulose hydrolysis. The basidiomycete Coprinus sp. appeared on the 10th day followed by Humicola insolens and Stilbella thermophila on the 15th and 17th days respectively. Although not an extensive flora the isolations were all cellulolytic with the exception of Humicola lanuginosa. The first conclusion to be drawn from this experiment was that, under natural conditions, there is a critical size for the mass of waste to rise to thermophilic temperatures. This critical size is probably due to several factors - the

surface area to volume ratio, the availability of utilisable substrates which can be used to generate heat energy, and the presence of a thermophilic flora able to take over at the upper limits of growth for the mesophiles and continue to create heat energy to encourage their own selective development. The second conclusion to be drawn is that thermophilic cellulolytic fungi became dominant and could be considered to be important cellulose decomposers of animal wastes under natural conditions.

The next stage was to investigate the parameters which might affect the biodegradation process to see whether improvements could be made over the natural system. An investigation into temperature revealed that at 48°C cellulolytic activity was greater than at 25°C. This may have been due to the presence of thermophilic fungi with greater activities than the mesophilic species present, or it may have been caused by the suppression of competition which would reduce the growth rate and cellulolytic activities of the species involved. In either case it was established that an increase of the operating temperature into the thermophilic ranges favoured greater cellulolytic activity and thus greater breakdown of the cellulose substrates present.

In order to increase the rate at which a microbial breakdown or conversion occurs it is sometimes worthwhile making additions of material known to contain an active

flora suitable for the breakdown process. Additions of 10% sewage sludge, 10% refuse, 10% soil, and 10, 25 and 40% recycled end-product were made to the waste before fermentation. The soil and 25% end-product gave the best two carbon decreases of 38% and 22% and cellulose reductions of 29.7% and 19.3% respectively after 17 days at 48°C. On increasing the temperature to 56°C the soil addition gave a carbon decrease of 33% and a cellulose decrease of only 2.4%. It can thus be seen that 48°C was the better temperature for optimum biodegradation of the cellulose.

The pH parameter was found to be important in controlling the release of gaseous ammonia which meant an irretrievable loss of nitrogen. Culp and Culp (1971) have shown that above pH 7 the tendency for gaseous ammonia to be released from solution was increased. The initial pH was important in determining the pH throughout the breakdown process. It was found that an initial pH of 4.0 kept the pH to this value for 7 days in which no microbial activity was in evidence. The pH then rose to 8.2 in the next 7 days and decreased to 7.8 by the 17th day. At pH 5.0 the pH slowly increased to 8.4 after 8 days and then stabilised out at 7.2 after 10 days. On the other hand, the process with an initial pH of 6.0 increased to 8.8 followed by a decrease to 7.2 after 17 days. In conjunction with these changes in pH nitrogen changes showed that only when the pH entered the alkaline ranges was there loss of nitrogen which stopped after the pH began to decrease to neutrality. This

investigation would suggest that the upper limit for the thermophilic stage should not exceed pH 7.. The isolation work has suggested that in the acid ranges Aspergillus fumigatus and Mucor pusillus are encouraged, and this has been supported by work done by Mills (1973) who found the pH optima for cellulolytic activity of this fungus were 4.3 and 6.3. It would thus seem that for minimal nitrogen loss and suppression of unwanted species - bacterial and fungal - the pH should be maintained at about neutrality. An initial pH of 5.0 gave the results nearest to this requirement. However, as pig waste starts off at about 7.5 and quickly increases to pH 9 - 9.5 it is necessary that some buffering agent would need to be added.

