THE MICROBIAL ECOLOGY OF COW SLURRY IN VERMICULTURE BEDS.

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THE UNIVERSITY OF ASTON IN BIRMINGHAM

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SUMMARY

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The suitability of cow slurry as a substrate for vermicomposting by Eisenia fetida was investigated. Particular attention was given to the effects of the earthworm on the decomposition and stabilisation of the slurry, and to the interactions between E. fetida and the microflora of the substrate.

Assessment of the chemical and microbiological changes in cow slurry stored under forced aeration, and subsequently in shallow trays, showed that neither method was suitable for the treatment of slurry.

A comparison of two methods of vermicomposting showed that top-feeding of slurry was more efficient in promoting earthworm growth and cocoon production than the mixing of slurry with solid materials. Management practices were found to have an important influence on the efficiency of the process.

An investigation of the effect of E. fetida on the decomposition of slurry indicated that the presence of this earthworm enhanced the stabilisation of the substrate and increased the plant-available nitrogen content.

Specific nutritional interactions were observed between E. fetida and micro-organisms in sand/cellulose microcosms. The earthworms were found to be feeding directly upon the cells of certain micro-organisms. Other species were found to be toxic to E. fetida. A technique was developed for the production of axenic E. fetida, and the use of such earthworms in feeding experiments confirmed the importance of some micro-organisms in earthworm nutrition. The seeding of vermiculture beds with one such micro-organism stimulated earthworm growth and consumption of the substrate.

Vermicomposted mixtures of cow slurry and spent mushroom compost were shown to have potential application as casing materials in mushroom cultivation.

The findings of this study indicate the suitability of vermicomposting as a method for the stabilisation of intensively-produced cow slurry, and give some indication of the importance of micro-organisms in the nutrition of E. fetida.

KEY WORDS

EISENIA FETIDA

COW SLURRY

VERMICOMPOSTING

EARTHWORM/MICROBE INTERACTIONS

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Taxonomy of *Eisenia fetida*.

Earthworm taxonomy has undergone considerable re-organisation in recent years, and one result of this has been the re-classification of many earthworm genera and species. The earthworm which has been used in experimental work in this study is included in these changes. In much of the published literature and virtually all of the studies quoted in this thesis, this earthworm is referred to as *Eisenia foetida*: the correct name is *Eisenia fetida*, as proposed by Savigny (1826). Two forms of this species occur together in nature, one having a distinct dorsal band of red pigment on each segment, the other being much darker in colour and uniformly pigmented. These forms were described by Avel (1937). Andre (1963) showed the two forms to be separate species, but named the striped form *Eisenia fetida* var. *unicolor*. Bouche (1972) correctly pointed out that these names were invalid, and renamed the uniformly pigmented earthworm *Eisenia fetida andrei*. This situation was reviewed by Sims (1983), who considered it difficult to apply the scientific nomenclature to these forms, since taxonomy is ultimately dependant on preserved type-specimens, and earthworm pigments do not survive preservation permanently.

For the purposes of this study, this earthworm will be considered as two separate sub-species; *Eisenia fetida* (striped) and *Eisenia fetida andrei* (unstriped). All

references to Eisenia foetida in the quoted literature are herein referred to as Eisenia fetida but it should be noted that the earthworms used in published studies may have been mixtures of the two forms. In all experiments involving earthworms which form part of this study, the striped form was used exclusively.

INTRODUCTION

In traditional farming systems, the application of animal manures to croplands is an integral part of mixed farming practice. In the developed countries, the adoption of intensive livestock production in recent years has led to the rearing of large numbers of animals on small areas of land. In the U.K. in 1979, 170 million tonnes of livestock excreta were produced, of which 60 million tonnes were voided indoors (Royal Commission, 1979). Most of the waste produced on intensive units is in the form of slurry, a mixture of faeces and urine. The large volumes produced may cause disposal problems, because insufficient land is available for land-spreading.

Excessive application of intensively-produced animal wastes damages soil fertility, causes water pollution and may present a health risk. The pollution problems from animal wastes have been well-documented. They include toxic effects on grazing animals (Gostick, 1982); inhibition of seedling emergence (Adriano et al, 1971); pollution of water courses by run-off (Magdoff et al, 1977); and pollution of groundwater with nitrates (Concannon and Genetelli, 1971).

Some form of treatment of animal wastes is therefore often necessary to make them suitable for safe disposal into the environment. Present methods of animal waste treatment have been the subject of several reviews (Loehr, 1971,1977; Mann, 1975; Vanderholm, 1979) and are based upon those employed in the treatment of sewage. Problems of treatment are greater, due to the higher B.O.D.,

C.O.D. and solids concentration of animal manures and slurries. In general the most efficient systems consume large amounts of energy and require a high initial investment.

The large increase in energy costs in recent years has promoted research into the application of low-cost, low-technology systems of animal waste management. Several biological methods have been investigated, including anaerobic digestion, single-cell protein production, solid state fermentation, and refeeding of wastes to animals. One method which has considerable potential is the utilisation of natural decomposition processes involving invertebrates. The commercial-scale use of earthworms in the treatment of organic wastes has been proposed, and the earthworm Eisenia fetida appears to be a suitable species, because of its rapid growth rate and reproductive potential, and occurrence in rich organic substrates in nature.

A review of the relevant literature revealed several areas in which further research and development were needed if the full potential of vermicomposting was to be realised. More information was required on the suitability of different animal wastes as substrates for vermicomposting, and on the parameters which determine this suitability. The changes brought about by earthworms in a substrate, and the properties of vermicomposted materials compared with material aged without earthworms were not well documented. This knowledge would enable comparisons to be made between vermicomposting and other methods of animal waste treatment, in terms of the

efficiency of the process and the quality of the treated waste. The utilisation of vermicomposted materials as a resource is another area which has received little attention. This represents a possible source of revenue for a waste treatment operation, and may be an important factor in determining the economic feasibility of large-scale systems.

An important area in which further work was needed was on the food source of earthworms in vermicomposting systems. Evidence in the literature suggested that E. fetida was able to selectively digest some micro-organisms, while others could remain viable on passage through the earthworm gut. However, these studies were performed in the presence of contaminating micro-organisms, and this may have affected the results obtained. The true feeding interactions of E. fetida with micro-organisms could only be determined precisely by studies involving axenic earthworms. If the microbial species associated with E. fetida in this way could be identified, management practices and vermicomposting systems could be adopted to increase the numbers of beneficial micro-organisms in animal and other wastes, and hence encourage the consumption of the materials by earthworms.

The main objectives of this study were therefore to determine the suitability of cow slurry as a substrate for vermicomposting; to investigate the changes brought about by E. fetida in the chemical and microbiological properties of cow slurry; to consider possible applications

of vermicomposted slurry; and most importantly, to develop techniques to enable the specific relationships between E. fetida and micro-organisms isolated from vermicomposting systems to be determined.

SECTION 1.

REVIEW OF RELEVANT LITERATURE

1.1. Composition and fertiliser value of cow slurry.

The large volumes of animal wastes produced in intensive livestock-rearing units present a disposal problem and possible pollution hazard. Most of the waste produced is in the form of slurry (a mixture of faeces and urine) and though this does have some plant nutrient value, indiscriminate landspreading may damage soil fertility, cause water pollution and present a health risk. The composition and fertiliser value of slurries may vary considerably due to such factors as the diet of the animals, and differences in handling and storage methods between farms.

In the case of cow slurry, several workers have quoted values for pH, dry matter and chemical constituents, based upon samples of varying size. The results give an idea of the range of values encountered when the major constituents of cow slurry are determined. pH has been found to range between 5.9 and 8.7; organic nitrogen from 0.16 - 5%; phosphorus from 0.04 - 2%; potassium from 0.25 - 5% (all on a dry weight basis); and solids content from 1-19%, on a fresh weight basis (Wedekind and Koriath, 1969; Staley et al, 1971; O'Connell, 1974; Jones and Matthews, 1975).

Plant availability of the nutrients in cow slurry is an important factor in determining the fertiliser value of the material. In the latest guidelines to U.K. farmers, the availability of the major nutrients to plants during the season of application is given as nitrogen 30-50%; phosphorus

50%; and potassium 90% (A.D.A.S.,1982). These figures show that cow slurry does not have a good nutrient balance for use as a fertiliser, being a good source of potassium and nitrogen but low in phosphates. Because of the imbalance, it may be impossible to achieve the full potential fertiliser value of the material. Dangers to grazing animals may arise through repeated applications of slurry containing a high proportion of potassium (Gestick, 1982). A build-up of this element in soil can reduce the uptake of magnesium in herbage and lead to a condition known as hypomagnesaemia in animals.

The figures given for the composition of slurry refer to freshly-voided material. However, significant losses of nutrients may occur on storage and immediately following landspreading. The major nutrient lost is nitrogen in the form of ammonia, which is readily volatilized. It has been found that initially 95% of the nitrogen in both faeces and urine is organic, with the remainder being ammonium nitrogen (Muck,1982). However,40-50% of the organic nitrogen fraction is in the form of urea, which is readily decomposed to ammonium nitrogen. The author estimated that almost 100% urea decomposition would occur in 24 hours at 10°C, so that half of the total slurry nitrogen would be quickly converted to the ammonium form. Substantial losses of nitrogen from slurry could therefore occur in the first few hours after production. Subsequent storage increases nutrient loss: Losses of nitrogen from different waste storage and treatment systems have been given as 30-90% for aerated systems; 10-75% for anaerobic

systems; and 25-99% for wastes stored on feedlot surfaces Vanderholm (1975). Up to 60% of total nitrogen present in dairy cow slurry has been found to be lost over 7 weeks storage under simulated field conditions (Chang and Johanson, 1977). A study of the losses of nitrogen from stored cattle slurry was carried out by Gracey (1979). Slurry samples were stored in an above-ground tank for 3-4 month intervals before land-spreading. The mean total nitrogen loss was 6%, and the mean soluble nitrogen loss was 8%. These figures are much lower than those in guidelines by A.D.A.S. (1982), who quote losses of 10-20% of initial nitrogen from slurry stored over winter. This difference may simply reflect the large variation found between farms, as the A.D.A.S. figures are based upon results from a large number of samples from different units, rather than repeated samples from the same system.

Further loss of nutrients may occur when animal wastes are spread onto land. Hoff et al (1981) found that 11.2-14.0% of surface-applied ammonium nitrogen in pig slurry was lost as ammonia within 4 days of application, whereas only 2.5% of ammonium nitrogen was lost when the slurry was injected below the surface. Flowers and Arnold (1983) found that very low losses of ammonium nitrogen occurred when pig slurry was thoroughly mixed with soil. In addition, it has been found that ammonium nitrogen was rapidly converted to nitrate nitrogen in slurry mixed with soil (Flowers and O'Callaghan, 1983). Similar results might be expected from the application of cattle slurry to the land, but further work is needed to

verify this. These results show that a significant proportion of the nutrients present in slurry are in a form which is readily lost. Careful storage and treatment of animal wastes before and during land application is therefore desirable if the maximum possible fertiliser benefit is to be obtained.

1.2. Microbiology of cow slurry.

Rhodes and Hrubant (1972) studied the microflora of cattle feedlot waste and runoff from a storage site. They found total counts of greater than 10 $\log_{10}$ micro-organisms g^{-1} dry weight of slurry. They stated that anaerobic bacteria formed the most numerous group present, but the isolation techniques used were not strictly anaerobic, and most of the organisms isolated were in fact facultative anaerobes. Gram-negative bacteria were also present in large numbers, with Bacillus species, fungi, yeasts and Streptomyces species also present in lower numbers. The composition of the microflora was found to be consistent throughout the year, though there was some increase in the numbers of Streptomyces during the summer months. In a study of Enterobacteria in feedlot waste, it was found that numbers of this group ranged from 4.4 to 6.8×10^7 g^{-1} dry weight of slurry (Hrubant et al, 1972). Of these, more than 90% were non-pathogenic strains of Escherichia coli. Citrobacter species and Enterobacter cloacae made up most of the remainder. Hrubant (1973) described a Corynebacterium isolate which was said to be consistently

associated with feedlot waste, being present as between 2 and 70% of the aerobic bacterial population. Numbers of this organism were found to decrease markedly on storage, and the author considered that it did not play an appreciable part in the overall decomposition of the waste. Several studies have been made on the incidence of pathogenic micro-organisms in cattle slurry and the survival of these organisms during storage and treatment (Jones and Matthews, 1975; Clinton et al, 1979). These papers describe the isolation of Salmonellae from cattle slurry and manure, but do not give actual numbers of Salmonella organisms present. Jones (1976) found that temperature affected the survival of Salmonella, a rapid decline in numbers occurring with increasing temperature. Jones et al (1977) studied the effect of the microflora of cattle slurry on the survival of Salmonella dublin. They isolated 13 genera of bacteria from cattle slurry, and these are listed in table 1.1.

When Salmonella dublin was seeded into samples of normal slurry, high mortality of this organism resulted. The authors concluded that this was due either to direct competition for nutrients, or the production of inhibitory compounds by other organisms. Ellis and McCalla (1978) suggested that there is a minimal disease potential from animal waste disposal, because of the short survival time of many pathogens once outside the host organism. This is not strictly true, as some pathogenic organisms are known to persist for long periods in the environment, but their numbers are usually too low to present a significant health hazard. There seems to be no reason why,

given the proper precautions, animal waste treatment and disposal should present a significantly greater disease hazard than any other waste treatment operation.

Table 1.1. Genera of bacteria isolated from cattle slurry (Jones et al, 1977).

Genus	No. of strains
Acinetobacter	9
Pseudomonas	7
Alkaligenes	6
Corynebacterium	6
Bacillus	5
Micrococcus	5
Aeromonas	3
Achromobacter	2
Flavobacterium	2
Kurthia	2
Escherichia	1
Serratia	1
Streptomyces	1

1.3. The role of earthworms in organic matter recycling.

The contribution of earthworms to soil fertility through their burrowing and feeding activities was recognised by White (1789). Charles Darwin (1881) showed that they play an important role in the disappearance of surface plant litter, and its breakdown and incorporation into the soil. More recent work on the ecology of earthworms has shown more precisely the importance of earthworms in the cycling of organic matter in terrestrial ecosystems, and their effects upon soil fertility.

Raw (1962) found that the earthworm Lumbricus terrestris removed more than 90% of the autumn leaf fall of an apple orchard during the following winter. This was calculated to be 1.2 tonnes dry weight of leaves per hectare. Edwards and Heath (1963) considered that earthworms may be responsible for up to 78% of total litter disintegration in woodlands. Calculated rates of consumption of litter and organic matter by earthworms are prodigious. It has been calculated that individuals of L. terrestris consumed 10-30% of their live body weight per day, and that the earthworm population of a deciduous wood could consume the annual leaf fall (3t. ha^{-1}) in approximately 3 months (Satchell, 1967). It was also calculated that the amount of dung produced by dairy cattle (6-7.5 tonnes $hectare^{-1}$) was only one quarter of the amount that a typical earthworm population could consume.

The direct contribution of earthworms to organic matter decay is, however, quite low. Barley and Kleinig (1964)

calculated that a population of Allolobophora caliginosa in a pasture would contribute only 4% of the total annual energy of decay of the organic matter present, and the energy of respiration of a woodland L. terrestris population was calculated as 8% of the total energy content of the annual leaf fall (Satchell, 1967). Lofty (1974) states that the direct contribution to soil metabolism by earthworms may be considered to be much less than that by other soil animal groups together.

The effect of earthworms upon the mineralization of nitrogen in soils has been the subject of much research, as this process can have a significant effect upon the fertility of soil. Needham (1957) considered that very little nitrogen was excreted in the faeces of earthworms, though Lunt and Jacobson (1944) and others, found considerably more nitrogen in casts than in the surrounding soil. This may be due to a concentration effect of the selective ingestion of litter by earthworms. However, Barley and Jennings (1959) found that 6.4% of the non-available nitrogen ingested by A. caliginea was converted to available nitrogen in castings. In a study of nitrogen transformations following the application of pig slurry to soil, it was found that ammonium and nitrite levels decreased, while nitrate-nitrogen levels increased simultaneously (Debry et al, 1983). The presence of earthworms in the soil was found to greatly increase the speed and efficiency of this transformation. Kaplan and Hartenstein (1977) found no evidence of nitrogen fixation or nitrite reduction in earthworms, and concluded that observed increases in available nitrogen

in soils containing earthworms must be due to sources other than their own metabolic activities. Syers et al (1979) produced data which supported this hypothesis. They found that 73% of the total nitrogen content of litter ingested by worms was accumulated in castings, and that most of the inorganic nitrogen excreted was in the form of ammonium. However 65% of this was rapidly nitrified to nitrate after voiding.

The role of earthworms in the breakdown of the more resistant ligno-cellulose fraction of organic matter is not completely defined. Tracey (1951) isolated cellulase enzyme from extracts of 17 species of British earthworm. Extracts from the foregut of L. terrestris contained the greatest amount of the enzyme, while little or no enzyme was found in the middle portion of the gut (the crop and gizzard), and the hindgut contained 10% of the levels in the foregut. However, the author was unable to exclude the possibility that the enzyme was produced by micro-organisms in the gut of the earthworm. Hartenstein (1982) isolated cellulase from earthworms, and found the maximum activity of the enzyme to occur at pH 4.6. Pearce and Phillips (1980) however, measured th pH of the earthworm gut as ranging from 6.5-7.2. Neuhauser et al (1978) also found that the earthworm Eisenia fetida was unable to degrade ground ^{14}C-labelled lignin after 10 days. These findings, together with the rapid gut transit times suggested by the consumption rates stated previously, indicate that any effect of earthworms upon the decomposition of these materials is

likely to be indirect, by the physical breaking up and mixing of the material with soil, and the stimulation of microbial breakdown.

Earthworms also have physical effects upon substrates. Earthworm casts contain more water-stable aggregates than the surrounding soil (Dawson, 1943). Soils containing earthworms have been found to drain 4-10 times faster than soils without earthworms (Hopp and Slater, 1949).

1.4. The potential of earthworms in waste treatment processes.

Knowledge of the role of earthworms in the recycling of organic matter in nature has led to considerable interest in the utilisation of certain species in the treatment of a wide range of agricultural and industrial wastes (vermicomposting). The theoretical advantages of this method over existing systems are that it would not require high technology processes or large energy inputs, and the products of the process (earthworm biomass and stabilised waste) have a potential resource value.

An early study of the application of earthworms to agricultural waste disposal was undertaken by Fosgate and Babb (1972). Neutralised bovine faeces was fed to *Lumbricus terrestris* in culture, and a 10% conversion efficiency of substrate to earthworm biomass was reported. The earthworms were found to contain 58% protein on a dry weight basis, and the manure was reduced in weight by 50% during the experiment. The digested residue was reported to be a satisfactory potting

soil for flowering plants. Graff (1974) considered E. fetida to have potential in waste conversion systems, on the basis of laboratory experiments on the growth and reproduction of this species on a cow dung/straw mixture. Support for this view came from a study of a field population of E. fetida (Watanabe and Tsukamoto, 1976). Mitchell et al (1977) compared the efficiency of L. terrestris and E. fetida in the decomposition of sewage sludge, and concluded that E. fetida is the most suitable, as it is a common inhabitant of rich organic substrates in nature.

Very little work has been published on the suitability of other earthworm species for waste treatment. Neuhauser et al (1979) assessed Eudrilus eugeniae in this respect, and Kaplan et al (1980) studied Amynthus hawayana and Amynthus rodericensis. In both cases, the growth and reproduction rates were found to be greatly below those of E. fetida on the same substrates.

Most of the detailed work on the application of vermicomposting has been carried out on E. fetida, and has concentrated on the treatment of sewage sludges. Mitchell et al (1980) and Horner and Mitchell (1981) studied the effect of this species on the decomposition of two sewage sludges. They found increased and prolonged oxygen consumption and decreased anaerobic decomposition in both sludges when earthworms were present.

The practical application of earthworms to the treatment of sewage sludges was discussed by Hartenstein et al (1979a)

and Neuhauser et al (1980a). It was concluded that such a process appeared to be feasible, but this was based on extrapolation from small-scale experimental data, and remains to be proven on a large scale.

Neuhauser et al (1980b) investigated the potential of E.fetida in the treatment of a wide variety of materials. It was found that earthworms were able to gain weight on organic manures and sludges, but not on simple nutrients (proteins, lipids and carbohydrates, including cellulose). The addition of soil to organic substrates was found to increase weight gain, and this was attributed to the inorganic fraction of the soil. It is possible that the soil was supplying trace nutrients which were limiting in the manures and sludges alone.

Hartenstein (1981) published figures for production efficiency of E. fetida and optimum management practices. It was stated that the optimum retention period of worms in a substrate was 6-8 weeks for biomass production. From the point of view of waste treatment, however, it may be more efficient to have longer retention times in the substrate, and have separate breeding beds which are transferred more often to maximise cocoon production. The maximum carrying capacity of a horse manure substrate was found to be 9.5g live weight of E. fetida on a surface area:volume ratio of 24 cm^2: 110cm^3. The maximum production of biomass at this density was 2g live weight in seven weeks. The author extrapolated this figure to give a U.S. potential production of 6685Kg earthworm

protein ha^{-1} yr^{-1}, which is 100 times higher than the current productivity of beef protein in the U.S.A. Until data are available from large scale vermicomposting systems, the accuracy of this extrapolation cannot be determined. However, it does indicate that the potential contribution of E. fetida to protein production may be significant.

In general, work on the application of earthworms to waste management has concentrated on the production of earthworm biomass, and there are few detailed reports on the changes brought about in wastes by the action of earthworms, though some general effects may be inferred from the literature quoted previously on the role of earthworms in organic recycling in soils. Many authors simply refer to the physical properties of materials after ingestion by earthworms. Some changes effected in sewage sludge by E. fetida were documented by Hartenstein and Hartenstein (1981). It was found that sludge respiration rate stabilised equally rapidly with or without earthworms present, but samples containing earthworms had a significantly higher overall oxygen consumption, and showed a greater degree of mineralisation than those without E. fetida. The presence of earthworms also caused a greater decrease in the C/N ratio of the stabilised sludge, due to assimilation of nitrogen by the earthworms. No data were presented on the forms of nitrogen present in the fresh vs stabilised sludges. Many of the effects of earthworms on the sludge were attributed to the stimulation of microbial activity by the tunnelling and casting activity of the worms, which served to increase

the aeration and surface area of the sludge. Hornor and Mitchell (1981) also found that the passage of sewage sludge through the gut of E. fetida stimulated microbial respiration, and reduced the production of volatile sulphur compounds by anaerobic bacteria. This produced a material which was said to be stable and free from offensive odours.

An economic assessment of the use of earthworms in waste treatment is difficult, as much of the data on earthworm growth rates and substrate turnover must be extrapolated from small-scale laboratory experiments. Pilot-scale treatment plants have been set up and operated sucessfully for short periods, for example the plant at Lufkin, Texas, though these have been unable to sustain long-term continuous operation (Green and Penton, 1981). Operational problems have been due to lack of knowledge of long-term management of the treatment operation, rather than inherent shortcomings in the process.

A detailed feasibility study into the economics of vermicomposting of municipal refuse was published by Camp, Dresser and McKee, Inc. (1980). This was based upon pilot-scale vermicomposting trials in Utah, U.S.A., in which 10 tons of solid municipal waste was vermicomposted. The estimated annual cost of vermicomposting the refuse from a community of 50,000 people was $1,065,000. This gave a unit cost of $24-32 per ton of waste. This compared unfavourably with the alternative methods considered. However, approximately half the cost of the process was incurred in the pre-treatment stage, including separation of ferrous

metals and shredding. If these costs were not taken into account, the cost of vermicomposting dropped to $9-17 per ton, which was comparable with incineration.

The authors of this report stated that because of the health aspect of feeding worms to animals, and the potential heavy metal content of worms and castings from this process, the potential markets for these products could not be economically assessed, but were not considered to be significant.

Not all vermicomposting systems would necessarily be uneconomical, however. Data has been presented which was based upon a study of the Lufkin plant (Pincince et al, 1980). This project used E. fetida to treat a proportion of the primary sludge produced by a sewage treatment plant. The total annual cost of a similar plant treating one dry ton of liquid sludge per day was put at $38-86,000. This was equivalent to a plant serving a community of 10,000 to 15,000 people. The cost per dry ton produced was $105-235, and the authors stated that these costs compared well with alternative treatment methods. They gave the cost of landspreading or dewatering and composting as $150-250 per ton. This assessment did not consider any possible revenue from the products of the process, regarding the operation purely as one of treatment of a potentially polluting waste.

These two analyses clearly demonstrate the importance of the correct choice of substrate for vermicomposting. Heterogenous solid wastes which require expensive pre-treatment, and provide sub-optimal growth conditions, are unsuitable

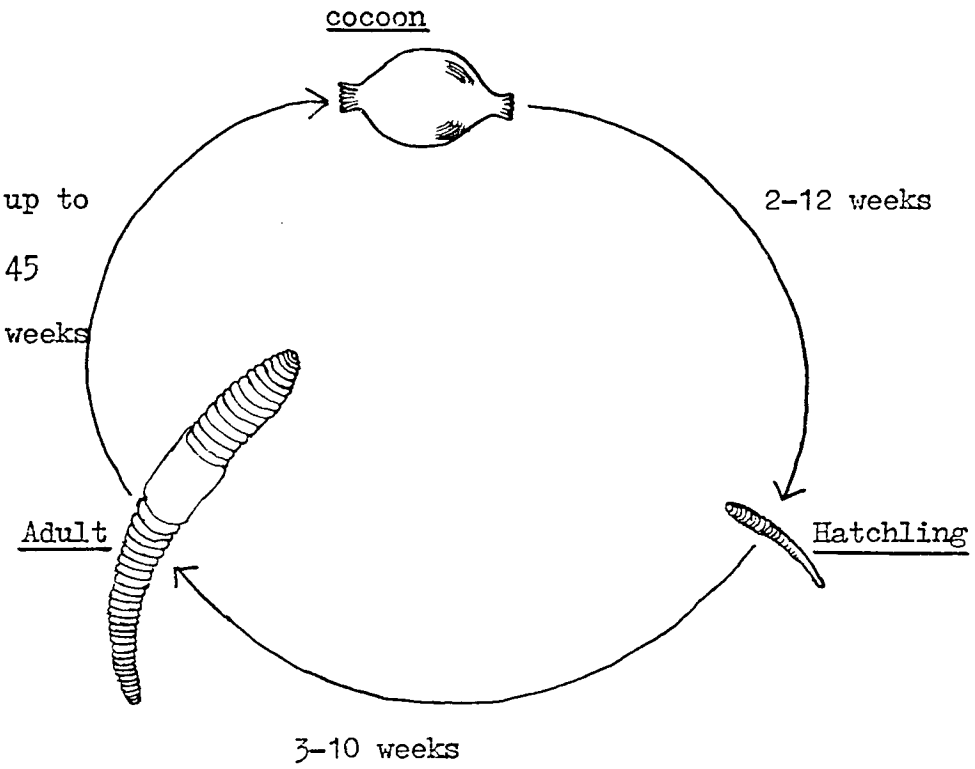
for treatment by earthworms. More homogenous wastes of better nutrient quality which require little or no pre-treatment are better suited to vermicomposting.

1.5. Life cycle of Eisenia fetida.

The life cycle of E. fetida is shown in Fig. 1.1.

The early work on earthworm biology was reviewed by Stephenson (1930). This book is still regarded as a classic reference work on Oligochaetes. Other detailed reviews of the subject include those by Satchell (1967) and more recently, Edwards and Lofty (1972). The latter authors commented upon the lack of detailed knowledge of the life cycles of even quite common earthworms. E. fetida is commonly found in habitats containing high concentrations of decomposing organic matter, such as compost and manure heaps, sewage sludge drying beds and under pastures receiving dung from grazing animals. It is a hermaphrodite and is not usually self-fertilising, so the normal method of reproduction is by copulation followed by cocoon production. Two adult worms come together with their ventral surfaces closely appressed, and their anterior ends pointing in opposite directions. The spermethecal openings of one worm come into close contact with the clitellar region of the other. These areas become constricted and come into close contact. Large quantities of mucus are secreted, so that the reproductive organs of each worm become covered with a slime tube. Seminal fluid is carried forwards from the male

Fig. 1.1. Life cycle of E. fetida.



pore to the clitellar region of each worm, and eventually enters the spermathecae of the opposing worm, though the exact mechanism of this transfer is not completely understood. Clasping and releasing movements of the clitellum are believed to assist this process.

Following copulation, the individuals separate, and the clitellum of each produces a secretion which eventually hardens over its outer surface. Each earthworm then moves backwards, drawing the tube over its head. The ends of the tube then close, to form the cocoon, which is lemon-shaped and measures on average approximately 4mm long and 3mm wide in this species (Evans and Guild (1947)). The cocoons are whitish in colour when formed, and gradually darken to yellow, then brown as they age. The walls of older cocoons become semi-transparent, and mature embryos are visible inside. Stephenson (1930) stated that up to 20 ova per cocoon are produced, though from these only 1-6 embryos develop. Evans and Guild (1948) recorded 1-8 hatchlings per cocoon, with most cocoons (30%) producing 2 hatchlings. Incubation time varies with temperature. Stephenson (1930) stated that most cocoons hatched within 2-3 weeks in the laboratory, but gave no data on culture conditions. Tsukamoto and Watanabe (1977) found a direct relationship between incubation time and temperature with mean incubation time varying from 85 days at 10°C to 19 days at 25°C. They calculated the theoretical minimum temperature for development of cocoons as 5.6°C. However, it was also found that the viability of cocoons

was reduced with increasing temperature. At 10°C 88% of cocoons hatched, whereas at 25°C, only 30% did so. Other workers have reported hatching rates of 81.5% and 85% at 25°C (Hartenstein et al,1979b; Graff, 1974). The reason for these differences is not clear: it may be due to the nutritive value of the substrate to the parent earthworms, as different substrates were used in these experiments; there may also be differences between strains of E. fetida from different sources.

Growth of E. fetida following hatching is also dependant upon environmental factors. Tsukamoto and Watanabe (1977) found that individuals grow from 6mg-680mg in 70 days at 25°C with unlimited food supply (in the form of compost) but at 10°C, the maximum weight achieved was 150mg in 200 days. However Neuhauser et al (1980b) achieved much higher growth rates at 15°C on a variety of substrates. Mean earthworm weights at 70 days were 871mg on horse manure, 1132mg on cow manure, and 1454mg on activated sewage sludge. Initial growth rate was found to be slow, regardless of food source, for 3 weeks, and this was followed by a period of rapid growth for 5-8 weeks. Growth rate then slowed, but weight was maintained provided that food supply was not limiting.

Onset of sexual maturity occurs between 3 and 10 weeks after hatching at 25°C, depending upon population density and food source (Hartenstein et al, 1979a). Most worms become mature between the ages of 4 and 6 weeks, and this appeared to be independant of the two variables mentioned above.

Evans and Guild (1948) found that reproduction in E. fetida

and other species, closely followed seasonal changes in soil temperature, and was continuous at soil temperatures greater than3°C. The total annual number of cocoons produced per worm was given as 11. The culture conditions used by these workers were however, chosen to simulate conditions in the soil, and this environment is not typical of the breeding habitat of E.fetida. These figures do not therefore, reflect the true reproductive potential of this earthworm. The breeding season of E. fetida was stated to be between March and November, but Stephenson (1930) points out that in active compost heaps, populations of E. fetida were able to continue breeding, even at quite low ambient temperatures, for an extended period. Active breeding populations of E. fetida have also been found in sewage sludge heaps in January under a covering of snow (unpublished observations). It is possible then, that under suitable conditions, field populations of E. fetida may be able to produce cocoons throughout the year. This is certainly the case for laboratory cultures. Graff (1974) reported a maximum cocoon production of 3 per individual per week at 25°C when earthworms were cultured in a mixture of cow manure, soil and straw. Hartenstein et al (1979b), who also worked with cultures at 25°C, calculated that at a low population density (3-5 adults in 300cc substrate) E. fetida should actively reproduce for 44 weeks. Their observations showed that cocoon production was still occurring in cultures after 45 weeks, though at a low level. The same workers calculated optimum population density for reproduction to be 8 adult E. fetida per 300cc.

of substrate at 25°C. They found that such a population would produce more than 500 cocoons in a 22 week breeding period, before food supplies became limiting. However these figures relate to a population from which all cocoons were removed as they were produced. Subsequently there was no competition with hatchlings for food. This would have the effect of reducing reproduction rate, as the data presented by the same authors for high population densities show . These figures do show the high potential fecundity of this species. Korschelt (1914) is quoted by Edwards and Lofty (1972) as having kept individuals of E. fetida in culture conditions for 4½ years.

1.6. Optimum physicochemical requirements of E. fetida.

The optimum environmental conditions for E. fetida have been documented by Kaplan et al (1980), who studied the growth of earthworms in response to a variety of parameters. The optimum temperature range was found to be 20-29°C, with high mortality and weight loss above this level. At temperatures below 20°C, worms gained weight down to 5°C, though a 30% mortality rate was observed at this temperature after 2 weeks. At moisture levels of 70-85%, maximum weight gain occurred, while at higher levels, mortality occurred. At lower moisture levels (down to 43%) no mortality was observed, but weight gain was reduced. The tolerable pH range was found to be 5-9, with maximum growth at pH7. The response of the

earthworms to salts was found to be variable. Ammonium ion was very toxic at a concentration of 0.1%, calcium carbonate and sulphate were non-toxic up to 50% concentration, whilst the other salts tested were toxic at the 5-10% levels. Overall electrical conductivity was also assessed, and it was found that animal manures alone, with conductivities of 1.5-3 mmhos do not affect weight gain, but manures which have been contaminated with urine, with conductivities up to 15 mmhos, are toxic to worms within 24 hours. E. fetida can tolerate conductivity up to 8 mmhos without mortality (R. Hartenstein, pers. comm.).

The data presented in section 1.1. on the composition of cow slurry, shows that the pH and moisture content of this material, though variable, fall within the range which is tolerable by E. fetida. The high ammonia content reported for fresh slurries suggests that problems may arise in the vermicomposting of this material, due to the toxicity of the ammonium ion to earthworms. Curry (1976) showed that fresh cattle and pig slurries applied to the land were toxic to earthworms on contact. The toxicity of pig slurry was found to decline rapidly, but cattle slurry remained toxic for 7-8 weeks of storage. The presence of ammonia and other volatile breakdown products in slurry was given as the main reason for this toxic effect.

1.7. Utilisation of the products of vermicomposting.

The products of vermicomposting are earthworm biomass and earthworm castings. In an animal waste treatment operation, the process may be run simply with a view to stabilising the waste for land application. However, potential markets exist for both of these products, though very little research has been carried out on the size of these markets or the financial returns to be expected from the sale of these materials.

The sale of earthworms to fishermen as bait is already an established practice, and the use of earthworms in bulk as food for animals and man has been proposed. Lawrence and Millar (1945) suggested that earthworms could be a source of protein for humans. They found that a mixed field population of earthworms contained 70-80% protein on a dry weight basis. McInroy (1971) investigated the nutrient content of E. fetida, and found that this species had a protein content equivalent to that of commercial fish meal.

Several workers have attempted to define the quality of earthworm protein. Schulz and Graff (1977) found that the essential amino-acids were present in earthworm meal in suitable concentrations for feeding to domestic animals, with the exception of methionine and cystine. Earthworm protein was found to be especially rich in lysine, and it was suggested that it could therefore be used to supplement grain diets, which are lysine-poor. In feeding trials on laboratory rats, the nett protein utilisation of earthworm meal was found to be

equal to that of high quality fishmeal. Sabine (1978) published results on the feeding of earthworm meal to poultry and young pigs, and also found that the protein quality of this food was equal to that of fishmeal. A recent study by Tacon et al (1983) has shown that A.longa and L. terrestris are a suitable food for rainbow trout. However, in the same study, E. fetida was found to be totally unacceptable to the fish. It was found that the coelomic fluid of the earthworms was the cause of this problem, and various treatment methods were described which would render the worm meal palatable. This problem was not described by the other workers quoted above, and it is possible that it is confined to trout. The necessity for additional treatment of earthworms before they can be used as a food would obviously affect any financial benefits to be obtained from the production of earthworm biomass, and further research into this finding is warranted.

A further problem which may arise in the feeding of eathworms to animals is the occurrence of heavy metals in earthworm tissues. It has been found that earthworms concentrate heavy metals from the surrounding environment within their tissues (Gish and Christenson, 1973). Hartenstein et al (1980) found this phenomenon in E. fetida grown on sewage sludge containing high levels of cadmium, zinc, nickel, lead and copper. Although a later study showed that the growth of earthworms would not be affected at the heavy metal concentrations normally encountered in sludges, the presence of heavy metal contamination would certainly preclude the use of earthworm

biomass as an animal food (Hartenstein et al, 1981). Animal wastes in general do not present a problem in this regard, though pig slurry is one possible exception, as copper is sometimes added to the diet of pigs as a growth promoter. Significant amounts of this metal are found in the faeces of such animals. Heavy metal content would not, of course, prevent the utilisation of earthworms solely for the production of stabilised castings from a waste.

Possible markets for earthworm castings were discussed by Pincince et al (1980). The good physical and structural properties of castings were emphasised, and it was suggested that the two most promising outlets for this material were as an organic soil amendment or as an ingredient in potting mixtures. Southgate et al (in press) considered the marketing of windrow-composted municipal sludge, but the general principles may equally be applied to earthworm castings. It was suggested that the sucessful marketing of such a material depends upon the maintenance of a consistently high quality of the final product. Variability of the product is less significant in landscaping and land reclaimation than in the horticultural market, and the former areas were suggested as the major outlets for composted material. However, the financial return from bulk sales would be much lower than those expected from the horticultural market.

Because of the variable nature of many animal wastes, which would be expected to be reflected in the vermicomposted material, the area of quality control is an important one, which merits

further research.

1.8. Possible application of earthworm castings in the mushroom industry.

One possible horticultural application of earthworm castings is in the mushroom industry, as a casing material. Mushroom cultivation is a significant horticultural industry: annual world production of fresh Agaricus mushrooms was estimated at 800,000 tons by Declaire (1978).

Recent general guides to the mushroom cultivation process include Atkins (1972, 1974); Hayes and Nair (1975) and Chang and Hayes (1978). Basically cultivation involves three stages. Firstly a suitable substrate is prepared by composting. This is then inoculated with a pure culture of Agaricus bisporus grown on cereal grain and termed spawn, and the compost is incubated to allow complete colonisation by the growing mycelium. A thin layer of soil, peat or other suitable material is then spread over the compost surface, and this casing layer, in combination with controlled environmental changes, induces the fungus to switch from vegetative to reproductive growth and produce fruitbodies.

The first crop, or "flush" of mushrooms is produced 16-24 days after casing, and further flushes are produced at 7-10 day intervals. Most commercial growers find it most cost-effective to produce four flushes from each batch of compost, after which the process is repeated with fresh compost.

The function and importance of the casing layer in mushroom production have been the subject of much research. Though some aspects still require clarification, the general properties of a good casing material are known.

The physical properties of a material which affect its suitability as a casing include water-holding capacity, pore space and particle size. Bels-Koning (1950) showed that casing materials with higher water-holding capacities produced better yields, and stressed the importance of good casing structure. Reeve et al (1959) concluded that a casing soil moisture between 60-90% was optimal for cropping.

The optimum pH of casing is 7.6, with a range from 5.5 to 8.0 within which pH is not a limiting factor (Lambert and Humfeld, 1939; Allison and Kneebone, 1963). The addition of calcium to casing does not only increase yields by improving pH: calcium-rich soils have a better structure which favours high moisture capacity and good aeration (Stoller, 1952b).

The gaseous environment of the casing layer has been found to be very important in the production of fruitbodies. Lambert (1933) found that high CO_2 concentrations in the air above mushroom beds adversely affected fruitbody morphology and yields. Stoller (1952a) emphasised the importance of sufficient air movement above mushroom beds to ensure that carbon dioxide concentrations were reduced to 0.01 - 0.03% during cropping.

The growing mycelium of A. bisporus has also been shown to produce a number of gaseous metabolites which can affect fruiting by producing abnormal fruiting bodies (Stoller, 1952b).

A possible indirect function of the casing layer in the removal of these metabolites has been suggested (Hayes et al, 1969). Certain casing bacteria were found to be able to utilise these volatiles as a sole carbon source.

The importance of micro-organisms in the casing layer on the production of fruitbodies has been explored by several workers. Hayes et al (1969) found that A. bisporus would not produce fruitbodies in sterile culture if cased with sterile casing. Use of unsterilised casing, or the addition of casing micro-organisms to sterile casing, allowed normal fruiting. The stimulatory organisms were identified as Pseudomonas putida and group IV Pseudomonads. Hume and Hayes (1972) consistently obtained high numbers of primordia in agar cultures of A. bisporus by the addition of agar plugs containing Ps. putida. Work by Arnold (1972) and Eger (1972) confirmed these results.

Although the requirement for bacteria in a casing material has been demonstrated, the exact role of micro-organisms in fruitbody formation remains unclear. Some workers have suggested that bacteria in the casing may absorb nutrients leaked from the mycelium of A. bisporus, so that the mycelium becomes "starved" and produces fruitbodies (Eger, 1961; Long and Jacobs, 1974). Other workers consider that it is the removal of inhibitory compounds by the casing microflora which causes fruiting to take place (Nair and Hayes, 1975; Hayes and Nair, 1976; Couvy, 1976).

Several studies have demonstrated that the casing layer does not appear to provide nutrients to A. bisporus (Stoller, 1952c;

Edwards, 1953 ; Flegg, 1958). Indeed other workers have suggested that the lower availability of nutrients in casing compared with compost is partly responsible for the initiation of fruiting (Mathew, 1961; Long and Jacobs, 1974).

