UTILISATION OF EARTHWORMS AND ASSOCIATED SYSTEMS FOR TREATMENT OF EFFLUENT FROM RED MEAT ABATTOIRS

AE De Jesus • PH Heinze • JR Müller •
GL Nortje

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Water Research Commission

UTILISATION OF EARTHWORMS AND ASSOCIATED SYSTEMS FOR TREATMENT OF EFFLUENT FROM RED MEAT ABATTOIRS

Report to the
WATER RESEARCH COMMISSION

by

A E DE JESUS P H HEINZE J R MÜLLER* G L NORTJE

*Multilog, ABAKOR
and Agriculture Research Council-Animal Nutrition and Animal Products Institute
(ARC-ANPI)

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EXECUTIVE SUMMARY

Abattoirs generate large amounts of solid waste and effluents such as rumen contents, blood and wash water. From data prepared for the Water Research Commission (Water Research Commission, 1990), a typical D-Grade abattoir (that slaughters up to 15 head of cattle per day) generates up to 1 ton of wet rumen contents and blood and up to 34.7 kl total wastewater per day.

Abattoir wastewater has high pollutant loads of organic and suspended solids. Abattoirs often have difficulties in disposing of the solid wastes and wastewater in an environmentally acceptable fashion and in many instances untreated rumen contents, blood and/or other abattoir effluents and wastewater are released into the environment. The resulting pollution not only causes problems related to odour, flies and hygiene, but surface and ground water can be polluted with pathogens and undesirable chemical compounds.

Earthworms are often used in large-scale intensive systems to reduce the obnoxious attributes of a variety of organic wastes including elimination of bad odours and rotting material. A considerable amount of information is available from the literature on vermicomposting and the use of *Eisenia fetida* for the breakdown and composting of solid wastes such as sewage sludge, municipal and industrial wastes. An important benefit of vermicomposting is that processing can take place *in situ* and that worthless or decomposing wastes need not be transported over long distances. The end products have value as fertilizer, compost, potting soil and as a protein source.

The objective of the project was:
The development of a robust system to treat effluent from abattoirs by the utilisation of earthworms and associated systems, which will be economically and practically feasible to apply to small and medium size rural and urban installations.

Previous South African studies were directed at the decomposition of solid abattoir wastes alone. With an extensive literature survey as basis, the purpose of the current project was to "clean up" wastewater in addition to solid wastes. It differs from the standard vermicomposting process in that large volumes of wastewater pass through the system. The main problem that had to be solved was to ensure that the earthworms remained sufficiently active to convert the solid effluent to vermicompost under conditions where large volumes of liquid effluent passed through the system.

The contract was awarded to ABAKOR (Multilog) and the ARC-ANPI was subcontracted to do the laboratory-scale studies using solid wastes and wastewater from the experimental D-Grade abattoir on the premises at Irene. Multilog as main contractor in the project was to have evaluated the results and build a pilot-scale plant. A Technical Sub-Committee met regularly to guide the project. This Sub-Committee was of great assistance and made valuable recommendations as to the design and operation of the system. Between February 1996 and July 1997, 5 detailed reports on the project were submitted to the Technical Sub-Committee and 2 annual reports were prepared for the Steering Committee of the Water Research Commission.

The original brief to the Technical Sub-Committee with regard to the project was that the laboratory-scale system consist of a cascade system of 3 containers with a high density of earthworms. However, problems with the concept of the 3-container system highlighted in the

laboratory-scale studies, led to the redesign of the system. A single container, adapted to ensure better filtration and harvesting of the vermicompost was designed and a laboratory-scale prototype built and evaluated.