As described in Chapter 5 the control of intestinal bacterial pathogens is very important when considering the use of the product as a feed supplement. An investigation into the effects of thermophilic temperatures and inocula additions on pathogen counts revealed the following:

at 56°C with no inocula additions of sewage sludge, refuse or soil all coliforms, non-lactose fermenting bacteria and the salmonellae were destroyed and did not return for the remainder of the 17 days. At 50°C although the coliforms were killed off within one day the salmonellae and non-lactose fermenting bacteria took 2 days. At 40°C the coliform count dropped to zero after 1 day, possibly due to the heat shock, but then recolonised to increase in numbers to 10⁸/ml. after 7 days which may be considered to be highly

dangerous from a public health viewpoint. The salmonellae and non-lactose fermenting bacteria did not show this initial decrease, the salmonellae reaching a plateau of about $10^{3.5}$ /ml. after 2 days and the non-lactose fermenters becoming stationary at about $10^{6.5}$ /ml after 3 days. The conclusions to be made from these results are that a temperature above 40°C is desirable for the removal of the type of bacterial pathogen described above. It is important that such bacteria which may not be detected owing to resistance in the animal are removed as they can increase in numbers at mesophilic ranges making the recycling of the waste impossible. It was not possible to determine the presence of other pathogenic or parasitic organisms such as the tapeworm and round worm, but it is likely that only the resistant cyst stages could survive $50 - 56^{\circ}\text{C}$ for 17 days. However, this would need further investigation to verify and determine its importance.

It was shown earlier that at 48°C the rate of biodegradation was greater than at 25 and 40°C . This investigation further supports the use of a thermophilic temperature in controlling selected intestinal pathogens.

An investigation into the addition of inocula showed that for 10% soil additions the rate of biodegradation could be enhanced. In parallel with the above investigation pathogen counts were carried out using sewage sludge, refuse, and soil additions to test the effects of these

on the pathogen population. As it was found that biodegradation was greater at 48°C than at 56°C the investigation was carried out at 48°C. The results showed that there was a characteristic fall in numbers to zero for all additions excepting the soil and that then the soil and refuse additions increased to a plateau of about 10^4 bacteria per ml. whilst the sewage sludge had increased to 10^6 bacteria per ml. after 11 days. These results showed that sewage sludge as an addition was less satisfactory than soil or refuse, but that all three did increase the numbers of viable coliforms present above the investigations without inocula additions. The counts, however, were still lower than those at 40°C without inocula additions.

It is desirable that bacteria be kept to a minimum during the thermophilic stage, not only because of their pathogenic activities, but also because of their competition which might suppress the thermophilic cellulolytic fungi. Thus, it may be that, as biodegradation was greater at 48°C due to fungi shown to be actively growing, the numbers of pathogens shown to be present did not compete for dominance. It may be more valuable to run the thermophilic stage at 48°C and then heat sterilise the product when the process is completed. Drying the material first would prevent further infection by any form of actively growing micro-organism.

The economics of any process may be dependant upon not

only the quality of the product but the length of that process. Although the quality of the product may improve with time the returns may be diminishing in relation to the input above a certain length of time. A laboratory-scale investigation was carried out to show whether the rate of biodegradation changed over longer periods of time than the 17 days employed. The results showed in fact that at 48°C the rate of cellulose breakdown with respect to time could be considered to be linear even after 38 days. This shows that time is not limiting on the thermophilic stage and that it is necessary to check on other aspects of the product such as nutritional value and operating costs. These could not be done until a pilot-scale plant was in operation so that enough product could be collected for nutritional evaluation, and the running costs be determined.

A discussion of the fungi present in pig waste revealed that there was a large number of cellulolytic species. Isolations made at 40°C and 50°C process fermentations with no added inocula showed cellulolytic thermophilic fungi were present together with actinomycetes. Torula thermophila, Humicola grisea and Humicola insolens, 3 cellulolytic species, were shown to succeed the initial short term colonisation by bacteria and Aspergillus fumigatus, of which the latter was only isolated for 2 days at 40°C. Apart from the absence of Aspergillus fumigatus at 50°C, possibly due to the more rapid colonisation by the other thermophiles, the succession was very similar. The addition

of inocula increased the numbers of thermophiles isolated. Chaetomium thermophile was found at the beginning of the fermentation, being replaced, in the case of the refuse addition, by Humicola lanuginosa. This succession has been postulated by Chang and Hudson (1967) who believe that Humicola lanuginosa may act as a secondary sugar utiliser taking up the soluble hydrolysis products of cellulose produced by Chaetomium thermophile. Similar successional patterns were noted for all three inocula. It was found that all the successions were pH dependant in that when the pH was at 9.0 or above bacteria dominated. Below 9.0 there was a succession of actinomycetes followed by colonisation by the fungi. The cellulolytic species colonised early probably due to the ecological advantage given to them by the large amounts of cellulose present.