A good casing material then, has a good physical structure and moisture holding capacity, and an optimum pH of 7.5. It should not contain a high concentration of soluble salts, and should allow adequate diffusion of gases from the compost to the air. It should preferably have some ability to immobilise or remove volatile metabolites produced by the growing mycelium. It should contain a suitable microflora and be relatively poor in nutrients compared with a compost.

The most widely-used casing material in mushroom production today is neutralised Sphagnum peat. Most of the peat used in the U.K. industry is imported from Eire. In recent years, as peat resources have begun to decline in that country, the price of peat has increased dramatically. This, coupled with the growth of mushroom cultivation in developing countries where peat is not readily obtainable, has led to increased research into the use of alternative casing media (Nair, 1977; Hayes and Shandilya, 1977; Bowden and Allen, 1978). Recently full-scale trials have established the suitability of an industrial waste from paper manufacture as a substitute casing material (Hayes et al, 1978).

1.9. Interactions between earthworms and micro-organisms.

It is generally accepted that earthworms do not posess an indigenous gut microflora. The species of micro-organisms isolated from the digestive tract of earthworms are essentially the same as those present in the surrounding substrate. Parle (1963a) found that types of bacteria and actinomycetes were the same in the gut of three earthworm species as in the surrounding soil. Khambata and Bhat (1957) isolated common soil-inhabiting bacteria from the gut of Pheretima species in India. Work by Marialigeti (1979) has suggested that there may be exceptions to this view: the species Eisenia lucens was found to have a gut microflora dominated by Gram-negative bacteria which he identified as members of the genus Vibrio.

Several workers have found very large differences in the numbers of micro-organisms in the earthworm gut and surrounding soil. Parle (1963a) found that the gut of L.terrestris, Allolobophora caliginosa and A. longa contained significantly higher numbers of bacteria and actinomycetes than the surrounding soil. It was shown that the numbers of both types increased exponentially between the fore-gut and hindgut, and that the transit time was approximately 20 hrs. Ghilarov (1963) also found higher numbers of micro-organisms in worm casts than in the surrounding soil in three different habitats, and greater numbers of nitrogen-fixing and cellulose-decomposing bacteria have been found in earthworm casts and burrows than in the soil

(Loquet et al, 1977)

The stimulatory effect of earthworms on soil micro-organism appears to differ between species. Kozlovskaya and Zhdannikova (1961) compared micro-organisms in the gut of a deep burrowing species Octolasium lacteum with those in the litter-feeding Lumbricus rubellus . It was found that the gut of O. lacteum contained approximately the same number of micro-organisms as the surrounding soil, while microbial numbers in the gut of L. rubellus were at least ten times greater than in the surrounding soil. However, castings of both species contained more fungi, actinomycetes and cellulose-decomposing and Clostridium-type bacteria. The effects of passage through the earthworm gut are to increase the surface area of soil for microbial colonisation, and to add nutrients in the form of earthworm waste products. The gut also provides a suitable medium for the proliferation of many micro-organisms. In the nutrient-poor deeper soil layers, a greater proportion of the microflora would be in the form of resting stages, and may not be able to take advantage of these effects during gut transit. The surface-litter microflora however, contains large numbers of active micro-organisms which would be able to respond much more rapidly to the environment in the earthworm gut.

The stimulation of microbial activity has been found to continue after castings are produced. Parle (1963b) found that numbers of yeasts, filamentous fungi and actinomycetes increased as castings aged, while bacterial numbers remained high. Oxygen consumption of castings decreased following

production, suggesting that an increasing proportion of the microflora formed resting stages. However, oxygen consumption remained higher in castings than in the surrounding soil for more than fifty days. The nitrate-nitrogen content of castings increased with age, while levels of ammonium nitrogen decreased. Barley and Jennings (1959) also found increased metabolic activity in experimental pots containing Allolobophora caliginosa. Half of the increases in O_2 consumption and nitrogen accumulation were attributed directly to the earthworms, the remainder to stimulation of the decomposer microflora. Mitchell et al (1982) found that E. fetida increased carbon fluxes in sewage sludges. This was again partly attributed to stimulation of microbial activity caused by communition and aeration of the substrate by the worms.

Earthworms may also act as dispersers of micro-organisms which survive passage through their gut. Plant pathogens, especially, may be spread in this way, though little research has been carried out on this subject. Khambata and Bhat (1957) found that spores of a plant-pathogenic Fusarium species remained viable after passage through the earthworm gut, and Hutchinson and Kamel (1956) also found that the presence of L.terrestris greatly increased the spread of fungi through sterilised soil, either by ingestion or by transport on the outer surface of the worm. The significance of this dissemination in the incidence and spread of plant diseases is unknown.

It has been suggested that at least some earthworms may

utilise micro-organisms as a food source, though much of the evidence to support this view is circumstantial. Dawson (1948) stated that the number of species of bacteria decreased following the ingestion of soil by earthworms, while fungal species were largely unaffected. This was attributed to the greater resistance of fungal spores to digestion. Solbe (1971) found populations of Eisenniella tetraedra and Dendrobaena rubida f. subrubicunda in a percolating filter, and concluded that the worms were grazing on the microbial biomass of the filter film. Miles (1963) suggested that the presence of bacteria, fungi and soil protozoa were all necessary for the normal growth and development of E. fetida, but these experiments were conducted in soil, which is not the preferred substrate of this species, and so may not reflect normal feeding behavior. Brusewitz (1959) found that Escherichia coli inoculated into soil was reduced in numbers in the presence of E. fetida, and Khambata and Bhat (1957) found E. coli only once in the gut of Pheretima species, even though these worms were common inhabitants of soil manured with human excrement. The latter authors suggested that intestinal secretions of the earthworms affected the growth of pathogenic bacteria. However, no data was presented on the numbers of these organisms in the soil from which the worms were collected. Brown and Mitchell (1981) found that survival of Salmonella in sewage sludge was greatly reduced by passage through the gut of E. fetida, but when Salmonella was inoculated into a mixed culture of bacteria isolated from earthworm faeces, similar reductions

in numbers of the pathogen occurred. This suggests that the reduction may have been largely due to competition from the indigenous microflora.

Evidence of selective feeding of earthworms on micro-organisms was presented by Cooke and Luxton (1980) and Cooke (1983). It was found that L. terrestris had definite preferences for filter paper discs inoculated with some species of fungi. Satchell and Lowe (1967) had earlier found leaves from some tree species to be more palatable than others, and attributed this to their phenolic content. Wright (1972), however, found that L. terrestris actively ingested leaves and filter paper discs spread with cells of the bacterium Pseudomonas aeruginosa, and suggested that the earthworms were chemically attracted to food colonised by micro-organisms. It is possible that other species which feed by ingestion of soils or organic matter selectively ingest parts of the substrate containing palatable micro-organisms. Dash et al (1979) suggested that earthworms fed selectively on soil particles which had been colonised by fungi.

Several workers have suggested that earthworms selectively digest micro-organisms. When Day (1950) inoculated soils heavily with cultures of Serratia marcesens and Bacillus cereus var. mycoides, then added L. terrestris, he found that Serratia was totally destroyed by passage through the earthworm gut, while numbers of Bacillus were greatly reduced. It was concluded that vegetative cells were destroyed, while spores could survive. Piearce and Phillips (1980) found that ciliate

protozoans were lysed in the midgut fluid of L. terrestris, while astomatous ciliates were unaffected. Dash et al (1980) found evidence of selective digestion of fungi by Enchytraeid worms. Trichoderma and Syncephalustrum species were digested or rendered non-viable after passage through the gut, while spores of Penicillium and Aspergillus species remained viable. Rouelle (1983) studied the fate of amoebae and bacteria in the gut of L. terrestris and E. fetida. In both worms it was found that non-encysted protozoa disappeared in the mid-gut region. The bacterium Rhizobium japonicum was isolated from all parts of the gut, and was apparently able to pass through unharmed, though numbers were not assessed quantitatively. The author suggests that the presence of organic matter in the gut of the earthworm may protect some micro-organisms from contact with digestive enzymes, and so allow them to pass through unharmed.

Another theory of earthworm nutrition is that it is the products of microbial metabolism, rather than the organisms themselves, which are the important food source for earthworms. If this is correct, then it would be expected that earthworms would grow better in the presence of live micro-organisms than dead ones. Neuhauser et al (1980b) published data which seem to support this theory, though the results obtained were variable, and there was a possibilty of interference from the presence of other micro-organisms.

SECTION 2.

MATERIALS AND METHODS

2.1. Chemical and microbial changes in decomposing cow slurry during storage.

2.1.1. Collection of slurry samples.

Supplies of dairy cow slurry were obtained from Meathop Park Farm, Grange-over-Sands, Cumbria. On this farm, slurry deposited in the milking shed was scraped into a holding pit during the cleaning operation immediately after every milking session. Every 2-3 weeks, the slurry from the pit was pumped into a tanker and sprayed onto the fields.

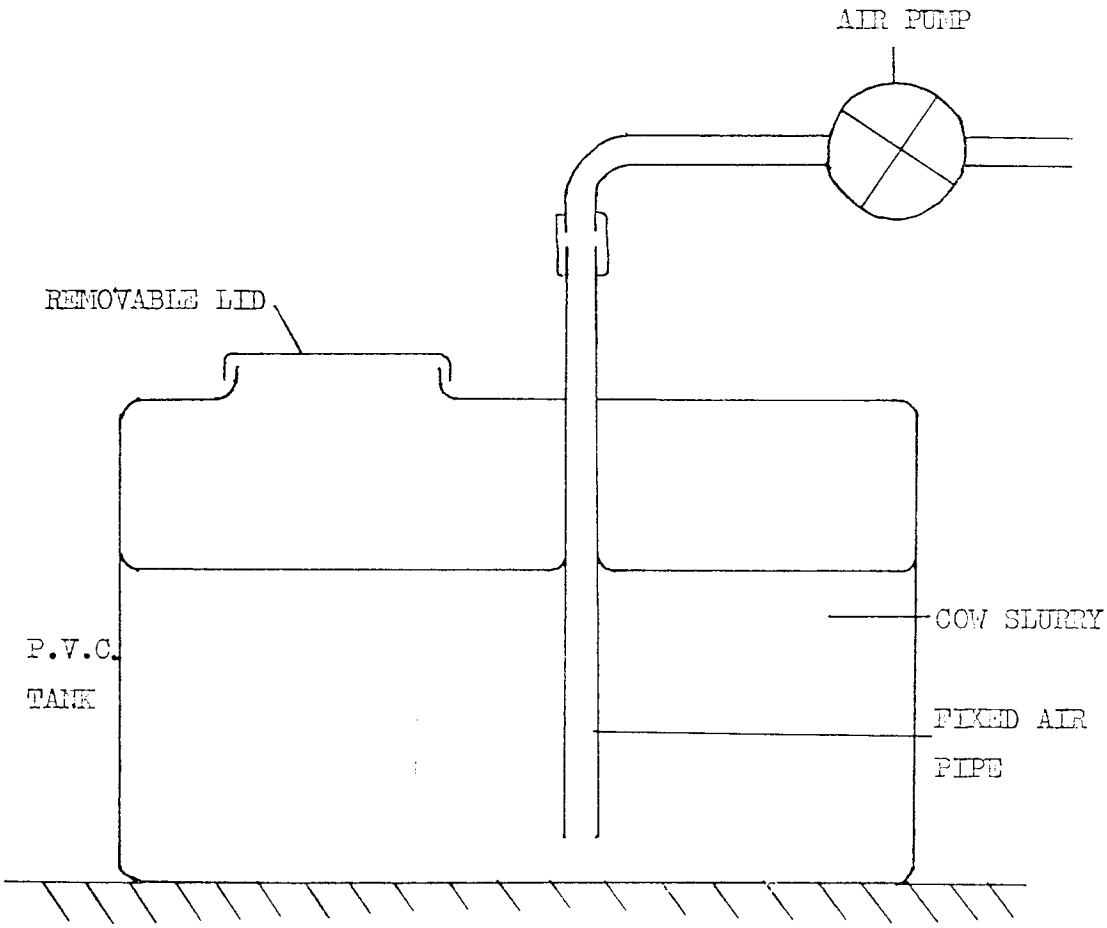
In order to obtain slurry in a fresh condition for experimental purposes, samples were collected during the scraping-out operation following the morning milking session. Slurry was shovelled into a 227l. P.V.C. water tank mounted on a vehicle trailer for transportation to the laboratory (Fig.2.1.)

2.1.2. Storage conditions.

Slurry was stored in the collecting tank, which was kept under cover at ambient temperature. To promote aerobic conditions in the slurry, an air pump was connected to the air inlet pipe shown in fig.2.1.and air at $1.4g.mm^{-2}$ pressure was blown through the slurry. The slurry was also thoroughly mixed every 12-24h to reduce settling.

As an alternative to aerated storage, a sub-sample of the slurry was spread in a layer 25mm deep in free-draining plastic

Fig. 2.1. Vessel for aerated storage of cow slurry.



propagating trays (350 x 220 x 50mm). The slurry in the trays was allowed to drain and age at ambient temperature.

2.1.3. Sampling of slurry for chemical and microbiological analyses.

Samples of decomposing slurry were taken from the tank at time 0 and every 2-3 days thereafter for 15 days after collection, then every week for the next two weeks. Samples were taken from this for chemical and microbiological analysis. Samples were also taken from the slurry trays after thorough mixing.

2.1.4. Estimation of aerobic microbial populations.

Numbers of bacteria, fungi, and actinomycetes were estimated by dilution plating (Brierley et al, 1927) and an agar film technique (Jones and Mollison, 1948).

(i) Dilution plates.

The diluent used throughout this study was 0.1% peptone water. This was found to cause less cell disruption and mortality than other diluents, such as Ringers solution or saline (Straka and Stokes, 1957).

Dilution blanks were prepared by dispensing 9ml aliquots of 0.1% peptone water into 20ml screw-capped McCartney bottles. The bottles were sterilized by autoclaving. To prepare a dilution series, a 10ml sample of slurry was added to 90ml of sterile diluent. The suspension was shaken at 100r.p.m. for 5 min., and allowed to settle for a further 5 min. A sterile 1ml pipette was rinsed out five times in the suspension, in order to minimise errors by adsorption of cells onto the pipette walls, and to further mix the sample. A 1ml aliquot was then transferred aseptically to 9ml of sterile diluent. A second pipette was used to repeat the operation, transferring 1ml from the first dilution blank. The sample was thus diluted ten-fold for each step in the series. Three replicate dilution series were prepared for each sample. Suitable dilutions (determined by preliminary experiments) were selected, and 1ml aliquots of these were pipetted aseptically into 75mm diameter petri dishes. For bacteria 10^{-6}–10^{-10} dilutions were prepared, and for fungi, 10^{-4}–10^{-7} dilutions were prepared. The plates were immediately poured with agar media.

Tryptone soya agar (Oxoid) and Bacto-nutrient agar (Difco) were used as culture media for both bacteria and actinomycetes. These were chosen because they are general-purpose media which support the growth of a wide range of organisms. Fungi were cultured on 2% malt extract agar (Oxoid), with the addition of Rose Bengal at $0.6g.l^{-1}$. Rose Bengal has been found by several workers (Martin, 1950; Ottow and Glathe, 1968) to prevent the growth of bacteria and to restrict the spread of fast-growing fungi which might swamp the plates.

Media at 45°C were added to each Petri dish and the dishes were rotated and moved backwards and forwards four times in each direction, in order to thoroughly mix and disperse the diluent in the medium. For each culture medium, three replicate plates were prepared per dilution series, giving nine replicates of each sample at each chosen dilution. The high dilutions were plated out for bacteria, and lower dilutions for actinomycetes and fungi.

All plates were incubated at 25± 0.5°C, and bacteria were counted after both seven and twenty-one days, to allow for slow growth of some species. Plates showing 30-300 bacterial colonies, and those showing 3-30 colonies of fungi and actinomycetes were counted. Counts were expressed as numbers of Colony-Forming Units (C.F.U.s) per gramme dry weight of slurry.

(ii) Bacterial counts using agar films

The agar film technique was devised by Jones and Mollison (1948) and subsequently modified by Thomas et al (1965) and Nicholas and Parkinson (1967). It was originally intended as a technique for estimating the length of fungal hyphae in soil samples, but may also be used for the estimation of numbers of bacteria of different size classes (Jenkinson, Powlson and Wedderburn, 1976). Agar films of standard thickness, containing a known amount of sample, are prepared and stained, and suspended bacteria can then be counted using a light microscope.

The method allows more precise counts than dilution plating,

as colony-forming units on plates may originate from clumps of many organisms. In addition, all media used in dilution plating are species selective to some extent. With dilution plating only viable organisms are counted, but these are not necessarily in an active form e.g. spores may germinate to give seemingly high counts for some species. Comparison of the results obtained from the two methods, however, can give some indication of the proportion of the microflora that is present in a viable, active condition.

Freshly collected samples of slurry were weighed into aluminium tins for dry weight estimation. At the same time 0.5g samples for agar films were placed in square-bottomed McCartney bottles, and 4ml of sterile distilled water was added. Single samples were homogenised in a Wareing blender at 14,000 r.p.m. for 3 min. The homogenate was then washed into Pyrex boiling tubes with a further 6ml of distilled water. Molten, filtered 1.5% Oxoid no.3 agar was then added to each tube in 40ml aliquots. The tubes were then capped and inverted slowly several times, to mix the suspension with minimal formation of air bubbles. The tubes were maintained in a water bath at 50°C until the agar films were prepared.

Immediately before preparation of the films, each tube was again inverted and allowed to settle for 10 sec. A small amount of the suspension was then taken with a sterile pasteur pipette, inserted just below the surface. The suspension was then pipetted onto the platform of a haemocytometer slide of 0.1mm depth. The well of the slide was immediately covered with a glass coverslip, and a 5g weight was added. When the agar suspension had solidified, it was immersed in a tray of

distilled water and the coverslip was carefully removed. Excess agar was trimmed away from the slide well with a razor blade. The agar film was then floated off the haemocytometer slide and onto a flat glass microscope slide. Manipulation of the film was carried out with a fine sable-haired brush.

The prepared slides were allowed to dry at room temperature in a covered container to exclude dust. The dried films were stained for 1h. with phenolic aniline blue, washed in absolute alcohol, and mounted in Euparal for storage until they could be counted. For each set of films prepared, control films of only agar and sterile distilled water were made. Three replicates of sample and control films were prepared. Counts were made at x1000 magnification, using an eyepiece grid. Five fields of view were chosen at random on each slide, and numbers of bacteria within the whole grid were counted for each field of view. Bacteria were separated into size classes similar to those used by Jenkinson, Powlson and Wedderburn (1976), but in these experiments only two classes were recorded: spherical organisms (cocci) less than 6µm in diameter; and cylindrical organisms (rods) less than 6µm in length. Control counts were subtracted from the experimental counts before analysis of the results.

2.1.5. Isolation of micro-organisms

Colonies of the most numerous bacteria, fungi and actinomycetes appearing on dilution plates were picked off using a flamed

nichrome wire needle, and subcultured on tryptone soya agar (for bacteria and actinomycetes), or 2% malt extract agar (for fungi), to check purity.

Pure cultures were then transferred to agar slopes for storage. Bacteria and actinomycetes were stored in duplicate on tryptone soya agar, and fungi were stored in duplicate on 2% potato dextrose agar. Stock cultures were maintained at 18°C and 6°C and subcultured at three-monthly intervals.

2.1.6. Identification of Micro-organisms.

Bacteria were classified using the A.P.I. 20B microbial identification system (A.P.I. Laboratory Products. Basingstoke, Hants.). This consists of a strip of 20 microtubules containing dehydrated substrates which enable a total of 22 biochemical tests to be performed on each isolate. Results are obtained after 24-48 hours incubation at 25°C. For purposes of identification, the results of these tests are combined with supplementary tests on Gram-staining, respiratory metabolism and microscopic examination of morphology. Isolates were identified by reference to Skerman (1959), Buchanan and Gibbons (1974) and Skinner and Lovelock (1979).

Fungi were identified using colony characteristics, especially the structure of reproductive stages, with reference to Barnett and Hunter (1972), Barron(1968), Pitt (1979), Raper,Fennell and Austwick (1977), Rifai (1969), Hermanides-Nijhof (1977), and Richardson and Watling (1968).

Actinomycetes were classified using the methods described by Williams *et al* (1969), with reference to Buchanan and Gibbons (1974).

2.2 Laboratory culture of Eisenia fetida.

Stock cultures of *Eisenia fetida* were maintained in the laboratory for use in experiments. Earthworms were obtained from natural populations in paper waste and sewage sludge. The worms were hand-sorted to exclude species other than *Eisenia fetida* (especially *Eisenia fetida andrei* and *Dendrobaena* spp., which are commonly found in association with *Eisenia fetida* in nature) and then placed in culture vessels.

Two types of culture vessel were used throughout this study. The first consisted of a large plastic funnel (0.65m x 0.47m diam) supported in a metal frame . The base was filled with coarse gravel for drainage, and a layer of coarse muslin material was placed over this to prevent the escape of earthworms. A thin layer (0.25m) of moist garden soil was then placed in the bottom of the funnel, and the sorted earthworms were added. The funnel was then filled to a depth of approximately 0.30m with fresh horse manure, obtained from a local stables, as a food source. The culture vessel was covered with thick polythene sheeting to reduce water loss by evaporation.

Consumption of the manure by the worms was assessed by visual examination, and fresh supplies of manure were added

to the surface of the beds at regular intervals. Every 3-4 months the bed was replenished. A thin layer of fresh manure was added to the surface, left for 2-3 days to allow worms to move up into it to feed, then the top 50-100mm layer of earthworm-rich material was scraped off. The remainder of the worm-worked material was removed from the funnel and hand-sorted to separate any remaining earthworms. The earthworm-rich layer was then replaced in the empty funnel to form the base of a fresh culture.

The second type of culture vessel used in this study was a large circular plastic tank with supporting legs (0.375m x 0.9m diam.). A 50mm diameter plastic pipe, with holes drilled at regular intervals along its length, was inserted into the centre of the tank to aid drainage and aeration of the bed, and further drainage holes were drilled into the bottom of the tank. All drainage holes were covered with squares of coarse muslin cloth to prevent escape of worms.

Paper tissue waste, obtained from Bowaters Paper Co. Ltd. Kemsley, Kent, was used as a bedding material. This was placed in the tank to a depth of 220mm, and earthworms were added. The food source used in this culture was cow slurry. This was obtained from the Royal Agricultural Showground, Stoneleigh, Warwickshire, and stored in a deep freeze until required. Slurry was applied to the surface of the culture bed to replace that consumed by the earthworms. The bed was covered with thick polythene sheeting to reduce water loss.

Replenishment of the worm bed was carried out every 3-4 months,

using a similar procedure to that described for the other culture system. The bed was refilled with fresh tissue waste over a thin layer of earthworm-rich material. Both types of culture vessel were kept in a darkened room at an air temperature of 25°C and a relative humidity of 70-80%

2.3. Vermicomposting of cow slurry.

2.3.1. Toxicity of raw slurry.

Fresh cattle slurry was found by Curry (1976) to be toxic to earthworms under both laboratory and field conditions, but in a vermicomposting system, it is desirable that the slurry should be available to earthworms as a food source as soon as possible after production. Different storage and treatment methods were therefore investigated, in an attempt to quickly reduce slurry toxicity.

2.3.2. Storage and treatment of slurry.

(i) Aeration.

The aeration system described in section 2.1.2. for storage of slurry was employed: compressed air was blown through the slurry, combined with regular thorough manual mixing to prevent build-up of anaerobic conditions.

(ii) Tray drying.

Slurry was spread in approximately 25mm layers in free-draining plastic propagating trays (350 x 220 x 50mm) and allowed to drain and age at room temperature.

(iii) Separation and dilution.

Physical separation of slurry into solid and liquid fractions was achieved by manually pressing it through fine muslin mesh in a 5mm soil sieve. The liquid fraction was collected in a container beneath the sieve. Both fractions were placed in shallow trays and stored at room temperature.

Fresh slurry was diluted 1:1, 1:5, 1:10 and 1:20 (v/v) by the addition of distilled water. The diluted slurry was immediately tested for its toxic effects upon earthworms.

Fresh slurry was mixed by trowel with freshly-dug woodland soil and several-month old dried cow dung. Soil/slurry and dung/slurry mixtures of 1:1, 2:1, and 3:1 (w/w) were prepared. The mixtures were aged in shallow trays at room temperature.

2.3.3. Toxicity testing.

Samples of stored slurry were taken every 7 days, and placed in small plastic pots. In the case of the liquid

fraction of separated slurry and the diluted slurry, the pots were filled with polystyrene packing "chips", and the liquid was poured over them. Five replicates of each sample were prepared. One E. fetida was added to each pot, and the pots were sealed with fine muslin cloth and incubated at 25°C for 24 hours. Toxicity of samples was assessed by recording earthworm mortality after 24 hours. The experiment was run for three weeks.

2.3.4. Assessment of different vermicomposting systems.

(i) Mixing of slurry with bedding materials.

Soil and peat were chosen as possible bedding materials for earthworm culture because they are readily available and have a good moisture absorbing capacity. They also provide sites for adsorption of toxic fractions of cow slurry. In addition to these materials, two types of waste from the paper industry were examined: paper mill by-product (P.M.B.), which is a solid material produced as a result of newspaper manufacture, and waste from the manufacture of paper tissues. Large volumes of these wastes are produced each year, and they represent a disposal problem to the paper industry.

All the materials were wetted to at least 60% moisture content and neutralised using powdered calcium carbonate. Fresh slurry was then mixed with the bedding materials in the proportions 1:1 and 2:1 (w/w) bedding:slurry. Controls were prepared by adding distilled water to the neutralised bedding materials.

The mixtures were then heaped onto polythene sheeting in the laboratory and tested for toxicity to E. fetida every day, as described in section 2.3.3.

When the materials became non-toxic to the earthworms, 50mm deep Petri dishes were filled with 100g wet weight of the material. Three replicates of each mixture were prepared, with a further three replicates of each control material. Large juvenile specimens of E. fetida were removed from laboratory stocks and washed several times in ditilled water to clean them. The worms were then blotted dry on filter paper and weighed in groups of five. One group was added to each prepared dish. The dishes were covered with standard Petri dish lids and incubated at 25°C in a humid environment. The worms were removed from the dishes weekly, washed, blotted, and reweighed. After five weeks, the number of cocoons present in each dish was counted.

(ii) Top-feeding of slurry onto worm beds.

In this system, cow slurry was applied to the surface of solid bedding materials containing earthworms. Soil and tissue waste were used as bedding materials in this experiment. All were moistened to at least 60% moisture, and neutralised with calcium carbonate.

A series of 50mm diameter plastic flower pots were filled with 100g wet weight of bedding material, ten pots containing soil and fifteen pots containing tissue waste were prepared,

Juvenile E. fetida were taken from laboratory cultures, washed and blotted, then weighed and added to the pots, five worms to each one. Five pots containing soil and five containing tissue waste had 10g of fresh slurry applied to the surface. A further five pots containing tissue waste received 20g of fresh slurry. The remaining five pots of each series were watered with 20ml of distilled water as controls. All pots were then covered with aluminium foil and secured with rubber bands. Small holes were punched into the foil with a dissecting needle to allow gaseous exchange. Pots were incubated at 25°C at a controlled humidity of 80%.

Experimental pots were examined visually each day for signs of feeding by earthworms on the applied slurry. Appropriate amounts of fresh slurry were added to the pots as needed. Control pots were watered weekly. Each week, the earthworms were removed from the pots with as little disturbance of the surface as possible, then washed and weighed. At the end of the experiment, the number of cocoons per pot was counted.

2.4. Chemical and microbiological changes during the vermicomposting of cow slurry.

2.4.1. Slurry mixed with a bedding material.

Twelve square plastic tubs were set up, each containing 500g of 2:1 (w/w) tissue waste:cow slurry mixture, prepared as described in the previous section. Six tubs were inoculated

with cleaned juvenile E. fetida, twenty-five worms to each tub. All the tubs were then covered with aluminium foil secured with rubber bands. The foil was pierced with a needle to allow aeration. The tubs were incubated at 25°C and 80% relative humidity.

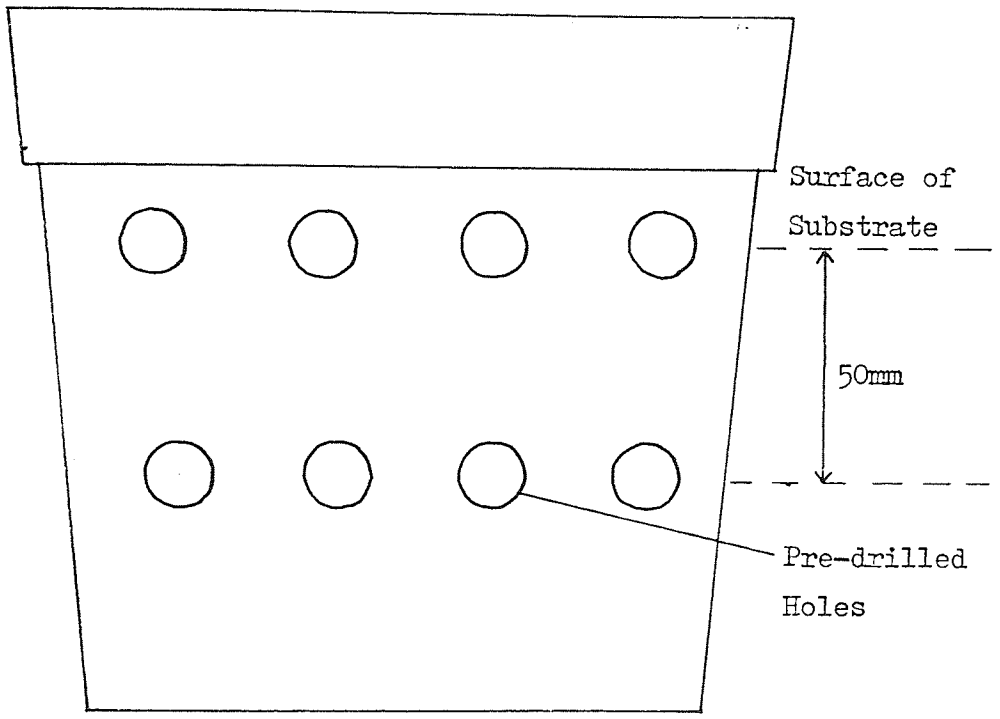
At the beginning of the experiment, three tubs were chosen at random from each of the treatments to be sampled for microbial analysis. At weekly intervals, the contents of each tub were tipped out onto sterile plastic trays and mixed with a sterile spatula. Three replicate 1g samples were removed from each tub for determination of numbers and types of micro-organisms present by dilution plating. A 20g sample was also removed from each tub for estimation of moisture content.

The remaining tubs provided samples for chemical analysis. The three tubs containing tissue waste:slurry mixture and earthworms were sampled at the beginning of the experiment, and subsequently after 2, 3 and 6 weeks of incubation. The tubs without earthworms were sampled at the beginning and end of the experiment. The tubs were emptied onto plastic trays and mixed. From each tub, 50g of material was removed, and samples from each treatment were combined to give a composite sample for chemical analysis.

2.4.2. Top-feeding of slurry to worm beds.

Holes were drilled in the sides of six plastic tubs as shown in fig. 2.2. . The holes were covered with insulating

Fig. 2.2. Top-feeding of cow slurry to worm beds- experimental boxes.



tape, and 500g of tissue waste was placed in each tub. Fifty weighed adult and juvenile E. fetida from laboratory stocks were added to each tub. Three tubs then had 250g wet weight of fresh cow slurry added as a thin layer on the surface. The remaining tubs were well watered with distilled water as controls. All the tubs were covered with aluminium foil and incubated at 25°C and 80% relative humidity.

At 0, 2, 5 and 7 days after application of cow slurry, horizontal samples were taken at the surface of the beds and at a depth of 50mm by inserting a sterilised cork borer through the pre-drilled holes in the tubs. Moisture content, pH, and electrical conductivity, ammonia volatilisation and ammonium-nitrogen content of the samples were determined, and microbial counts were also taken. Dilution plates were used to estimate the numbers of fungal propagules present, while bacteria and actinomycetes were enumerated by the droplet technique (Sharpe and Kilsby, 1971; Sharpe et al., 1972). This is a miniature pour-plate method. Each dilution was plated out as a series of 0.1ml agar droplets containing suspended bacteria. After 48 hours incubation, colonies in each droplet were counted using a magnifying viewer. In this study the Colworth Droplette apparatus was used for both dispensing and viewing of droplets. Numbers of micro-organisms isolated from each sample were recorded.

2.5. Interactions between earthworms and micro-organisms.

2.5.1. Choice of experimental substrate.

For initial screening of micro-organisms for their nutritive value to E. fetida, it was considered necessary to minimise any secondary effects arising from the experimental conditions. Several relatively inert materials were therefore tested for their suitability as substrates for E. fetida. The main criterion for this was that the sterilised materials should allow survival and normal behavior of the earthworms for at least seven days and preferably longer.

Materials tested were; shredded filter paper, sand mixed with shredded filter paper 50/50 (v/v), sand mixed with ∝-cellulose powder 5:1 (v/v), neutralised peat, and neutralised "perlite". Wetted test materials were placed in glass tubes (200 x 50mm diameter) to a depth of 100mm. The tubes were plugged with non-absobent cotton wool, and autoclaved at 121°C for 20 min. Three tubes of each material were prepared, and five adult earthworms were added to each. Survival and activity of the earthworms was monitored daily for fourteen days.

2.5.2. Reduction of microbial contamination of earthworms.

In order to achieve meaningful results from studies of earthworm feeding preferences, it was considered necessary to achieve a large reduction in the level of microbial

contamination of earthworms. Two approaches were taken to this problem: the first involved the treatment of earthworm cocoons with chemical agents and antibiotics with subsequent sterile culture of hatchlings. The second approach was to apply chemical and antibiotic treatments to juvenile worms, which could then be used directly in feeding experiments.

(i) Treatment of earthworm cocoons with chemical sterilants.

Cocoons taken from stock cultures of E. fetida were washed clean in distilled water, then immersed in several concentrations of chemical sterilants. The sterilants used were: sodium hypochlorite 0.5%; formalin 1%; mercuric chloride 0.05%; and cetrimide 5%. In addition, a solution containing 2.5% sodium hypochlorite and 0.5% Teepol in distilled water was also tested. This has been suggested as a surface sterilising agent for earthworm cocoons (J.Rouelle, pers. comm.). Exposure times of the cocoons in the sterilising agents were 5, 10, 20, 30 and 60min. Control cocoons were immersed in sterile de-ionised water for 60min. At the end of the contact time, cocoons were removed and washed in sterile de-ionised water for 5 min. Six cocoons from each treatment were then placed singly in Petri dishes, washed with 1ml of sterile de-ionised water, then replaced. Three dishes from each treatment were then over-poured with nutrient agar, the remainder with malt extract agar and Rose Bengal. Plates were incubated for 14 days at 25°C, and the level of contamination of treated cocoons was compared with

controls, as a measure of the effectiveness of the treatments.

Cocoons were transferred in pairs to Petri dishes containing sterile filter paper moistened with sterile de-ionised water. The dishes were enclosed in plastic bags and incubated at 25°C in a humid incubator for 8 weeks. During this period, numbers of hatchlings produced were noted, and these were removed aseptically, and placed in large pyrex boiling tubes containing sterile tissue waste.

(ii) Antibiotic treatment of earthworm cocoons.

Initial experiments were run to determine the effect of different concentrations of antibiotics on the hatchability of cocoons. From the results of these trials, a concentration of 50μg.ml^{-1} of tetracycline, streptomycin, cycloheximide and nisin was chosen for use in further experiments, as these concentrations did not significantly affect the number of hatchlings produced compared with control cocoons.

Cocoons from laboratory stocks were washed in distilled water, then immersed in sterile antibiotic mixtures. The mixtures were as follows: cycloheximide + each of the other antibiotics in turn; cycloheximide + combinations of two of the other antibiotics; and a mixture of all four antibiotics. In all treatments, the final concentration of each antibiotic in the mixture was 50μg ml^{-1}. Controls contained sterile de-ionised water only.

Twenty cocoons were used per treatment. They were soaked

in the antibiotic solution for 10 hours, then transferred in pairs to Petri dishes containing sterile filter paper moistened with the appropriate solution. The Petri dishes were then placed in plastic bags and incubated at 25°C under humid conditions for 8 weeks. During this period, hatchlings which emerged were aseptically transferred to tubes containing sterile paper tissue waste. At the end of the incubation period, cocoons were removed, and the dishes were overpoured with nutrient or malt extract agar, and incubated at 25°C for 14 days. Numbers of contaminating micro-organisms on treatment and control plates were compared.

(iii) Treatment of earthworms with chemical sterilants.

Large juvenile E. fetida from stock cultures were washed in distilled water and immersed in different concentrations of the chemical sterilants sodium hypochlorite, mercuric chloride, formalin and cetrimide. Immersion times were 30 sec., 1, 2, 3, 5, and 10 min. Control worms were immersed in de-ionised water for 10min. The worms were then rinsed in de-ionised water, and placed singly in 10ml sterile peptone water for 30mins. Dilutions up to 10^4 were prepared in nutrient and malt extract agars, and incubated at 25°C for subsequent counting. Earthworms were kept under observation for several hours for assessment of the long-term effects of the treatments upon earthworm survival.

(iv) Antibiotic treatment of earthworms.

Following initial experiments to determine the toxicity of several antibiotics to earthworms the following antibiotic mixture was chosen for further investigation: cycloheximide at 100$\mu g.ml^{-1}$ concentration; aureomycin (chlortetracycline hydrochloride) at 6$\mu g.ml^{-1}$; and nisin at 50$\mu g.ml^{-1}$.

The effectiveness of this mixture in reducing microbial contamination of E. fetida was investigated. Pyrex 250ml conical flasks containing de-ionised water were sterilised by autoclaving. Concentrated antibiotic solutions were filter-sterilised and added to the flasks in appropriate amounts to give 100ml of the final antibiotic mixture.

Large adult E.fetida were removed from laboratory stocks, washed clean in de-ionised water, and immersed in fresh sterile de-ionised water for 24 hours to encourage voiding of the gut contents. Earthworms were then transferred to fresh de-ionised water and rinsed for two hours. Individual earthworms were then transferred aseptically to the flasks containing the antibiotic mixtures and stored in darkness at room temperature.

At the beginning of the experiment, and every 24 hours for the next four days, three worms were removed from the flasks and rinsed in sterile de-ionised water for 2 hours. Each worm was then placed in a McCartney bottle with 10ml sterile peptone water and shaken vigorously for 1 min. The worms were left in the bottles for 30 min., then removed aseptically. The peptone water was then used to prepare dilution series. Dilutions up

to 10^{-4} were mixed with nutrient and malt extract agars. Plates were incubated at 25°C in Petri plates for subsequent counting. The results of this experiment enabled a standardised method to be developed for cleaning earthworms prior to their use in feeding experiments.

Filter-sterilised stock antibiotic solutions were made up and added aseptically to sterile de-ionised water in 250ml conical flasks, to give 100ml of final mixture. Large juvenile E. fetida were washed clean in de-ionised water, then immersed in de-ionised water for 24 hours. After rinsing in fresh de-ionised water for 2 hours, worms were blotted dry on sterile filter paper and weighed in batches of five. Each group of five worms was then transferred to a flask containing the antibiotic mixture. Flasks were stored in darkness at room temperature for 24 hours, then the earthworms were transferred in batches to sterile de-ionised water for a further 2 hours as a final rinse.

2.5.3. Feeding of earthworms with pure cultures of micro-organisms.

Initial screening experiments were performed in microcosms. These consisted of glass tubes, 50mm in diameter and 200mm deep, filled with 150g of a 5:1 (v/v) mixture of fine acid-washed sand and powdered α-cellulose, at a moisture content of 75%. The tubes were sterilised by autoclaving at 121°C for 1 hour. Antibiotically treated earthworms of known weight were then added to the tubes, five earthworms per tube.

The following micro-organisms were isolated and enumerated from cow slurry and cow slurry/tissue waste mixtures:

Acinetobacter lwoffi

Acinetobacter citroalkaligenes

Alkaligenes faecalis

Arthrobacter simplex

Arthrobacter tumescens

Enterobacter cloacae

Flavobacterium lutescens

Micrococcus luteus

Micrococcus varians

Pseudomonas fluorescens

Pseudomonas putida

Rhodotorula sp.

Aspergillus fumigatus

Aureobasidium pullulans

Fusarium oxysporum

Gliocladium deliquescens

Gliomastix murorum var. *felina*

Mucor plumbeus

Penicillium purpurogenum

Trichoderma harzianum

Scopulariopsis sp.

Trichoderma hamatum

Nocardia salmonicolor

Streptomyces sp.

Pure cultures of these isolates were grown up in shake culture at 25°C for 48 hours in tryptone soya broth (for bacteria and actinomycetes), or for 72 hours in malt extract broth (for fungi). The resulting biomass was harvested by centrifugation at 5,000 r.p.m. for 10 min. Pellets were re-suspended in sterile peptone water and re-centrifuged. The supernatant was discarded, and two further washings were performed. The pellets were finally re-suspended in peptone water. The cell suspensions were then divided into two halves, and one half was autoclaved at 110°C for 20 min. to kill the organisms.

Live and heat-killed micro-organisms were presented to the earthworms as a food source by pipetting 5ml of the appropriate suspension onto the surface of the substrate in each of five tubes. Controls consisted of tubes watered with sterile peptone water only. Experiments were run for 7 days at 25°C. During this period, tubes were re-fed with the appropriate cell suspensions every 48 hours.