Results

- Typically from a feed with a COD of 1325 mg/l an 80 % (±10 %) reduction was achieved.
- Both the solids collecting on the surface of the container and the effluent were efficiently deodorised.
- The earthworm systems effectively removed the suspended solids. On standing hardly any solids settled out from the effluent. The low level of suspended solids is advantageous for subsequent treatment of the effluent.
- The original concept of using 3 stacked containers did not prove have any advantage for the removal of solid effluents and deodorisation of the effluent. After passing through the top container, no further improvement in the COD values or removal of suspended solids were observed when the effluent percolated through the middle or bottom container of a stack. This trend was observed for small and medium containers and also for large trays.
- A laboratory-scale prototype apparatus was designed in which solutions to previously experienced problems were incorporated. This apparatus was able to deal with about a fifth of the rumen solids from a cattle unit per day (0,25 m^2 surface area). Previous problems with sufficiently rapid drainage of the liquid, crust and slurry formation, harvesting of the vermicompost and distribution of the liquid effluent were successfully addressed. Solid wastes were manually placed into the apparatus.
- The earthworm ecosystem could be adapted to tolerate addition of blood provided it was not too concentrated (blood 0,7 % of the feed liquid). COD values of the effluent with blood were higher than without blood (COD values up to 900 mg/l and 450 mg/l respectively). The reduction of COD with added blood was on average 73 ± 10 %.
- Provided the water could drain away within hours (about 3 hours in the present series of experiments), the earthworms were able to maintain a good speed of composting (10-15 cm per week) even when large volumes of water (similar to the amount of effluent from an abattoir) passed through the system.

General conclusions and recommendations

- In general it can be stated that the use of earthworms for cleaning up abattoir wastes is technically feasible. It is an attractive system, especially for odour removal and prevention of putrefaction at small (D-Grade and smaller) abattoirs.
- The system works well provided the layer of added solids does not exceed 2-3 cm per day; the liquid drains away fairly fast and aerobic conditions are maintained. The system is simple but adequate supervision to ensure a well maintained system is required.
- The present process opens up the possibility to rid abattoir effluent of solids and to make the resultant liquid effluent more amenable to further treatment with existing systems. The effluent from the earthworm plant is not yet sufficiently clean to be released into the environment without further cleaning and polishing.
- Successful application in abattoirs will only become a sustainable proposition once it also becomes financially rewarding for the abattoirs to sell the vermicompost and/or to avoid financial penalization for pollution of the environment.

- It can be concluded from the study that the following aspects still need attention:
  - Testing of the system on a larger (pilot-plant) scale.
  - Determination of sustainable conversion rates by the earthworms, including effect of population density (harvesting).
  - Determining the chemical composition of the effluent from the prototype apparatus. The effluent is brown (even without blood in the feed).
  - Factors that play a role regarding utilisation of blood by the earthworm ecosystem. These could include the maximal amount of blood the system can tolerate and the influence of congealed blood on the system.
  - The chemical composition of the vermicompost. It is foreseen that the large volumes of water passing through the system may leach components out of the vermicompost and that the composition may differ from typical vermicompost from solid abattoir wastes.
  - Biological quality of the vermicompost. The vermicompost may occasionally contain plant/animal/human pathogens or viable plant seeds.
  - Cost-effective ways of pasteurizing (partially sterilising) the vermicompost need to be explored.

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UTILISATION OF EARTHWORMS AND ASSOCIATED SYSTEMS FOR TREATMENT OF EFFLUENT FROM RED MEAT ABATTOIRS

The Steering Committee responsible for the project consisted of the following persons:

Mr G N Steenveld (Chairperson)	Water Research Commission
Mr Z T Ngcakani (previous Chairperson)	Water Research Commission
Dr N P Mjoli (previous Chairperson)	Water Research Commission
Mr G D Hefer	Dept. of Forestry and Water Affairs
Mrs W J Meyer	WormCo, Potchefstroom
Dr G V Kriel	Red Meat Abattoir Association
Prof P D Rose	Rhodes University, Grahamstown
Dr S P Greyling	Earlybird Farms
Dr H Bouwman	ARC-Plant Protection Institute
Mr J R Müller	Multilog, ABAKOR Ltd
Dr G L Nortjè	ARC-ANPI
Dr A E de Jesus	ARC-ANPI
Prof P J Jooste	ARC-ANPI

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LIST OF ABBREVIATIONS

COD	Chemical oxygen demand
Effl	Effluent
E_h	Oxidation-reduction potential
LT_{50}	Lethal temperature (temperature killing 50 % of the individuals)
SD	Standard deviation of the mean
SS	Suspended solids
wrcu	Water related cattle unit (the number of non bovine animals equivalent to one bovine animal in terms of water use during processing)

1. INTRODUCTION

1.1 Industry background

Abattoirs generate large amounts of solid waste and effluents such as rumen contents, blood and wash water. From data prepared for the Water Research Commission (Water Research Commission, 1990), a typical D-Grade abattoir (slaughtering up to 15 cattle units per day) generates up to 1 ton of wet rumen contents and blood and up to 34.7 kl total wastewater discharge per day. Abattoir wastewater have high pollutant loads of organic and suspended solids with typical national mean values of 5.5 kg chemical oxygen demand (COD) and 1.6 kg suspended solids (SS) discharged per water related cattle unit (wrcu).