The laboratory-scale investigation of the thermophilic stage has thus determined the following parameters for biodegradation to be optimised:-

1. Biodegradation is at its optimum at 48°C.
2. At 48°C the numbers of intestinal pathogens are reduced, although not completely removed, when inocula additions were made.
3. The pH should be kept between 5.5 and 7.5 to reduce loss of ammonia, bacterial growth and the associated odours.

4. The addition of a 25% end-product or soil inoculum has been shown to enhance the rate of biodegradation.

5. Although in most of the process runs stabilisation of the pH was complete within 17 days and the product had a pleasant texture, biodegradation continued at the same rate for 38 days. Thus, the process time may be, based on laboratory-scale work, between 17 and 38 days.

The results above have been able to demonstrate the contribution that the solid fraction makes to the biodegradation process. The slurry fraction, a mixture of faeces and urine, with no added straw bedding, may affect the biodegradation rate, in that it may contain a high number of bacteria, its buffering capacity may be higher and it may contain additives such as copper which may retard microbial growth. Experiments using a respirometer to measure oxygen uptake by a mixed flora showed that, although at 48°C the rate of oxygen uptake was less than at 30°C, it was present the lower figure probably being due to firstly the lower number of organisms able to tolerate 48°C, and secondly, especially noticeable at acid pH's where fungi would have been dominant, due to the lower respiration rates of the fungi. It was found that by adding sterilised slurry the rate of

respiration and hence utilisation of the slurry at both temperatures was increased over the endogenous respiration of the soil.

Pure culture work using thermophilic fungi grown on a pig slurry agar further supported the mixed culture work. Growth was pH dependant, being greatest at pH 5.6 and less in the alkaline ranges although still present. Torula thermophila, Chaetomium thermophile, Humicola grisea and Humicola insolens all cellulolytic species, grew very well at pH 5.6. All these fungi, however, had a one day lag period which was probably due to substrate adaptation. Such an observation might be important in considering early colonisation and fast initial growth.

The addition of extra nitrogen to lower the carbon to nitrogen ratio suppressed growth the lower the ratio was. This suggested that the addition of extra nitrogen sources was probably not necessary, and that growth might be enhanced in ad-mixtures of slurry and solid waste where the carbon from the straw added to the slurry would keep the C:N ratio above 20:1 which was considered to be the enhancement threshold.

It now seems likely that under the right conditions outlined above a thermophilic biodegradation of the solid fraction of pig waste with added straw can be achieved and enhanced over the natural composting breakdown process. In order to test the parameters on a larger scale a tower

was constructed and designed to operate with a 'beneath pen' filtration system using straw as the filtration agent. The results from the filtration show straw to have good filtration characteristics, holding back the solid fibrous fraction of the waste, and allowing the liquid to trickle through. By building up layers of straw and solid waste the filter could be kept without changing for up to 6 weeks, and only then was it limited by its volume which could increase no more in the experimental pens. Solids reductions from 6% to 1% were obtained and up to 80% BOD₅ reductions which were considered to be good for a primary filtration operation. A preliminary operation with the thermophilic tower has shown that thermophilic temperatures can be attained. The material in the tower reached 47°C in 2 days and remained above 40°C for about 10 days. The resultant product after 20 days was odourless, could be pulled apart showing that the straw fibres had undergone breakdown, and was a light brown colour in contrast to the black to grey colouring which is characteristic of anaerobicity in animal wastes. Although it is too soon to make comments on the thermophilic stage, it has been shown in the filtration stage that straw filtration not only offers a simple and passive method of solid waste separation, but also serves to quickly reduce the polluting ability of the liquid.