At the end of the experiment, the earthworms were retrieved from each tube, washed in de-ionised water, blotted, and re-weighed. The interaction of E. fetida with test micro-organisms was assessed as the mean weight change of fed worms compared with that of unfed controls. Five replicates of each experimental treatment were performed, with nine replications of the control treatment. The results were analysed using Dunnetts analysis of variance.

2.5.4 Production of axenic earthworms.

Round flasks (1.1)containing 400ml of de-ionised water were autoclaved at 121°C for 1 hour, then allowed to re-oxygenate by standing for two days. Antibiotics were then added to give the following concentrations: Aureomycin 6μg.ml^{-1}, Cycloheximide 50μgml^{-1}; Nisin 50μgml^{-1}; Novobiocin 50μgml^{-1}.

Large adult and juvenile E. fetida were taken from laboratory stocks and washed in de-ionised water. The earthworms were then placed in an autoclaved sand/cellulose mixture for two days, then washed and transferred to fresh autoclaved mixture for a further two days. The earthworms were washed in sterile de-ionised water for 1 hour, then placed in the flasks containing antibiotics. Five worms were added to each flask, and flasks were stored in the dark at room temperature.

Earthworms were transferred to fresh antibiotic mixture every day for five days, and every two to three days subsequently. Beginning on day seven, 0.1ml. aliquots of waste antibiotic mixture were pipetted into Petri dishes after each transfer, and the dishes were overpoured with molten culture media at 45°C. Replicate plates of nutrient and malt extract agar plates were prepared from each flask. Three replicate plates of nutrient and malt extract agars from each flask were incubated at 25°C and 52°C aerobically, and a further three nutrient agar plates were incubated at 25°C under anaerobic conditions. Plates which produced between 30 and 300 colonies were counted.

When counts were reduced to less than 10 C.F.U.s per plate,

a different plating technique was employed. Individual worms were removed from each flask and transferred aseptically to 10ml. of sterile peptone water in McCartney bottles. The worms remained in this solution for 30 min. with regular shaking. The earthworms were then aseptically transferred to fresh antibiotic mixture. Aliquots of 0.5ml. of the peptone water were used to prepare dilution plates as described above.

When colony-free plates were produced using this method, one worm was removed from each flask and dissected under sterile peptone water. The gut was removed and suspended in 10ml of peptone water, and 1ml aliquots were used to prepare 5 dilution plates of nutrient agar. These were incubated at 25°C. Three 1ml aliquots of the peptone water were also spread onto the surface of plates of protozoan medium (Anon, 1958) seeded with Enterobacter aerogenes and examined hourly for the presence of excysted protozoa. 0.1ml drops of peptone water were also examined microscopically under phase-contrast illumination. When microbial contamination of the earthworms could no longer be detected by the above methods, the earthworms were transferred aseptically into individual Pyrex boiling tubes containing sterilised paper tissue waste. After 24 hours, the worms were aseptically removed and placed in sterile Petri dishes to produce faeces. The earthworms were then placed into fresh sterile tissue waste.

1ml of peptone water was added to the faecal material in each Petri dish, and the dishes were swirled to mix the contents. Dishes were then filled with 15ml of molten nutrient

agar and incubated at 25°C for 48 hours. Any earthworms which produced contaminated faeces were discarded. The remaining earthworms were utilised in further feeding experiments.

2.5.5. Feeding of pure cultures of bacteria to earthworms in axenic culture.

Concentrated suspensions of the following live bacteria in peptone water were prepared as described in section 2.5.3.

Acinetobacter lwoffi

Alkaligenes faecalis

Enterobacter cloacae

Pseudomonas fluorescens

Three 1ml aliquots of each suspension were added to large Pyrex boiling tubes containing 40g of sterilised tissue waste. Ten replicate tubes were prepared for each treatment. Control tubes were watered with peptone water only. The tubes were left for 3 days at room temperature to equilibrate.

Individual earthworms, prepared as described above, were weighed aseptically and transferred to the Pyrex tubes. The tubes were incubated at 25°C for 21 days. Every seven days, a further 1ml of the appropriate bacterial suspension was added to the tubes. At the end of the experimental period, the worms were removed from the tubes, rinsed in de-ionised water, blotted, and weighed. Mean weight change of worms fed with different bacteria was compared with control worms without bacteria.

2.5.6. Seeding of vermiculture beds with pure cultures of micro-organisms.

Following the initial screening tests, several micro-organisims were chosen for testing as a food source for earthworms in an environment similar to that found in vermiculture beds. The following micro-organisms were tested:

Acinetobacter lwoffi

Enterobacter cloacae

Pseudomonas fluorescens

Nocardia salmonicolor

Streptomyces sp.

Aspergillus fumigatus

Trichoderma hamatum

Experimental units were 50mm diameter plastic flower pots containing shredded paper tissue waste. The tissue waste was wetted to 75% moisture and autoclaved in bulk at 121°C for 1 hour on two consecutive days. 100g amounts were then added to each experimental pot, and the pots were then covered with aluminium foil and autoclaved at 121°C for 15 min. Live suspensions of the test micro-organisms were prepared as before and watered onto the surface of the substrate in the pots in 5ml aliquots. Control pots were watered with sterile peptone water. An additional series of pots was also set up, containing non-sterile tissue waste watered with sterile peptone water.

The pots were then incubated at 25°C for four days to allow micro-organisms to colonise the substrate.

Earthworms were cleaned using antibiotics and weighed in groups of three. One group of worms was added aseptically to each pot, with five replicates of each treatment. Pots were incubated at 25°C for 14 days, with re-feeding every four days. At the end of this time, the earthworms were recovered from the pots, washed and weighed. Weight change of fed worms compared with controls was once more used as an indication of the suitability of the micro-organisms as food foe E. fetida.

In a further experiment, unsterilised shredded tissue waste was added to ten 75mm diameter pots. Half of the pots were watered with a suspension of Acinetobacter lwoffi in peptone water, the other half were watered with peptone water only. Pots were covered with aluminium foil and incubated at 25°C for three days. Earthworms from laboratory stocks were washed in de-ionised water, blotted, and weighed in groups of three. One group was added to each pot. Pots were incubated at 25°C for a further three weeks. The pots were watered every five days with 5ml of bacterial suspension or with peptone water. Each week the earthworms were removed from the pots and weighed. The degree of breakdown of the substrate was also noted visually, by estimating the percentage of the tissue waste which had been converted to wormcasts.

2.6. Utilization of vermicomposted material.

(i) Experiment 1.

A 3:1 (w/v) mixture of paper tissue waste and cow slurry was prepared and placed in three 0.5m^2 wooden trays to a depth of 150mm. A further three trays were filled with the same volume of tissue waste only. Approximately 200 *E. fetida* were added to each box, and the boxes were incubated at 25°C and 85% relative humidity. After 6 weeks, the material had been completely vermicomposted, and the boxes were tipped out and handsorted to remove the earthworms. The treatments were bulked and sieved through a 5mm mesh. The following casing mixtures were prepared: vermicomposted tissue waste; vermicomposted tissue waste/slurry mix; and commercial peat. All treatments were nuetralised where necessary with powdered calcium carbonate, and pasteurised at 60°C ± 5°C for 30 min.

The materials were then applied to boxes each containing 1kg. of spawn-run mushroom compost. Numbers and weights of fruitbodies produced were recorded for four flushes.

(ii) Experiment 2.

Spent mushroom compost was obtained from a commercial grower, and leached with running water at 2 litres $min.^{-1}$ for 3 hours to reduce the soluble salts content to a level tolerable to *E. fetida*. After drainage the leached compost

was placed in three 0.5m^2 wooden trays and 200 E. fetida were added to each. The trays were each top-fed with 2.5 litres of cow slurry, and the boxes were incubated at 25°C and 85% humidity for 6 weeks. The boxes were then tipped out and the worms separated from the vermicomposted material. The material was bulked and sieved through a 5mm mesh. Four casing treatments were prepared: vermicomposted spent compost/cow slurry mix; vermicomposted compost/cow slurry mixed 50:50 with moist peat; leached unwormed spent compost; and commercial peat. All treatments were neutralised and pasteurised as in section 2.6. (i) then applied to boxes containing 1kg of spawn-run mushroom compost. Four boxes were cased with each test material, and numbers and weight of fruitbodies produced were recorded for four flushes.

2.7. Physical properties and chemical analysis.

Samples for analysis were oven-dried at 40°C unless otherwise stated.

2.7.1. Dry weight as % fresh weight.

Between 10g and 20g of fresh sample was weighed and oven-dried to constant weight at 105°C.

$$\text{Dry weight \%} = \frac{\text{oven dry weight (g)} \times 100}{\text{initial sample weight (g)}}$$

2.7.2. pH.

A mixture of 10g fresh sample and 20ml de-ionised water was prepared and thoroughly mixed by stirring for 5 min. The mixture was allowed to stand for 30 min. in a water bath at 20°C. The pH of the sample was then determined at this temperature using a glass electrode and a Pye Unicam pH meter.

2.7.3. Electrical conductivity.

Electrical conductivity of a sample is a measure of its dissolved salt content. In this study the method of Flegg (1958) was adopted. This allowed pH to be determined on the same suspension.

10g fresh samples were mixed with 20ml of de-ionised water and stirred for 5min. The sample was then placed in a water bath at 20°C for 30 min, after which the conductivity of the suspension was measured using a portable conductivity measuring bridge type MC3 (Electronic Instruments Ltd.). The result was corrected for dilution and dry weight of the sample, and conductivity expressed as mmhos cm^{-1}. The mean of three replicate measurements per sample was determined.

2.7.4. Gas concentration.

The concentration of volatile gases at the surface of stored slurry and above vermiculture beds, was measured using a Draeger gas detector. This consists of a bellows unit which is used to suck a measured volume of air through a calibrated glass tube containing a suitable reagent. Gas concentration (ppm) was read directly from the tube.

Samples were taken at the same time each day, before any disturbance of the material. The gas sampling tube was held within 20mm of the surface of the material. The mean of three replicate measurements per sample was determined.

2.7.5. Soluble Carbohydrates.

The colorimetric method of Allen *et al* (1974) was employed. The 50mg samples were extracted by boiling under reflux before estimation. The extracts and water blanks given the same treatment, were then immediately used in the colour development procedure, using anthrone-thiourea mixture.

A series of standard glucose solutions, containing between 0 and 0.1gm of glucose.ml^{-1}, , was also developed. Colour intensity was read at 625nm on a Unicam colorimeter and a calibration graph prepared from the standards. The amount of carbohydrate in the sample aliquot was read directly from the graph. Where necessary, blank determinations were subtracted before calculation.

If Cmg = glucose obtained from the graph, then

$$\text{Soluble carbohydrates (\%)} = \frac{C(mg) \times \text{extract volume (ml)}}{10 \times \text{aliquot (ml)} \times \text{sample wt. (g)}}$$

2.7.6. Ammonium and nitrate plus nitrite nitrogen.

The distillation method of Allen et al (1974) was used. Fresh samples were pre-extracted in 6% sodium chloride before determination. 150ml of extract were pipetted into a round-bottomed macro-Kjeldahl flask. In the first stage of distillation, 0.2g of magnesium oxide were added to the distillation flask, and 50ml were distilled over and collected in 10ml of saturated boric acid solution. The flask was then removed, 0.4g Devarda's alloy (approximately 45% aluminium; 50% copper; and 5% zinc) was added to the extract, and the flask was quickly replaced. A further 50ml of distillate was collected in 10ml of saturated boric acid solution. The collected distillates were titrated against M/140 HCl using Tashiro's indicator (methyl red 2.0 g l^{-1}; methylene blue 1.0g l^{-1}; in absolute alcohol). Ammonium nitrogen was determined from the first collected distillate and nitrate plus nitrite nitrogen from the second distillate.

$$\left.\begin{array}{l}\text{Extractable} \\ NH_4 - N(mg.100g^{-1})\end{array}\right\} = \frac{\text{titre (ml)} \times \text{extractant volume (ml)} \times 10}{\text{aliquot (ml)} \times \text{sample wt. (g)}}$$

$$\left.\begin{array}{l}\text{Extractable } NO_3\text{- N} + \\ NO_2 - N\ (mg.100g^{-1})\end{array}\right\}$$

Results were expressed as the mean of three replicate determinations, on a dry weight basis.

2.7.7. Organic nitrogen.

Total organic nitrogen was estimated by the semi-micro Kjeldahl digestion procedure, followed by steam distillation as described by Allen (1974), using 0.10g of sample. Reagent blanks were also prepared. Digested samples were stored at 1°C and distilled within 24 hours. Distillate was collected in 10ml of saturated boric acid solution and titrated against M/140 HCl using Tashiro's indicator (methyl red 2.0g.l^{-1}; methylene blue 1.0g.l^{-1}; in absolute alcohol).

Digestion blanks were subtracted from the sample titrations, and results were expressed as the mean of three replicate determinations.

If Tml M/140 HCl are required for the titration then:

$$\text{Organic N}(\%) = \frac{T(\text{ml}) \times \text{solution volume (ml)}}{10^2 \times \text{aliquot (ml)} \times \text{sample wt (g)}}$$

2.7.8. Organic carbon.

Organic carbon was determined according to the wet oxidation/titration method of Tinsley (1950). The indicator solution was 5g $BaCl_2 . 2H_2O$ and 0.3g barium diphenylamine-sulphonate dissolved in 100ml de-ionised water. Finely ground 0.05g samples were used. After reflux, 5ml of indicator

solution were added, and the suspension was titrated with ferrous ammonium sulphate, to determine the amount of unused dichromate present. A further 2.5ml of dichromate mixture were added when the first colour change occurred, and the titration was completed dropwise. If Tml = ferrous ammonium sulphate used in the titration, then

$$\text{Organic carbon (\%)} = \frac{(27.5 - T)\ \text{ml} \times 0.12}{\text{sample wt (g)}}$$

2.7.9. Carbon/nitrogen ratio.

The C:N ratio was calculated using the values obtained for organic carbon in 2.7.8. and organic nitrogen in 2.7.7.

$$\text{C:N ratio} = \frac{\text{g. organic carbon/100g dry sample}}{\text{g. organic nitrogen/100g dry sample}}$$

2.7.10. Organic matter content.

Organic matter was expressed as percentage loss on ignition. Porcelain crucibles were pre-heated to 500°C in a muffle furnace for 15 min. The crucibles were then cooled in a desiccator and weighed. 1g of ground sample was added to each crucible, and samples were placed in a cool muffle furnace and heated to 450°C for 4 hours. Samples were then removed and cooled sufficiently to allow transfer to a desiccator. When completely cooled samples were weighed.

$$\text{Organic Matter (\%)} = 100 - \frac{\text{final sample wt (g)}}{\text{initial sample wt (g)}} \times 100$$

2.7.11. Holo-cellulose.

The sodium chlorite delignification procedure of Wise et al (1946) was used, as described in Allen (1974).

1g finely-ground samples were used. After 4 hours extraction the flask was removed and cooled immediately in iced water. Contents were filtered through a weighed No. 2 Pyrex sintered crucible, and washed with ice cold water, acetone and ether. The sinter funnel was dried in an oven at 105°C for 30 min, then cooled in a desiccator and weighed.

Samples were corrected for ash and crude protein content (N x 6.25) of the holo-cellulose before calculation.

$$\text{Holo-cellulose (\%)} = \frac{\text{Corrected holo-cellulose (g)} \times 10^2}{\text{Sample wt (g)}}$$

2.7.12. Alpha-cellulose.

The procedure of Allen (1974) was used for determination of alpha-cellulose, using holo-cellulose samples obtained in section 2. 7. 11. above.

0.1g holo-cellulose samples were used. After KOH digestion for 2 hours, the residue was filtered and washed through a weighed no. 2 glass sintered crucible. The crucible plus sample was then dried at 105°C for 30 minutes, cooled

in a desiccator, and the weight of alpha-cellulose determined. Corrections for ash and crude protein content were subtracted before calculation.

2.7.13. Lignin.

The acid hydrolysis method of Allen (1974) was followed. A 1g sample was pre-extracted in glass fibre paper in a Soxhlet apparatus for 6 hours, then dried and weighed. About 0.80g from the extracted sample was accurately weighed into a tall 600ml Pyrex beaker. Samples were extracted in sulphuric acid, then filtered and washed through a weighed no. 2 sintered glass crucible and contents were then dried at 105°C for 3 hours, cooled and weighed. Ash and protein content were determined on sub-samples, and subtracted before calculation of lignin content.

$$\text{Lignin \%} = \frac{\text{corrected lignin (g) x total ether extracted sample (g) x } 10^2}{\text{wt takin for water extraction (g) x sample wt (g).}}$$

2.8. Satistical analysis.

2.8.1. Analysis of variance.

This procedure was used to test the data obtained from experiments with more than two treatments. It showed whether any significant difference existed among treatment means.

Variance ratio (F) was calculated as follows:

$$F = \frac{\text{between treatments mean square}}{\text{error mean square}}$$

2.8.2. Least significant difference (L.S.D.).

Where ANOVAR indicated the existence of a significant difference, estimation of L.S.D. was used to locate which of the means caused the difference. L.S.D. is the smallest difference which can exist between two significantly different means.

$$\text{L.S.D.} = \sqrt{\frac{2}{n} \times \text{EMS} \times F_{n1\ n2}}$$

n= no. of replicates EMS = Error mean square

$F_{n1\ n2}$ = F value at P = 0.05, where n1 and n2 = error d.f.

2.8.3. Dunnett's test (Dunnett, 1955).

Data from feeding of pure cultures of micro-organisms to earthworms was subjected to Dunnett's analysis of variance. This test allowed several treatments to be compared in turn with a control treatment, so that significantly different treatments could be identified. A standard analysis of variance was performed on the data, and t-values obtained from Dunnett's tables were used to calculate L.S.D. between means.

$$L.S.D. = t \times \sqrt{\frac{2 \times E.M.S.}{n}}$$

t = Dunnett's t-value

E.M.S. = Error Mean Square

n = number of replicates.

2.8.4. t-Test.

The t-Test was employed in the comparison of two treatments. t is a measure of the difference between the sample means.

$$t = \frac{\Sigma x_1/n_1 - \Sigma x_2/n_2}{\sqrt{\left(\frac{\Sigma x_1^2 - \left[(\Sigma x_1)^2/n_1\right] + \Sigma x_2^2 - \left[(\Sigma x_2)^2/n_2\right]}{n_1 + n_2 - 2}\right)\left(\frac{n_1 + n_2}{n_1 n_2}\right)}}$$

with $n_1 + n_2 - 2$ degrees of freedom.

where: x_1 = sample 1.

x_2 = sample 2.

n_1 = no. of scores in sample 1.

n_2 = no. of scores in sample 2.

SECTION 3.

RESULTS

3.1. Chemical and Microbiological changes in cow slurry during storage.

3.1.1. Physical and chemical composition of cow slurry stored under forced aeration and in shallow trays.

The results of the analysis of stored cow slurry are shown in Figs. 3.1 to 3.5.

The moisture content of slurry stored in a tank under forced aeration remained relatively stable during the sampling period. When the slurry was transferred to shallow trays after 24 days, some loss of moisture occurred, though insufficient data were available for statistical analysis. The slurry tended to form a surface crust in the trays which was broken up during mixing at each sampling time.

The pH of the aerated slurry fell slightly over 24 days. A small increase in pH occurred when the slurry was placed in shallow trays, after which the pH remained stable for the remainder of the experiment.

Electrical conductivity of the slurry under forced aeration was initially stable. A significant ($t < 0.05$) decrease occurred between days 9 and 14 of the experiment, followed by a further significant fall between days 22 and 24. When the slurry was placed in shallow trays, conductivity continued to decline, stabilising after four days of tray storage. Overall a significant decrease ($t < 0.01$) in conductivity occurred during the experiment.

Fig. 3.1. Moisture content and pH of cow slurry stored under forced aeration and then in shallow trays.

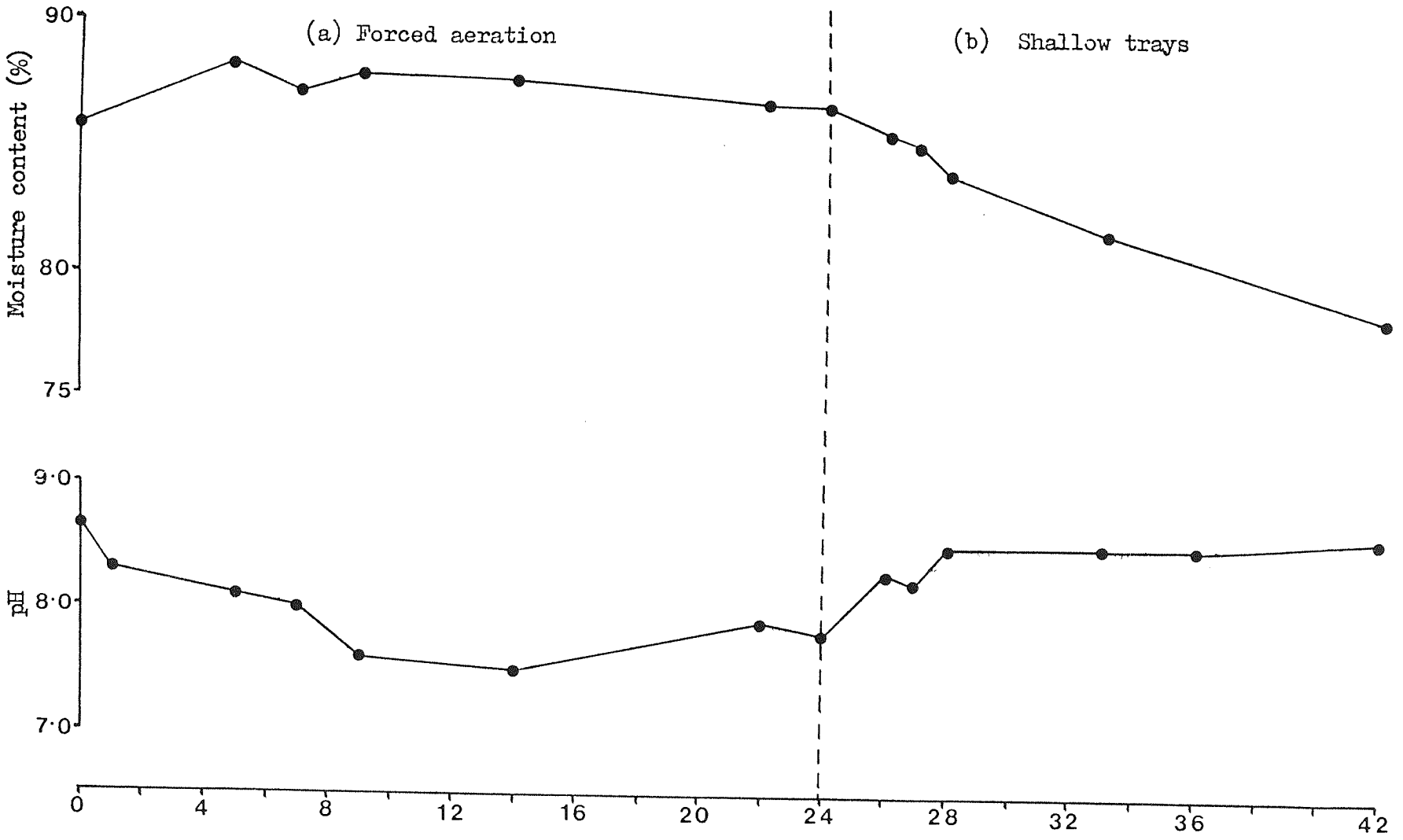
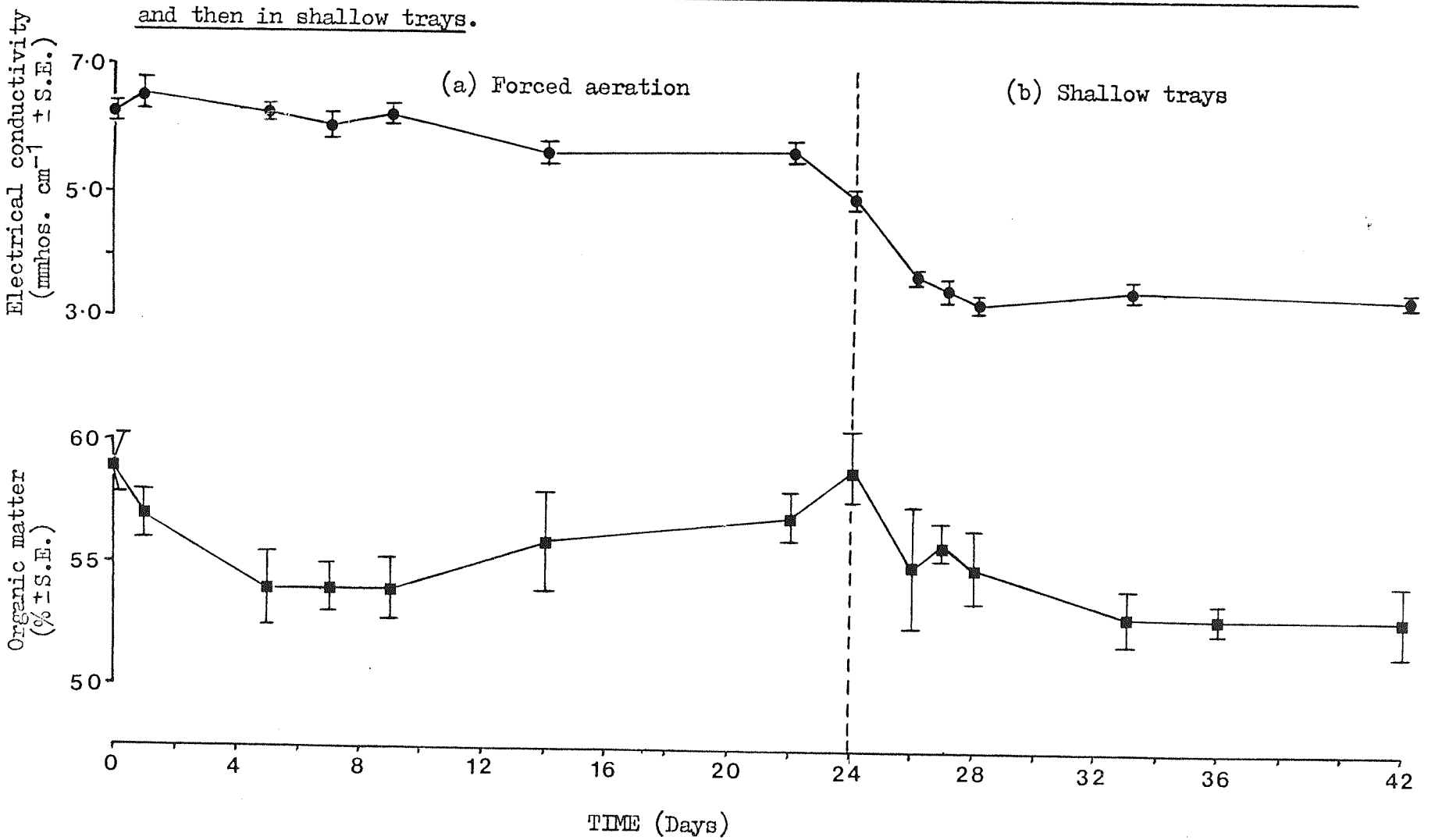


Fig. 3.2. Electrical conductivity and organic matter content of cow slurry stored under forced aeration and then in shallow trays.



The organic matter content of the stored slurry showed no significant change during the experimental period. The variability of the results obtained reflects the imprecision of the method of determination employed in this study.

A large significant ($t < 0.01$) fall in ammonia volatilisation from the slurry occurred during the first 24 hours of aerated storage, followed by gradual stabilisation at a steady rate of ammonia loss. A further fall ($t < 0.05$) in ammonia volatisation occurred when a sample of slurry was transferred to tray storage, and ammonia was not detectable at the surface of the slurry after 18 days of tray storage.

The organic nitrogen content of the stored slurry showed a small decrease during aerated storage, but this was not significant ($t > 0.05$). The level of organic nitrogen in slurry placed in shallow trays remained relatively constant throughout the sampling period.

A significant ($t < 0.05$) fall in the organic carbon content of the cow slurry occurred during the first day of forced aeration. No significant change in organic carbon occurred during the next 23 days of storage. In tray-stored slurry, a further significant fall in organic carbon content between days 4-9 of storage was followed by further stabilisation to the end of the experiment. The carbon:nitrogen ratio of the slurry showed a small overall decrease over the whole experiment, the largest fall occurring during storage in shallow trays.

Fig. 3.3. Volatilisation of ammonia and organic nitrogen content of cow slurry stored under forced aeration and then in shallow trays

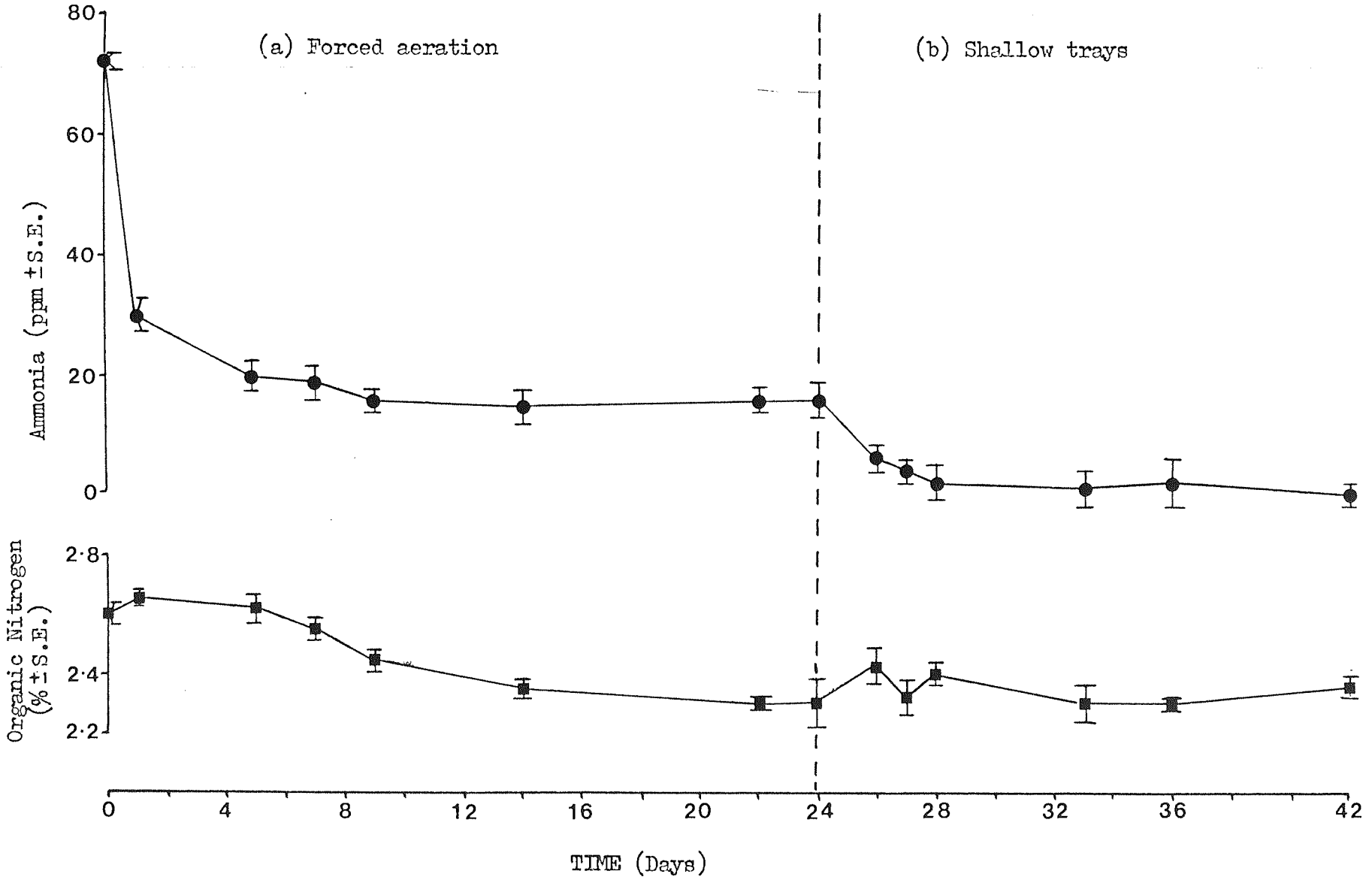
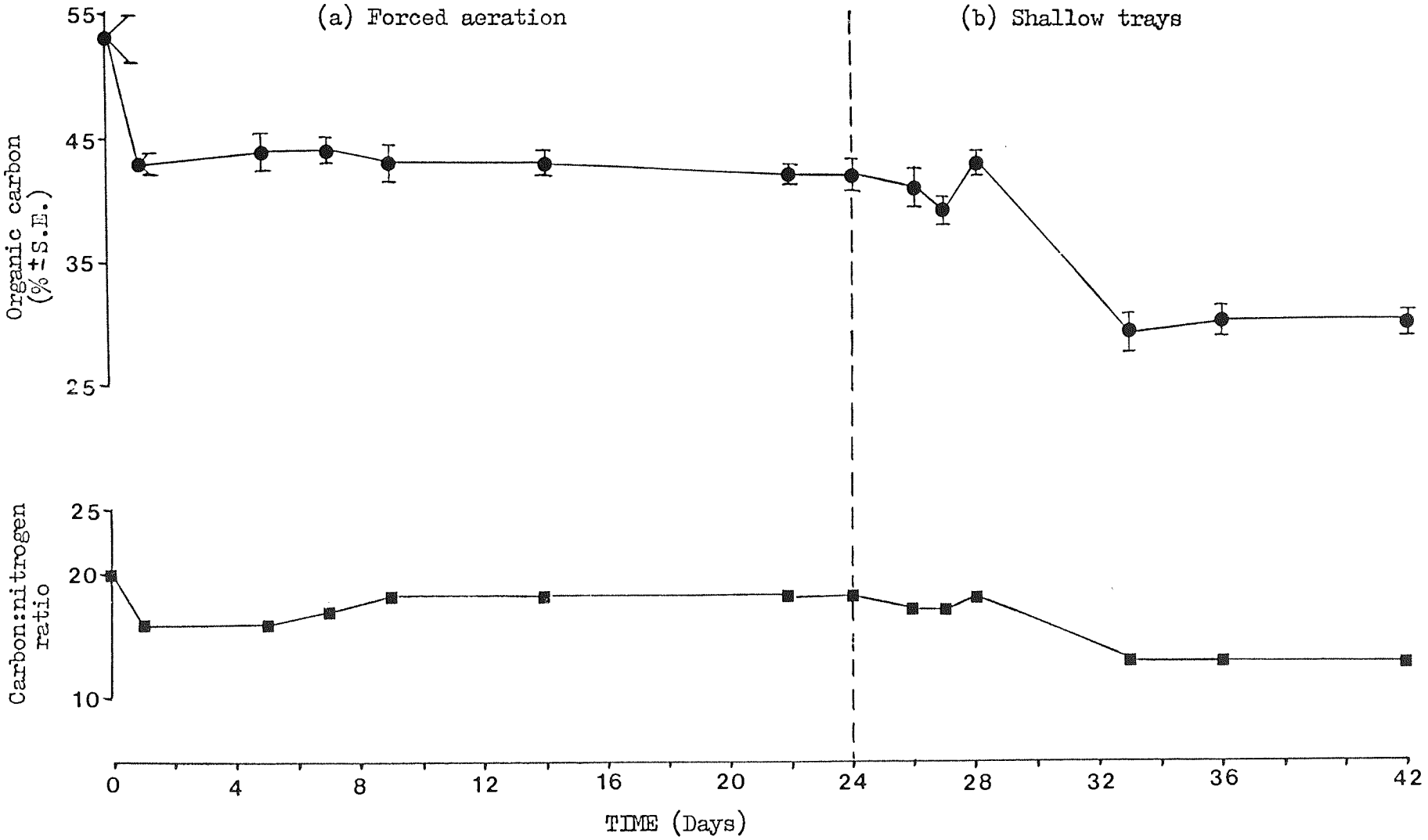


Fig. 3.4. Organic carbon content and carbon:nitrogen ratio of cow slurry stored under forced aeration and then in shallow trays



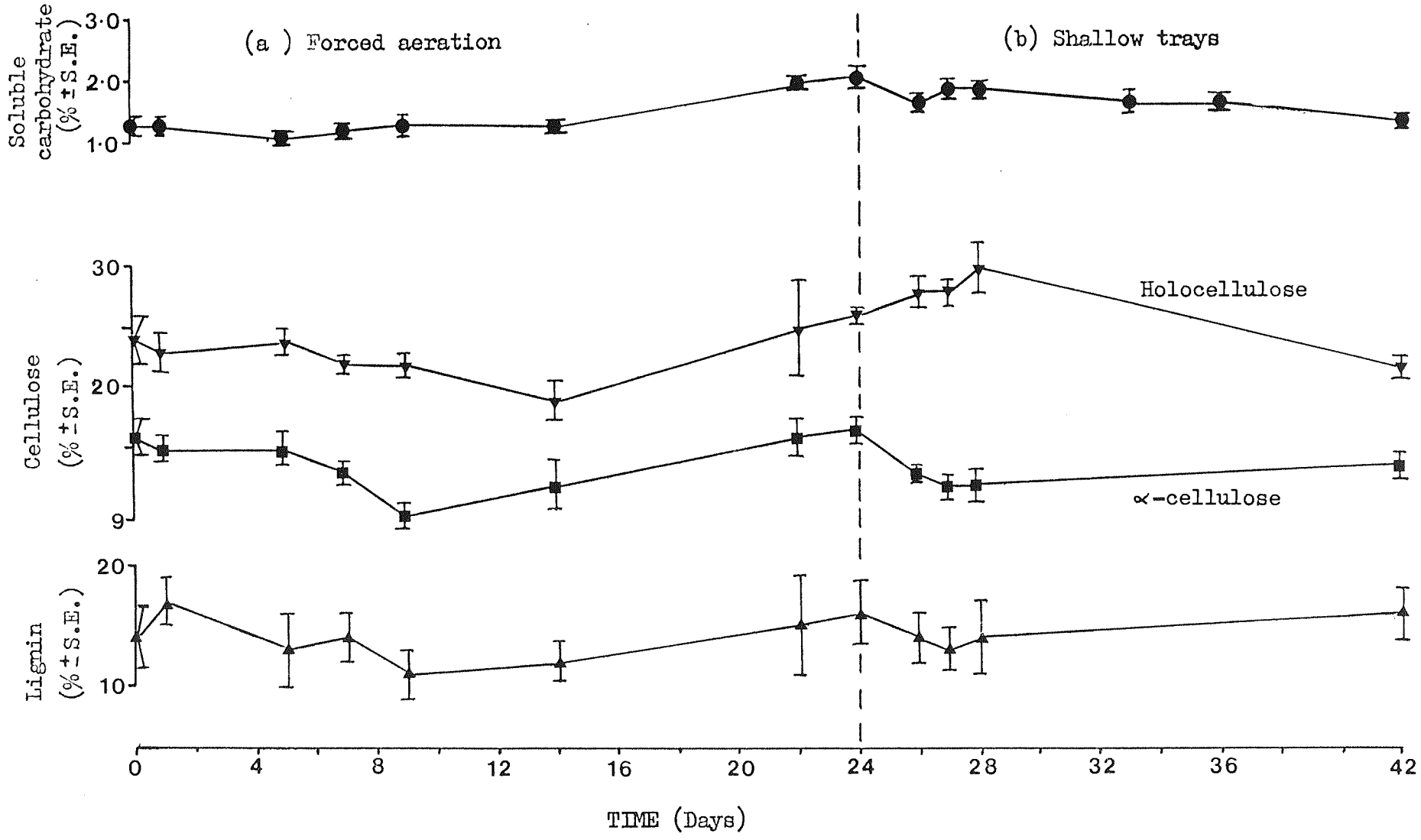
The soluble carbohydrate content of the cow slurry was low, and showed no significant change during forced aeration and tray storage.

The results of the determinations of the polysaccharide content of the stored slurry showed considerable variation. In the aerated slurry, the overall trend was for a small increase in the proportion of holocellulose but this was not significant. The proportion of α-cellulose in the total polysaccharide fraction remained relatively constant over the 24-day aerated storage period, suggesting that no preferential breakdown of α- or hemi-cellulose took place. When the slurry was transferred to shallow trays, the results showed an initial fall in the proportion of α-cellulose, though this was not significant, followed by a significant ($t < 0.05$) decrease in the proportion of holocellulose present, suggesting decomposition of hemicellulose under this storage method. Overall, however, little cellulose decomposition occurred during the experimental period.

Lignin determinations were also variable, though no significant change in the proportion of lignin present occurred over the timescale of this experiment.

It can be seen from these results that the major chemical changes in slurry stored under forced aeration were the initial rapid loss of nitrogen as ammonia from the slurry, and the initial large decrease in the organic carbon content, which suggests an initial high level of microbial

Fig. 3.5. Soluble carbohydrate, cellulose, and lignin content of cow slurry stored under forced aeration and then in shallow trays.



respiration which is subsequently greatly reduced. The pH of the aerated slurry decreased during aerated storage, while the electrical conductivity of the material also declined during the experiments. No significant change occurred in the other parameters measured .

When slurry was transferred to shallow trays, ammonia volatisation decreased further, eventually falling to zero. The organic carbon content also decreased during storage, but the largest fall occurred after some days, suggesting a lag period before micro-organisms were able to exploit the new conditions created by the storage method. The moisture content of the slurry in shallow trays decreased during the sampling period as expected. Electrical conductivity also declined initially, while pH increased slightly. Evidence of hemicellulose decomposition was also observed during tray storage of slurry.