Abattoirs often have difficulty in disposing of the solid wastes and wastewater in an environmentally acceptable fashion (Water Research Commission, 1990). The existence of problems with disposal of wastes from small abattoirs was highlighted by a tour of the country undertaken in 1995 by members of the Water Research Commission, Multilog (ABAKOR), the National Directorate of Veterinary Public Health of the National Department of Agriculture, the University of Pretoria and the Red Meat Abattoir Association. In many instances untreated rumen contents, blood and/or other abattoir effluents and wastewater were seen to be released into the environment. The resulting pollution not only causes problems related to odour and flies but even more importantly, surface and ground water can be polluted with pathogens and undesirable chemical compounds.

1.2 Application of vermiculture

The use of earthworms in waste management is known as vermicomposting or vermistabilisation. Earthworms have been used in large-scale intensive systems to reduce undesirable effects of a variety of organic wastes such as elimination of bad odours and rotting material. A considerable amount of information is available from the literature on vermicomposting and the use of *Eisenia fetida* for the breakdown and composting of solid wastes such as sewage sludge, municipal and industrial wastes (Lee, 1985, Edwards, 1988; Hartenstein & Bisesi, 1989). An important benefit of vermicomposting is that processing can take place *in situ* and that worthless or decomposing wastes need not be transported over long distances. The end products have value as fertilizer, compost, potting soil and as a protein source.

Vermiculture has become an industry with both "low technological" and "high technological" facets. In its "low technology" form it is cheap and can be applied on a small scale at individual, household or village level. In its "high technology" form it implies high levels of financial input, high levels of scientific interest and high levels of risk (Sabine, 1988).

The "low technology" facet seems particularly appropriate for application under the present conditions in South Africa. The application of earthworms to the management of solid abattoir wastes as is presently practised in South Africa (cf. Rieckert, 1990) is "low technology". The aim is to apply the "low technology" approach in cleaning up solid wastes and wastewater from abattoirs.

1.3 Vermiculture at South African abattoirs

Use of *Eisenia fetida* for vermicomposting of abattoir wastes has been the subject of considerable research at Potchefstroom University during the previous two decades but has since declined considerably. From 1987 to 1988, ABAKOR Ltd sponsored a vermicomposting trial at Pyramid abattoir to decompose rumen solids. It was established that vermicomposting of cattle rumen contents can be achieved under field conditions (Rieckert, 1990). Recently, the firm WormCo from Potchefstroom together with other interested parties ran a project at Springs abattoir for the decomposition of abattoir wastes.

1.4 The project and its objective

The previous South African vermiculture studies were directed at the decomposition of solid abattoir wastes alone. With the current project the "cleaning up" of wastewater in addition to solid wastes received attention. It differs from the standard vermicomposting process in that large volumes of bloody water pass through the system. Although earthworms are able to survive in water, they are not active and do not convert the material in the presence of excess water. The main problem to be addressed in this project on abattoir effluents is to ensure that the earthworms remain sufficiently active to convert the solid waste to vermicompost under conditions where large volumes of liquid effluent also pass through the system.

The contract was awarded to ABAKOR (Multilog) and the ARC-ANPI was subcontracted to do the laboratory-scale studies using solid wastes and wastewater from the experimental D-Grade abattoir on the premises at Irene. Multilog as main contractor in the project was to evaluate the results and build a pilot-scale plant. A Technical Sub-Committee with Mr. J R Müller (Multilog, ABAKOR) and Dr G L North (ARC-Irene) as joint chairpersons as well as Ms W J Meyer (WormCo, Potchefstroom), Mr. W van Staden (Enviro Metsi (Pty.) Ltd, Pretoria), Mr. S W Nieuwoudt (ARC-AII, Irene), Dr P H Heinze (ARC-ANPI) and Dr AE de Jesus (ARC-ANPI) met regularly to guide the project. The Technical Sub-Committee greatly assisted and made valuable recommendations as to the design and operation of the system. Between February 1996 and July 1997, five detailed reports on the project were submitted to the Technical Sub-Committee and two annual reports were prepared for the Steering Committee of the Water Research Commission.