It was discussed above that nutritional factors may be important in determining the length of the thermophilic

stage. A preliminary analysis for amino acids in samples from the laboratory-scale experiments showed that a wide range of basic, neutral and acidic amino acids was present. It was further shown that those samples which underwent greatest biodegradation did meet the Agricultural Research Council's recommended levels of essential amino acids (lysine, methionine, tryptophan, threonine and isoleucine) with the exception of methionine which has been found to be the limiting essential amino acid in other fungal and plant preparations. Although not conclusive the investigation does show that levels of amino acids can be reached which may be of use to animals in their feedstuff. What it has not shown is the digestibility and availability of the amino acids which is more important in evaluating a feedstuff.

The original proposals were to investigate the treatment of intensively produced pig waste using a two stage process consisting of an ambient temperature mechanical filtration system to separate out the solid waste from the liquid waste. In this way the polluting ability of the waste would be reduced and valuable carbonaceous and nitrogenous nutrients would be reclaimed. Also the liquid fraction would be reduced in solids content so that it could be more easily handled. The second stage was to take the filtered solid together with the straw filter and to subject these to thermophilic breakdown using thermophilic fungi under controlled conditions. In this way the straw and the reclaimed nutrients - both considered waste products - could

be utilised for the growth of thermophilic fungi and the product could be upgraded to increase its protein content and value as a recycled feedstuff. Such recycling of nutrients may be considered as an aid to the recycling of nutrients via the soil and, as such, may prevent waste treatment as becoming one of the limiting factors in intensive meat production.

The investigations into the mechanical filtration stage have shown that the solid fraction can be retained, and that the liquid filtrate is reduced in solids and polluting ability. It was thus concluded that straw might provide a cheap filter as well as providing a source of cellulose for the breakdown in the second stage. As straw is considered to be a waste product and is burnt each year, terminating any recycling through the soil back to the animals via their feedstuffs, it is important that if a use be found for the straw it is one where the nutrients can be utilised and recycled either through the soil or straight back to the animals.

An investigation of the thermophilic stage found that above 40°C breakdown of cellulose proceeded at a greater rate than below this temperature, and that thermophilic fungi were the dominant species responsible. It was found that control of the temperature and pH would be necessary to encourage quick establishment of a cellulolytic thermophilic flora and thus speed up the breakdown process. Very low

aeration rates controlled odours from bacteria and it was decided that aerobic conditions were sufficient to keep odours under control. When the pH of the thermophilic stage was not controlled the process took longer because bacteria became established first and the pH remained alkaline. However, fungi finally took over and the breakdown rate increased. A pilot-scale thermophilic stage has further indicated in its preliminary stages that breakdown of the waste and odour removal by passive aeration can be achieved over a period of about 20 days.

In the light of the above discussion, and the conclusions to be drawn from it, it is now possible to gauge the extent to which the results from this thesis can be used to solve the problems of intensive animal waste disposal. In Chapter 1 eight problems which face farmers concerning waste management were listed. These were:-

1. Odour production.
2. Disease hazards
3. Pollution of waters
4. Large bulk for storage
5. Handling problems
6. Cost of treatment equipment
7. Large areas of land required for safe disposal
8. Low value of waste as fertiliser or resaleable product

It has been observed that low aeration rates such as

allowing the passage of free air through both the filtration stage and the thermophilic stage is sufficient to reduce odours and encourage the aerobic fungi to become established. By encouraging a thermophilic stage air is drawn up through the waste and a circulation may be set up. Also, a thermophilic stage has been found to reduce the numbers of intestinal pathogens, which would not only make the product safer but, from an ecological viewpoint, would decrease the competition and help the fungi to become established.