Neither method of slurry storage produced significant decomposition of the waste, the pattern in both cases being one of initial loss of nitrogen (as ammonia) and organic carbon, followed by stabilisation of the material, though the tray storage method allowed some decomposition of hemicellulose during the experiment.

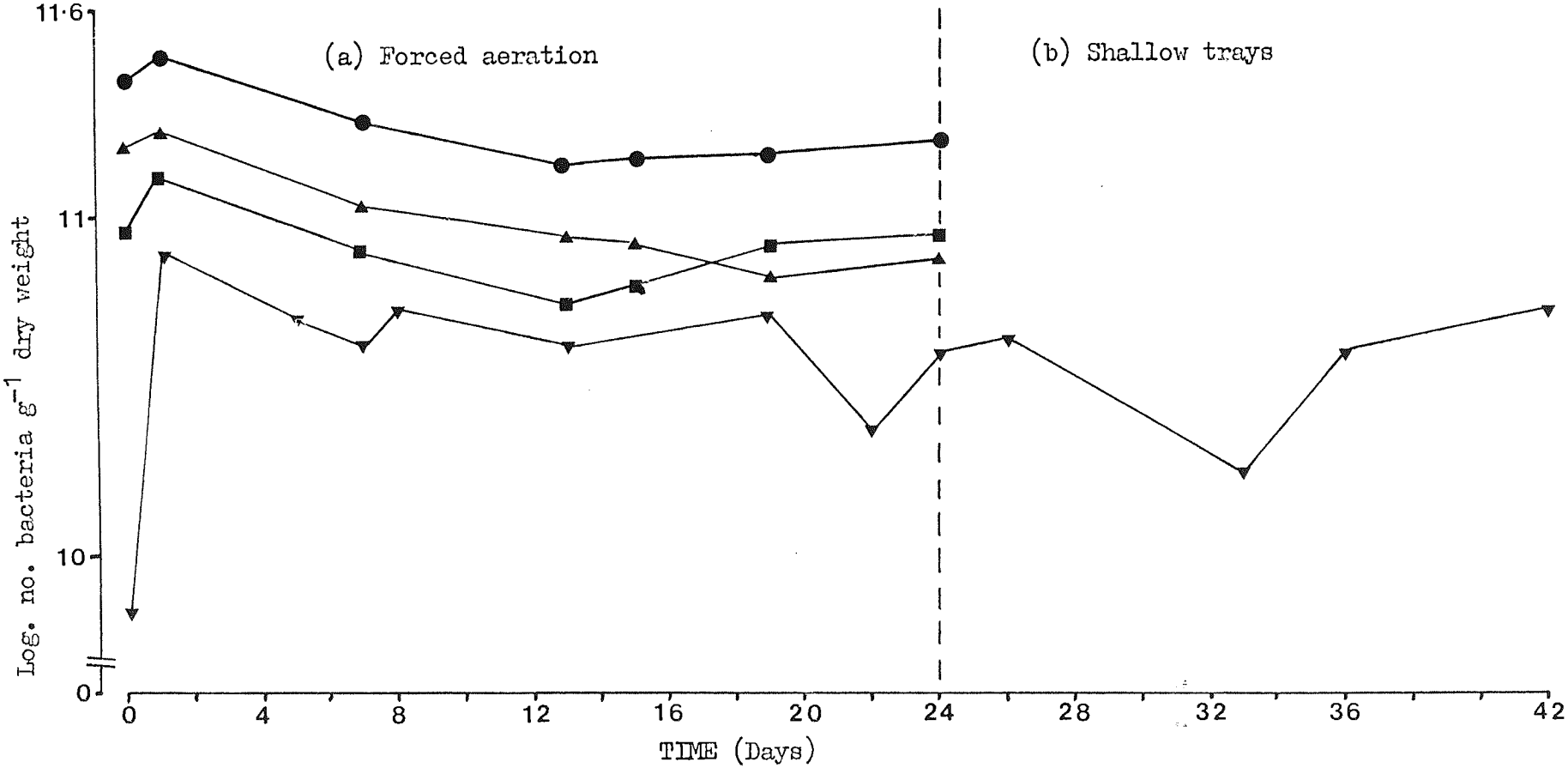
3.1.2. Microbiological changes in cow slurry stored under forced aeration and in shallow trays.

Fig. 3.6. shows the total numbers of the most abundant micro-organisms in cow slurry during storage. During forced aeration, total counts obtained by the agar film and dilution plate techniques were recorded, and in addition, the numbers of bacterial rods and cocci present were estimated from agar films. Over the first 24 hours of aerated storage, numbers of bacteria estimated from dilution plates showed a ten-fold increase, indicating rapid multiplication of organisms under favourable environmental conditions. Thereafter numbers declined slowly until day 22, when a temporary fall in numbers was recorded.

Total numbers of bacteria estimated by the agar film technique were consistently higher than counts from dilution plates. The initial increase in numbers shown by this method was much smaller than that estimated from dilution plates. Numbers slowly declined following this initial increase, stabilising after day 13. Rods and cocci were present in similar numbers throughout the experiment. The latter were more numerous until days 15-19 when rods became numerically dominant, this situation continuing until the end of the sampling period.

The total numbers of aerobic bacteria in cow slurry stored in shallow trays were estimated by the dilution plate

Fig. 3.6. Total numbers and morphological groups of the most abundant micro-organisms in cow slurry stored under forced aeration and then in shallow trays.



-●- = Total numbers estimated from agar films: -▲- = Number of cocci (from agar films): -■- = Number of rods (from agar films): -▼- = total numbers estimated from dilution plates.

technique. Numbers declined between the second and ninth days of tray storage, then increased to the end of the sampling period.

Fig. 3.7. and appendix 1.1., show the changes in the abundance of the commonest micro-organisms isolated from slurry stored under forced aeration. Of the species isolated from fresh slurry, most increased in numbers over the first few days of aeration. However, two species were isolated on only one sampling date; Flavobacterium lutescens on day 0, and Arthrobacter tumescens on day 1. Another Arthrobacter species, A. simplex, and a grey Streptomyces sp. were initially numerous, but numbers of these fell rapidly after day 7. After 7 days of storage, Acinetobacter lwoffi was the most abundant species present until day 24, with a Rhodotorula sp. and Micrococcus luteus present in relatively low numbers for most of this period. Alkaligenes faecalis was isolated in large numbers from day 22 to the end of the sampling period, and Pseudomonas putida was the most abundant species in the slurry at the end of the experiment.

Fig. 3.8. and appendix 1.3. show the changes in the relative abundance of bacteria when slurry was transferred to shallow trays following 24 days of aerated storage. The initial microflora was similar to that of the source slurry in the aeration tank. Ps. putida and Alk. faecalis were initially most numerous, with Ac. lwoffi present in lower numbers. This pattern continued throughout the sampling period, though numbers of the two most abundant species fell

Fig. 3.7. Relative abundance of the most frequent micro-organisms in cow slurry stored under forced aeration.

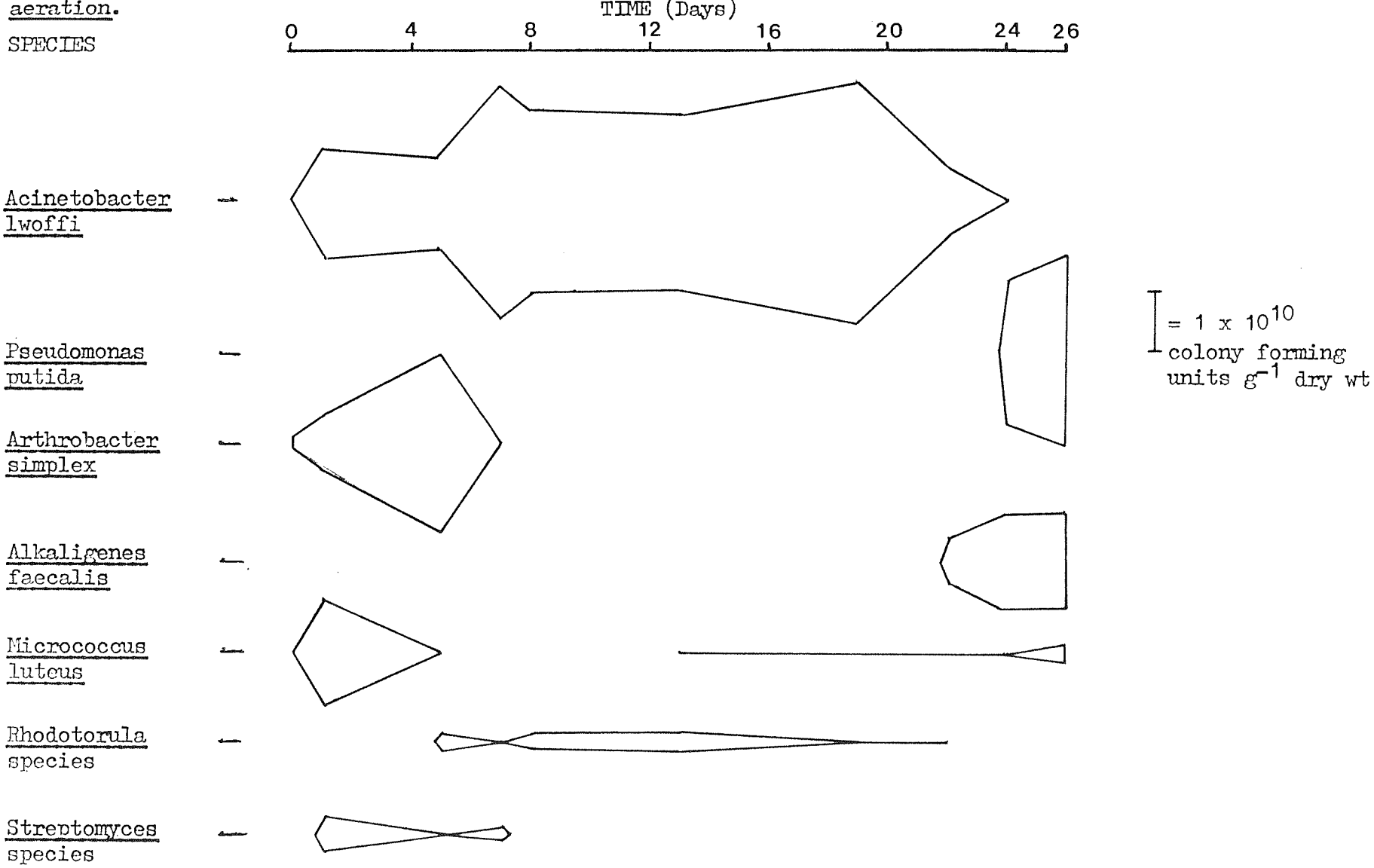
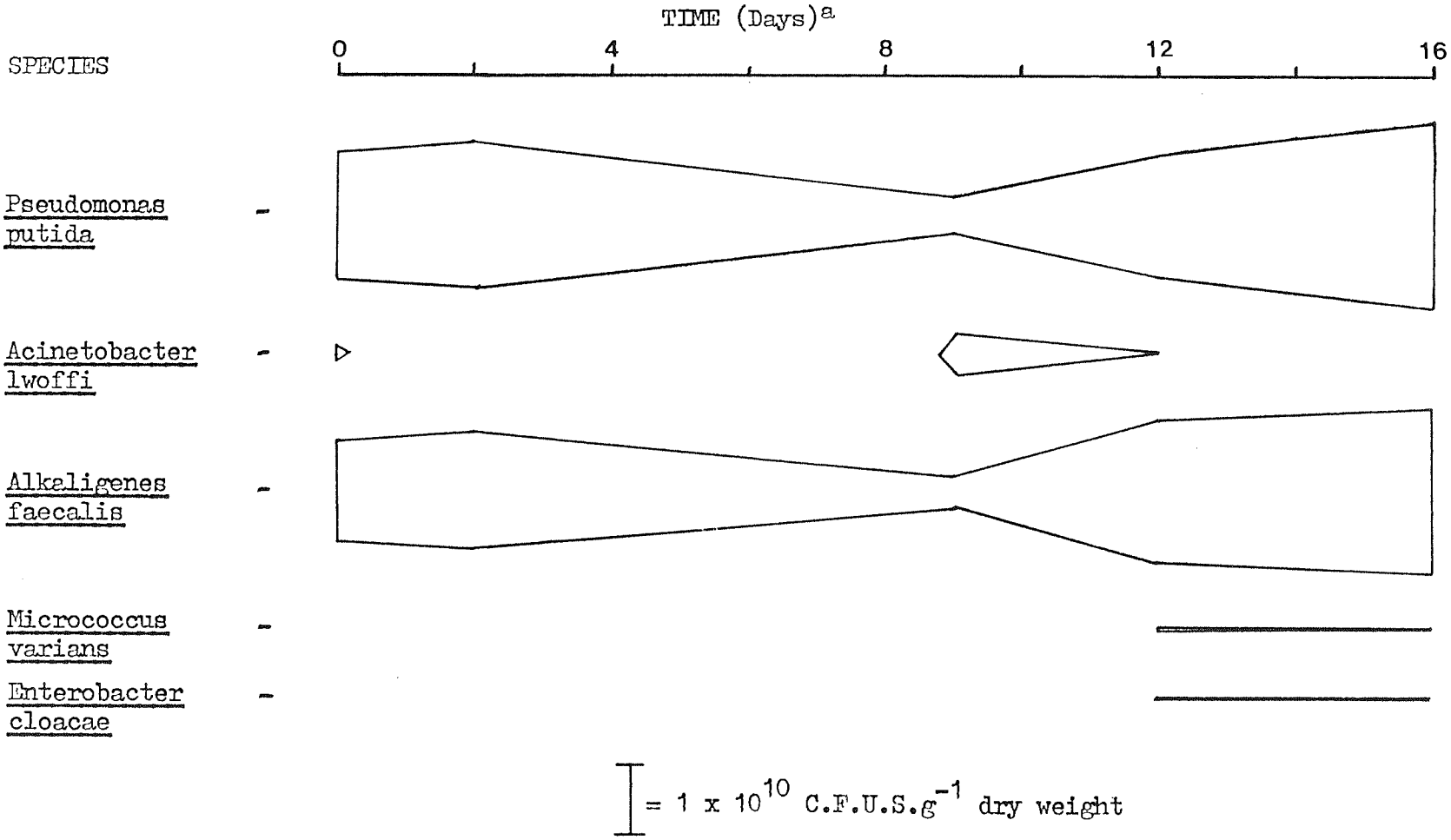


Fig. 3.8. Relative abundance of the most frequent micro-organisms in cow slurry stored in shallow trays.



[a]Slurry 24 days old at beginning of experiment.

at day 9 and recovered by day 12, with Ac. lwoffi showing the opposite trend. This suggests a temporary change in environmental conditions at this time which favoured Ac. lwoffi at the expense of the other species. After 12 days of tray storage, Micrococcus varians and Enterobacter cloacae appeared, and were isolated in low numbers for the remainder of the sampling period.

The overall trend represented by these results is one of an initially diverse microflora which was soon dominated by one species. This was in turn replaced by other micro-organisms after approximately 3 weeks of aerated storage. These species then remained numerically dominant during the period of tray storage.

Fig. 3.9. shows the total numbers of fungi in stored slurry, estimated from dilution plates. In aerated slurry, numbers declined slowly for the first 8 days of storage, then more rapidly up to day 13. This was followed by an increase in numbers to the end of the sampling period. When the slurry was transferred to shallow trays, a slow increase in numbers of fungi occurred over the first 9 days followed by a more rapid increase to the end of the experiment.

Fig. 3.10. and appendix 1.2. show the changes in the relative abundance of fungi in slurry under forced aeration. In the early stages of the experiment, the most frequent fungi were Aureobasidium pullulans, Cladosporium herbarum, and two Penicillium species. However, the Penicillia were not

Fig. 3.9. Total numbers of fungi in cow slurry stored under forced aeration and then in shallow trays, Estimated from dilution plates.

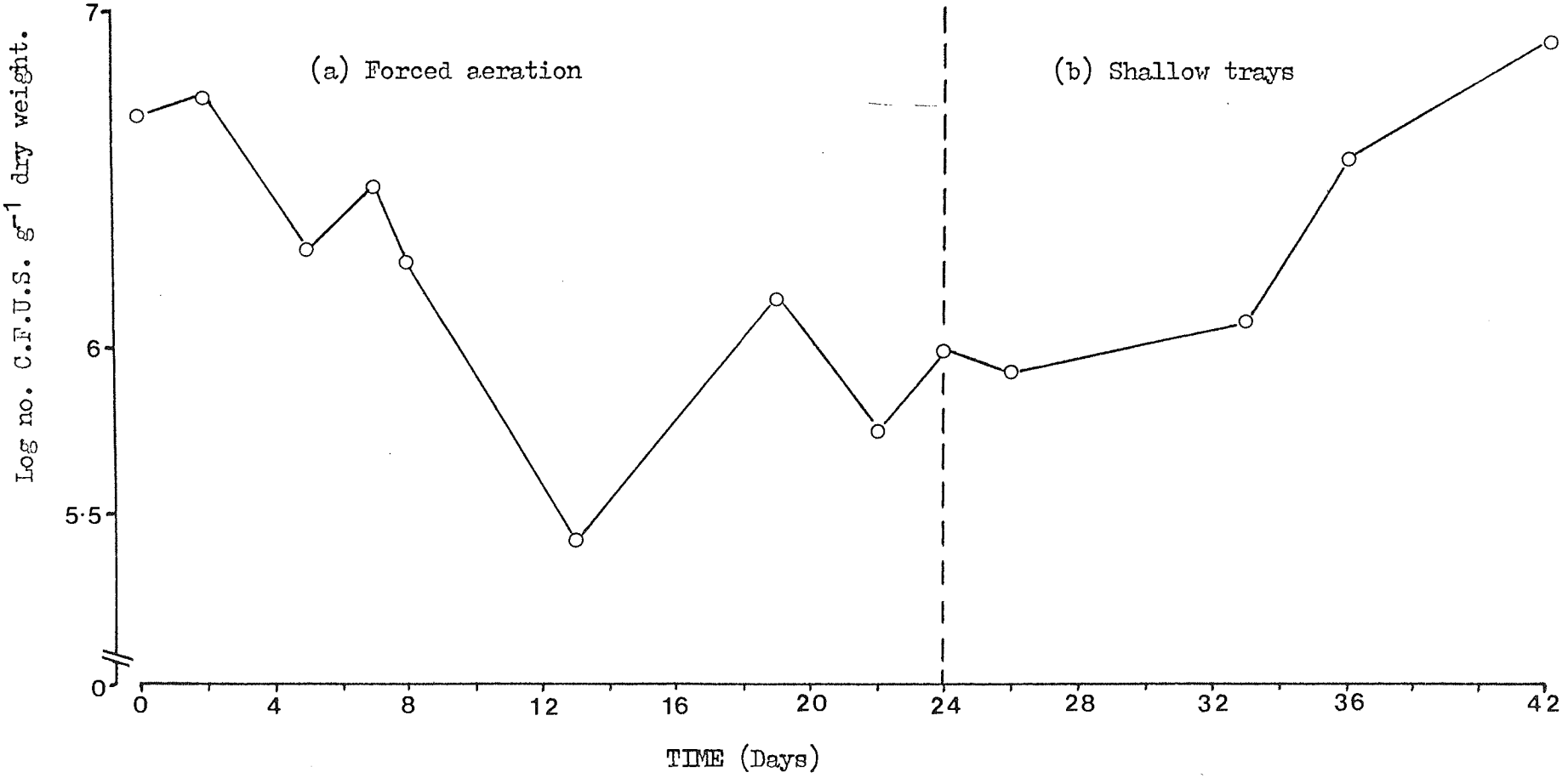


Fig. 3.10. Relative abundance of fungi in cow slurry stored under forced aeration.

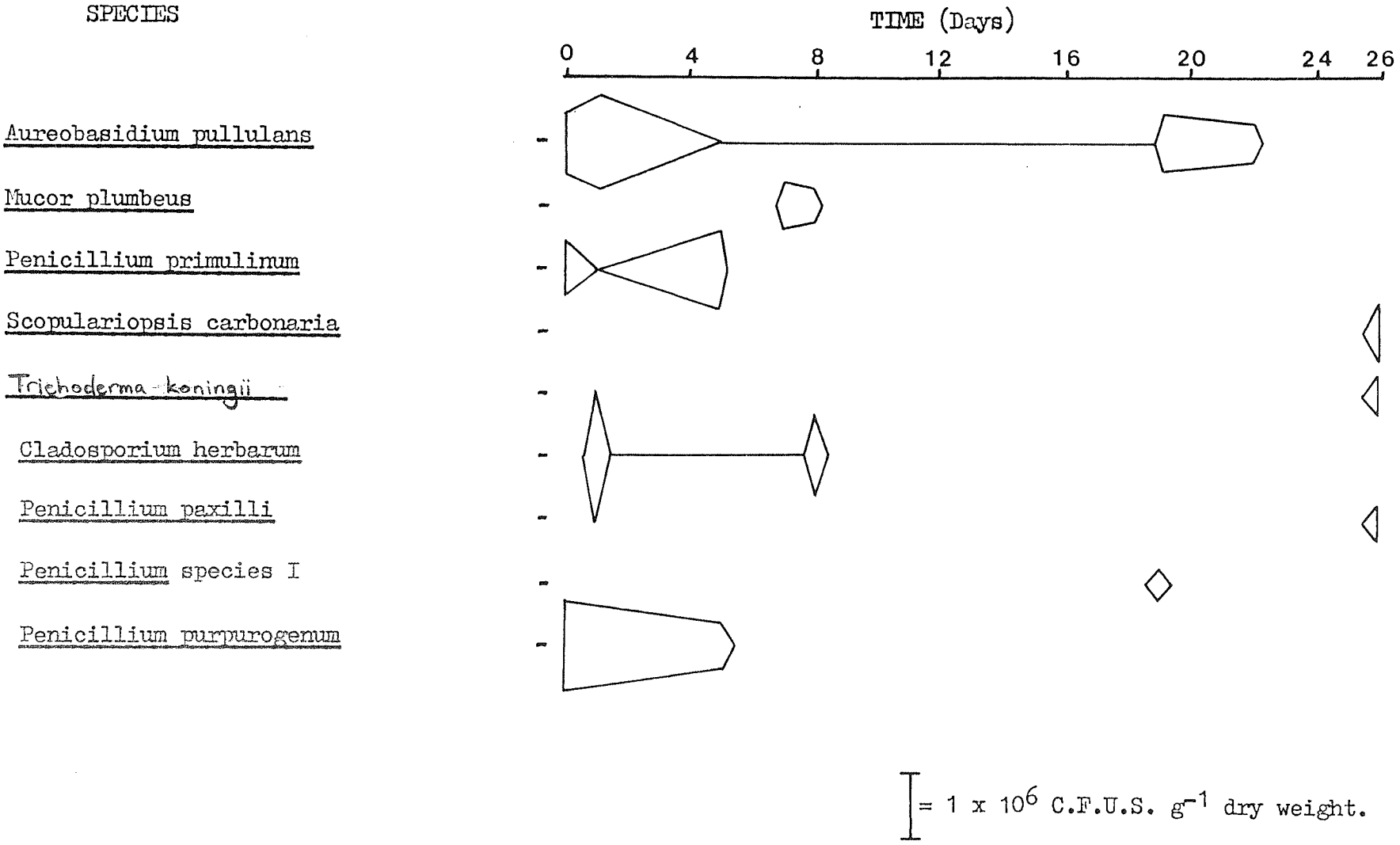
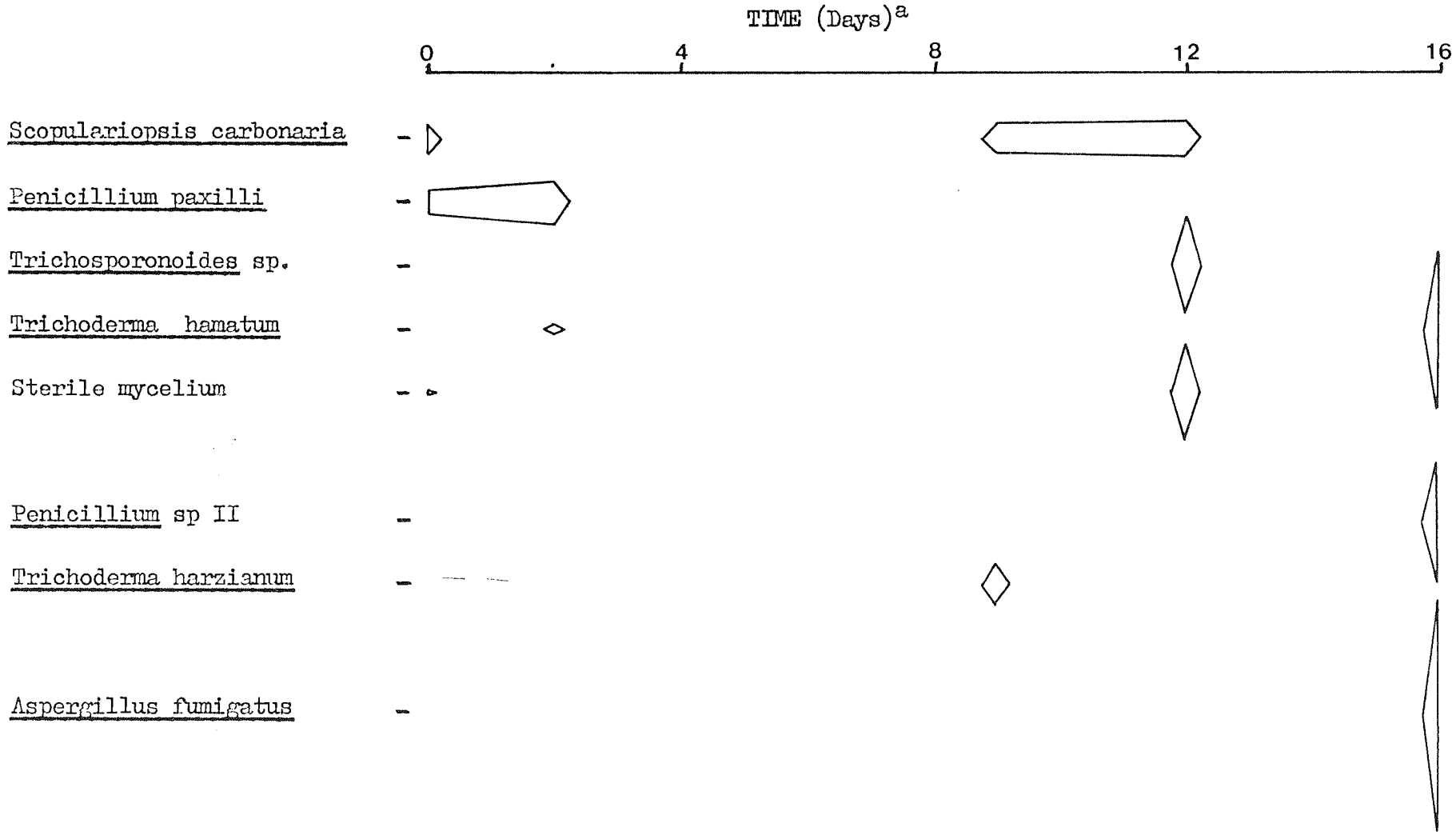


Fig. 3.11. Relative abundance of fungi in cow slurry stored in shallow trays.



[a]Slurry 24 days old at beginning of experiment.

I = 1 x 10^6 C.F.U.S.g^{-1} dry weight.

isolated after day 5 of the experiment. C. herbarum was not found in high numbers after day 8, and A. pullulans was reduced in numbers by day 5, but persisted at a low level until days 19-22 when numbers of this species increased once more. At the end of the sampling period, the most numerous fungal species present were isolated for the first time, the initial microflora having been totally replaced.

Fig. 3.11. and appendix 1.4. show the sucession of the most abundant fungal species in cow slurry in shallow trays. Two of the initial most numerous species, Scopulariopsis carbonaria and Penicillium paxilli were isolated from the source slurry under aerated storage. In addition, an unidentified floccose, hyaline, non-sporulating species was isolated at this time. The Penicillium species declined in numbers after 2 days, while S. carbonaria was not isolated on day 2, but re-appeared in relatively high numbers between days 9-12. The unidentified hyaline species was not re-isolated until day 12 when it re-appeared in high numbers. Another species which was present in the early stages of the experiment and re-appeared in large numbers later was Trichoderma hamatum. This species, Aspergillus fumigatus and Penicillium species II were the most abundant fungi at the end of the experiment. Trichoderma harzianum and a Trichosporonoides sp. were each isolated on only one sampling date.

The pattern of fungal succession during the aerated storage of cow slurry was of the initial isolation of several species which may have been present as spores which passed through the gut of the cows, and which quickly declined in numbers. This was followed by the persistence of one species at a relatively low level for some time with sporadic appearance of heavily sporing species which are common members of the airspora.

When the slurry was transferred to shallow trays, the sporadic appearence of members of the airspora continued, and numbers of isolated species tended to increase during the experiment, suggesting that conditions in the tray-stored slurry were becoming more favourable for fungal growth.

3.2. Growth and reproduction of Eisenia fetida in vermicomposting systems.

3.2.1. Reduction of cow slurry toxicity.

The results of this investigation are summarised in tables 2.1., 2.2. and 2.3.. Slurry stored under forced aeration remained highly toxic for three weeks. Tray-stored slurry showed a small decrease in toxicity after three weeks, but even after this period only one earthworm out of five survived 24-hour contact with the material. Crude separation of the slurry into solid and liquid fractions had no immediate effect upon toxicity, though the solid

Table: 2.1. Survival of earthworms in cow slurry during forced aeration, tray drying, and separation and ageing.

Slurry Treatment	No.earthworms surviving in slurry following storage for: 0 days	7 days	14 days	21 days
Forced aeration	0	0	0	0
Tray--drying	0	0	0	1
Separation and ageing (i) Solid fraction	0	0	0	1
(ii)Liquid fraction	0	0	0	0

Initial n = 5 for all treatments.

Table 2.2. Survival of earthworms in cow slurry following dilution and aerated storage.

Dilution (v/v)	No.earthworms surviving in slurry following storage for: 0 days	7 days	14 days	21 days
Undiluted	0	0	0	0
1:1	0	0	0	0
1:5	0	0	0	0
1:10	1	1	1	3
1:20	5	5	5	5

Initial n = 5 for all treatments.

Table 2.3. Survival of earthworms in cow slurry mixed with soil or aged cow manure.

Mixture (w/v)	No. earthworms surviving in mixtures following storage for: 0 days	7 days	14 days	21 days
Soil/slurry 1:1	0	0	0	1
Soil/slurry 2:1	0	0	3	3
Soil/slurry 3:1	0	1	5	5
Manure/slurry 1:1	0	0	3	5
Manure/slurry 2:1	5	5	5	5
Manure/slurry 3:1	5	5	5	5

Initial n = 5 for all treatments.

fraction was slightly less toxic following three weeks of ageing, suggesting that the toxic factors were concentrated in the liquid fraction.

Dilution of slurry with water was effective in reducing toxicity at a 1:10 dilution, and the slurry was apparently de-toxified at a 1:20 dilution. This effect was immediate, and the toxicity of the 1:10 dilution was further reduced after 14 days of storage.

These results suggest that the toxic fractions present

in cow slurry persisted in the material over several weeks of storage, and were present in concentrations which required considerable dilution to reduce their adverse effect upon E. fetida.

The mixing of cow slurry with solid materials (Table 2.3.) was more effective in reducing the toxicity of slurry. In the case of soil/slurry mixtures, a reduction in toxicity was observed, especially in the 3:1 soil/slurry mix, which was completely de-toxified after 14 days. When the slurry was mixed with aged, dried cow manure, de-toxification occurred more rapidly. The 2:1 and 3:1 manure/slurry mixtures were de-toxified immediately, while the 1:1 mixture became totally de-toxified after 21 days. The immediate de-toxification of the slurry when mixed with larger volumes of dry cow manure suggests that the effect may have been due to absorption and adsorption of the toxic slurry fraction by the dry solid.

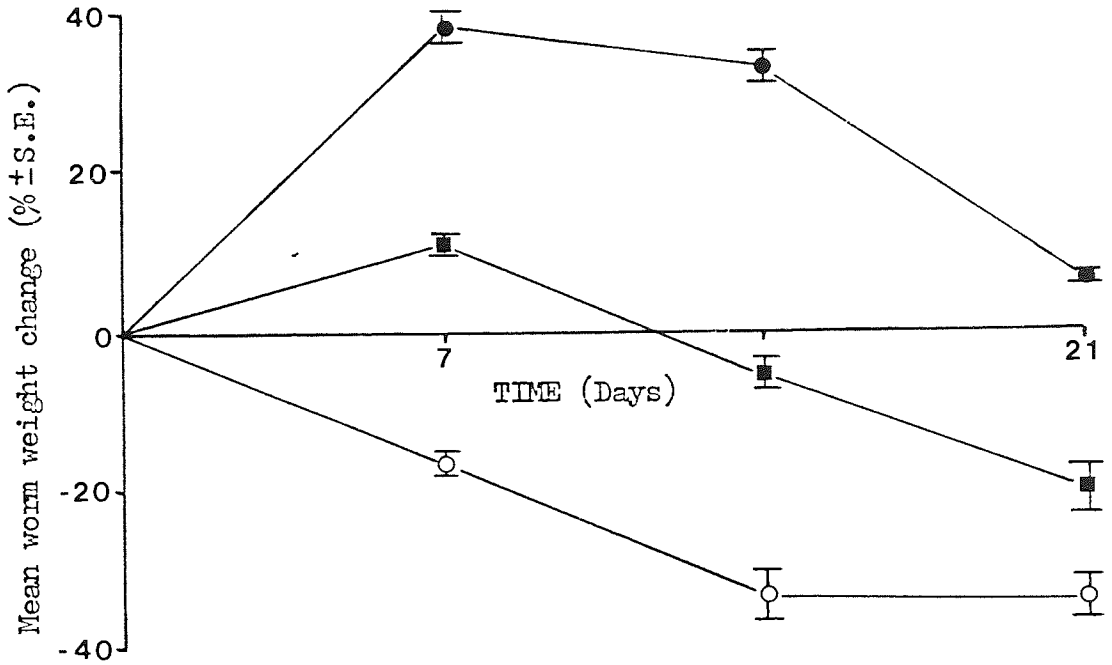
3.2.2. Mixtures of cow slurry with different bedding materials.

Slurry was not quickly de-toxified by mixing with soil or P.M.B.

(i) Weight change of E. fetida.

The weight change of E. fetida in different peat/slurry mixtures is shown in Fig. 3.12. and appendix 2.1.. Peat alone provided little or no nutrients to E. fetida. Worms in this substrate lost weight rapidly for 14 days, then maintained weight at a low level, presumably as they reached

Fig. 3.12. % Weight change of E. fetida with time in different peat/cow slurry mixtures.



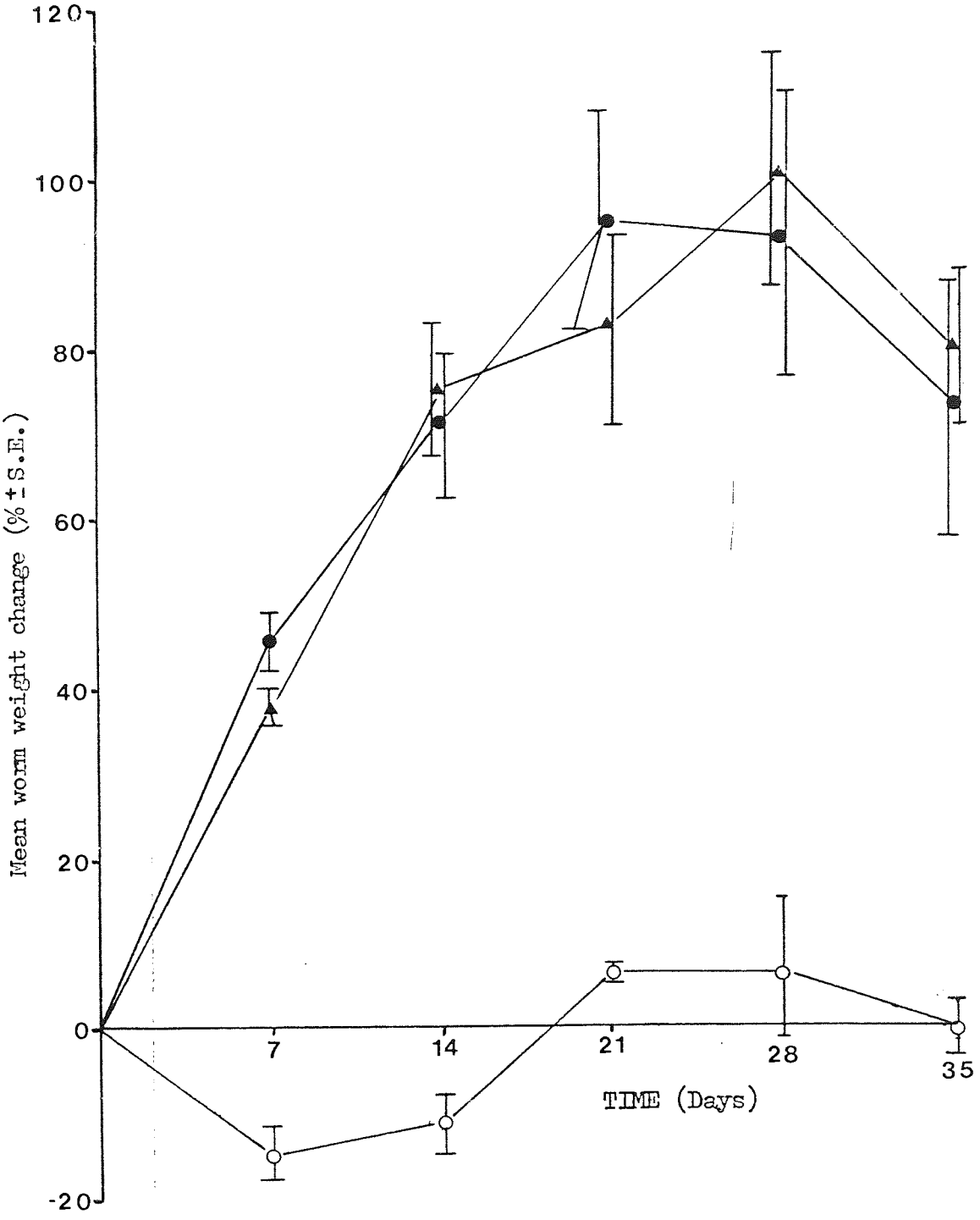
—●— = 1:1 peat/cow slurry mixture
—■— = 2:1 peat/cow slurry mixture
—○— = control: peat only

starvation point. In the 2:1 (w/w) peat/slurry mixture, a significant initial worm weight gain occurred, but between 7 and 14 days weight loss occurred at the same rate as the control worms, and by day 21, earthworm weights in this treatment were not significantly different from controls. This suggests that the slurry fraction was quickly consumed, and the worms were then unable to obtain further nutrients from the substrate. The 1:1 peat/slurry mixture supported significantly greater earthworm weight gain than the control with maintenance of weight for a short time, but this was followed by rapid weight loss between days 14-21, to a level which was not significantly different from the control.

The rate of ingestion of cow slurry in the 1:1 peat: slurry mixture was calculated as 17.6g slurry g^{-1} earthworm day^{-1} (fresh weight). However, the conversion of slurry to worm biomass was low, at 0.3%.

The use of paper tissue waste in mixtures with slurry is shown in Fig. 3.13. and appendix 2.2.. In controls containing tissue waste only, initial earthworm weight loss was comparable to that in peat, but this weight loss subsequently slowed, and a small weight increase was recorded during the later part of the experiment. The addition of slurry in different proportions produced large significant worm weight increases. The proportion of slurry in the mixture did not appear to affect the growth rate of the worms. When feeding on tissue waste:slurry mixtures, the ingestion rate of E. fetida was much lower than on peat mixtures, with

Fig. 3.13. % Weight change of E. fetida with time in different tissue waste/cow slurry mixtures.



—●— = 1:1 tissue waste/cow slurry mixture

—▲— = 2:1 tissue waste/cow slurry mixture

—○— = control: Tissue waste only

Table: 2.4. Reproduction of E. fetida in cow slurry mixed with two bedding materials. Mean number of cocoons after 35 days. (n = 3).

Treatment	mean no cocoons per worm (n=3)	S.E.
Peat control	0	-
2:1 peat/slurry	0.4	±0.12
1:1 peat/slurry	0.7	±0.17
Tissue waste control	1.8	±0.18
2:1 T.W./slurry	4.2*	±0.28
1:1 T.W./slurry	6.1*	± 0.37

S.E. = Standard error of the mean.

* = Treatment significantly different from control: $P < 0.05$

a maximum value of 4.8g slurry g^{-1} earthworm day^{-1}. Conversion of slurry to earthworm biomass was, however, considerably higher at 0.7%.

(ii). Cocoon production.

Table 2.4. shows the numbers of cocoons produced by E. fetida after 35 days in peat:slurry and tissue waste:slurry mixtures.