The objective of the project was:
The development of a robust system to treat effluent from abattoirs by the utilisation of earthworms and associated systems, which will be economically and practically feasible to apply to small and medium size rural and urban installations.

The original brief to the Technical Sub-Committee for the project was for the laboratory-scale system to consist of a cascade system of 3 containers with a high density of earthworms. Solid wastes and wastewater were to be added to the top container and liquids then allowed to percolate through to the second and third containers by gravity. However, problems with the concept of the 3-container system highlighted in the laboratory-scale studies and described in the appropriate section (Section 4), led to the redesign of the system to ensure better filtration and building of a laboratory-scale prototype. The idea was to thoroughly monitor the system on a laboratory-scale before proceeding to pilot-plant trials. At the July 1997 meeting, the Technical Sub-Committee recommended that a pilot-plant could be erected at the ARC-ANPI abattoir and that pilot trials should be carried out at the ARC-Irene abattoir instead of at the Johannesburg Abattoir as originally intended.

The present report deals with the laboratory-scale and up-scaled prototype experiments. In Section 4 the stacked 3-container system and the results obtained by this system in smaller and larger containers are described. Section 5 deals with experiments using redesigned single containers. The first experiments with single containers were on a small scale (first in small, then in a medium container) that did not allow for regular harvesting of vermicompost. A prototype apparatus capable of dealing with effluent from a fifth of a wrcu and that also allows harvesting of the vermicompost, was designed and built. The prototype apparatus performed successfully and results obtained with the apparatus will be described in Section 5. Design recommendations for a pilot-plant are given in the Appendix.

2. LITERATURE SURVEY

The use of earthworms for effluent treatment is not a topic previously handled by the WRC (e.g. no available information on earthworms was recovered from Waterlit). The following extensive literature survey on the characteristics and use of earthworms was prepared for the inaugural meeting of the Steering Committee (5 August 1996).

Vast amounts of organic wastes are produced all over the world. Edwards (1988) calculated that in the United Kingdom, farmed livestock produced about 120 million tonnes of excreta during 1980; plant and vegetable wastes amounted to 11.6 million tonnes of dry matter annually. With the rapidly expanding human population in the world, growing use of intensive farming and expanding food industries coupled with increasingly stringent waste disposal legislation, problems in disposing of organic wastes in effective and environmentally friendly ways are becoming more acute.

Earthworms in dense cultures and in large quantities are able to dispose of animal, vegetable and industrial organic wastes like effluents from intensively housed animals, activated sewage sludge, arable crops, effluents from food industries like breweries, sugar mills, paper pulping (Edwards, 1988; Hartenstein & Bisesi, 1989) and even dairy waste sludge cake (Hatanaka *et al*, 1983). Of particular advantage is their ability to clear up such wastes relatively quickly and eliminate offensive odours. That earthworms indeed have an important role to play in the degradation of sewage sludge was shown by Neuhauser *et al*, (1988) who found that earthworms have an important effect on the destruction of volatile solids in aerobically digesting sludge. After 20 days, double the amount of sludge solids was destroyed in the presence as compared to the absence of earthworms. In recent years earthworms are increasingly seen as important agents in waste and environmental management (cf. Edwards & Neuhauser, 1988).

In order to understand the application of earthworms for the management of abattoir wastes and wastewater, some characteristics that influence the growth and behaviour of earthworms will be discussed.

2.1 Earthworm anatomy and life-cycle

The basic "design" of the earthworm is simple. They are cylindrical animals, the body is tapered at both ends with the tail end being the blunter of the two. Essentially the lumbricid earthworms consist of two concentric tubes (the body wall and the gut), separated by a fluid filled cavity (the coelom) and divided into segments by septa. They have a closed vascular system. Some internal organs, including the excretory organs (nephridia) are duplicated in each segment. Most segments have setae (stiff bristles) which can be extended or retracted. The body wall of the earthworm is composed of an outer layer of circular muscles that decreases the body diameter and an inner layer of longitudinal muscles that shorten the body. Movement is achieved through alternative contraction and relaxation of opposing muscles while setae provide anchorage. The muscle systems are overlain by a skin of epidermal tissue and a cuticle. Respiration takes place through the thin moist cuticle. Oxygen passes readily into the blood as the skin is well supplied with blood vessels.