The pollution of streams and rivers has been shown to be reduced in two ways. Firstly by simply reducing the amount of liquid through absorption by the straw pad. Calculations have shown that the pigs produced only 1.2 litres of liquid per day as opposed to the 5 litres which is quoted as a working average. Thus, if the liquid has to be stored it will be longer before the storage capacities are reached and seepage into streams occurs and thus removal can be spread over longer periods. The second way that the pollution problem has been reduced is by reducing the polluting ability of the liquid in terms of its BOD and nitrate content. Unfortunately it has not been shown that phosphate removal is possible and more work would need to be done on the filter to determine the reasons for this. However, reducing the polluting ability means that more liquid can be spread per unit area of land before the land becomes overloaded and before the liquid run-off to streams reaches gross polluting concentrations. Of course more

research would be needed in terms of dosing fields with the liquid but it would be expected that greater doses would be possible.

By reducing the straw and cellulosic components of the waste for conversion to fungal energy and cell material a reduction in the bulk can be achieved. It has been shown that over 60% of the cellulose can be removed in 38 days and that the product can be ground to a powder. Also separation of the solid and liquid fractions reduces the bulk. Removal of water from the waste will reduce the weight and size of the waste considerably.

Associated with all of the above problems is the one of handling. Thus, a reduction in odour makes the waste more aesthetically pleasing to handle and reduces flies in the waste. The liquid will be easier to handle by pumping. A reduction in solids means that there is a lessened possibility of clogging, and if the liquid is aerobic odours are considerably reduced. A reduction in bulk and weight will save time and energy on the part of the intensive unit owner, and the solids, if dried 'in situ' would be easier to handle.

A very rough costing of the capital equipment has shown that for what it achieves it is very competitive with other systems. However, it would be too early to make any statements on running costs and returns from the products.

The filtration system has been designed to fit in with existing farm buildings and this would reduce the capital costs, perhaps appealing to the farmer more than a system which might mean large-scale conversion of buildings and equipment.

At the present time large areas of land are needed for safe disposal of animal wastes from intensive units. A reduction in bulk and pollution would mean that more treated liquid waste could be applied to smaller areas of land. With the exception of poultry waste other animal wastes have a low retail value as a fertiliser. Although an investigation of the fertiliser value of the products from the thermophilic stage has not been possible, an amino acid determination has shown that upgrading and an increase in the protein content may increase the value of the waste as a feed supplement. However, much work would be needed to be done before the material could be marketed as such.

Taking a broad view of the two stage system proposed in this thesis it can be seen that there is much large-scale practical work to be done before final conclusions can be arrived at concerning the value of the process in solving intensive waste management problems. However, it has shown that controlled microbiological processes together with a cheap filtration system may be able to contribute towards solving waste problems. The desire to increase meat and dairy production and the decrease in available farming land makes the solving of these problems paramount.