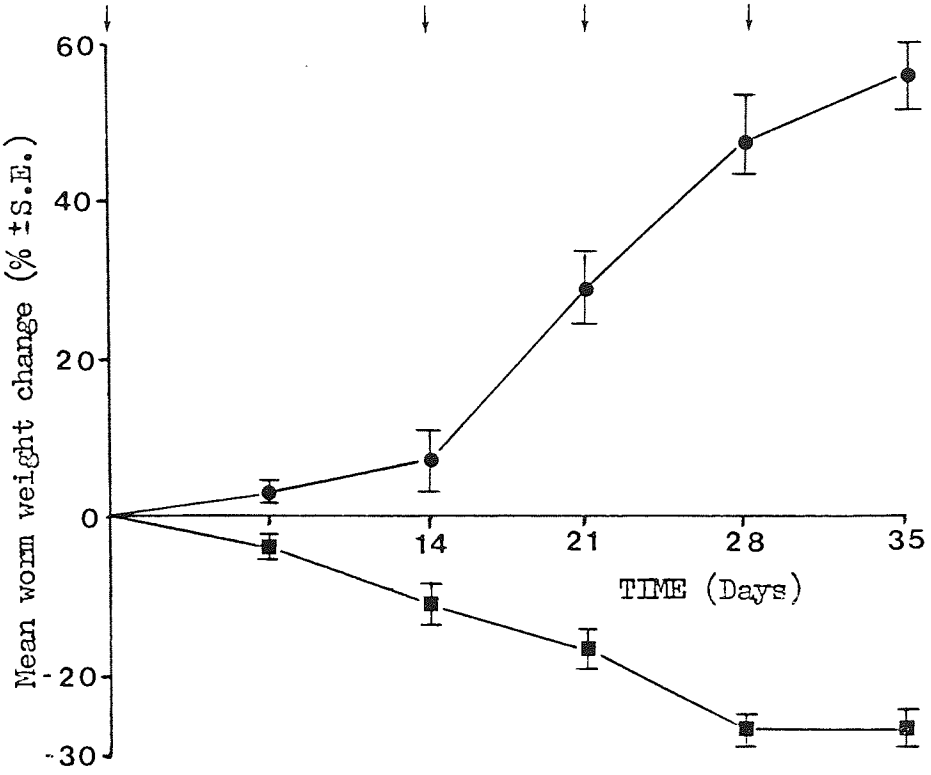
Cocoons were first observed in peat/slurry mixtures after two weeks, and in the tissue wate/slurry mixtures after four weeks. In both cases this marked the onset of earthworm weight loss in the treatments producing cocoons. In the case of peat/slurry mixtures, few cocoons were produced overall, and only the 1:1 peat/slurry mixture produced significantly more cocoons than the control. More cocoons were produced in the tissue waste/slurry mixtures, indicating the greater nutritional value of this substrate to E. fetida. Both 2:1 and 1:1 tissue waste/slurry mixtures produced significantly more cocoons than controls, with the largest numbers produced in the 1:1 mixture.

3.2.3. Surface addition of cow slurry to bedding materials containing earthworms.

(i) Weight change of E. fetida.

The weight change of E. fetida in soil with surface addition of cow slurry is shown in Fig. 3.14. and appendix 2.3.. Control earthworms in soil only lost weight in a similar manner to worms in peat: a gradual weight loss for some weeks, followed by maintenance of a much reduced weight. In soil with regular applications of cow slurry, a small earthworm weight increase for 14 days was followed by a significant weight increase for the remainder of the experiment. Initially, slurry was consumed slowly, but

Fig. 3.14. % Weight change of *E. fetida* in soil top-fed with cow slurry (10% w/v).



Cow slurry applied to soil at times marked (↓)

—●— = soil plus slurry

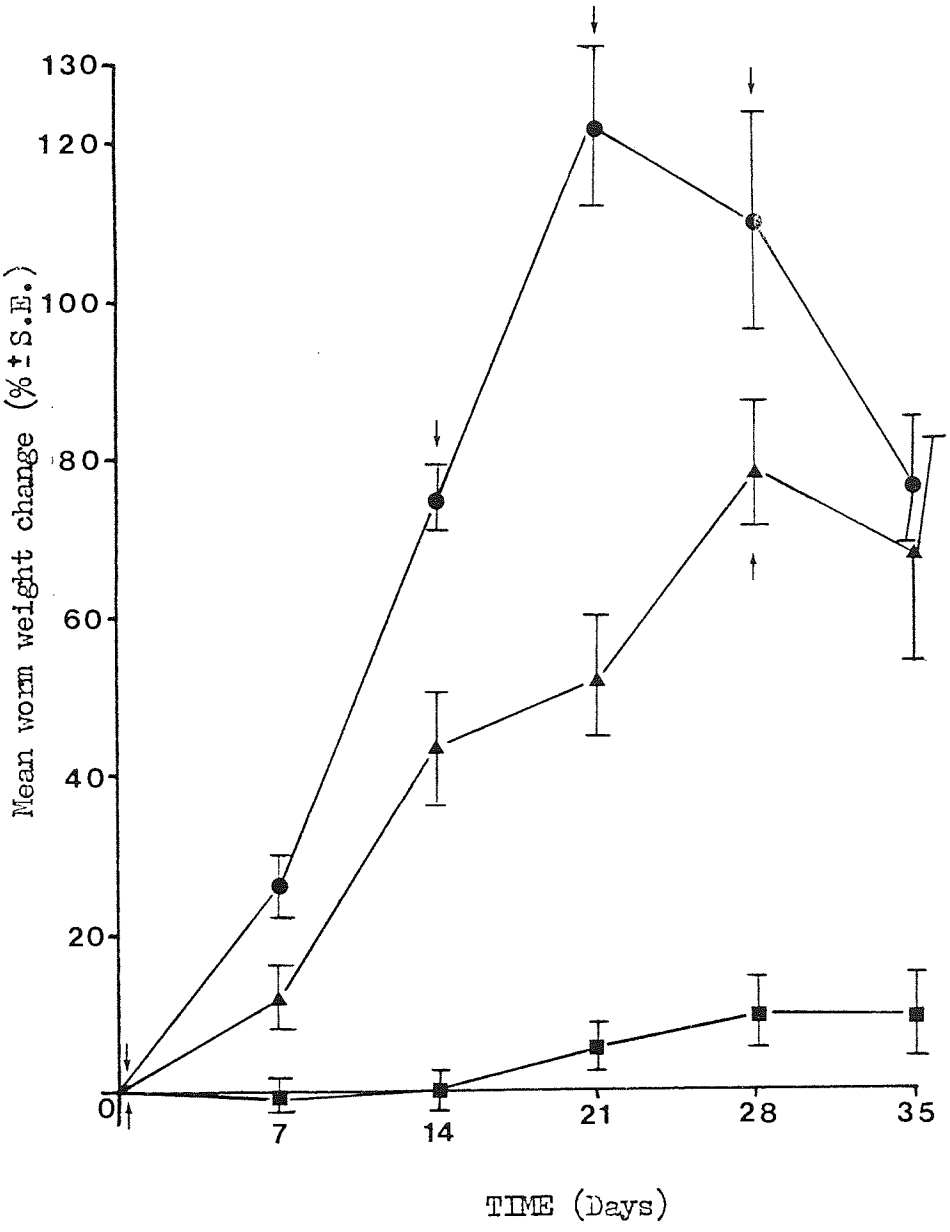
—■— = control: soil only

pots required weekly additions of cow slurry from day 14 onwards. Overall, the rate of consumption of slurry by E. fetida in this system was lower than in the peat:slurry mixtures, at 4.1g slurry g^{-1} earthworm day^{-1}. However, a similar conversion efficiency of 0.4% was calculated.

Weight change of E. fetida in tissue waste with added cow slurry is shown in Fig. 3.15. and appendix 2.4.. Earthworms in tissue waste alone showed little weight change for 14 days followed by a small weight increase to the end of the experiment. Pots with 10% cow slurry additions produced significantly higher worm weight gains than controls between days 7 and 28. Evidence of feeding by earthworms on the slurry was observed 2-3 days following application. Earthworms began to lose weight after 21 days, and this weight loss was not reversed by further additions of cow slurry. This treatment produced the greatest assimilation efficiency of the systems tested, at 1.3%, and 4.3g slurry g^{-1} worm day^{-1} were consumed during the experiment.

Earthworms in pots with 20% slurry additions showed much lower weight gains than those fed at the 10% level. Feeding by earthworms on the applied slurry was observed after 5 days. Slurry was consumed slowly and incompletely: slurry remaining on day 28 had formed a dry crust on the surface of the pots. Addition of fresh slurry at this time did not prevent subsequent earthworm weight loss.

Fig. 3.15. % Weight change of E. fetida in tissue waste top-fed with cow slurry (10 and 20% w/v).



Cow slurry applied at times marked (↑ ↓)

—●— = slurry added at 10% w/v

—▲— = slurry added at 20% w/v

—■— = control: tissue waste only

Table 2.5. Reproduction of E. fetida in worm beds top-fed with cow slurry. Mean number of cocoons after 35 days. (n = 5).

Treatment	Mean no. cocoons per worm (n=5)	S.E.
Soil control	0	-
Soil+10% (w/w) cow slurry	0.8	±0.22
Tissue waste control	1.8	±0.16
Tissue waste + 10% (w/w) slurry	6.3★	±0.44
Tissue waste + 20% (w/w) slurry	4.3★	±0.48

S.E. = standard error of the mean.

★ = Treatment significantly different from control: $P < 0.05$

(ii) Cocoon production.

After 5 weeks of incubation, the number of cocoons in each pot was counted, and the mean number of cocoons produced per worm during the experiment was calculated. Cocoon production during the experiment was higher in tissue waste pots with added slurry than in soil pots with added slurry. Cocoon production in the pots is shown in Table 2.5. Cocoons were first observed in the soil pots with slurry added after

four weeks. No cocoons were produced in control pots during the experiment. Cocoons were first observed in all pots containing tissue waste after four weeks. The earthworms fed with added cow slurry produced significantly more cocoons than the control earthworms. Pots with slurry added at the 10% level produced more cocoons than those with slurry added at the 20% level.

The results of earthworm growth rate and reproduction from the mixing and top-feeding systems (sections 3.2.2. and 3.2.3.) allowed comparisons to be made between the two vermicomposting systems. Cow slurry was quickly de-toxified when mixed with certain solid materials, or when added to the surface of earthworm beds in thin layers, and formed a suitable food source for the growth and reproduction of E. fetida. Peat/slurry mixtures were vermicomposted more quickly than tissue waste/slurry mixtures, though the earthworms gained weight more quickly and produced more cocoons in the latter substrate. When slurry was applied to the surface of earthworm beds, equivalent amounts of slurry were vermicomposted per unit of time in both soil and tissue waste beds, though earthworm growth and cocoon production were again higher in tissue waste.

3.3. Chemical and microbiological changes in cow slurry during vermicomposting.

3.3.1. Chemical and microbiological changes in a 2:1 (w/w) tissue waste/cow slurry mixture with time.

(i) Chemical Changes.

Fig. 3.16. shows the changes in the moisture content of a 2:1 (w/w) tissue waste:cow slurry mixture with time, in the presence and absence of E. fetida. In both cases, an increase in moisture content occurred over 6 weeks, but this increase was greater in the presence of earthworms.

The organic matter content of the substrate in the presence of earthworms is shown in Fig. 3.17.. The rate of organic matter decomposition was greatest at the beginning of the experiment, but had slowed considerably after 6 weeks.

Fig. 3.18. shows that the ammonium-nitrogen content of the mixture with time was almost identical in the presence and absence of earthworms. Most of the initial ammonium-nitrogen was lost by day 14. This corresponds with the period of maximum ammonia volatisation from stored slurry (Fig. 3.3.) and it is therefore possible that a significant proportion of the slurry ammonium-nitrogen is normally lost from the mixture in the form of ammonia gas. However, Fig. 3.19. shows the changes in nitrate plus nitrite nitrogen content of the mixture. This rose from an initial low level

Fig. 3.16. Changes in moisture content of a 2:1 (w/w) tissue waste/cow slurry mixture.

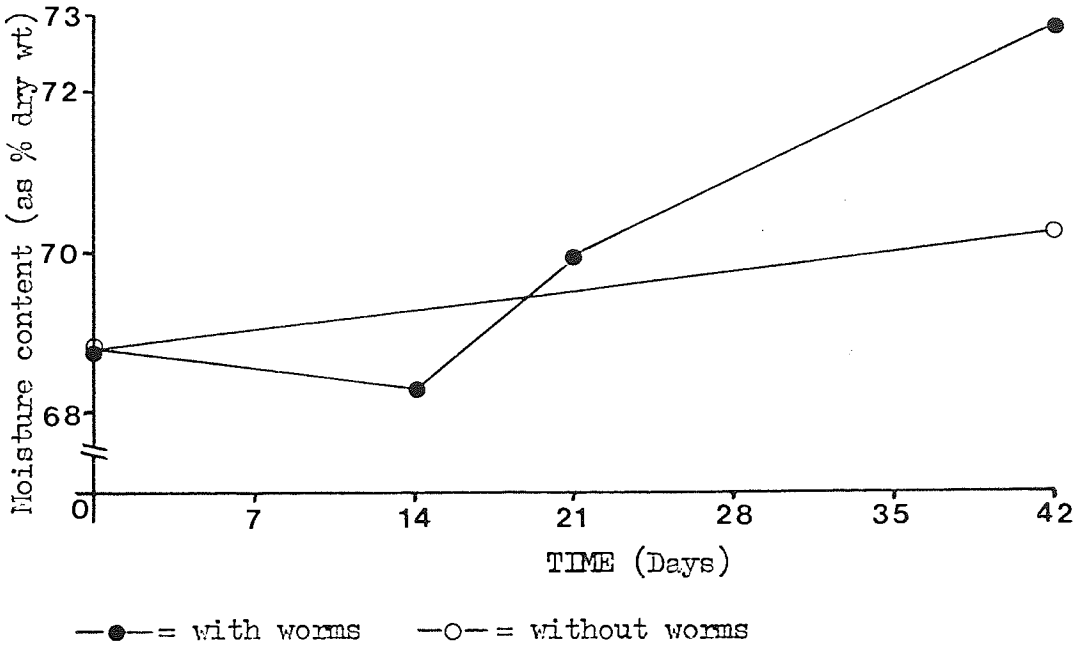


Fig. 3.17. Organic matter content of a 2:1 (w/w) tissue waste/ cow slurry mixture.

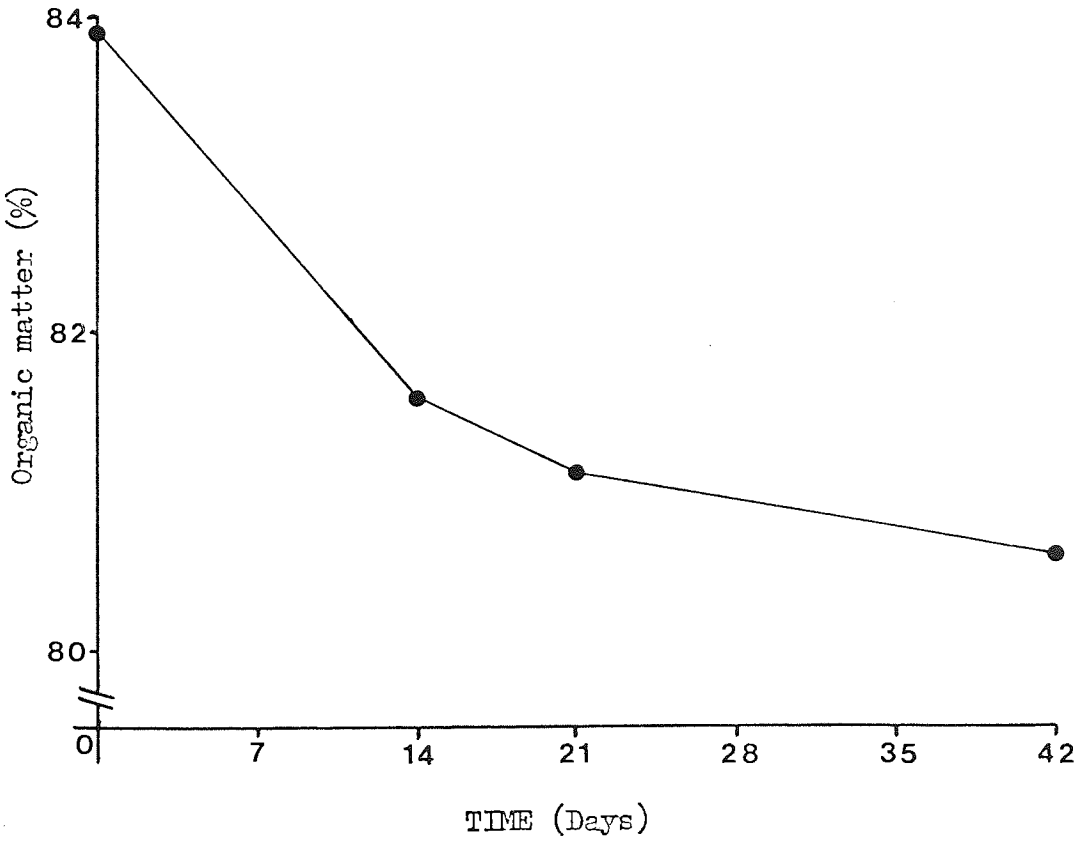


Fig. 3.18. Changes in NH_4-nitrogen content of a 2:1 (w/w) tissue waste/cow slurry mixture.

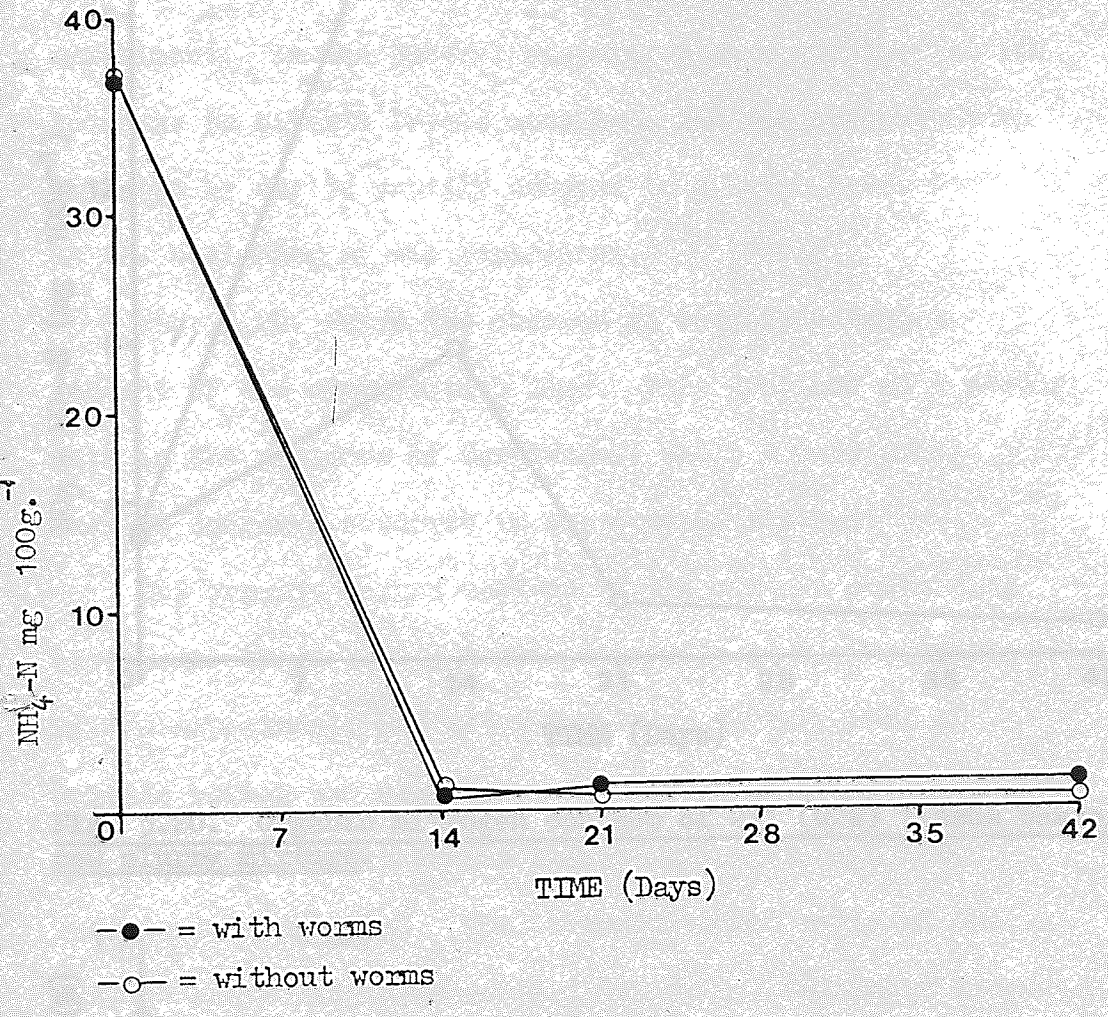


Fig. 3.19. Nitrate + Nitrite-nitrogen content of a 2:1 (w/w) tissue waste/cow slurry mixture.

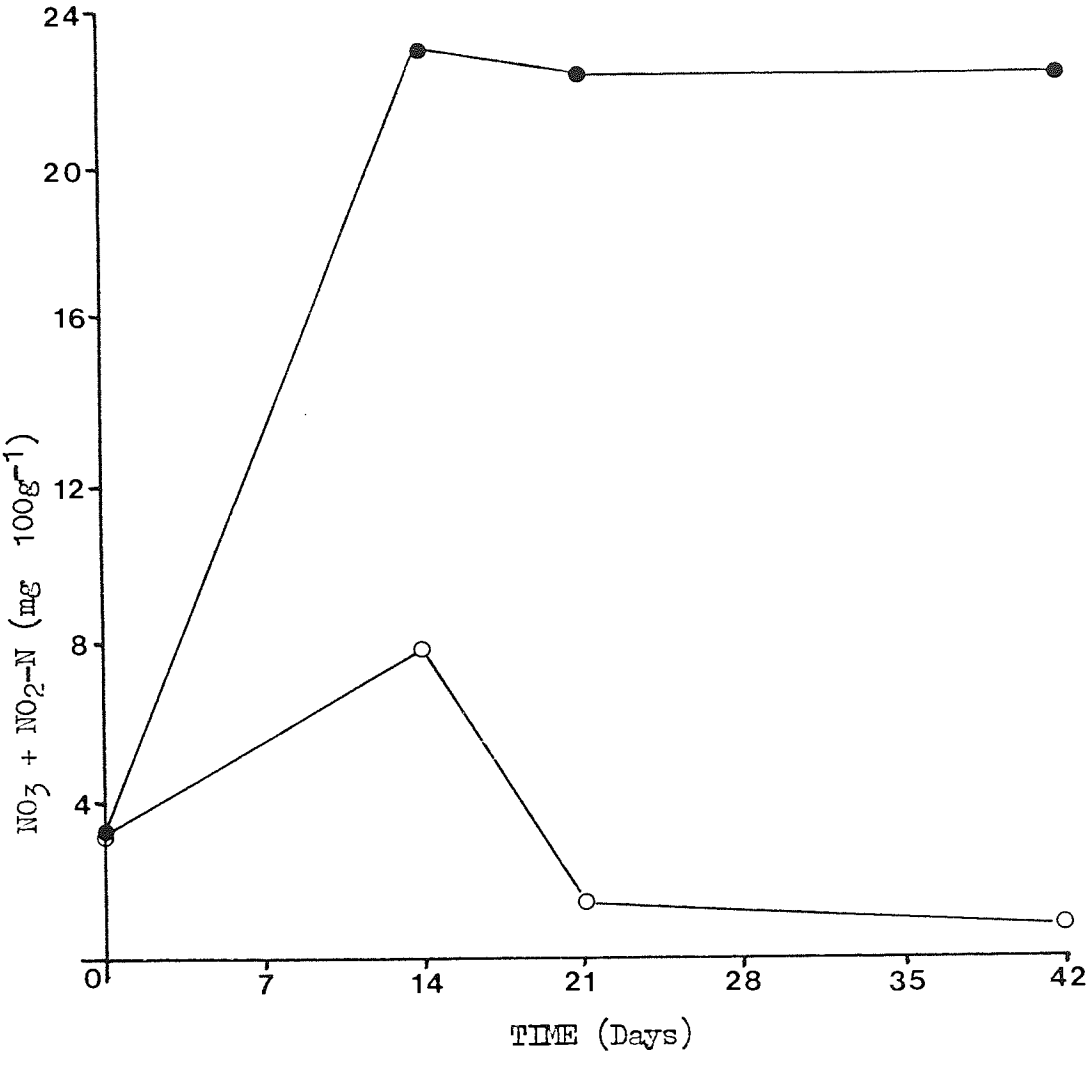
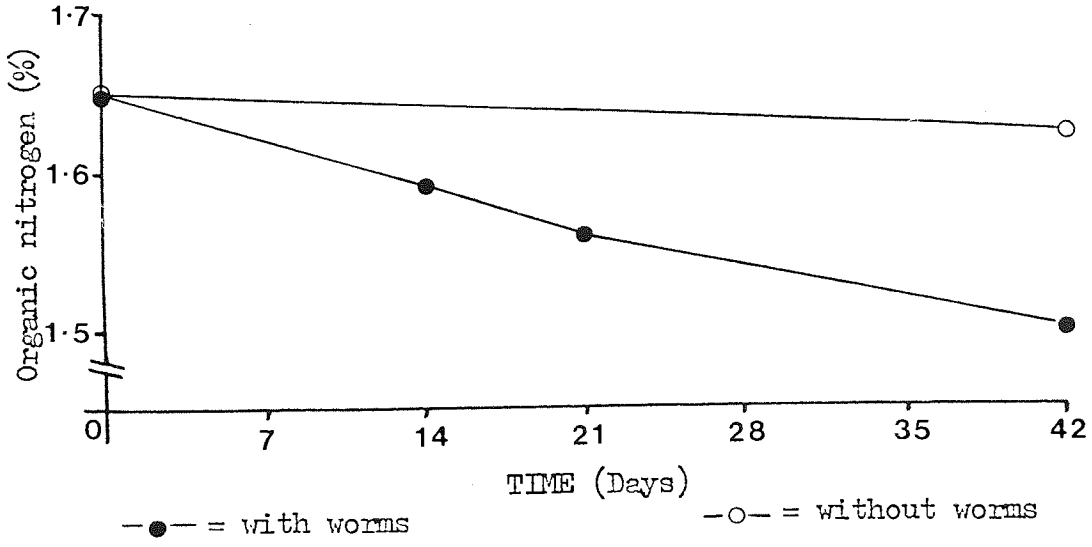


Fig. 3.20. Organic nitrogen content of 2:1 (w/w) tissue waste/ cow slurry mixture.



in both treatments at the same time as ammonium nitrogen was being lost from the mixture, suggesting that some of the ammonium nitrogen is converted to nitrate. In the treatment containing earthworms, a large increase in nitrate nitrogen content was observed in the first 14 days, and this level was subsequently maintained to the end of the experiment. In the control mixture, a much smaller initial increase in nitrate levels occurred, and the concentration achieved by day 14 rapidly dropped to a level below that at the beginning of the experiment.

Fig. 3.20. shows the changes in organic nitrogen content of the mixture with time. This declined at a steady rate in the presence of earthworms, while a very small overall decrease occurred in the control mixture.

The organic carbon content of the mixture containing earthworms (Fig. 3.21.) declined rapidly at the beginning of the experiment, but after day 21 no further loss of organic carbon was recorded. The mixture without earthworms had a higher organic carbon content after 42 days than that containing worms. The carbon:nitrogen ratio of the mixture containing earthworms (Fig. 3.22.) initially decreased rapidly as organic carbon was lost from the substrate. The ratio then stabilised, and finally rose slightly, due to the continued loss of organic nitrogen. The control mixture has a slighty lower C:N ratio than the mixture containing earthworms after 6 weeks.

Fig. 3.23. shows that the holo-cellulose content of

Fig. 3.21. Organic carbon content of a 2:1 (w/w) tissue waste/ cow slurry mixture.

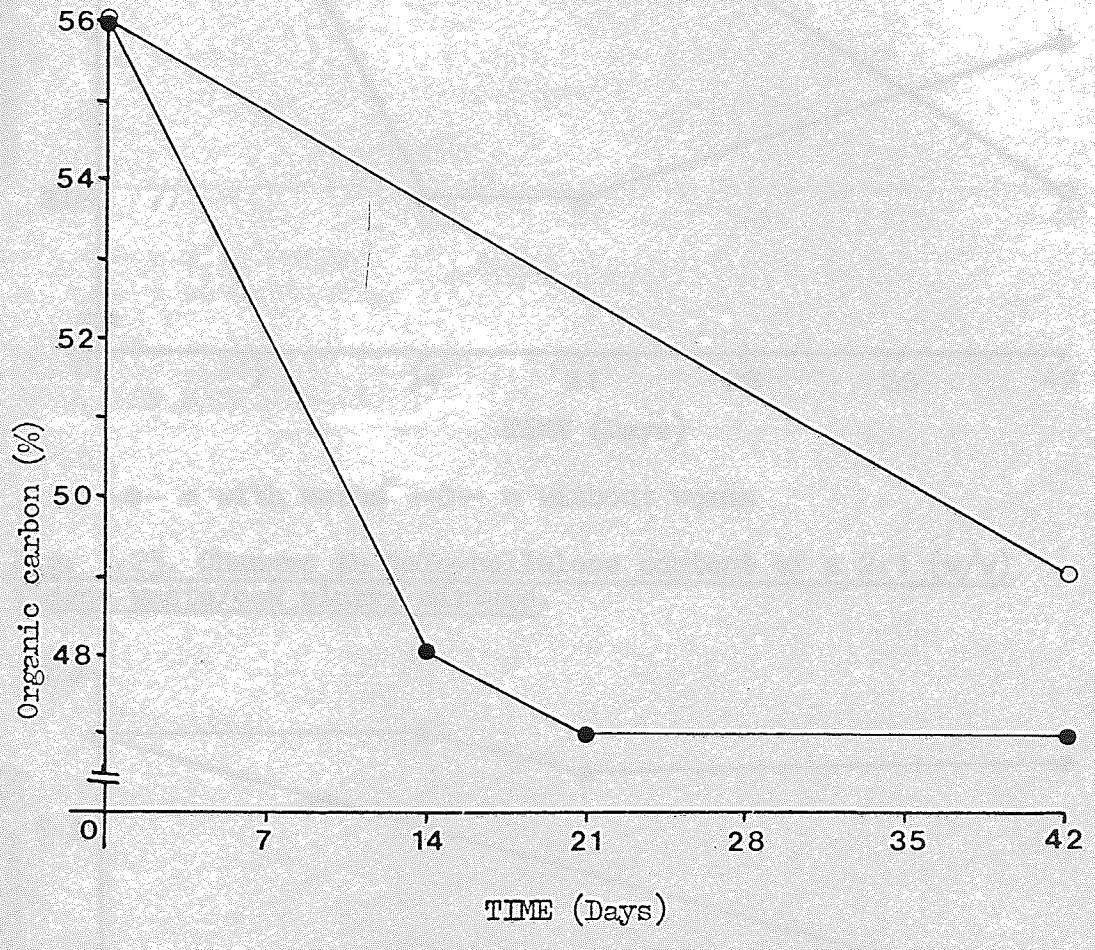


Fig. 3.22. Carbon: nitrogen ratio of a 2:1 (w/w) tissue waste/ cow slurry mixture with time.

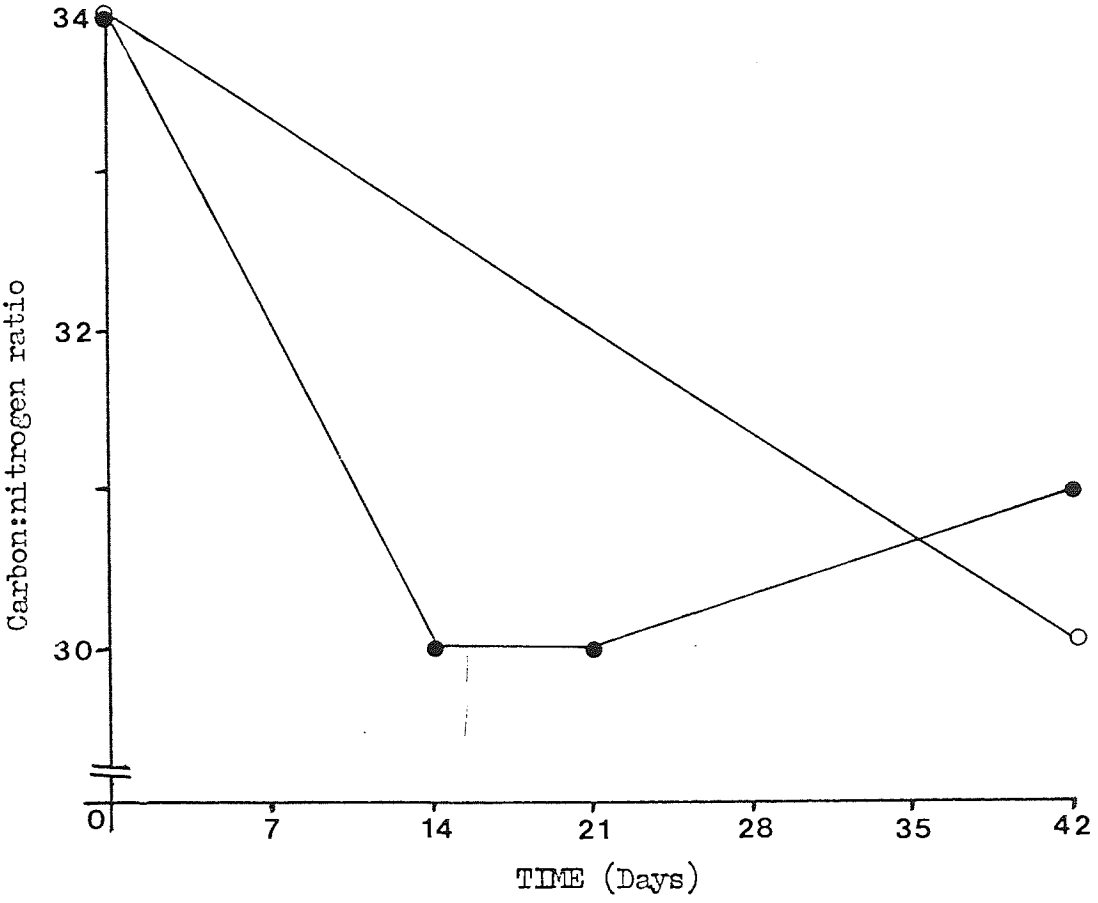


Fig. 3.23. Changes in holo-cellulose content of a 2:1 (w/w) tissue waste/cow slurry mixture.

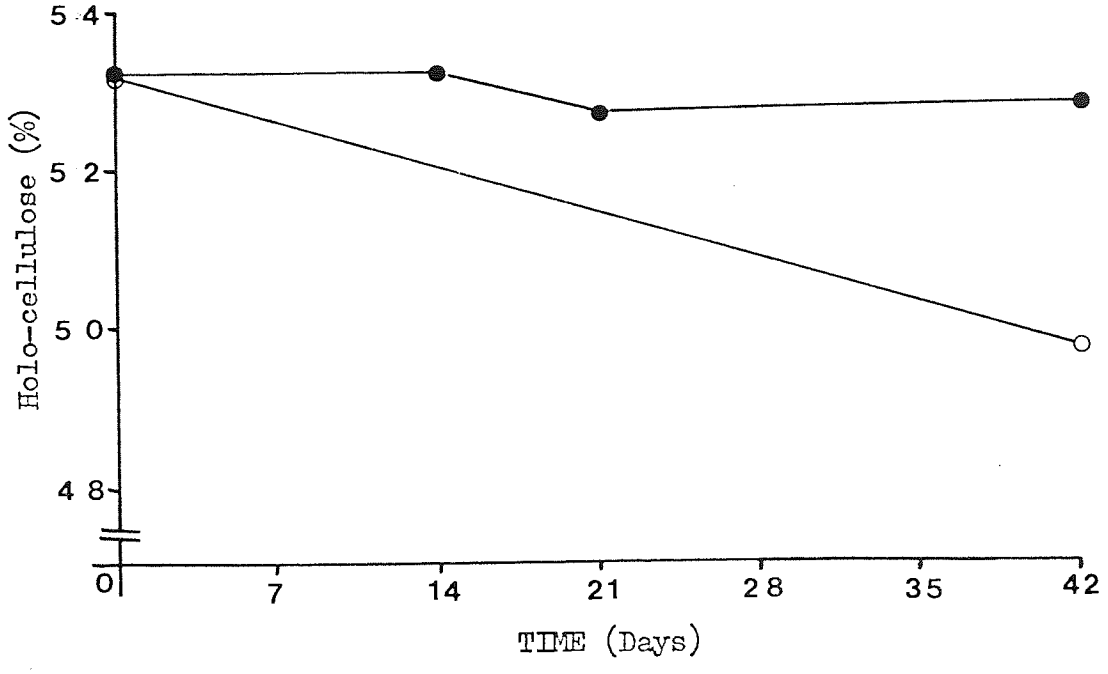


Fig. 3.24. ∝-cellulose content of a 2:1 (w/w) tissue waste/cow slurry mixture with time.

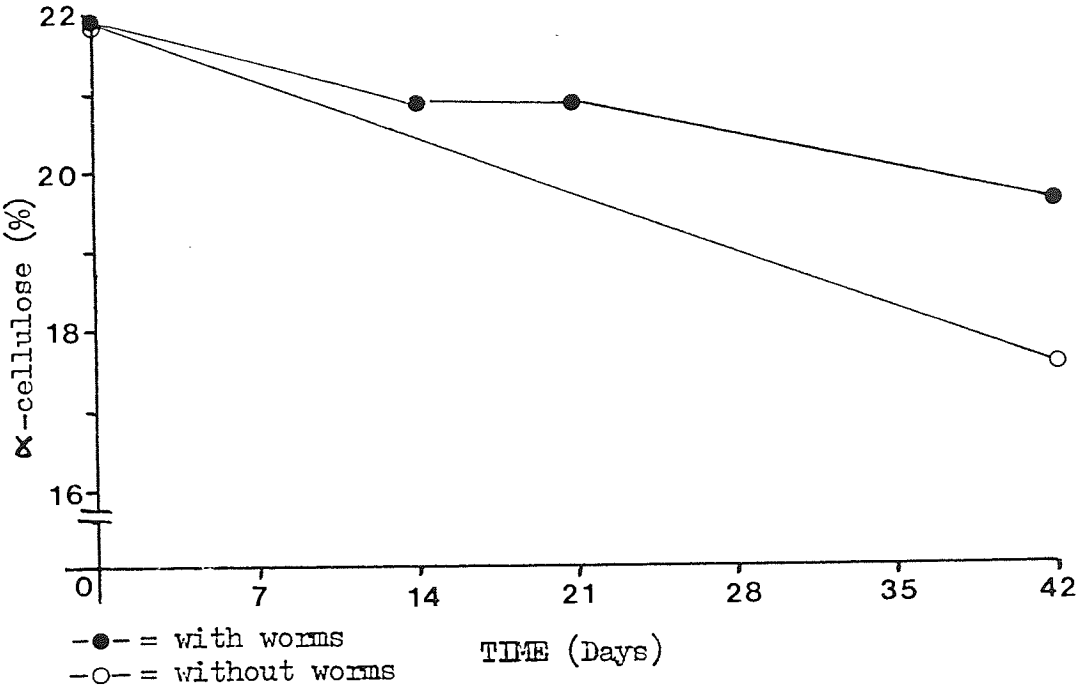
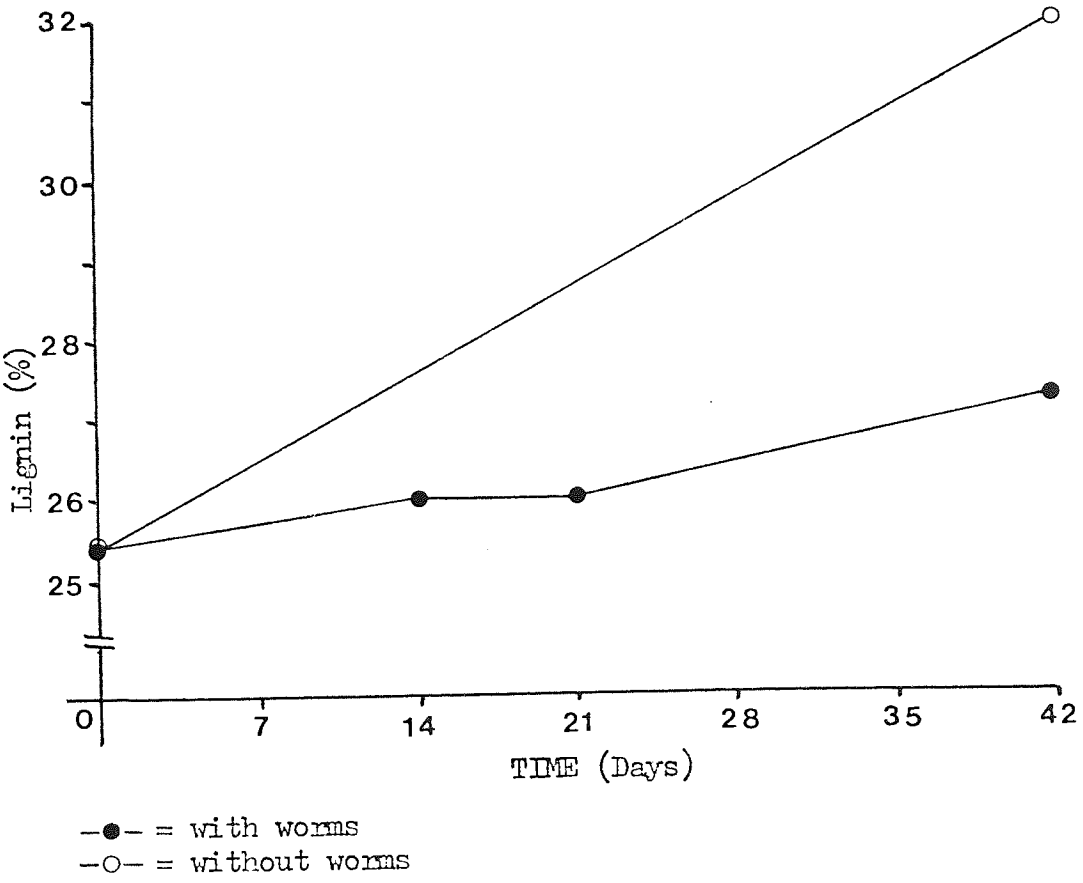


Fig. 3.25. Changes in Lignin content of a 2:1 (w/w) tissue waste/cow slurry mixture.



the mixture containing earthworms showed no significant change over 6 weeks. However, in the mixture without worms, evidence of cellulose decomposition was observed. This trend was also observed in the levels of α-cellulose (Fig. 3.24.), which were lower in the control mixture than in the presence of worms after 6 weeks.