Sensory organs consist of a wide variety of specialised epidermal and sub-epidermal cells and free nerve endings in the epidermis associated with reception of tactile, positional, chemical and light stimuli and are scattered over the body surface (Lee, 1985).

The lifecycle is uncomplicated, with worms producing eggs from which new worms develop. Although earthworms are hermaphroditic, the eggs of one individual are fertilised by the sperm of another individual. During mating the earthworms are bound together with a mucous band. This mucous band slips off and in the process collects eggs and sperm. A sealed cocoon is formed in which the eggs are fertilised and from which the hatchlings emerge (Lee, 1985).

2.2 Choice of earthworm species for biotechnological applications

Estimations put all earthworm species to be in excess of 3 000 but only about 5 % of these have been studied to any extent (Sabine, 1988). The potential choice of earthworm species for use in the treatment of abattoir wastes is, therefore, wide and it is not considered impossible that better species than those presently in use may be found. It is most important to choose the correct earthworm for a particular purpose.

Earthworms can be divided into a number of categories depending on their vertical distribution in soils. The two categories relevant as far as their application for the management of organic wastes is concerned are epigeic and endogeic.

Epigeic species like *Eisenia fetida* are surface dwellers, produce neither recognisable casts nor burrows and show no seasonal rhythms of activity. They are commonly referred to as "red worms". They are particularly suited to the management of solid biological wastes because they are able to convert agriculturally related wastes, activated sewage sludge and solid organic wastes from food and other industrial processes (brewing, potato processing, mushroom industries, sugar mills, paper pulping) into compost at the same time reducing offensive odours (Lee, 1985; Hartenstein & Bisesi, 1989).

Although the epigeic earthworms are able to burrow into soil, they are primarily slit crawlers who slither in and out of crevices in the organic matter present on and in the superficial layers of the soil. This ability to move away and 'hide' in the soil is particularly useful when changes in the physical and chemical conditions in the upper layers (light, humidity, temperature, pH, presence of poisonous substances) adversely affect their well being.

Hartenstein & Bisesi (1989) relate their ability to eliminate offensive odours rapidly to the abundance of sensory organs on the body surface and particularly in the region round the mouth. These organs enable them to locate food. In the course of feeding they also produce aerobic conditions, which reduces the potential formation of substances such as cadaverine, agmatine, skatole, hydrogen sulfide and other malodorous compounds.

Endogeic earthworms are true burrowers and are commonly present in the top layers of the soil from about 2 cm below the soil surface. They can co-exist with epigeic species. They create an extensive network of burrows oriented mainly horizontally and obliquely to the soil surface thereby aerating the soil and providing conduits for water. They mix leached microbial and other organic matter with clay, silt and sand particles and in this way *improve the texture and structure of soil.*

The use of endogeic earthworms for the decomposition of biological wastes has not received much attention in western countries but in India, Bhawalkar (1995) claims spectacular success with the utilisation of these earthworms for the degradation of biological wastes. Since his theories are somewhat "different" by western standards and since his thesis is only privately sold (US $ 300), a short summary of his approach follows.

Bhawalkar refers to treatment of biological wastes with "red worms" as "biotreatment" and that with endogeic earthworms (or "earthworms") as "bioconversion". He classifies "red worms" with scavengers such as flies, cockroaches, rats, ants, crows, foxes, vultures, etc. able to deal with "biological fires". "Biological fires" occur when large amounts of biological material accumulate. He, however, favours "bioconversion" of biological wastes with "earthworms" in an ecosystem including plants for which he supplies schematic drawings (his Figure 1). Unfortunately he supplies very little technical data on conversion rates, etc. He also supplies a photograph of solid wastes from chicken abattoirs (including chicken feet) ready for "bioconversion" in such a system. It is not clear to what extent his ecosystem is applicable to systems such as abattoir wastes and wastewaters from cattle abattoirs. (In the present author's opinion, production of large amounts of abattoir wastes could probably be classified as a potential "biological fire"). He does warn though that organics with more that 25 % proteins, soon become anaerobic and generate bad odours and flies (which serve as warning signals) (Bhawalkar, 1995, page 31). Organics with 15 - 20 % proteins pose no problem but when in combination with inorganics, their disposal becomes tricky with the nature of the inorganics determining the impact. He also maintains that heavy metals and metal ions inhibit the system, especially the bacteria, severely. He defines "palatability" as a ratio of simple organics to a sum of complex organics that is, $BOD \times (COD\text{-}BOD)^{-1}$. He mentions that dilute wastewaters (such as sugar-mill wastewater with only 0.1 % sugar) are 'tricky' and often not suitable for bioconversion processes. To quote him (Bhawalkar, 1995, page 32 - his bold type): "**Current bioconversion techniques are mostly based on liquid volume or liquid flow and would need excessive reactor volumes to process such dilute wastewaters**. We need an improved technique for utilisation of these organic resources and water streams. **Adsorptive bed bioreactor with immobilised organisms would be the ideal choice**. Such a bioreactor **would provide a high solid residence time (SRT) without increase in liquid residence time (HRT). Also, the immobilised organisms would utilise the bio-products thus avoiding inhibition as well as the**