9.2 PROPOSALS FOR FUTURE WORK

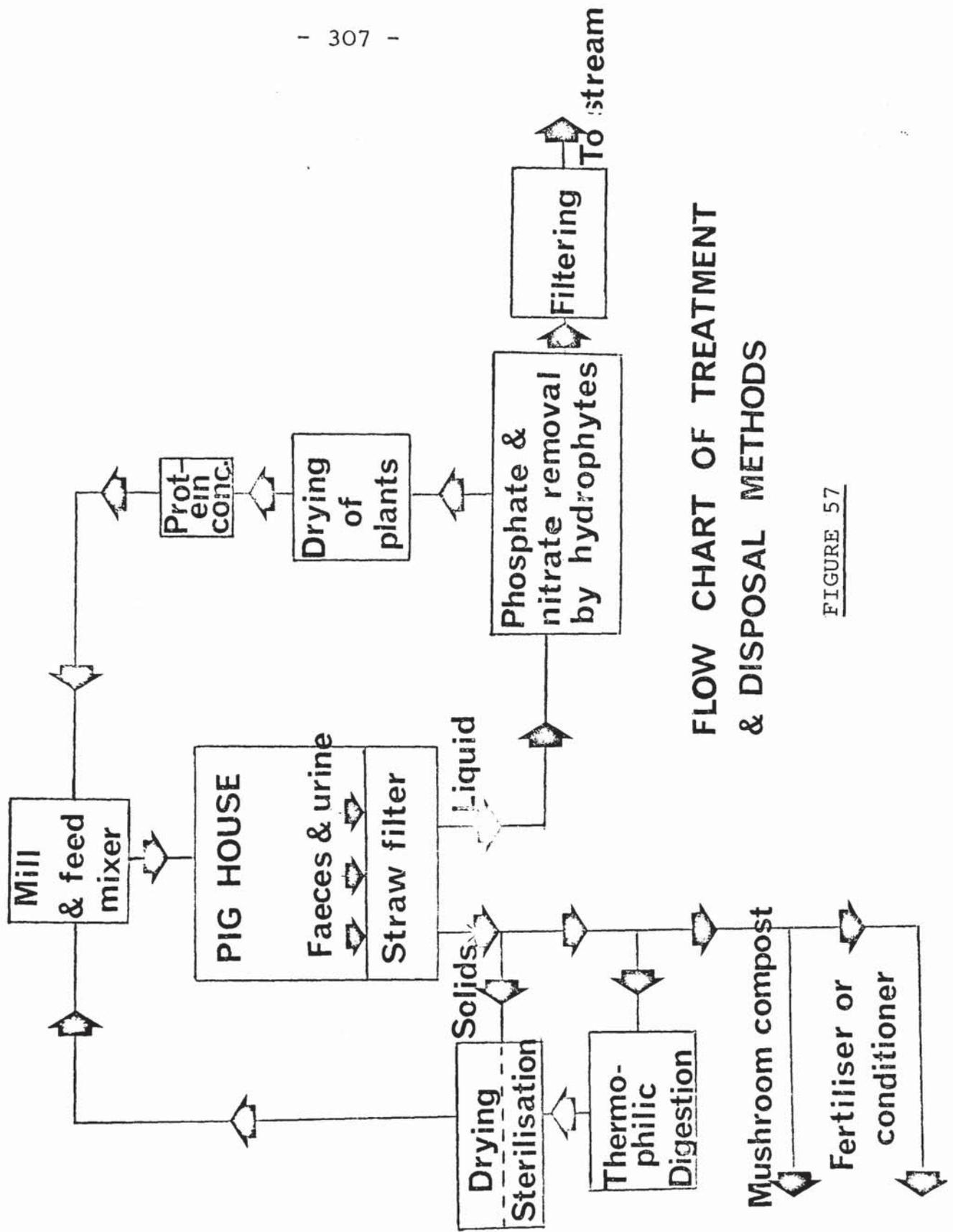
The above conclusions were by no means complete and, for this reason, the following future work needs to be undertaken to increase the knowledge of the two stage system:-

1. Characterisation and optimisation of the pilot-scale thermophilic stage.
2. Toxicity investigations on individual thermophilic fungi.
3. A nutritional evaluation of the products from the thermophilic stage.
4. Drying and sterilising procedures for the product.
5. Incorporation of product into compounded feedstuffs.
6. Other uses of product such as fertiliser or as a mushroom compost.
7. Methods of further treatment for the liquid fraction to further reduce its BOD and reclaim any nutrients present by filtration or use of hydrophytes (see Chapter 8).

8. Incorporation of large-scale filtration system into existing farm buildings and the evaluation of various types of equipment for handling the straw pads after use.
9. An economic evaluation of the whole process.

A summary of a complete system is shown in figure 57. This shows the work already achieved and the work which would need to be done. Although the recycling of the solid waste fraction is shown as going back to the animal from which it was produced it is probable that another animal would be used to prevent the build-up of nutritional deficiencies and to increase the efficiency of digestion. For example the waste product from the pig containing cellulose would be better utilised by a ruminant which was able to convert the cellulose and digest its breakdown products.

The fate of the liquid run-off may simply be to spray on fields. With its lowered solids content and polluting ability it is easier to pump and spray larger quantities onto land without causing overloading. An investigation into the safe limits for application would indicate whether this could be the fate of the liquid after filtration through the pad. An alternative solution might lie in the use of hydrophytes, as discussed in the previous Chapter. The extra protein gained from harvesting these plants might make the process worthwhile. However, a very careful



FLOW CHART OF TREATMENT & DISPOSAL METHODS

FIGURE 57

economic evaluation would need to be done as well as a determination of the efficiency of the plants in a temperate climate to remove the polluting nitrates and phosphates.