Fig. 3.25. shows that the proportion of lignin increased slightly with time in the mixture containing worms, but showed a much greater increase after 6 weeks in the control mixture.

(ii) Microbiological Changes.

Fig. 3.26. shows the changes in the total numbers of the most abundant micro-organisms in a 2:1 (w/w) tissue waste:cow slurry mixture with time, in the presence and absence of E. fetida. Total numbers in the two treatments were similar for 6 weeks, and followed the same trend of a slow decline. Between weeks 6 and 7 however, the number of bacteria in the mixture containing earthworms fell sharply compared with the control mixture.

Figs. 3.27. and 3.28. and appendices 3.1. and 3.2. show the relative abundance of the most numerous bacteria isolated from the mixtures. The pattern of succession was very similar in both treatments. Numbers of Acinetobacter lwoffi and Micrococcus luteus were initially high, but fell gradually. Numbers of these isolates in the control mixture

Fig. 3.26. Changes in total numbers of aerobic bacteria in a 2:1 (w/w) tissue waste/cow slurry mixture.

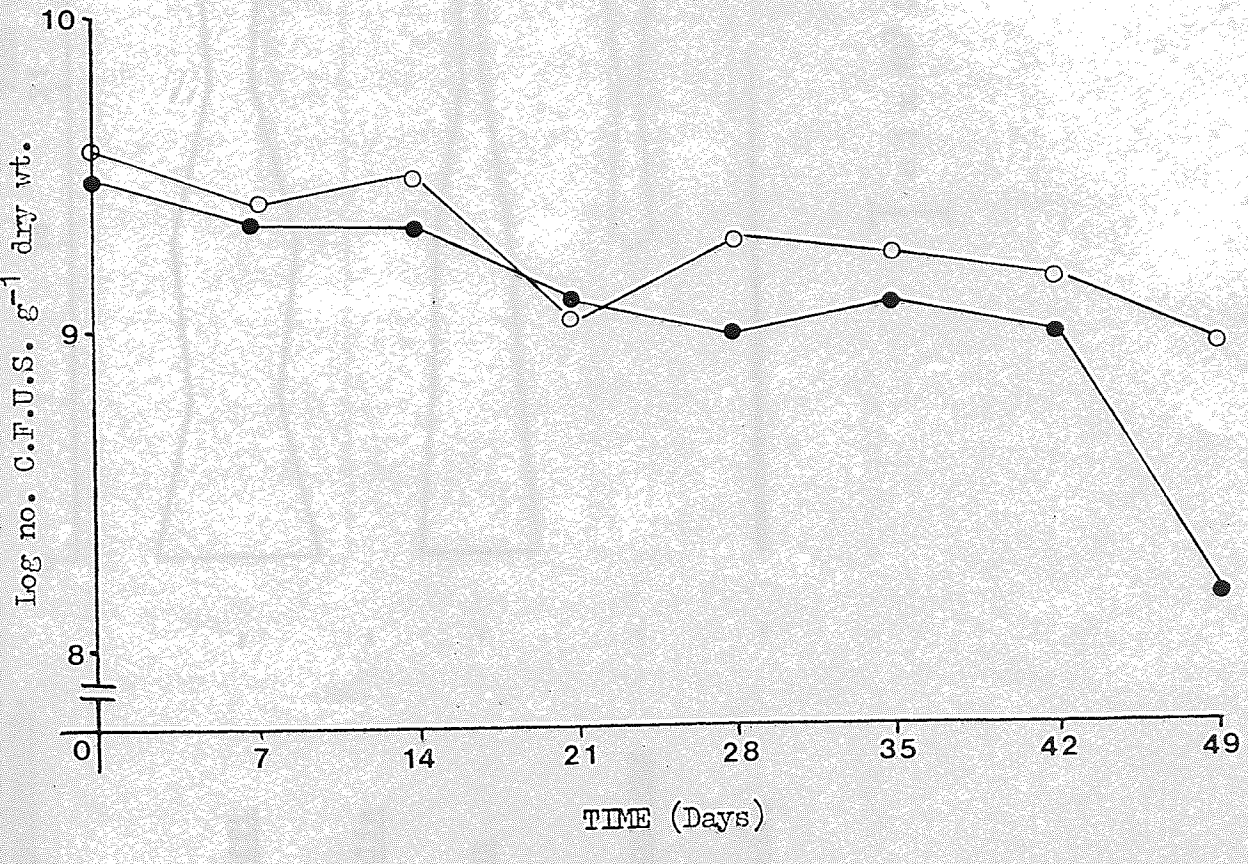


Fig. 3.27. Relative abundance of aerobic bacteria in a 2:1 (w/w) tissue waste/cow slurry mixture without earthworms.

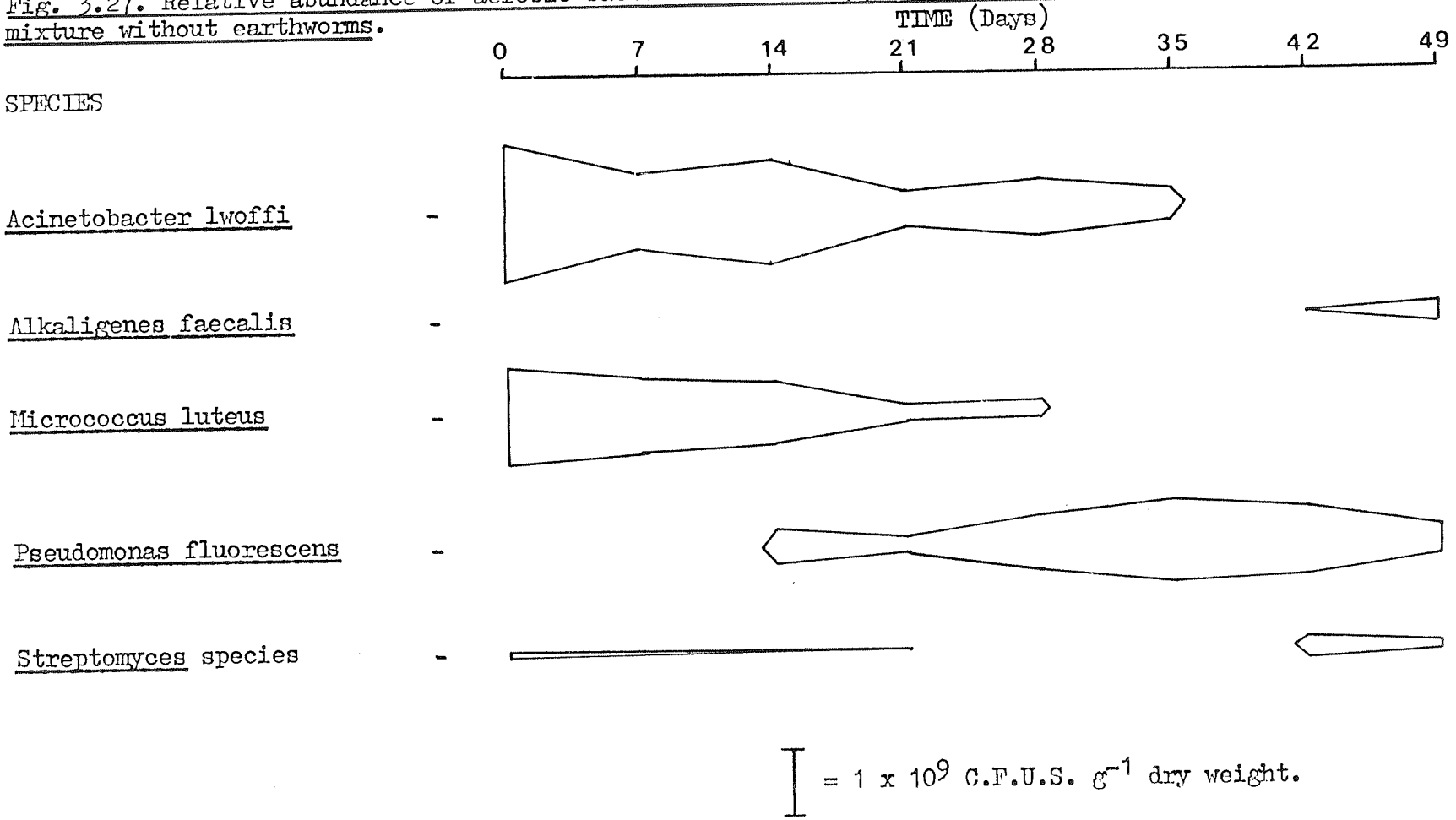
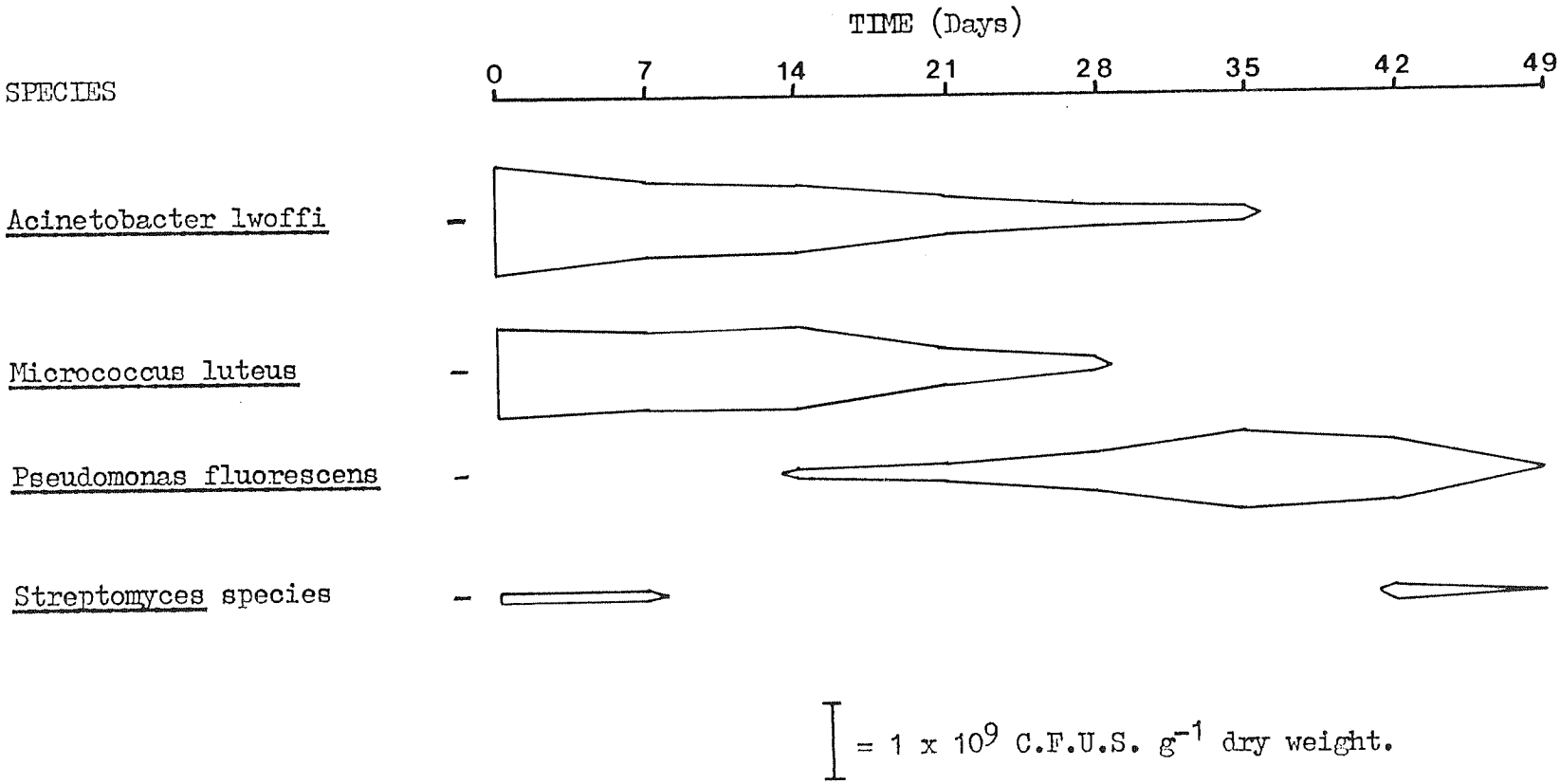


Fig. 3.28. Relative abundance of aerobic bacteria in a 2:1 (w/w) tissue waste/cow slurry mixture containing earthworms.



were slightly higher throughout than in the mixture containing earthworms. Pseudomonas fluorescens was isolated in large numbers from both treatments from day 14 onwards, and numbers of this species rose to a maximum after 5 weeks and subsequently declined. Again, numbers of this species were slightly higher in the control mixture than in the mixture with earthworms. A Streptomyces species was found in both treatments in relatively low numbers at the beginning and end of the experiment.

In the control mixture, Alkaligenes faecalis was isolated after 6 weeks, and had increased in numbers by the end of the experiment. The same species, and Flavobacterium lutescens, were isolated in slightly lower numbers from the mixture containing worms at the end of the experiment.

Fig. 3.29. shows the total numbers of fungi isolated from the tissue waste/cow slurry mixture with time. As in the case of the bacteria, numbers were similar in tubs with and without earthworms for the first 6 weeks of the experiment The total numbers of propagules showed a small increase over the first four weeks, followed by a decline in numbers. In the control tubs, this trend was reversed over the last week of the experiment, while numbers in the tubs containing earthworms continued to decline at the same rate.

Changes in the relative abundance of the most numerous fungal species with time are shown in Figs. 3.30. and 3.31. and appendices 3.3. and 3.4. Again the pattern of change in relative numbers was very similar between the two treatments.

Fig. 3.29. Changes in total numbers of fungi in a 2:1 (w/w) tissue waste/cow slurry mixture.

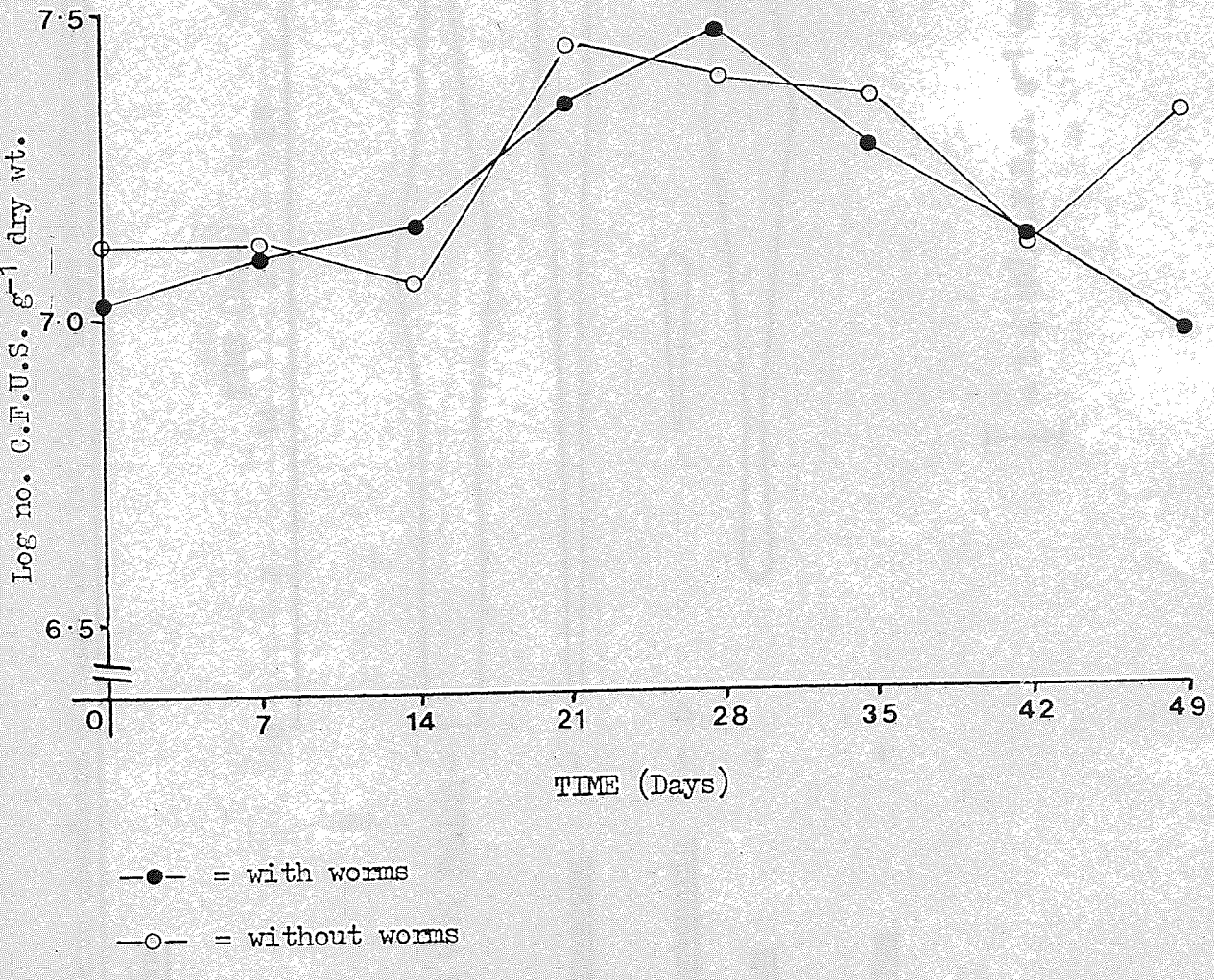


Fig. 3.30. Relative abundance of fungi in a 2:1 (w/w) tissue waste/cow slurry mixture without earthworms.

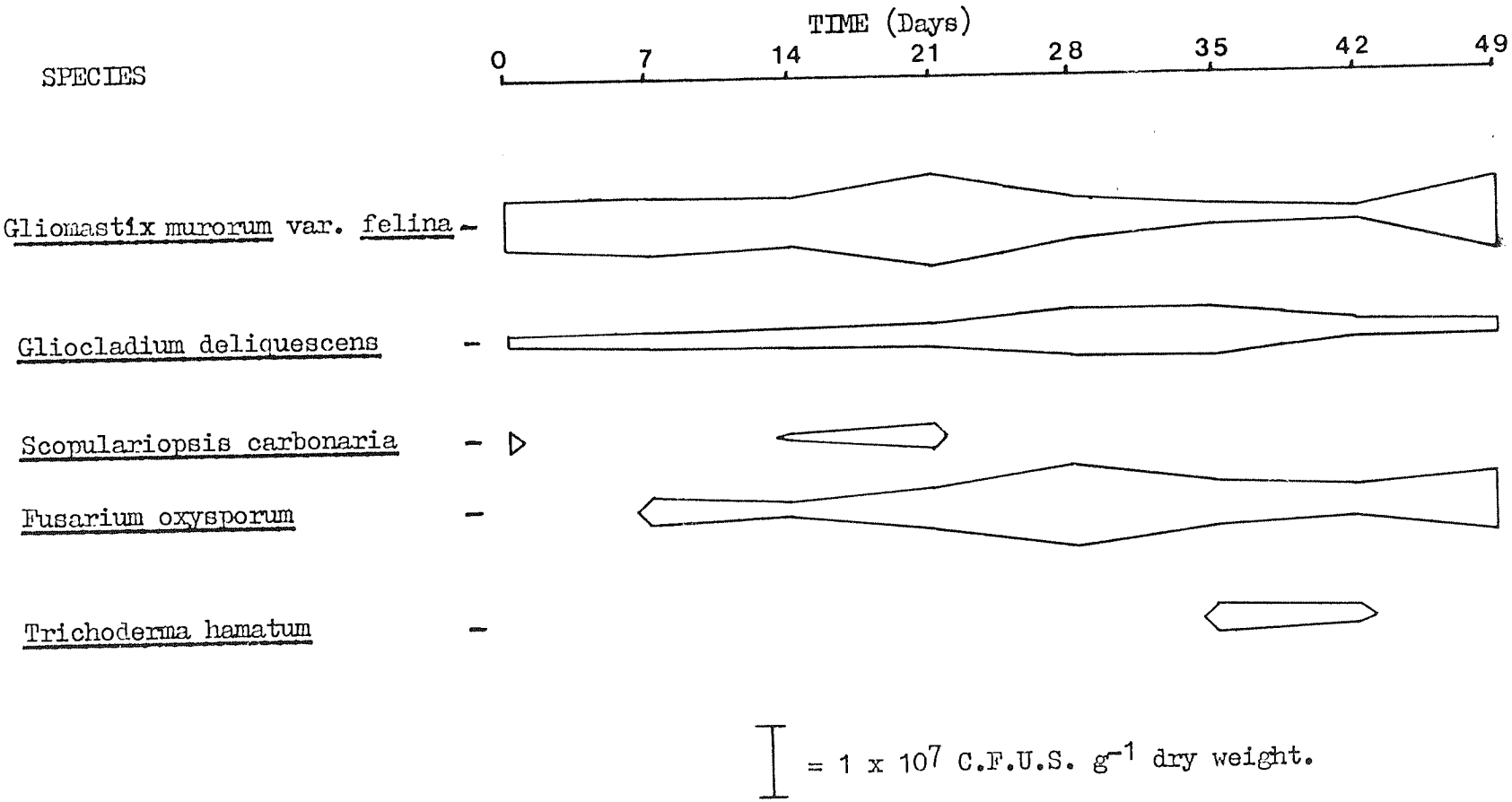
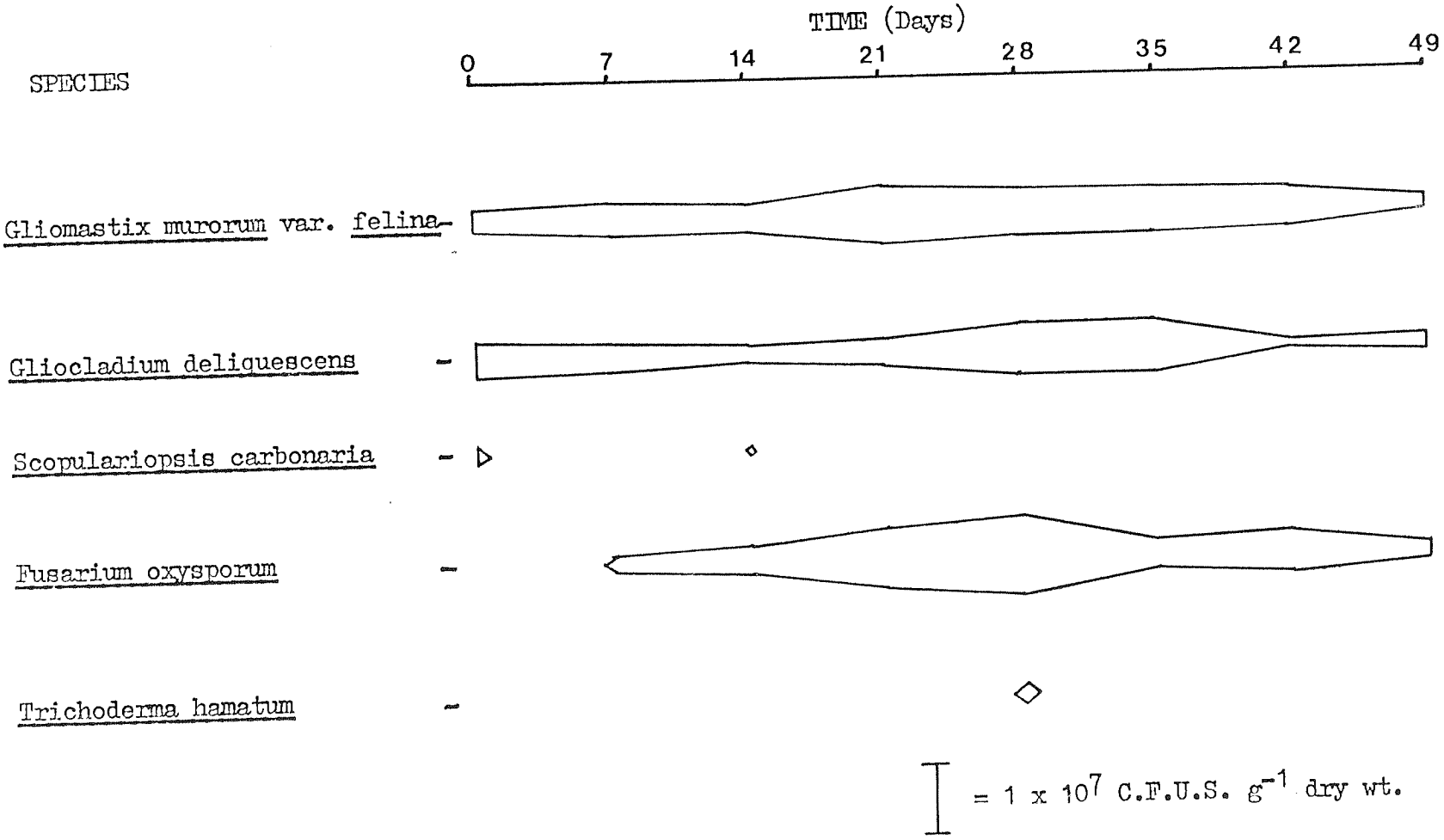


Fig. 3.31. Relative abundance of fungi in a 2:1 (w/w) tissue waste/cow slurry mixture containing earthworms.



The same fungal species were most numerous throughout the experiment in both treatments. Trichoderma hamatum was isolated in significant numbers from the mixture without earthworms between weeks 5 and 6. The same species was isolated only once in large numbers from the mixture containing earthworms at day 28. Numbers of all species in the mixture containing worms fell at the end of the experiment, but no other significant differences in numbers of the species isolated were observed.

3.3.2. Surface addition of cow slurry to paper tissue waste containing Eisenia fetida.

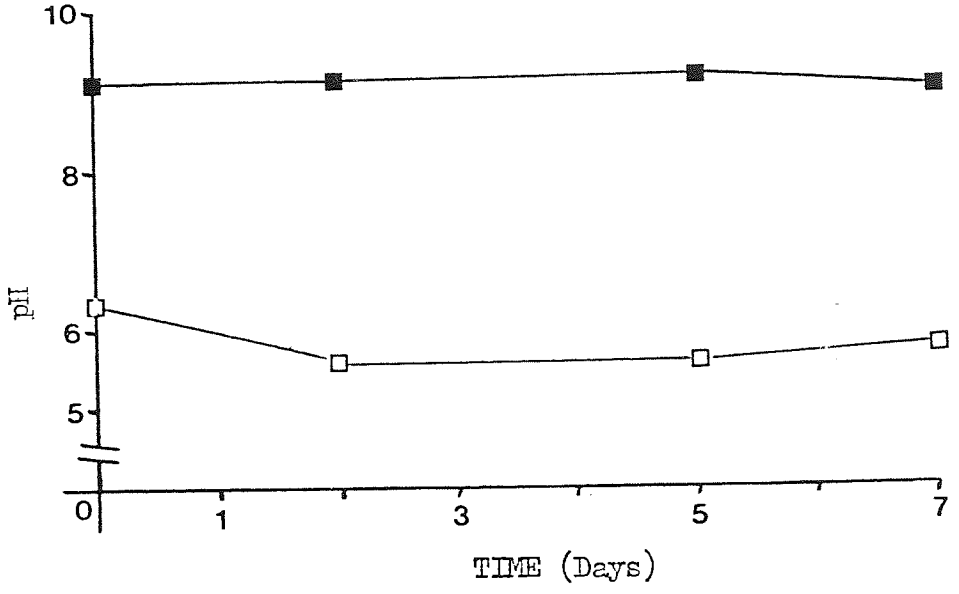
(i) Physical and chemical changes.

Figs. 3.32. to 3.35. show the physical and chemical changes which occurred at the surface of earthworm beds containing tissue waste following the application of cow slurry to experimental beds.

The surface pH of beds with slurry applied (Fig. 3.32.) was higher than that of controls throughout the experiment, and did not change significantly during this time.

The initial high electrical conductivity at the surface of beds with applied slurry quickly declined, becoming equal to that in control beds by day 5 (Fig. 3.33.). Ammonia volatisation at the surface of experimental pots was constant for 2 days, then declined over the remainder

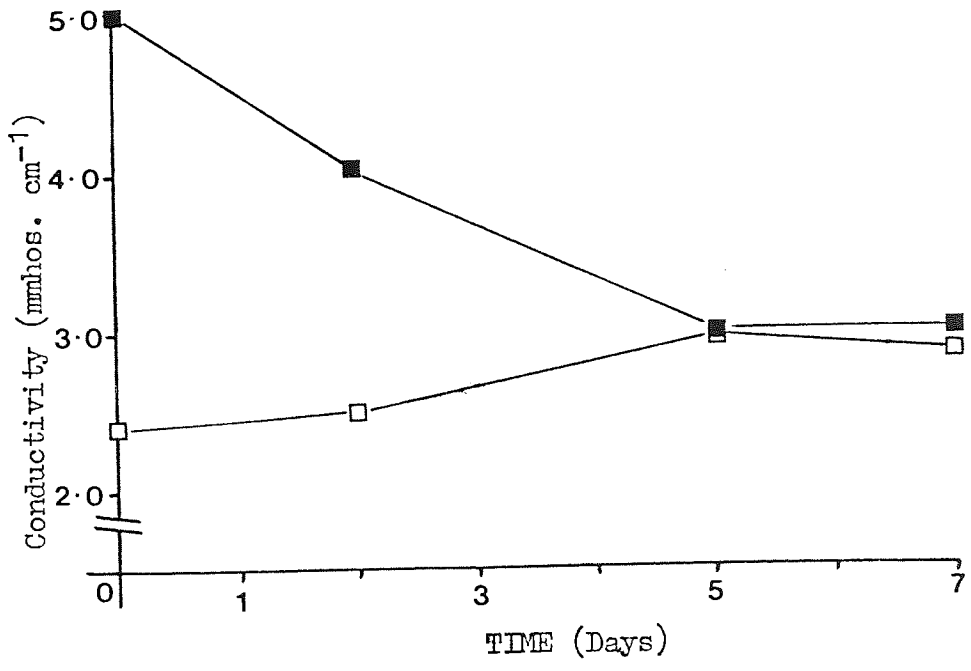
Fig. 3.32. pH at the surface of earthworm beds containing tissue waste.



—■— = with cow slurry

—□— = control: no slurry added

Fig. 3.33. Electrical conductivity at surface of earthworm beds containing tissue waste.



—■— = with cow slurry

—□— = control: no slurry added

of the experiment (Fig. 3.34.). No ammonia volatisation was detected at the surface of control beds without applied slurry.

The ammonium nitrogen content of slurry at the surface of the experimental beds (Fig. 3.35.) declined slowly throughout the experiment. Ammonium nitrogen concentration at the surface of control beds was not determined.

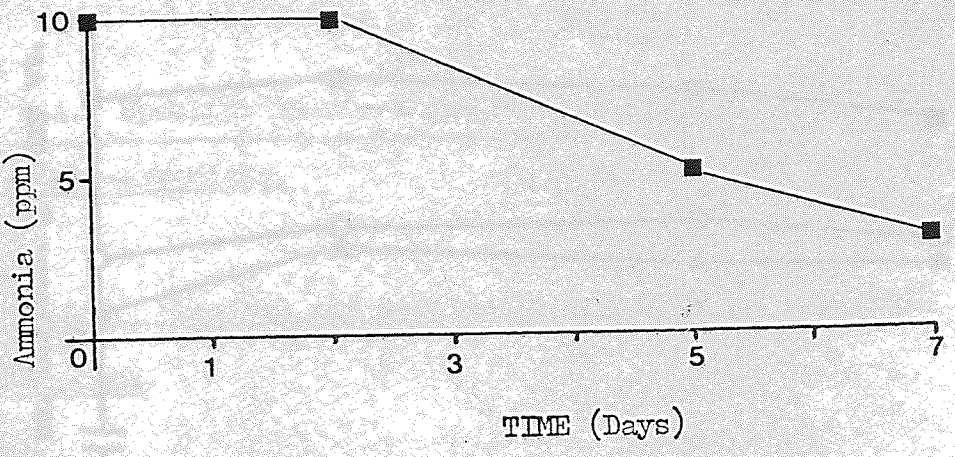
(ii) Microbiological changes.

Figs. 3.36. and 3.37. show the total numbers of aerobic bacteria and fungi at the surface and at 50mm depth in tissue waste earthworm beds with and without surface applications of cow slurry.

As expected, the surface of beds with applied cow slurry had higher bacterial populations than control beds. Samples taken at 50mm depth in beds with applied slurry contained similar numbers of bacteria to samples from the control beds, suggesting that little or no mixing of the surface slurry with the substrate at this depth took place during this experiment. Numbers at each sampling point showed no significant change over 7 days.

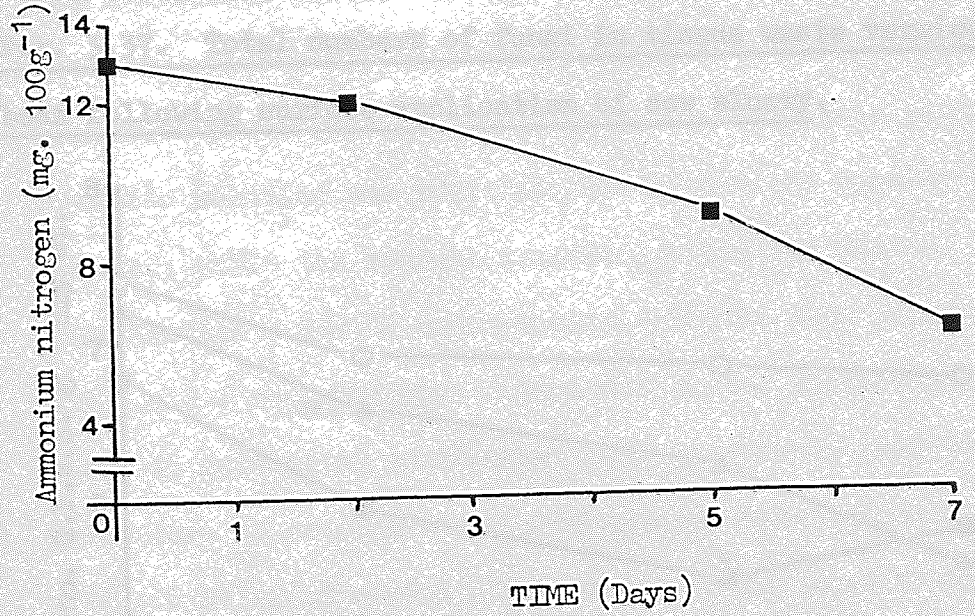
Numbers of fungi in all samples initially declined. Numbers at the surface of control beds became stable after 2 days, while numbers at the surface of beds with applied slurry declined over 5 days, then increased slightly to the end of the experiment. At 50mm depth in the beds with

Fig. 3.34. Ammonia volatilisation at surface of earthworm beds containing tissue waste.



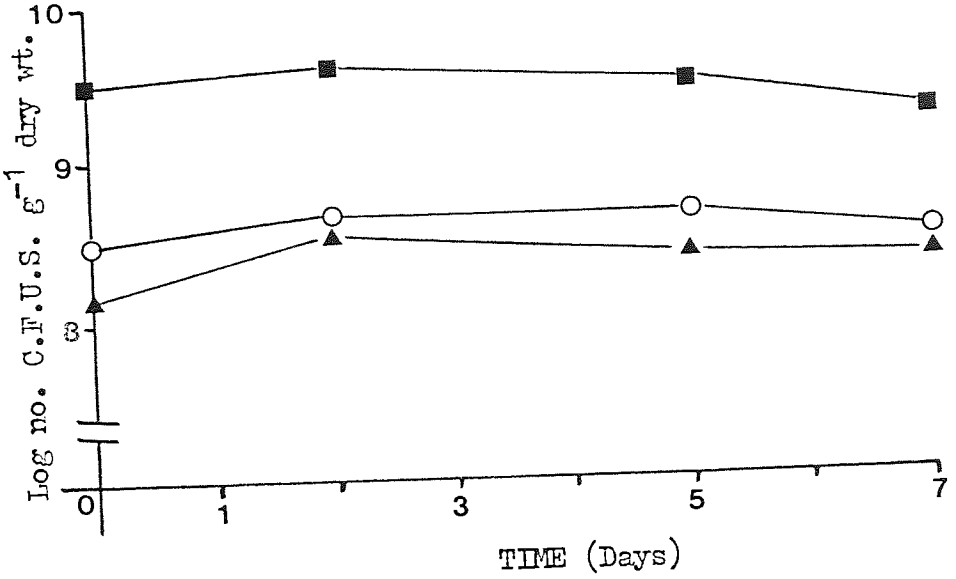
—■— = cow slurry added

Fig. 3.35. Ammonium nitrogen at surface of earthworm beds containing tissue waste.



—■— = cow slurry added

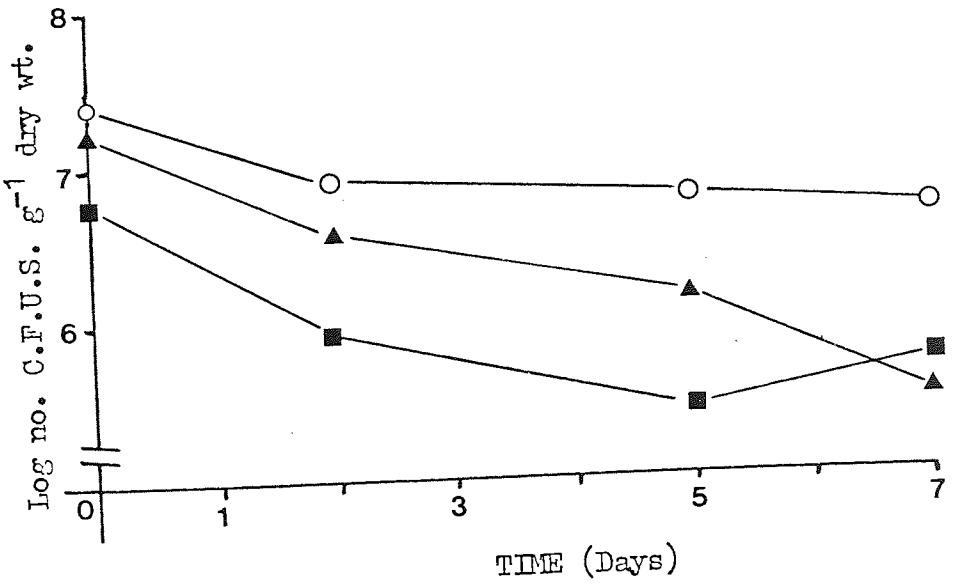
Fig. 3.36. Total numbers of aerobic bacteria in tissue waste beds following surface application of cow slurry.



—■— = with slurry (0mm depth) —▲— = with slurry (50mm depth)

—○— = control: no slurry (0mm depth)

Fig. 3.37. Total numbers of fungi in tissue waste vermiculture beds following surface application of cow slurry.



—■— = with slurry (0mm depth) —▲— = with slurry (50mm depth)

—○— = control: no slurry (0mm depth)

applied slurry, numbers of fungi declined throughout the sampling period.

3.4. Specific interactions between Eisenia fetida and Micro-organisms.

3.4.1. Chemical and antibiotic treatment of earthworm cocoons.

(i) Chemical treatment.

Table 4.1. shows the effect of chemical treatment upon the hatching and microbial contamination of cocoons. The sterilants tested varied in their toxic effects upon cocoons. Sodium hypochlorite produced viable cocoons after immersion for 60 min., as did the de-ionised water control. Formalin solution was toxic at immersion times greater than 30 min., while the maximum immersion times for sodium hypochlorite + Teepol and mercuric chloride were 15 min. and 5 min., respectively. Cetrimide proved immediately toxic to developing earthworms. All treatments reduced the percentage of cocoons which subsequently hatched compared with controls. The number of hatchlings per cocoon was also reduced, but these differences were not found to be significant when tested by analysis of variance (Section 2.8.1.).

At the maximum tolerable immersion time, the effects of the sterilants in reducing microbial contamination of

Table 4.1. Effect of chemical sterilants on hatchability and microbial contamination of earthworm cocoons.

Sterilant	Max immersion time producing viable cocoons (min)	% cocoons hatching	Mean no hatchlings per cocoon[a] (±S.E.)	Microbial contamination (no. C.F.U.S per cocoon) Bacteria	Fungi	Actinomycetes
De-ionised Water (control)	60	67	1.17 (±0.37)	1.9×10^5	14	3.0×10^4
Sodium hypochlorite	60	17	0.33 (±0.30)	4.2×10^4	10	1.8×10^4
Formalin	30	17	0.17 (±0.15)	1.8×10^4	10	0
Sodium hypochlorite + Teepol	15	33	0.33 (±0.19)	5.0×10^4	14	3.0×10^3
Mercuric chloride	5	50	0.67 (±0.30)	1.4×10^4	5	0
Cetrimide	0	0	0	-	-	-

[a] Mean of six replicates.