downstream processing expenses". Bhawalkar does not give any specifics and his approach will not be considered further in this report.

Edwards (1988) reported on some results from a laboratory experiment in which five different earthworm species and nine different substrates were screened to assess their biological and economic potential. Earthworm species were: the tropical species *Eudrilus eugeniae* and *Perionyx excavatus* and the species *Eisenia fetida*, *Dendrobaena veneta* and *Lumbricus rubellus* from temperate climates. The organic substrates used were pig, cattle, duck, turkey, poultry, potato, brewery and paper wastes and activated sewage sludge. Results confirmed the preferred use of *Eisenia fetida* for the breakdown of organic wastes. Although not the best performer in every respect such as maximum reproductive rate, shortest life-cycle and highest biomass production, overall *Eisenia fetida* performed well in all these respects and was able to maintain a sustainable population. It also has a wide temperature tolerance, can live in a range of moisture contents, is tough, ubiquitous, naturally inhabits organic wastes, is readily handled and becomes dominant in mixed cultures. The tropical species were found to have poor temperature tolerance. The lower LT_{50} (temperature killing 50 % of the individuals) for *Eudrilus eugeniae* was found to be 7.5 °C (Lee, 1985). However, Edwards (1988) mentioned that although *Eudrilus eugeniae* is a large worm that grows extremely rapidly and is reasonably prolific, its main disadvantages are that it has poor handling characteristics and cannot tolerate temperatures above 30 °C and below 9 °C (slightly higher than mentioned by Lee, 1985).

Neuhauser *et al* (1988) also evaluated five species of earthworms (*Eudrilus eugeniae*, *Perionyx excavatus*, *Eisenia fetida*, *Dendrobaena veneta* and *Pheretima hawayanus*) for their ability to grow in sewage sludge. They determined their response to temperature, cocoon production and its viability, growth rates, etc. and found that both *Eudrilus eugeniae* and *Eisenia fetida* performed well under the conditions of testing. In mixed culture, volatile solids reduction was slightly more and biomass gain was slighly reduced compared to cultures of single species. The presence of both *Eudrilus eugeniae* and *Eisenia fetida* tended to increase gain in biomass and production of volatiles but differences were not sufficient to advocate the use of mixed cultures.

An important consideration in the preferred use of *Eisenia fetida* is that a vast body of international literature is available on the basic characteristics of and requirements for the application of *Eisenia fetida* in organic waste management (cf. Edwards, 1988; Hutha & Haimi, 1988; Morgan, 1988; Jeffries & Audsley, 1988). Moreover, *Eisenia fetida* is considered a suitable earthworm species for use under South African conditions (Reinecke & Venter, 1985).

2.3 Growth

Many growth studies have been reported for *Eisenia fetida* under different conditions and growth (biomass gain) usually follows the normal sigmoid growth curve (Lee, 1985; Neuhauser *et al*, 1988; Edwards, 1988; Hutha & Haimi, 1988; Morgan, 1988).

Biomass production of *Eisenia fetida* is difficult to establish from the literature. Reported biomass production varies for different media and under different conditions. Rieckert (1990) quotes average mass gain for *Eisenia fetida* the first 60 days of an experiment as being 2.4 mg per worm per week with gains up to 16 mg between day 60 and day 90. From results obtained by Neuhauser and his colleagues (Neuhauser *et al.* 1988) with sewage sludge (Figure 6), *Eisenia fetida* produced 2600 mg of worm biomass in 10 weeks.