Finally, but probably of fundamental importance, is to produce a system of waste treatment which the farmer is willing to use and operate. The design of the system which has been described in Chapter 8 sets out to do this using normal farming equipment and procedure and trying to upset very little of the routine which is vital to an intensive farming concern.

Much more work, whether it be with this system or others involving animal waste treatment, must look towards conserving and recycling the vast amounts of waste nitrogen, carbon and phosphorus which are continually being produced in excess of the capacity with which natural recycling processes can cope. It thus seems likely that by enhancing and controlling recycling processes in the treatment of animal wastes, either by returning to the land or by utilisation as a feed supplement, a saving of raw materials and energy in short supply, which may be costly, can be achieved.

In conclusion it can be said that the problems of animal waste treatment are so wide that interdisciplinary co-operation is necessary to achieve solutions to the waste problem. It is hoped, though, that this thesis has contributed towards

solving these problems, and that many questions, for which answers must be found, have come to light which would otherwise have never been revealed until gross pollution dictated urgent research on the subject.

A P P E N D I C E S

A P P E N D I X 1

Eggins & Pugh (1962) Cellulose Agar

Potassium dihydrogen orthophosphate	1.0g
Ammonium sulphate	0.5g
Potassium chloride	0.5g
Yeast extract	0.5g
L-asparagine	0.5g
Magnesium sulphate	0.2g
Calcium chloride	0.1g
Oxoid agar No. 3	15.0g
Ball milled 2% cellulose suspension	250ml.
Rose Bengal, 670 μ g/litre	15ml.
Distilled water	up to 1 litre

The agar was autoclaved at 10 lb/sq. in. for 20 minutes

For the glucose-starch medium the cellulose suspension was substituted with 5g. glucose and 5g. starch per litre.

A P P E N D I X 2

Preparation of McIlvaine's Citrate Phosphate Buffer

Solutions of 0.2M disodium hydrogen phosphate and 0.1M citric acid were prepared and used according to the following table. Figures for sodium salt and citric acid are expressed as number of ml. per litre of solution to be buffered.

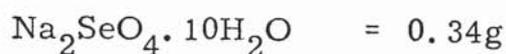
<u>pH</u>	<u>Na₂HPO₄</u>	<u>Citric Acid</u>
4.0	7.71	12.29
4.2	8.28	11.72
4.4	8.82	11.18
4.6	9.35	10.65
4.8	9.86	10.14
5.0	10.30	9.70
5.2	10.72	9.28
5.4	11.15	8.85
5.6	11.60	8.40
5.8	12.09	7.91
6.0	12.63	7.37
6.2	13.22	6.78
6.4	13.85	6.15
6.6	14.55	5.45
6.8	15.45	4.55
7.0	16.47	3.53
7.2	17.39	2.61
7.4	18.17	1.83
7.6	18.73	1.27
7.8	19.15	0.85
8.0	19.45	0.55

A P P E N D I X 3

The Micro-Kjeldahl Method for the Determination of Nitrogen

Reagents

1. Catalyst



These chemicals were mixed and ground to a fine powder.

2. Concentrated sulphuric acid ('Analar' grade).
3. 40% (w/v) sodium hydroxide.
4. 4% (w/v) boric acid.

About 0.15g dried ground sample were accurately weighed and transferred to a 30 ml. digestion flask. 4ml. conc. H_2SO_4 and sufficient catalyst to cover the end of a micro-spatula were added. The mixture was digested for about 3 hours until the liquid was colourless and then it was allowed to cool. The nitrogen in the form of ammonia was liberated using 40% sodium hydroxide in a Markham still and collected in 10 ml. boric acid with 1 drop of bromo-cresol purple indicator. The boric acid was titrated against $\frac{N}{50}$ HCl and the following equation used to determine the nitrogen:-

$$\% \text{ Nitrogen in sample} = \frac{\text{titre vol.} \times 0.028}{\text{sample wt.}}$$