Number of hatchlings not significantly different from controls: $P > 0.05$

the cocoons differed. All treatments produced an immediate ten-fold reduction in the numbers of bacteria present. The effect upon numbers of fungi was less marked, with only mercuric chloride causing an appreciable reduction in numbers. Formalin and mercuric chloride treatments produced the largest reduction in the numbers of actinomycetes associated with the cocoons.

On subsequent incubation of all cocoons, it was found that many of the cocoons which did not produce viable hatchlings became covered in heavy fungal growth. Moreover, when hatchlings from viable cocoons were transferred to sterile tissue waste, all died within 24 hours.

(ii) Antibiotic treatment.

Tables 4.2. and 4.3. show the effect of antibiotic treatment on the subsequent hatching of cocoons. Large variations between treatments were observed, both in the percentage of cocoons which hatched, and in the mean number of hatchlings produced. Numbers of hatchlings produced after treatment were, however, not significantly different from controls when tested using analysis of variance (Section 2.8.1.).

When nutrient and malt agar plates were examined following incubation of cocoons, high levels of contamination, especially by fungi were observed in all treatments and the control.

Table 4.2. Effect of sequential mixtures of antibiotics on hatchability of cocoons of *E. fetida* (n = 20).

Treatment	% Cocoons hatching	Mean number Hatchlings/cocoon (± standard error)
Control (water)	50	0.6 ± 0.36
Cycloheximide + Nisin	33	0.3 ± 0.19
Cycloheximide + streptomycin	25	0.5 ± 0.28
Cycloheximide + Tetracycline	54	0.7 ± 0.21

All antibiotics at 50μg ml^{-1} concentration.

Numbers of hatchlings were not significantly different from controls, $P > 0.05$.

When hatchlings were transferred to sterile tissue waste the same pattern of mortality as found after chemical treatment was observed, with death of hatchlings occurring within 24 hours.

Table 4.3. Effect of combinations of antibiotics on hatchability of cocoons of E. fetida (n = 20).

Treatment	% Cocoons hatching	Mean no. hatchlings/cocoon (± standard error)
Control (water)	36	0.4 ± 0.13
C + S + N	71	0.9 ± 0.21
C + S + T	55	0.9 ± 0.20
C + T + N	67	0.9 ± 0.19
C + T + N + S	25	0.3 ± 0.15

C = Cycloheximide; N = Nisin; S = Streptomycin;
T = Tetracycline (all at 50μg ml^{-1}).
Numbers of hatchlings were not significantly different from controls, $P > 0.05$

3.4.2. Chemical and antibiotic treatment of juvenile earthworms from laboratory stocks.

(i) Chemical treatment.

Table 4.4. shows the maximum survival time of juvenile E. fetida when immersed in different concentrations of sterilants and the microbial contamination of earthworms after treatment for these times. Effective concentrations

Table 4.4. Maximum survival time and microbial contamination of earthworms immersed in chemical sterilants (n = 3).

Sterilant (%)	Max. survival time (min)	Microbial contamination (C.F.U.s/ worm) Bacteria	Fungi	Actinomycetes
Formalin (0.1)	10+	1.2×10^5	0.8×10^3	6.0×10^4
Formalin (0.5)	1		Not determined	
Formalin (1.0)	0.5		Not determined	
Sodium hypochlorite (0.1)	10+	9.6×10^4	4.8×10^3	1.1×10^4
Sodium hypochlorite (0.25)	1		Not determined	
Sodium hypochlorite (0.5)	0		Not determined	
Mercuric chloride (0.05)	0.5		Not determined	
Cetrimide (0.075)	0		Not determined	
Control (De-ionised water)	10+	3.7×10^5	2.6×10^3	4.3×10^4

Table: 4.5. Effect of immersion in antibiotic mixture on microbial contamination of *E. fetida*. (n = 3).

Immersion time (Days)	Microbial contamination (C.F.U.s/worm)		
	Bacteria	Fungi	Actinomycetes
0	8.9×10^4	1.0×10^3	3.9×10^1
1	9.6×10^2	4.2×10^2	2.6×10^1
2	6.8×10^2	5.3×10^2	Not detected
3	3.6×10^2	1.6×10^2	Not detected
4	3.2×10^2	2.4×10^2	Not detected

Results are the mean of three replicate samples.

Antibiotic mixture: Aureomycin, 6µg ml^{-1}; Cycloheximide 100µgml^{-1}; Novobiocin 50µg ml^{-1}; Nisin 50µg ml^{-1}.

of the sterilants were quickly toxic to the earthworms. Toxicity was reduced in lower concentrations of all the sterilants except cetrimide, which was immediately toxic down to the lowest concentration tested, 0.075%. The concentrations which were tolerable by earthworms were ineffective in reducing microbial contamination.

(ii) Antibiotic treatment.

Table 4.5. shows the levels of microbial contamination of the earthworms during prolonged immersion in the following antibiotic mixture: cycloheximide 100µg ml^{-1}; aureomycin 6µg ml^{-1};

novobiocin 50μg ml^{-1}; nisin 50μg ml^{-1}. The greatest reduction in microbial numbers occurred in the first 24 hour period. Subsequently, numbers of bacteria showed a further decrease, while numbers of actinomycetes fell below detectable levels.

No earthworm mortality occurred during the experimental period.

Because the greatest reduction in microbial numbers occurred in the first 24 hours of immersion, this period was adopted as the standard treatment in the preparation of earthworms for investigation of feeding interactions with micro-organisms.

3.4.3. Pure cultures of micro-organisms as a food source for E. fetida.

A suitable substrate for use in the screening of micro-organisms for their nutritive value to E. fetida was chosen as described in section 2.5.1.. Table 4.6. shows the survival of earthworms in the substrates under test after 14 days. A 5:1 (v/v) mixture of acid-washed sand and α-cellulose powder allowed survival and normal behavior of E. fetida, but provided minimal nutrition to the earthworms, so this substrate was adopted for use in further experiments.

In initial screening experiments, the feeding interaction of E. fetida with test micro-organisms was assessed by comparison of weight change of earthworms fed with

Table: 4.6. Earthworm survival in potential substrates for use in feeding experiments. Initial n = 15.

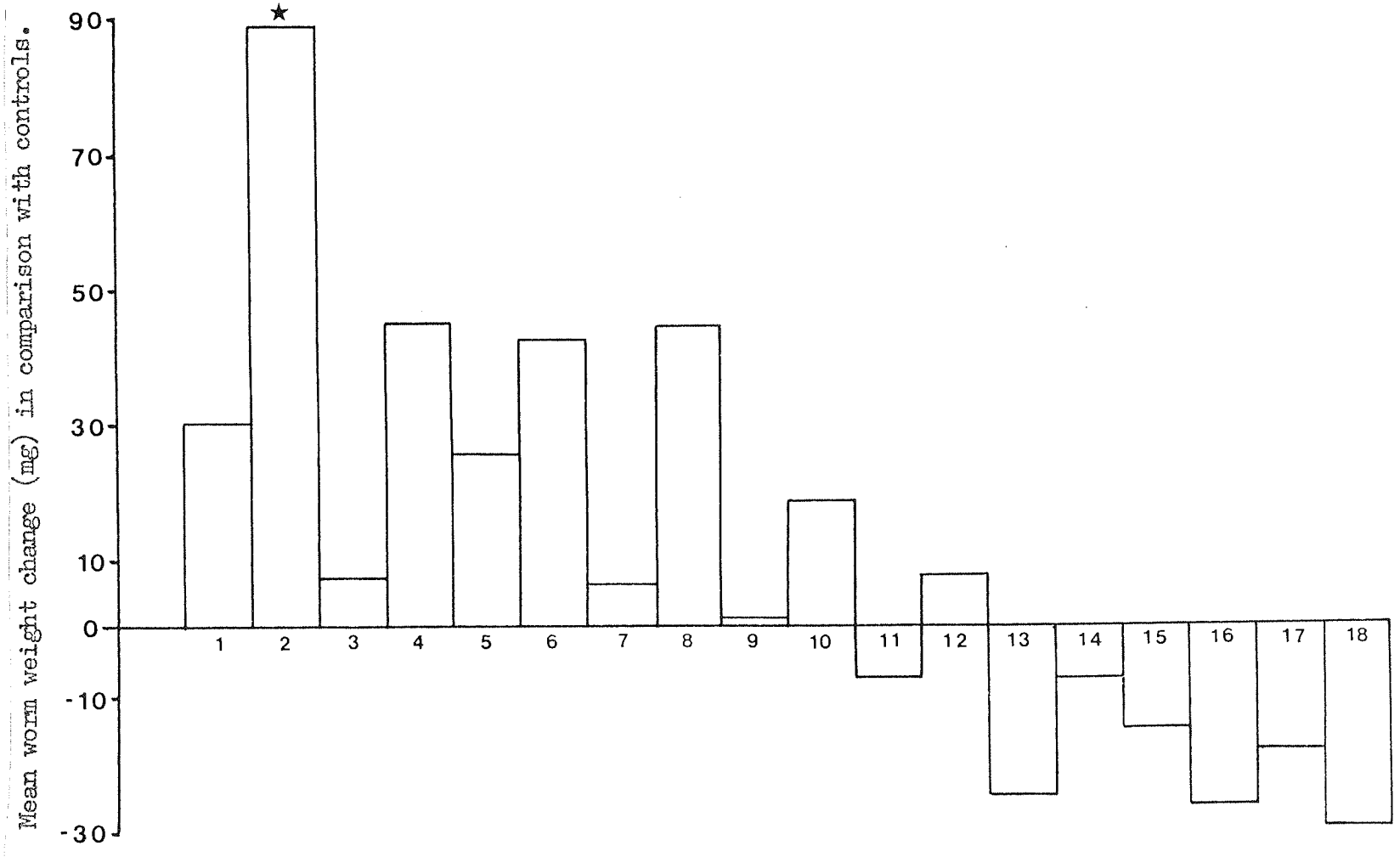
Substrate	Earthworm survival after 14 days.
Sand + ∝-cellulose (5:1v/v)	15
Sand + Filter paper (1:1 v/v)	5
Filter paper	4
Sand	0
"Perlite"	0

pure cultures, with weight change of unfed controls. Significance of the results was determined using Dunnett's test (Section 2.8.3.).

The following four species tested were found to be toxic to E. fetida: Flavobacterium lutescens; Pseudomonas fluorescens; Ps. putida; and a Streptomyces species. High mortality of the earthworms occurred within seven days when these species were fed as both live and heat-killed cells.

The effects of feeding single species of non-lethal bacteria and an actinomycete to E. fetida are shown in Fig. 3.38. and appendix 4.1. Both control and experimental earthworms lost weight during the experiment. Positive, negative, and indifferent effects of micro-organisms on earthworm nutrition were observed. Heat-killing of the

Fig. 3.38. Effects of feeding individual species of bacteria and actinomycetes to E. fetida.



★ = mean significantly different from control at p = 0.05

(For least significant differences see Appendix 4.1.).

Figure 3.38 (Continued).

1 = Acinetobacter lwoffi (L).

2 = Ac. lwoffi (H)*.

3 = Ac. citroalkaligenes (L)

4 = Ac. citroalkaligenes (H)

5 = Alkaligenes faecalis (L)

6 = Alk. faecalis (H)

7 = Nocardia salmonicolor (L)

8 = N. salmonicolor (H)

9 = Enterobacter cloacae (L)

10 = E. cloacae

11 = Micrococcus varians (L)

12 = M. varians (H)

13 = Arthrobacter simplex (L)

14 = A. simplex (H)

15 = Micrococcus luteus (L)

16 = M. luteus (H)

17 = Artrobacter tumescens (L)

18 = A. tumescens (H)

* = Mean significantly different from control, P = 0.05.

L = Live cells.

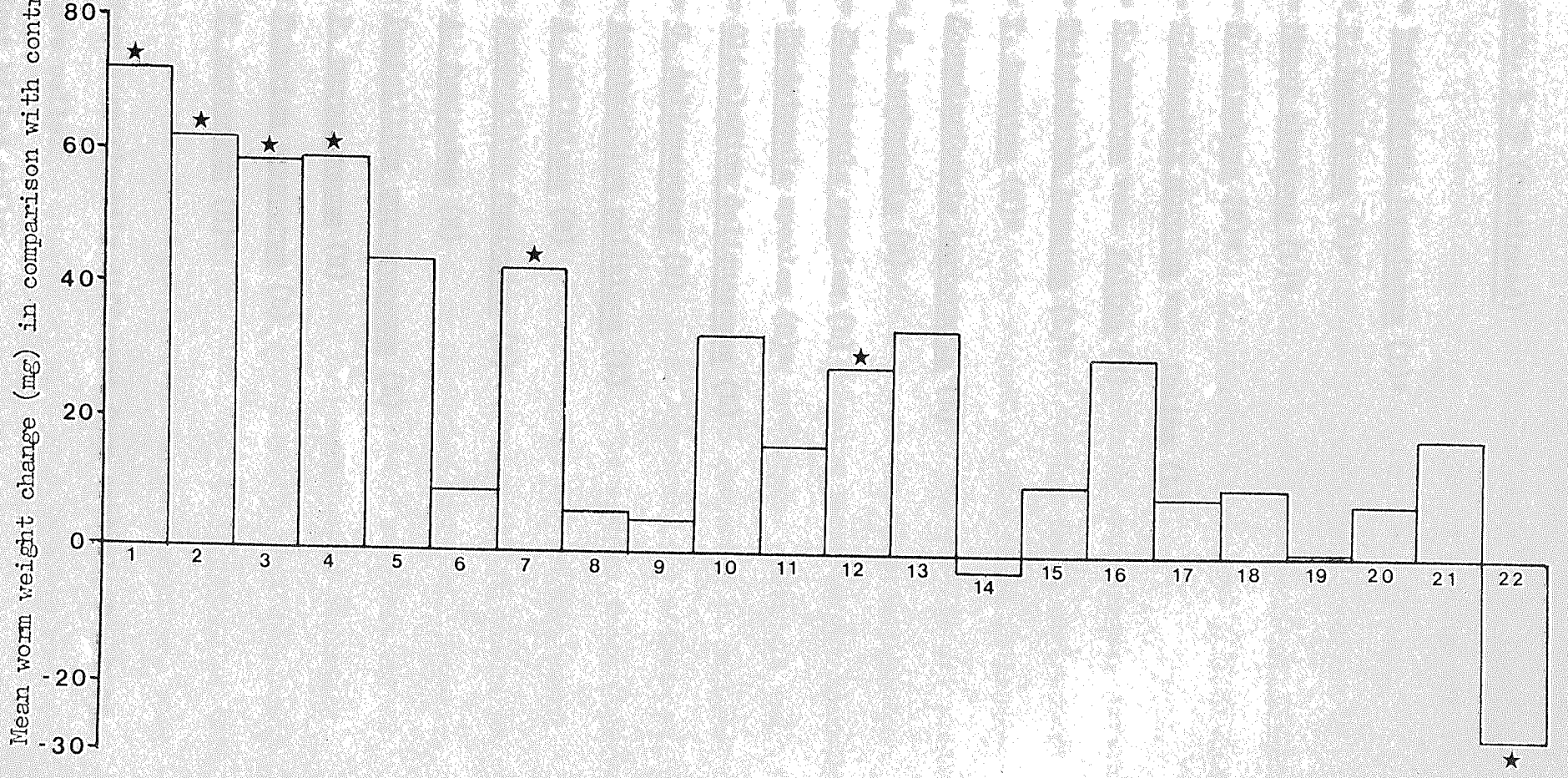
H = Heat killed cells.

microbial cells tended to enhance both increased and decreased weight loss of earthworms compared with controls. The positive effects of Acinetobacter lwoffi, Ac. citroalkaligenes and Nocardia salmonicolor were thus considerably enhanced by heat-treatment. Live cells of Enterobacter cloacae produced a weight change identical to that of control worms, while heat-killed cells produced an increased positive effect. The negative effect of Arthrobacter tumescens was enhanced by heat-killing.

Fig. 3.39. and appendix 4.2. show the results of feeding experiments using pure cultures of fungi, including a yeast species. Again, control and experimental worms generally lost weight over 7 days, though feeding of Mucor plumbeus and Trichoderma harzianum produced weight gain of E. fetida.

In all but two cases, the test fungi produced positive effects upon worm nutrition. The exceptions were heat-killed cells of Fusarium oxysporum and Gliocladium deliquescens. However, live cells of these two species produced definite positive effects. The effect of heat-killing on the remainder of the species tested was less marked than that observed with bacteria. Most of the test species small positive effects on earthworm growth when heat-killed. However the nutritional value of Gliomastix murorum var. felina and Trichoderma hamatum was greatly reduced by autoclaving, while that of T. harzianum was reduced to a smaller extent.

Fig. 3.39. Effects of feeding individual species of fungi to E. fetida.



★ = mean significantly different from control at p = 0.05
(For least significant differences see Appendix 4.2.).

Figure. 3.39 (Continued)

1 = Trichoderma harzianum (L)*

2 = T. harzianum (H)*

3 = Mucor plumbeus (L)*

4 = M. plumbeus (H)*

5 = Gliomastix murorum var. felina (L)

6 = G. murorum var. felina (H)

7 = Trichoderma hamatum (L)*

8 = T. hamatum (H)*

9 = Scopulariopsis carbonaria (L)

10 = S. carbonaria (H)

11 = Rhodotorula species (L)

12 = Rhodotorula species (H)*

13 = Gliocladium deliquescens (L)

14 = G. deliquescens (H)

15 = Aurebasidium pullulans (L)

16 = A. pullulans (H)

17 = Aspergillus fumigatus (L)

18 = A. fumigatus (H)

19 = Penicillium purpurogenum (L)

20 = P. purpurogenum (H)

21 = Fusarium oxysporum (L)

22 = F. oxysporum (H)*

* = Mean significantly different from control, P = 0.05.

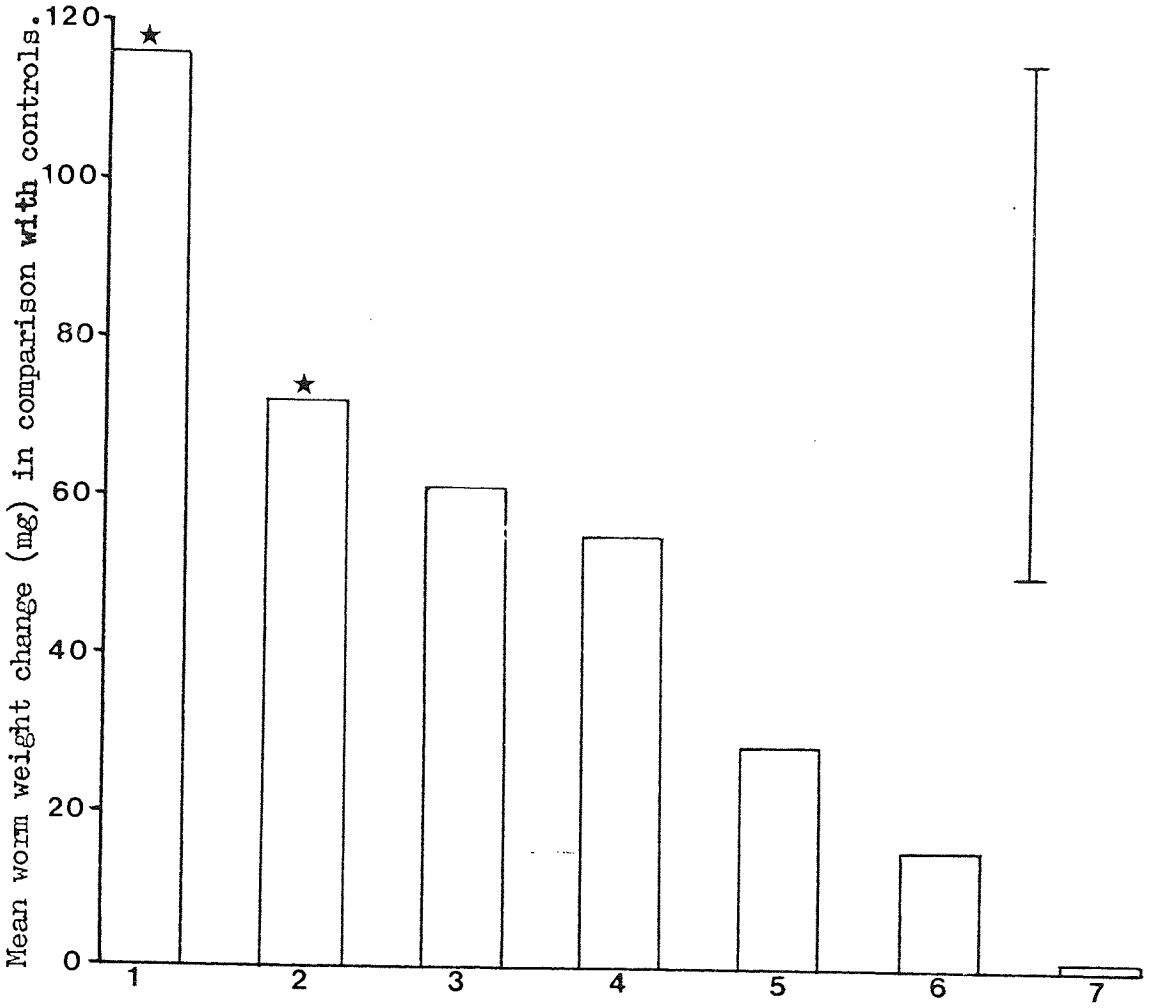
L = Live cells.

H = Heat killed cells.

Apart from the effect of autoclaving on T. hamatum, the two Trichoderma species tested showed similar effects upon earthworm nutrition. The greatest significant positive effects upon earthworm growth were produced by Trichoderma harzianum (live and heat-killed); Mucor plumbeus (live and heat-killed); T. hamatum (live), and Rhodotorula sp. (heat-killed).

Following these initial screening experiments, several micro-organisms were chosen for testing in an environment which rather more closely resembled that found in large-scale commercial vermiculture beds. The results are presented in Fig. 3.40. and appendix 4.3. As expected, earthworms in unsterilised tissue waste showed a large weight increase over unfed controls in sterile substrate. Controls and earthworms fed with micro-organisms suspended in peptone water lost weight over 14 days. However, the effects of the species tested were generally the same as those observed in the initial screening experiments, differences being of magnitude only. Most of the species tested showed an enhanced positive effect on earthworm nutriion. However, Trichoderma harzianum produced a reduced positive effect in this system, possibly due to production of secondary metabolites by this species during growth on tissue waste. Pseudomonas fluorescens was not toxic to E. fetida in this experiment, though weight loss of the earthworms was very similar to that of controls. It is possible that the earthworms in this experiment were able to avoid contact

Fig. 3.40. Effects of the addition of different micro-organisms to sterile tissue waste containing E. fetida.



1 = non-sterile tissue waste

2 = Acinetobacter lwoffi

3 = Aspergillus fumigatus

4 = Nocardia salmonicolor

5 = Trichoderma harzianum

6 = Enterobacter cloacae

7 = Pseudomonas fluorescens

★ = Mean significantly different from control at p = 0.05.

Vertical bar indicates least significant difference at p = 0.05.

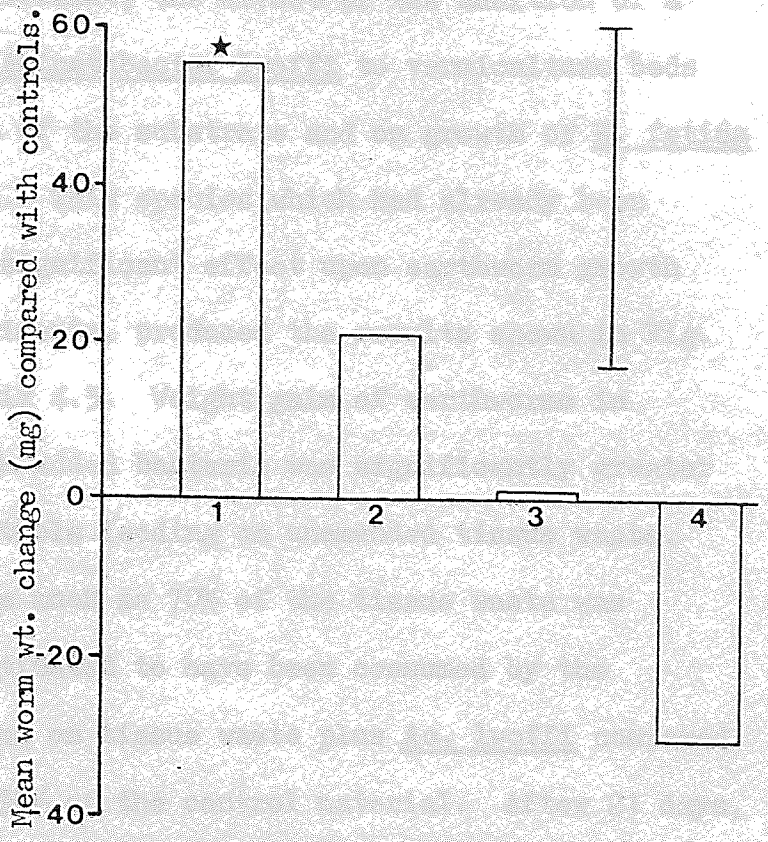
with the toxic factor associated with this species, but were consequently unable to gain nutrition in this system.

3.4.4. Feeding of single species of bacteria to earthworms in axenic cultures.

The feeding of bacteria to axenic earthworms in sterile culture was investigated to determine if the results of the initial screening experiments represented true interactions between E. fetida and the test micro-organisms. The results are shown in Fig. 3.41. and appendix 4.4. Both unfed controls and earthworms fed with bacteria lost weight during the experiment, indicating that E. fetida was unable to utilize the species tested as a sole source of nutrition. The observed effects were similar to those obtained in the initial feeding experiments. Acinetobacter lwoffi produced a significant weight change compared with controls, indicating the importance of this species in earthworm nutrition. The effects of Alkaligenes faecalis and Enterobacter cloacae on earthworm weight change were very similar to those produced in the initial screening experiments, suggesting that the results of the screening experiments provided an accurate indication of the type of interactions occurring between E. fetida and the species tested.

Pseudomonas fluorescens produced a larger weight loss of earthworms than the controls, and some earthworm mortality

Fig. 3.41. Effects of feeding bacteria to axenic E. fetida.



1 = Acinetobacter lwoffi

2 = Alkaligenes faecalis

3 = Enterobacter cloacae

4 = Pseudomonas fluorescens

★ = Mean significantly different at p = 0.05.

Vertical bar represents least significant difference at p = 0.05.

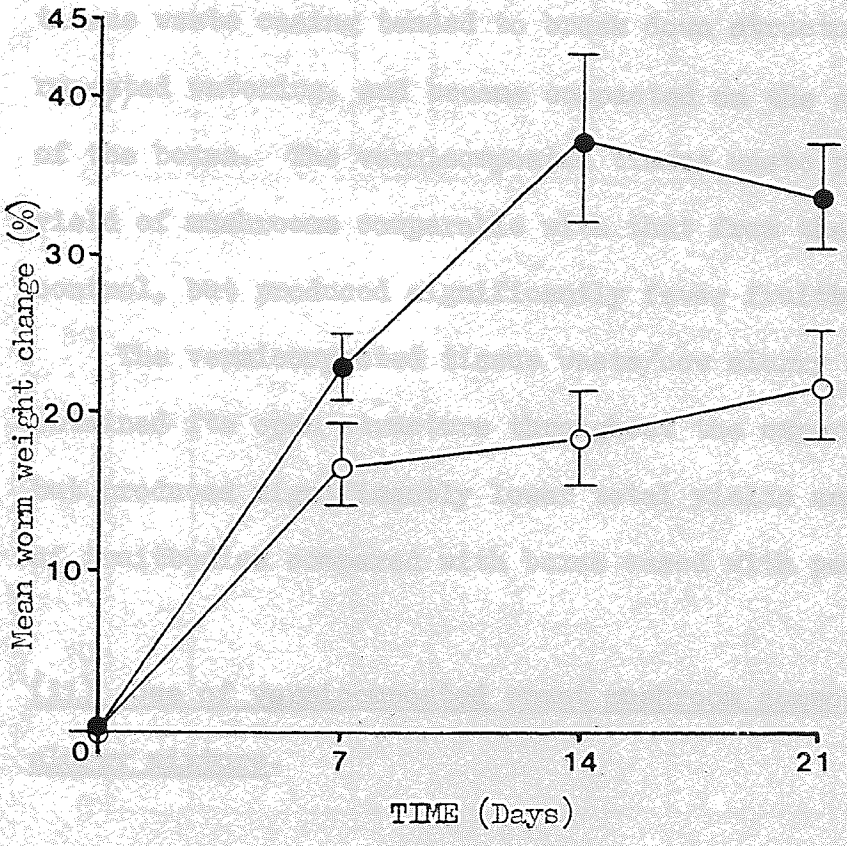
occurred in this treatment during the experiment, confirming that close proximity of this species to E. fetida produces toxic effects.

3.4.5. Seeding of vermiculture beds with a pure culture of a micro-organism.

In this experiment, the effect of the addition of a pure culture of Acinetobacter lwoffi to vermiculture beds on the breakdown of the substrate and on growth of E. fetida was investigated. This species, which had already been found to have a significant effect upon earthworm growth in feeding experiments, produced the results shown in Fig. 3.42. and appendix 4.5. Weight gain of earthworms in tissue waste with added bacteria was significantly greater than that of controls feeding on unamended tissue waste. After 14 days, as much as 70% of the tissue waste was subjectively estimated to have been consumed by the earthworms feeding on tissue waste plus Ac. lwoffi compared with only about 30% of the control material. After 21 days, 90-95% of the tissue waste with added Ac. lwoffi was estimated to have been consumed, compared with 50% of the tissue waste in the controls.

These results provided further evidence of the importance of Ac. lwoffi in the nutrition of E. fetida.

Fig. 3.42. Effect of adding Acinetobacter lwoffi to earthworm beds on the growth of E. fetida.



Vertical bars indicate standard error of the mean.

-●-= seeded with bacteria

-O- = control: unseeded

3.5. Utilisation of vermicomposted material as a casing soil in mushroom cultivation.

(i) Use of vermicomposted tissue waste/cow slurry mixture.

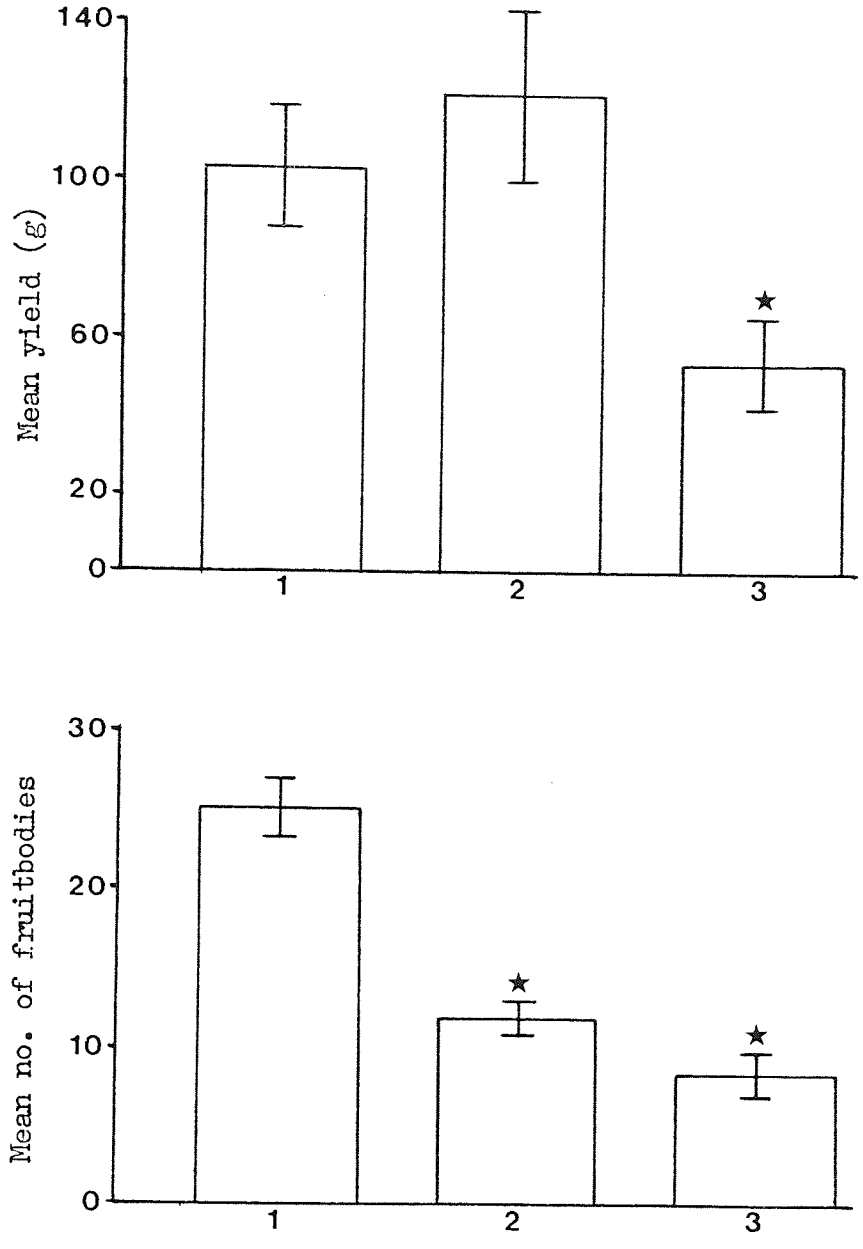
Fig. 3.43. shows the total yields and numbers of fruitbodies obtained when vermicomposted tissue waste and tissue waste/slurry mixture were used as casing soils in the production of mushrooms. During the experiment, the tissue waste casing tended to break down structurally with repeated watering, and became compacted on the surface of the boxes. The vermicomposted tissue waste produced a yield of mushrooms comparable with that from the peat control, but produced significantly fewer fruitbodies.

The vermicomposted tissue waste/cow slurry mixture retained its open structure throughout the experiment, but produced significantly lower total yields and numbers of fruitbodies compared with boxes cased with peat.

(ii) Use of vermicomposted spent mushroom compost/cow slurry mixture.

The results obtained using vermicomposted spent mushroom compost and cow slurry as casing materials are shown in Fig. 3.44. Some overgrowth of *A. bisporus* mycelium occurred at the surface of boxes cased with spent compost. Fruitbodies did not develop where overgrowth

Fig. 3.43. Mean weight and number of A. bisporus fruitbodies harvested from compost cased with vermicomposted tissue waste and cow slurry. (n = 5)



1 = peat/chalk mixture (control)

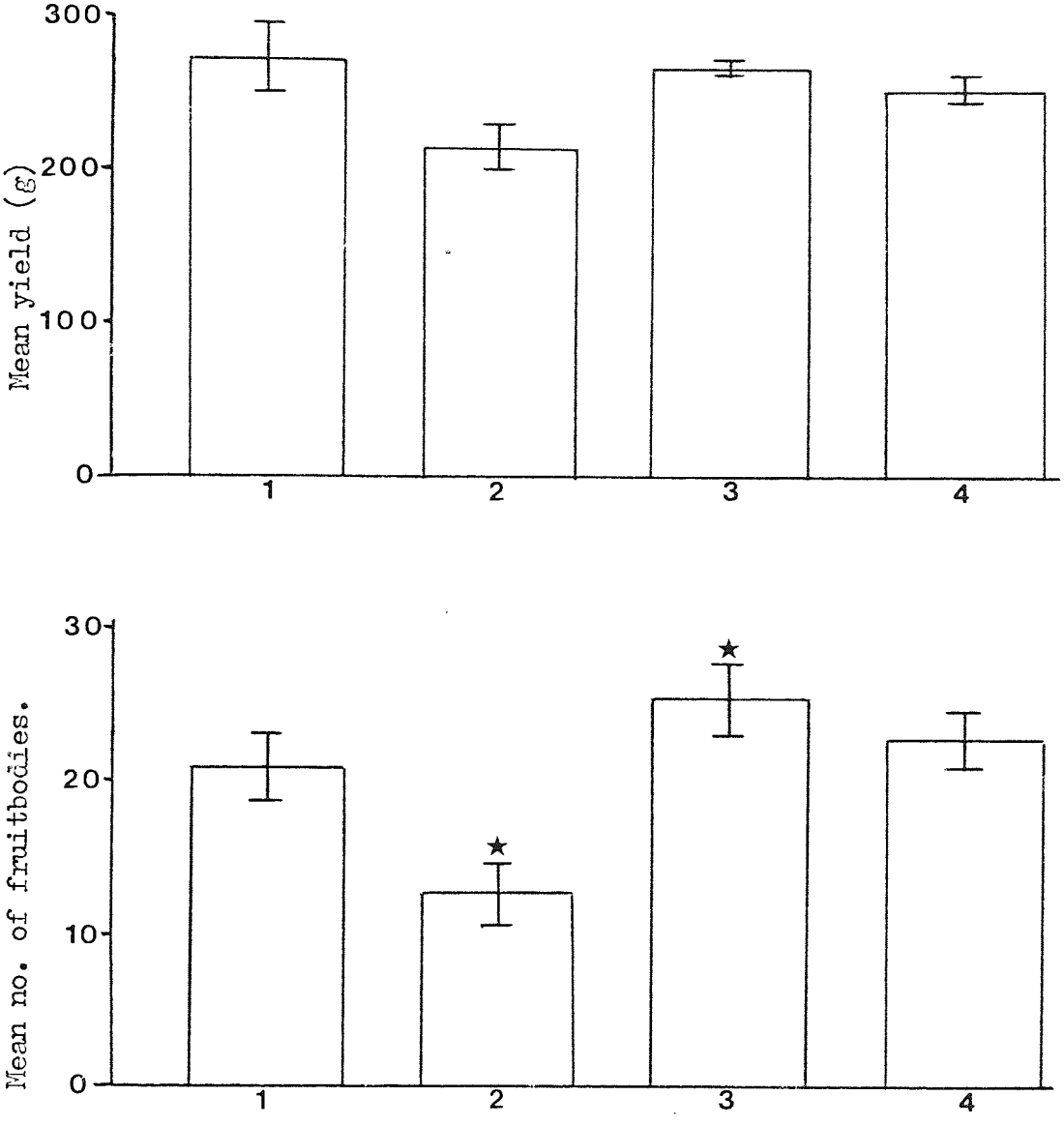
2 = vermicomposted tissue waste

3 = vermicomposted tissue waste:cow slurry mixture

Vertical bars indicate standard error of the mean

★ = treatment significantly different from control, p= 0.05.

Fig. 3.44. Mean weight and number of A. bisporus fruitbodies harvested from compost cased with vermicomposted mushroom compost and cow slurry (n = 4).



1 = peat/chalk mixture (control)

2 = Vermicomposted spent mushroom compost

3 = Vermicomplsted spent compost/cow slurry mixture

4 = 50:50 vermicomposted spent compost/cow slurry:peat mixture

Vertical bars indicate standard error of the mean

★ = Treatment significantly different from control, p = 0.05.

occurred, and this treatment produced significantly lower numbers of fruitbodies than the peat control. Total yield of fruitbodies was also slightly lower, but this difference was not significant. The other casing mixtures tested gave total yields and fruitbody numbers which were comparable with those obtained from the boxes cased with peat. Dilution of the spent compost/cow slurry mixture with peat had no significant effect upon the total yield or number of fruitbodies produced.

SECTION 4.

DISCUSSION AND CONCLUSIONS

4.1. Chemical and microbiological changes in cow slurry during storage.

Despite increasing problems of animal waste disposal and utilisation in recent years, little detailed research has been carried out on the effects of different storage methods on intensively-produced wastes. The ultimate disposal of the majority of treated and untreated animal wastes is onto land as a fertiliser or soil conditioner. Although different storage and treatment methods are known to produce considerable differences in the plant nutrient value of wastes (Vanderholm, 1975), the reasons for these differences are not fully understood.

The experiments described in section 3.1. were conducted with the aim of quantifying the main chemical and micro-biological changes in stored cow slurry, and assessing how rapidly cow slurry became acceptable as a substrate for E. fetida. As anaerobic wastes were known to be toxic to this earthworm (Neuhauser, et al, 1980), storage under forced aeration was first employed, and when this was found to be ineffective in de-toxifying slurry, storage in shallow trays was also considered.

The initial values recorded here for slurry moisture content, pH and chemical constituents were within the ranges quoted in the literature (Wedekind and Koriath, 1969; Staley et al, 1971; O'Connell, 1974; Jones and Matthews, 1975), showing that the sample studied was representative of slurry

generally produced on farms. The pattern of chemical change was found to be one of initial rapid decomposition followed by a period of relative stability, when little further breakdown occurred. As cow slurry is largely made up of plant residues which have survived passage through the digestive tract of cattle, it would be expected to contain a relatively poor supply of available nutrients which would quickly be assimilated by micro-organisms.

A major loss of ammonium nitrogen from the slurry occurred during this study, in agreement with the findings of Vanderholm (1975) and Chang and Johanson (1977). The high initial pH of the slurry provided conditions which favoured ammonia volatilisation. The initial high rate of ammonia loss probably represented the rapid conversion of urea from the urine fraction of the slurry to ammonia. As the concentration of ammonium present fell, and the pH fell below 8.0, volatilisation was reduced to a steady rate. The further losses which occurred when the slurry was placed in shallow trays were probably due to disturbance of the material and the increase produced in the surface area/volume ratio.