APPENDIX 4

FATTENING FEEDSTUFF FORMULA

	<u>Cwts</u>	<u>lbs</u>
Barley	7	28
Wheat	7	0
Wheatings	2	0
Fish-meal 65%	1	56
Soya-bean 46%	1	0
Whey powder 12%	1	0
Vitamins, minerals, trace elements	<u>0</u>	<u>28</u>
TOTAL	20 cwts	0 lbs

Chemical analysis

	<u>%</u>
Oil	1.95
Protein	16.80
Fibre	3.75
Digestible protein	13.70
Total digestible nitrogen	71.00
Calcium	0.90
Phosphorus	0.67
Salt	0.55
Lysine	0.88
Methionine)	0.59
)	
Cystine)	
Copper	180 ppm

APPENDIX 5

THE DETERMINATION OF NITRATE

Before the determination of nitrate by colorimetric means it is necessary to prepare the sample to remove colour and convert any interfering nitrite to nitrate.

Preparation

2 ml. of the raw sample were added to 18 ml. distilled water in a 100 ml evaporating dish. To this were added 2 ml. of a 0.5% chalk suspension in water. The mixture was evaporated on a water bath to a volume of about 10 ml. Then 1 ml. of 30% hydrogen peroxide was added, the basin covered with a watchglass, and digested on a steam bath for 2 hours. The watchglass was then removed and mixture evaporated to dryness. The dry residue was heated for an extra 30 minutes to decompose the peroxides.

Determination of nitrate

Reagents

1. Phenol-disulphonic acid, about 25% w/v in concentrated sulphuric acid.
2. Ammonia solution containing 10% w/v NH_3 .

To the dried residue from above was added 1 ml. phenol-disulphonic acid, making sure that the acid made contact with all of the residue. This was allowed to stand for 10 minutes and then 10 ml. water were added, the solution allowed to cool. 10 ml. ammonia were then added, cooled and finally the mixture diluted to 25 ml. A blank was prepared at the same time substituting water for the sample. The resulting colour was determined using the Lovibond Comparator and a Comparator disc 3/17 which covered the range from 5 - 100 μ of nitrate nitrogen, equivalent to between 1 and 20 ppm on a 5 ml. sample. The comparator tubes were filled with 10 ml. of the sample and 10 ml. of the blank. The blank was placed in the left-hand compartment of the Comparator and the sample in the right-hand one. The Comparator was held up to north daylight and the disc turned until the colours in both tubes matched. This gave a wavelength reading of between 5 and 100 μ .

APPENDIX 6

THE DETERMINATION OF PHOSPHATE

Preparation

A 1 in 40 dilution, at least, was required for this determination, and this gave an almost clear solution. To convert pyro- and meta-phosphates to orthophosphate it was necessary to boil the sample with 10% sulphuric acid for 20 minutes.

Determination of phosphate

Reagents

- | | | | |
|----|---------------------------------------|-------|--------|
| 1. | Ammonium molybdate ('Analar') | | 10g |
| | Distilled water | up to | 100 ml |
| | When dissolved add slowly to a cooled | | |
| | mixture of sulphuric acid | | 150 ml |
| | and distilled water | | 150 ml |

Store in brown glass bottle

- | | | | |
|----|-------------------|--|--------|
| 2. | Stannous chloride | | 2.5 g |
| | Glycerol | | 100 ml |

This will keep for several weeks.

The prepared solution was further diluted to 45 ml. and decolourised using activated charcoal. It was then transferred to a flask immersed in a water bath at 25°C. 1 ml. ammonium molybdate was added to the equilibrated sample and mixed thoroughly. Then 0.15 ml. stannous chloride were added and the solution diluted to 50 ml. at 25°C. The colour was allowed to develop over 5 minutes. The Comparator disc 3/7 which covered a range of 2 - 22 ppm P_2O_5 on a 10 ml. sample was used, the technique used being the same as for Appendix 5.

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