The large decrease in organic carbon content observed in the first day of aerated slurry storage probably represents losses of carbon dioxide due to microbial respiration. Subsequent organic carbon loss was minimal, suggesting that the slurry microflora entered a stationary phase of low metabolic activity, until the slurry was disturbed and placed in shallow trays. The further loss of organic

carbon some days after the beginning of tray storage suggests a further short phase of rapid microbial decomposition.

The initial high rate of slurry decomposition probably reflects the replacement of the largely anaerobic gut microflora of the cow by proliferating aerobic micro-organisms. These may have been environmental contaminants, or facultative anaerobes from the gut of the cows (Bryant, 1959). The nutrients released by the death of anaerobic micro-organisms would represent a readily available nutrient supply for the microflora which replaced them.

The stability observed in the organic nitrogen content of the slurry suggests that cycling of this nutrient took place: nitrogen initially released from dead microbial cells quickly becoming immobilised in the cells of the proliferating microflora.

The pattern of change in the cellulose fraction of the stored slurry showed that little decomposition of this fraction took place over the aerated storage period. Storage of slurry in shallow trays provided a more favourable environment for hemicellulose decomposition, though little significant loss of cellulose occurred over the timescale of this experiment.

As expected, no decomposition of lignin occurred during the experiment, as the aromatic structure of this fraction makes it highly resistant to chemical and microbial degradation.

The two methods of estimation of bacterial numbers in stored slurry produced a predictable difference in the results obtained. However, the difference was not so great as is sometimes reported for soil samples in the literature (Clark , 1967). The microflora of a substrate such as cow slurry may contain a higher proportion of viable cells than soils, which are usually comparatively poor in nutrients. The results suggest that in this case, plate counts gave a reasonably accurate estimate of the number of viable bacteria present. The large initial differences between plate and agar film counts were probably due to counting of moribund anaerobic bacteria by the latter technique. This highlights a major disadvantage of the agar film technique: it cannot provide information on the importance of micro-organisms counted in the decomposition of the substrate.

The use of dilution plates for estimation of numbers of fungi also has disadvantages: the counts obtained do not indicate whether the colonies produced developed from hyphae or spores, and fragmented hyphae may give rise to many more colonies than the biomass of fungi in the substrate reflects. Heavily-sporing fast-growing types are favoured over others by this method. Many fungi have been directly observed growing heavily on a substrate, but are never detected on dilution plates. During this experiment, regular direct observation of slurry samples indicated that fungal spores rather than hyphae were more prevalent in the stored slurry.

The data presented on the relative abundance of bacteria in aerated slurry showed the initial proliferation of species which are commonly found in the environment, so that it is not possible to categorically state which, if any, may have originated from the rumen. The species isolated in large numbers were all members of genera reported from slurry by Jones et al (1977). The Arthrobacter species isolated from slurry during the first few days of storage may correspond to the unidentified Corvneform bacterium reported by Hrubant (1973) to be consistently present in slurry but quickly reduced in numbers during storage.

The pattern of bacterial succession in aerated slurry showed that an initial diverse microflora developed which was quickly replaced by the numerical dominance of Acinetobacter lwoffi, which persisted for some time, until in turn replaced by other species. The role of Acinetobacter lwoffi in slurry decomposition is unclear. This species is generally biochemically unreactive, and may be able to utilise compounds which other organisms are unable to assimilate, being thus able to remain abundant when nutrients have become limiting for other species.

A similar pattern of fungal succession occurred in the aerated slurry, with an initial growth of a diverse microflora followed by dominance of one main type. The initial high numbers of several of the fungal species recorded suggest that they were present in the slurry when it was produced, and probably originated from the cattle feed, surviving

passage through the animals as spores. Support for this hypothesis comes from a study of fungi on cattle feeds by Bonner and Fergus (1959), who found many of the species recorded here in cattle feed. In addition, Cladosporium herbarum and Aureobasidium pullulans are commonly isolated from vegetation.

The decline in species of fungi present in slurry after the first few days of storage may reflect a reduction in the presence of readily-available nutrients, or a drop in the viability of the fungal spores under the storage conditions. Aureobasidium pullulans is a member of the so-called "yeast-like fungi", and the growth form of this species may be more suitable for growth in slurry than the ramification of hyphae. This may explain the continued presence of this species in high numbers. The rapid rise and fall in numbers of other species during slurry storage may represent brief growth of airborne contaminants which enter the slurry.

The greatest rate of slurry decomposition measured by chemical analysis occurred during the early part of this experiment, during the period of greatest diversity of the microflora. It appears that following this period the microflora enters a resting phase when little further decomposition takes place.

The increased rate of decomposition which was observed when aged aerated slurry was placed in shallow trays (Section 3.1.3.), was probably due to aeration and mixing of the

slurry during handling. The bacterial microflora which was dominant in slurry in trays reflected that of the aerated slurry at the time of removal, and it is possible that this represented types which were able to utilise more recalcitrant fractions of the substrate. The physical condition of the slurry was changed by the tray storage procedure, the material rapidly becoming semi-solid due to loss of moisture. This change appeared to favour the development of fungi, as the numbers of these rose during the experiment, largely due to increases in Scopulariopsis and Trichoderma species. The increase in fungal numbers co-incided with a reversal of the slow decline of the numbers of dominant bacteria, and a considerable decrease in the hemicellulose fraction of the slurry. A wide range of micro-organisms are able to utilise hemicellulose, but it is probable that the fungi were largely responsible for the decomposition of this fraction in this experiment. The bacteria may have been utilising the by-products of hemicellulose decomposition for growth.

4.2. Growth and reproduction of E. fetida in vermicomposting systems.

The experiments described in section 3.2.2. showed that neither of the tested methods of slurry storage de-toxified the material sufficiently for direct vermicomposting. However, the mixing of slurry with solid materials was

effective in reducing toxicity to E. fetida. The mechanism for this may be the adsorption of the toxic fraction by the solid material. Curry (1976) suggested that ammonium is the most toxic constituent of fresh slurry, and Kowalenko and Cameron (1976) reported that this ion is adsorbed by clay particles. Peat, which contains many sites for adsorption, was found in this study to be effective in reducing slurry toxicity, while materials such as straw and polystyrene granules were much less so (unpublished observations).

The experiments described in section 3.2. show that de-toxified cow slurry provided a suitable food source for E. fetida, both in mixtures with solid materials and when applied to the surface of material containing earthworms.

In mixtures of peat and slurry, earthworm weight gain was proportional to the amount of cow slurry present in the substrate. The 1:1 peat/slurry mixture produced the highest slurry ingestion rate, but the lowest conversion efficiency of slurry to earthworm biomass of any of the systems tested. Cocoon production was also low in peat/slurry mixtures. Peat appears to act as an inert carrier of cow slurry, providing no nutrition to E. fetida. The high slurry consumption rates in this system would entail frequent replenishment of vermicomposting beds. In addition, the low reproductive rate of E. fetida in this system would be insufficient to maintain earthworm stocks in a vermicomposting operation.

When supplied with mixtures of tissue waste and cow

slurry as a food source, the ingestion rate of E. fetida was much lower than in peat/slurry mixtures, though conversion efficiency and reproduction rate were considerably higher. Cocoon production in tissue waste/slurry mixtures approached the maximum levels reported by Graff (1974). Although the proportion of slurry in the substrate did not affect the growth rate of E. fetida, the number of cocoons produced was proportional to the amount of slurry available. It is therefore possible that earthworm growth rate was maximal in the 2:1 tissue waste/slurry mixture, and increased amounts of slurry were utilised by the earthworms for reproduction. Neuhauser et al (1980a) observed a similar effect on earthworm growth when sewage sludge was added to soil containing E. fetida: little difference in growth rate was observed on the addition of 10-100% sludge.

Although the mixing of slurry with solids was found to be effective in producing a suitable substrate for vermicomposting, the process would incur high handling and labour costs on a large scale, which may render the system uneconomic.

The surface addition of slurry to soil beds containing E. fetida produced a lower slurry consumption rate than peat/slurry mixtures, but with a similar conversion efficiency. This system allowed the earthworms to feed upon a concentrated substrate compared with slurry mixed with peat, so that the energy budget of E. fetida top-fed with slurry would be more favourable. The reproduction of the earthworms in this

system was similar to that in the peat/slurry mixtures. This is at variance with work by Neuhauser *et al* (1980), who found that the addition of soil to organic wastes increased cocoon production. However, until a specific factor is identified which is resposible for this effect, no valid comparisons may be made between soils from different sources.

The increased conversion efficiency, slurry consumption rate and cocoon production of earthworms in tissue waste top-fed with slurry compared with those in soil top-fed at the same rate, and the fact that earthworms in tissue waste only maintained weight, suggests that tissue waste has some nutritional value to *E. fetida*. It is also possible that tissue waste enhanced the de-toxification of slurry so that it was consumed more quickly after application, and therefore when it contained more nutrients, than in other systems.

The lower weight gain of *E. fetida* in the beds with the higher loading rate of slurry may have been due to slower de-toxification of slurry when a larger volume was applied, with a corresponding greater loss of available nutrients before feeding by earthworms could begin. The greater rate of cocoon production at the lower rate of slurry application supports the suggestion that slurry fed at this rate had a greater nutrient value to *E. fetida*.

4.3. Chemical and microbiological changes in cow slurry during vermicomposting.

The experiments reported in section 3.3.2. were designed to elucidate the chemical and microbiological changes brought about by the presence of E. fetida in a mixture of tissue waste and cow slurry. Much research has been carried out in the past on the effects of earthworms on decomposition of organic matter in terrestrial ecosystems, but the applicability of this knowledge to the study of vermicomposting of animal wastes is largely unknown.

The aims of the experiments in section 3.3.3. were to study closely the changes which occur in cow slurry following application to earthworm beds, and to determine the most significant of these changes in relation to de-toxification of the slurry and the commencement of feeding by E. fetida.

The overall effect of the presence of earthworms was to cause a faster decomposition of organic matter in the substrate during the experiment. This effect has been noted by other workers (Hartenstein and Hartenstein,(1981); Mitchell et al, (1982)), and was attributed partly to the uptake of nutrients by worms, and partly to stimulation of the decomposer microflora.

The presence of earthworms had a significant effect upon nitrogen transformations in the substrate. Nitrogen mineralisation was greater in the presence of worms, and

this mineral nitrogen was retained in the nitrate form. This suggests that E. fetida produced conditions which favoured the action of nitrifying bacteria. Earthworms digest organic nitrogen, and excrete approximately equal amounts of nitrogen as ammonium and muco-proteins (Needham, 1957). This is the probable cause of the reduction in organic-N in the substrate containing worms. The fact that ammonium nitrogen levels were not elevated in the presence of earthworms suggests that excreted ammonium-N was rapidly converted into nitrate. This effect has been noted by several workers to occur in soils (Syers et al 1979; Parle, 1963b), and following application of slurries to land (Debry et al 1982). The maximum level of nitrate nitrogen recorded here in the presence of earthworms compared well with that found by Lunt and Jacobson (1944) in worm casts in soil. Kaplan and Hartenstein (1977) found no evidence that earthworms were capable of nitrogen fixation, which further suggests a microbial role in this transformation.

The fluctuations in nitrate content of the substrate without earthworms may have been due to immobilisation of nitrogen by the microflora. Ammonium is the preferred nitrogen source for micro-organisms and is preferentially utilised over nitrate nitrogen. However, the losses of ammonium from the substrate in the first 14 days may have necessitated a switch to nitrate utilisation by the microflora. Some denitrification may also have occurred in the substrate without worms, as low oxygen tension, which favours this process, may have developed at the centre of a number of the large

undisturbed particles of material. E. fetida also appeared to inhibit cellulose decomposition in this substrate, with a greater effect on hemicellulose decomposition than α-cellulose. The much greater increase in the proportion of lignin in the absence of earthworms reflected the greater decomposition of cellulose in this treatment.

A study of the literature on the effects of earthworms on soil micro-organisms suggests that E. fetida should increase the number of microbes in a substrate as it was converted to castings. In this experiment, however, little change was observed in total numbers of micro-organisms in the presence and absence of earthworms until the end of the experiment, when a large reduction in numbers of micro-organisms occurred in the presence of earthworms. This may have been due to competition with E. fetida for available nutrients, or more complete decomposition of the substrate containing earthworms. Satchell (1967) pointed out that the effect of earthworms in readily degradable organic matter, which naturally contains a high population of micro-organisms, is likely to be less significant than in soils. The absence of a significant effect of E. fetida on microbial numbers may have been due to predation by the earthworms on the microflora. This has been suggested by several workers (Day, 1950; Cooke and Luxton, 1980; Rouelle, 1983). This experiment did not produce direct evidence to support this view: however, the rapid fall in numbers of

the initially dominant species of bacteria during days 14-35 correspond to a weight loss of earthworms, suggesting that these species may be of nutritional importance to E. fetida.

The same species of fungi remained dominant in the substrates with and without worms, though numbers were again slightly reduced in the presence of earthworms. The dominant species were not those found in stored cow slurry, suggesting that conditions in this substrate favoured other species: these have been found to originate from the tissue waste, rather than the slurry fraction of the mixture. Trichoderma hamatum was found in greater numbers in the substrate without worms. This species is able to decompose cellulose, and may therefore have been responsible for some of the cellulose breakdown observed in this experiment. T. hamatum was isolated less frequently from the mixture containing earthworms. This may have been due to the physical action of the worms in disturbing the substrate and inhibiting fungal growth, or to grazing by worms on this species.

The experiment described in section 3.3.3. showed that the main chemical changes in surface-applied cow slurry were a reduction in ammonium-nitrogen levels, accompanied by a fall in electrical conductivity. Earthworms were observed feeding on the slurry after 3 days, when levels of these constituents were higher than those quoted in the literaure as the maximum tolerable by E. fetida (Kaplan

et al, 1980). However those figures refer to earthworms in intimate contact with materials. In this experiment earthworms were free to move away from the toxic environment, and were probably able to tolerate short periods of contact with the slurry. No gross microbiological changes were observed in the slurry which might have explained the reduction in toxicity. Ammonium ion concentration may have been largely responsible for the toxicity of fresh slurry to E. fetida, but the levels of this ion quickly reduced to a tolerable level following application of the slurry to the surface of earthworm beds.

4.4. Specific interactions between E. fetida and micro-organisms.

The results presented in sections 3.4.1. and 3.4.2. show that both chemical and antibiotic treatment of cocoons were unsatisfactory for the production of large numbers of earthworms with reduced microbial contamination. Rapid mortality of hatchlings when placed in sterile tissue waste occurred following all treatments, including controls. This may have been caused by production of a toxic substance in the substrate during autoclaving, but adult and juvenile E. fetida were able to tolerate the same substrate. Mortality was possibly due to rapid starvation of hatchlings with little or no food reserves in a nutritionally poor substrate.

Chemical treatment of juvenile earthworms was also found to be unsuitable, because of the toxicity of most of the sterilants tested. Concentrations of the sterilants which were tolerable by earthworms were too low to be effective in reducing the numbers of contaminating micro-organisms.

Immersion of juvenile E. fetida in antibiotic solution was found to be the most effective method of reducing microbial contamination without causing mortality of earthworms. This method was therefore adopted for the preparation of large numbers of earthworms for use in the initial screening of micro-organisms.

In this study, definite nutritional interactions were found to exist between E. fetida and micro-organisms. These interactions appeared to be highly species-specific, supporting the findings of Day (1950), and Rouelle (1983).

With bacteria, the types of interaction observed appeared to be correlated with cell structure. Bacteria are classified according to their Gram-reaction, which differs according to cell wall composition. In these experiments, bacteria with a positive effect upon earthworm nutrition were generally Gram-negative, while those producing indifferent or negative effects were generally Gram-positive. The composition of the cell wall of bacteria therefore seems to be an important factor in determining their digestibility by E. fetida

Heat-killing of the cells by autoclaving undoubtedly

affects the cell structure and composition to some extent. The fact that this process generally appeared to increase their nutritional value to E. fetida suggests that the earthworms were feeding directly upon the bacteria, rather than on products of the living cells. These findings disagree with those of Neuhauser et al (1980): however, in the latter study, bacteria were added to earthworms in non-sterile substrate mixtures, so that secondary effects may have occurred due to the presence of large numbers of other micro-organisms.

The observed negative effects of Micrococcus varians and Arthrobacter simplex may have been due to active feeding of E. fetida on the live cells which were subsequently indigestible by the earthworms. The reduced negative effect when the cells were heat-killed may reflect a small increase in digestibilty caused by autoclaving.

The negative effects of Micrococcus luteus and Arthrobacter tumescens were increased when the cells were heat-killed, suggesting some toxic effect of these species on E. fetida which was enhanced by the autoclaving conditions employed. However, no earthworm mortality occurred in these treatments during the experimental period. Several other species were highly toxic to E. fetida, causing earthworm mortality within 7 days. Of these species, the Pseudomonas and Streptomyces species are known to produce anti-microbial compounds, and it is possible that these were responsible for the observed toxic effects, though the exact nature

and mechanism of action of the toxic factors was not established. The toxic effect was not reduced by the autoclaving conditions employed in this experiment, suggesting that the agents responsible were not readily heat-labile. Toxicity of Pseudomonas species and other bacteria to earthworms has been reported by Rao et al (1983).

Fungal species appeared to provide a more readily available food source than bacteria, as several of the species tested produced a positive effect upon earthworm nutrition. The interactions were once more species-specific, in agreement with work by Cooke and Luxton (1980) and Cooke (1983).

The effect of heat-killing on fungi was more variable than that on bacteria. The autoclaving of some species produced marked differences in their effect upon the nutrition of E. fetida, whereas with other species little difference was observed between live and heat-killed cells. Heat treatment may release nutrients from less easily digested species, and destroy the nutritional value of others. Some species may be easily digested by E. fetida, so that autoclaving has little effect upon their nutrient value.

One species, Fusarium oxysporum, produced a significant weight reduction compared with control when presented to E. fetida as heat-killed cells. This effect may have been due to the production or release of some toxic factor on autoclaving.

The testing of pure cultures as food for E. fetida in a more complex sterile substrate produced results which were similar to those observed in the initial screening experiments. This suggests that the interactions observed in the simplified system reflect the type of interactions which may occur in vermiculture beds. Trichoderma harzianum produced a lower positive result than in the screening experiments. In this system, the test micro-organisms were probably growing and producing metabolites. T. harzianum is able to decompose cellulose, so could readily utilise tissue waste as a substrate. It is possible that breakdown products of this process had the observed effect on earthworm nutrition. Toxicity of Pseudomonas fluorescens was not apparent in this system. The earthworms may have been able to avoid contact with the toxic factor associated with this species.

The significant earthworm weight gain over the control produced by the feeding of Acinetobacter lwoffi provided further evidence of the importance of this species in the nutrition of E. fetida. The fact that non-sterile tissue waste produced the greatest increase in earthworm weight over the sterile control suggests that the true feeding relationship of E. fetida with the microflora of a substrate is probably complex, involving interactions between several species.

The development of a technique for the production of

axenic E. fetida allowed the testing of micro-organisms as a food source in the absence of secondary interactions due to contaminants. This technique may have a significant application in future studies of earthworm/microbe interactions in a variety of substrates.

The effects of the species tested in this experiment were similar to those observed in experiments with non-axenic earthworms. This is in agreement with the generally-accepted view that earthworms do not possess a specific gut microflora which has an important nutritional role (Edwards and Lofty, 1972).

Pseudomonas fluorescens once more produced a toxic effect upon E. fetida, though toxicity was reduced in comparison with the initial screening experiments. This reduction in toxicity was also noted in the previous experiment using tissue waste as an experimental medium. It is possible that the bacterial suspension is less widely distributed through this substrate than through the sand/cellulose mixture, so that the earthworms were able to avoid contact with the toxic factor.

The addition of a suspension of Acinetobacter lwoffi to tissue waste in vermiculture beds stimulated both earhtworm growth and the consumption of the waste. This result differs from that observed in a previous experiment (section 3.4.3.) in which Acinetobacter lwoffi was fed to worms in sterile tissue waste. This suggests that at least

part of the enhanced effect was due to interactions between the established microflora of the tissue waste and the added species. Ac. lwoffi may have had a stimulatory effect upon other micro-organisms in the substrate which resulted in an increase in the amount of food available to the earthworms within the system. The increased rate of consumption of the substrate in the presence of added Ac. lwoffi may have been due to enhanced palatability of the tissue waste: Wright (1972) observed that materials coated with a bacterial paste were more readily consumed by earthworms.

The finding that earthworms ceased to gain weight when most of the tissue waste had been consumed, despite further additions of Ac. lwoffi, is similar to results observed in other vermicomposting systems in this study (Section 2.3.4. (ii)). This effect may have been due to a build up of earthworm waste products and/or toxic micro-organisms in the substrate. Numbers of Pseudomonas fluorescens were found to increase during the vermicomposting of tissue waste/cow slurry mixture (Section 3.3.1. (ii)), the period of greatest abundance of this species corresponding to a period of weight loss of E. fetida in the substrate (Section 3.2.2. (i)). It is possible that the migration of earthworms out of a depleted substrate may be due, in part, to the presence of large numbers of toxic micro-organisms (R. Hartenstein, personal communication).

The technique of seeding wastes with beneficial micro-organisms has a potential application in the

vermicomposting of many agricultural and industrial wastes which are naturally poor food sources for E. fetida. A system could easily be developed whereby wastes were seeded with food micro-organisms before or during vermicomposting in order to enhance the speed and efficiency of the process. Such a system would allow the upgrading and safe utilisation of many waste materials which currently present a disposal problem.

4.5. Utilisation of vermicomposted material.

The utilisation of vermicomposts in mushroom cultivation showed that some of the materials are potential alternatives to peat as casing soils. Vermicomposted tissue waste produced problems of water management and breakdown of physical structure. The consequent compaction of the casing layer reduced the number of fruitbodies produced, compared with peat. The reduced yields and numbers of fruitbodies when vermicomposted tissue waste/cow slurry mixture was used as a casing may have been caused by chemical or microbiological factors. The mixture may have been too high in nutrients, especially nitrogen, or may have contained an unsuitable microflora, both of which are known to affect the transformation from vegetative to reproductive growth in A. bisporus.

The overgrowth of mycelium on the surface of mushroom boxes cased with vermicomposted spent compost suggests that

this material was also too rich in nutrients to function as an ideal casing medium. The lower yields and fruitbody numbers produced compared with controls were similar to results obtained from a study on the use of leached spent compost as a casing (Nair, 1977).

Mixtures of vermicomposted cow slurry and spent mushroom compost produced numbers of fruitbodies and yields which were comparable with peat controls. Vermicomposted slurry/compost mixture in fact produced a larger number of smaller mushrooms than controls. Smaller mushrooms are popular with consumers and therefore attract a higher price. These results suggest that the nutrient balance and microflora of the cow slurry/compost mixtures were suitable for their use as a casing. The mixing of cow slurry with spent compost probably improved the nutrient balance of the latter which would allow more complete decomposition of this substrate, producing a more suitable product than vermicomposted spent compost.

Dilution of the slurry/compost casing with peat produced yields which fell between those from the whole mixture and the peat controls. Further dilution would be expected to produce yields which approached more closely those from peat only, though none of the measured differences in yield were significant. The price of peat is currently high, and not expected to fall in the future, so these results suggest that even partial replacement of this material with vermicompost could produce a substantial saving to growers, without affecting quality or yields. Further experimentation

may produce yields from vermicomposts which are better than those obtained from peat casing.

4.6. Conclusions.

Much of the nutrient content of cow slurry was found to be immobilised in the form of microbial cells, or present as ligno-cellulose which was not easily biodegradable. Major losses of ammonia-nitrogen occurred from stored cow slurry. Cow slurry contained high numbers of micro-organisms, with bacteria present in the highest numbers. Following an initial large increase in the number of aerobic bacteria, little significant change in total numbers of micro-organisms occurred during storage. The pattern of microbial succession observed in stored slurry was one of an initially diverse microflora, which became dominated by one species of bacteria. This was in turn replaced by other species.

Aerated storage caused some chemical stabilisation of the slurry, but the process was not complete, and appeared to be limited by nutrient availability. Subsequent tray storage produced further short-term decomposition, which was apparently due chiefly to micro-organisms which were able to degrade hemicellulose. Aerated storage consumes energy while tray storage requires a large area. Both methods proved unsatisfactory in the treatment of cow slurry for disposal, and neither storage method de-toxified slurry for direct use in vermicomposting.

The major toxic element in cow slurry was possibly ammonium. Slurry was de-toxified by mixing with solids or by application to the surface of earthworm beds. Two vermicomposting systems utilising these methods were developed and compared. Cow slurry was found to be a suitable substrate for treatment using earthworms. The top-feeding of slurry to earthworm beds was found to be the most efficient vermicomposting system in terms of the rate of slurry consumption and reproduction of E. fetida. Management practices were found to be an important factor in the efficiency of vermicomposting: frequent small applications of cow slurry were consumed more rapidly than larger, less frequent applications.

The presence of E. fetida in a tissue waste/slurry mixture was found to produce greater organic matter decomposition, and conservation of nitrogen as nitrate. Little effect was observed on the microflora of the substrate during decomposition, though numbers of bacteria and fungi were reduced in completely vermicomposted material. The overall effect of E. fetida was to enhance the stabilisation of the substrate and cause conservation of plant-available nitrogen.

Specific nutritional interactions were found to exist between E. fetida and micro-organisms isolated from its substrate. Positive, negative and indifferent interactions were identified. The earthworms were found to be feeding directly upon the cells of certain micro-organisms. In

general, fungal species appeared to form a more readily available food source than bacteria. The cell wall structure of bacteria was correlated with their effect upon earthworm nutrition, Gram-negative species producing a positive effect. Toxicity of some microbial species to E. fetida was also observed.

A technique was developed for the production of axenic earthworms which may have application in future studies of earthworm/microbe interactions. Feeding experiments conducted on axenic earthworms confirmed the direct interactions of E. fetida with certain microbial species. In particular Acinetobacter lwoffi was found to consistently produce a positive effect upon earthworm nutrition. The true feeding relationship of E. fetida in a substrate is probably complex, involving interactions between several species of micro-organisms. The single-culture studies described here represent an imporant first step in defining the interactions between this earthworm and the microflora of materials which it inhabits. Further studies are required in order to define the combinations of micro-organisms which are important in earthworm nutrition.

The seeding of tissue waste vermiculture beds with Acinetobacter lwoffi stimulated both earthworm growth and consumption of the waste. This technique may have an application in the vermicomposting of other agricultural and industrial wastes.

Vermicomposted mixtures of cow slurry and spent mushroom

compost were found to be suitable replacements for traditional casing materials in mushroom cultivation. Provided that such vermi-composts could be marketed at a lower price than that of peat, the mushroom industry represents a large potential market for the products of the stabilisation of cow slurry by E. fetida.

SECTION 5.

APPENDICES

Appendix 1.1. Relative abundance of micro-organisms in cow slurry stored under forced aeration.

Species	Storage time (days) 0	1	5	7	8	13	19	22	24	26
Flavobacterium lutescens	9.23									
Acinetobacter lwoffi	8.93	10.25	10.17	10.58	10.48	10.47	10.60	10.05	9.20	
Arthrobacter simplex	9.23	9.95	10.48	8.58						
Arthrobacter tumescens		10.25								
Streptomyces sp.		9.77	8.87	9.18						
Micrococcus luteus	8.93	10.25				8.56	8.90	8.88	8.37	9.20
Micrococcus roseus								9.18		
Pseudomonas fluorescens					10.17	9.16				
Pseudomonas putida									10.37	10.50
Alkaligenes faecalis								9.88	10.20	10.20
Rhodotorula sp.			9.35		9.35	9.56		8.88		

(Nos. expressed as log no. C.F.U.S.g^{-1} dry weight.)

Appendix 1.2. Relative abundance of fungi in cow slurry stored under forced aeration.

Species	Storage time (days) 0	1	5	7	8	19	22	26
Aureobasidium pullulans	6.0	6.17				5.98	5.78	
Mucor plumbeus				5.88	5.72			
Penicillium primulinum	5.93		6.1					
Scopulariopsis carbonaria								5.98
Trichoderma koningii								5.80
Penicillium paxilli								5.70
P. waksmanii	6.06							
Trichosporon sp.				6.36				
Mucor racemosus		5.87						
Cladosporium herbarum		6.34			6.10			
Penicillium species I						5.67		
Penicillium purpurogenum	6.15	6.12		5.87				

(No.s expressed as log no. C.F.U.s.g^{-1} dry weight).

Appendix 1.3. Relative abundance of aerobic bacteria in cow slurry ageing in shallow trays.

Species	Storage time (days)[a] 0	2	9	12	16
Pseudomonas putida	10.27	10.32	9.74	10.25	10.44
Alkaligenes faecalis	10.17	10.24	9.64	10.31	10.38
Acinetobacter lwoffi	9.17		9.74	8.61	
Micrococcus luteus	8.35	8.84	7.74	7.71	
Micrococcus varians				8.91	8.47
Enterobacter cloacae				8.82	8.89

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight).

[a] Slurry 24 days old at beginning of experiment.

Appendix 1.4. Relative abundance of fungi in cow slurry ageing in shallow trays.

Species	Storage time (days)[a] 0	2	9	12	16
Penicillium paxilli	5.59	5.84			
Scopulariopsis carbonaria	5.71		5.74	5.71	
Sterile mycelium	4.87			6.19	
Trichoderma hamatum		5.14			6.40
Trichoderma harzianum			5.82		
Trichosporonoides species				6.19	
Aspergillus fumigatus					6.55
Penicillium sp. II					6.25

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight)

[a] Slurry 24 days old at beginning of experiment.

Appendix 2.1. % Weight change of *E. fetida* in peat/slurry mixtures. Mean values ± standard error. (n = 3).

Treatment	After 7 days	14 days	21 days
Peat only (Control)	-15.3 ± 0.5	-33.6 ± 3.2	-33.9 ± 3.0
2:1 (w/w) Peat/slurry	+10.9* ± 0.6	-5.7* ± 1.9	-20.3 ± 3.1
1:1 (w/w) Peat/slurry	+38.4* ± 2.1	+33.2* ± 1.9	+6.6 ± 0.9

* = mean significantly different from control ($P < 0.05$)

Appendix 2.2. % Weight change of *E. fetida* in tissue waste/slurry mixtures.

Mean values ± standard error (n = 3).

Treatment	After 7 days	14 days	21 days	28 days	35 days
Tissue waste only (Control)	-14.3 ± 3.1	-11.1 ± 3.4	+6.6 ± 0.9	+6.7 ± 8.3	-0.2 ± 3.3
2:1 (w/w) Tissue waste/slurry	+37.9* ± 2.0	+75.2* ± 7.8	+82.7 ± 11.2	+101.2 ± 13.7	+80.1 ± 9.1
1:1 (w/w) Tissue waste/slurry	+45.9* ± 3.0	+71.1* ± 8.6	+95.5 ± 12.9	+93.6 ± 16.8	+73 ± 15.1

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

Appendix 2.3. % Weight change of *E. fetida* in soil beds top fed with cow slurry (10% w/v)

Mean values ± standard error (n = 5).

Treatment	After 7 days	14 days	21 days	28 days	35 days
Control	-3.5 ± 1.35	-10.6 ± 2.2	-17.0 ± 2.4	-27.2 ± 1.9	-27.1 ± 2.4
Slurry added	+3.8 ± 2.05	+7.1 ± 3.9	+28.8* ± 4.7	+48.4*** ± 5.1	+55.8*** ± 4.2

Significance of t value: * $P < 0.05$; *** $P < 0.001$.

Appendix 2.4. % Weight change of *E. fetida* in tissue waste top fed with cow slurry.

Mean values ± standard error (n = 5).

Treatment	After 7 days	14 days	21 days	28 days	35 days
Control-without slurry	-0.4 ± 2.13	+0.1 ± 2.4	+5.5 ± 2.9	+9.8 ± 4.5	+9.8 ± 5.1
10% (w/v) slurry added	+25.3* ± 3.6	+75.3*** ± 3.9	+122.1*** ± 9.9	+110.0*** ± 14.0	+77.0 ± 8.3
20% (w/v) slurry added	+12.0 ± 4.2	+43.6*** ± 7.0	+52.5 ± 7.6	+79.6 ± 7.9	+68.3 ± 14.2

* = mean significantly different from control $p < 0.05$

*** = mean significantly different from control $p < 0.001$.

Appendix 3.1. Relative abundance of aerobic bacteria in cow slurry/tissue waste mixture (1:2 w/w)

Species	Time (days) 0	7	14	21	28	35	42	49
Acinetobacter lwoffi	9.30	9.04	9.20	8.73	8.89	8.73		
Micrococcus luteus	9.19	9.04	8.98	8.34	8.29			
Streptomyces species	8.03	8.07	7.53	7.64			8.47	7.94
Pseudomonas fluorescens			8.68	8.34	8.89	9.08	8.99	8.64
Alkaligenes faecalis							8.11	8.41

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight)

Appendix 3.2. Relative abundance of aerobic bacteria in cow slurry/tissue waste mixture (1:2 w/w) containing *E. fetida*

Species	Time (days) 0	7	14	21	28	35	42	49
Acinetobacter lwoffi	9.19	9.01	8.97	8.71	8.47	8.35		
Micrococcus luteus	9.09	9.01	9.04	8.71	8.23			
Streptomyces species	7.99	8.07					8.21	7.43
Pseudomonas fluorescens			8.21	8.41	8.71	8.99	8.89	7.73
Alkaligenes faecalis								7.60
Flavobacterium lutescens								7.13

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight).

Appendix 3.3. Relative abundance of fungi in cow slurry/tissue waste mixture (1:2 w/w).

Species	Time (days) 0	7	14	21	28	35	42	49
Gliomastix murorum var. felina	6.85	6.90	6.83	7.12	6.80	6.52	6.23	6.98
Scopulariopsis carbonaria	6.55		5.83	6.55				
Gliocladium deliquescens	6.15	6.29	6.43	6.55	6.80	6.87	6.53	6.41
Fusarium oxysporum		6.60	6.23	6.73	7.07	6.82	6.63	6.89
Trichoderma hamatum						6.61	6.41	

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight).

Appendix 3.4. Relative abundance of fungi in cow slurry/tissue waste mixture (1:2 w/w) containing *E. fetida*.

Species	Time (days) 0	7	14	21	28	35	42	49
Gliomastix murorum var. *felina*	6.51	6.71	6.60	6.93	6.83	6.85	6.75	6.25
Gliocladium deliquescens	6.72	6.60	6.51	6.63	6.88	6.91	6.21	6.43
Scopulariopsis carbonaria	6.42		5.91					
Fusarium oxysporum		6.50	6.60	6.93	7.04	6.65	6.75	6.43
Trichoderma hamatum					6.53			

(Nos. expressed as log no. C.F.U.s g^{-1} dry weight).

Appendix 4.1. Effect of feeding single species of bacteria and actinomycetes to *E. fetida*.

Test organism	Mean worm wt. change (mg) (± S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
1 *Acinetobacter lwoffi* (L)	-19.03 ±16.8	+30.27	78.9
2 *Ac. lwoffi* (H)	+39.4 ±6.2	+88.7*	78.9
3 *Ac. citroalkaligenes* (L)	-78.6 ±25.0	+7.0	78.9
4 *Ac. citroalkaligenes* (H)	-40.9 ±11.7	+44.7	78.9
5 *Alkaligenes faecalis* (L)	-60.3 ±11.3	+25.3	78.9
6 *Alk. faecalis* (H)	-43.1 ±27.0	42.5	78.9
7 *Nocardia salmonicolor* (L)	-66.1 ±12.0 -66	+6.0	60.9
8 *N. salmonicolor* (H)	-27.1 ±17.01	+45.0	60.9
9 *Enterobacter cloacae* (L)	-69.3 ±25.0	+1.1	71.3
10 *E. cloacae* (H)	-52.1 ±6.1	+18.3	71.3
11 *Micrococcus varians* (L)	-54.6 ±11.7	-7.4	53.2
12 *M. varians* (H)	-39.7 ±26.9	+7.5	53.2
13 *Arthrobacter simplex* (L)	-91.7 ±8.7	-22.9	53.4
14 *A. simplex* (H)	-76.7 ±15.4	-7.9	53.4

Appendix 4.1. (Continued).

Test organism	Mean worm wt. change (mg) (±S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
15 *Micrococcus luteus* (L)	-84.0 ±6.8	-15.2	53.4
16 *M. luteus* (H)	-95.1 ±15.2	-26.3	53.4
17 *Arthrobacter tumescens* (L)	-59.9 ±22.8	-18.4	34.4
18 *A. tumescens* (H)	-48.4 ±18.6	-29.4	34.4

n = 5 (treatments); n = 9 (controls)

S.E. = Standard error of the mean

L.S.D. = Least significant difference

H = Heat killed cells; L = Live cells

* = treatment significantly different from control: $P < 0.05$.

Appendix 4.2. Effect of feeding single species of fungi (including yeasts) to E. fetida.

Test organism	Mean worm wt. change (mg) (± S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
1 Trichoderma harzianum (L)	+54.7 ±6.3	+72.2*	35.7
2 T. harzianum (H)	+44.5 ±6.2	+62.0*	35.7
3 Mucor plumbeus (L)	+31.0 ±14.9	+48.5*	35.7
4 M. plumbeus (H)	+30.9 ±3.9	+48.4*	35.7
5 Gliomastix murorum var. felina (L)	-27.0 ±12.0	+43.4	71.3
6 G. murorum var. felina (H)	-61.5 ±3.0	+8.9	71.3
7 Trichoderma hamatum (L)	-33.1 ±5.2	+42.2*	40.3
8 T. hamatum (H)	-69.4 ±14.6	+5.9	40.3
9 Scopulariopsis carbonaria (L)	-42.6 ±30.4	+4.6	53.2
10 S. carbonaria (H)	-14.5 ±5.6	+32.7	53.2
11 Rhodotorula sp. (L)	-41.8 ±5.5	+15.8	24.6
12 Rhodotorula sp. (H)	-29.9 ±6.1	+27.7*	24.6
13 Gliocladium deliquescens (L)	-15.7 ±23.6	+33.6	78.9

Appendix 4.2. (Continued).

Test organism	Mean worm wt. change (mg) (±S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
14 *Gliocladium deliquescens* (H)	-51.7 ±32.4	-2.4	78.9
15 *Aureobasidium pullulans* (L)	-37.0 ±18.8	+10.2	53.2
16 *A. pullulans* (H)	-17.4 ±11.6	+29.8	53.2
17 *Aspergillus fumigatus* (L)	-39.8 ±12.0	+8.4	35.4
18 *A. fumigatus* (H)	-38.3 ±9.5	+10.0	35.4
19 *Penicillium purpurogenum* (L)	-47.4 ±2.3	+0.8	35.4
20 *P. purpurogenum* (H)	-40.4 ±6.5	+7.8	35.4
21 *Fusarium oxysporum* (L)	-39.7 ±14.0	+17.9	24.6
22 *F. oxysporum* (H)	-84.5 ±17.8	-26.9*	24.6

n = 5 (treatments); n = 9 (controls).

S.E. = Standard error of the mean

L.S.D. = Least significant difference

H = Heat killed cells; L = Live cells

* = Treatment significantly different from control: $P < 0.05$.

Appendix 4.3. Effects of the feeding of single species of micro-organisms to *E. fetida* in tissue waste. (n = 5)

Treatment	Mean worm wt. change (mg) (± S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
1 Unsterilised tissue waste	+39.0 ±25.0	+116.0*	65.3
2 *Acinetobacter lwoffi*	-5.0 ±6.0	+72.0*	65.3
3 *Aspergillus fumigatus*	-15.9 ±3.0	+61.1	65.3
4 *Nocardia salmonicolor*	-22.0 ±20.0	+55.0	65.3
5 *Trichoderma harzianum*	-48.5 ±12.0	+28.5	65.3
6 *Enterobacter cloacae*	-62.0 ±4.0	+15.0	65.3
7 *Pseudomonas fluorescens*	-76.0 ±20.0	+1.0	65.3

S.E. = Standard error of the mean

L.S.D. = Least significant difference

* = Mean significantly different from control: $P < 0.05$.

Appendix 4.4. Effect of feeding axenic *E. fetida* with single cultures of bacteria.

Test organism	Mean worm wt. change (mg) (± S.E.)	Mean worm wt. change v control (mg)	L.S.D. (P = 0.05)
1 *Acinetobacter lwoffi*	-36.9 ± 7.9	+55.5*	42.7
2 *Alkaligenes faecalis*	-71.7 ± 12.1	+20.9	42.7
3 *Enterobacter cloacae*	-90.7 ± 9.1	+1.7	42.7
4 *Pseudomonas fluorescens*	-122.9 ± 13.4	-30.5	42.7

S.E. = Standard error

L.S.D. = Least significant difference at P = 0.05.

* = Mean is significantly different from control at P = 0.05.

Results are the mean of 10 replicates, except *Pseudomonas fluorescens* result, which is the mean of 6 replicates.

Appendix 4.5. % Weight change of *E. fetida* in tissue waste seeded with *Acinetobacter lwoffi*.

Mean values ± standard errors (n = 5).

Treatment	After 7 days	14 days	21 days
Tissue waste control	+16.7 ± 2.6	+18.4 ± 3.1	+21.9 ± 3.4
Tissue waste + *Acinetobacter lwoffi*	+22.9 ± 2.1	+37.6* ± 5.4	+34.0 ± 3.3

* = Mean significantly different from control,: $t < 0.05$.

SECTION 6.

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