In order to maintain the population over the longer term, it is necessary to consider the effect of different factors on both biomass gain and reproductive capability (cocoon production, incubation time, hatching rate, length of life, worm density) (cf Venter & Reynecke, 1988; Reinecke & Viljoen, 1990 a and b). However, optimal conditions for these processes may not be the same. For instance, Neuhauser *et al* (1988) found that in aerobically digested sludge containing different amounts of solids, the optimal range of solids for mass gain was lower than that for cocoon production. Optimal biomass gain occurred between 9.3 % to 15.9 % solids while optimal cocoon production occurred at 11.9 % to 18.6 % solids. No cocoon production occurred at concentrations from 6.3 % to 7.9 % and 20.5 % to 25.1 % solids. It is thus necessary to select conditions representing the best compromise to satisfy all these different requirements.

A population model for *Eisenia fetida* was developed by Jefferies and Audsley (1988). The model takes into consideration the interrelated three stages of growth - cocoons, small worms and adult worms - and the influence of changing conditions on population biomass. Although the model is derived from laboratory experiments, which probably simulate optimal conditions, the authors nevertheless regard the model as supplying a convenient and quick way to examine the effect of changing conditions on sustainable worm growth, productivity and maintenance of the population.

2.4 Factors affecting earthworm growth

2.4.1 Temperature

Temperature is probably one of the most important parameters for earthworm performance. Earthworms are poikilotherms and take on the temperature of their direct environment. At temperatures away from the optimal temperature for growth, increase or decrease in temperature has a significant effect. The important effect of temperature on the growth of *Eisenia fetida* is illustrated by Edwards (1988). *Eisenia fetida* tolerated temperatures between 0 °C and 35 °C and the optimal temperature for biomass production was 25 °C. Biomass production at 20 °C and 15 °C was somewhat lower than at 25 °C. However, at 10 °C biomass production was only about a quarter of that produced at 25 °C.

Reproduction is also strongly influenced by temperature and a highly significant difference in cocoon production occurred between a constant temperature of 20 °C and a mean temperature of 20 °C (Reinecke & Kriel, 1981).

The temperature limits for survival vary between species but in general they can survive for long exposure times only within the range 0 °C to 35 °C. Tropical species have higher temperature optima than species from the temperate zones. However, some acclimatisation to higher temperatures can be achieved. Lee (1985) quotes some experiments in which the LT_{50} could be increased 0.3 °C for every 1 °C rise in conditioning temperature but only at low temperatures (4 °C to 15 °C conditioned for 1 month) and under specific conditions.

2.4.2 pH

Earthworms are able to grow in soils with pH between pH 5 and pH 9 (Lee, 1985). They are relatively tolerant regarding pH in this range. Edwards (1988) found that when given a choice in a pH gradient between pH 4 and pH 9, *Eisenia fetida* moves towards the more acid material with a preference for pH 5.

2.4.3 Oxygen requirements

It is generally agreed that aerobic conditions favour the elimination of bad odours and that anaerobic conditions encourage the production of malodours. Earthworms are known for their ability to create aerobic conditions and to eliminate foul odours. They can obtain oxygen for respiration both from oxygenated water and from the air. Absorption from the air requires maintenance of a moist surface layer and earthworms readily become desiccated. They are able to tolerate much higher concentrations of carbon dioxide than normally occuring in soils.

Results of studies summarised by Lee (1985) showed that the carbon dioxide concentration of the air in soil appears to have no effect on respiration of *Eisenia fetida* at a concentration of 25 % and only a slight effect at a 50 % concentration. This means that earthworms can tolerate much higher concentrations in culture and can readily survive in closed containers. The concentration of the respiratory pigment erythrocruorin in the blood of different species increases with reducing oxygen supply in their respective niches: *Lumbricus rubellus* which lives near the soil surface has a level of 3.1 mg erythrocruorin/g of blood, *Eisenia fetida* from compost a level of 4.2 mg erythrocruorin/g of blood and *Eiseniella tetraedra* from wet soils and flooded ground a level of 4.2 mg erythrocruorin/g of blood. More of the respiratory enzyme is therefore required when conditions are less aerobic.

Earthworms are influenced by the degree of aerobiosis/anaerobiosis of a substrate as indicated by the oxidation-reduction potential (E_h) of the substrate. The oxidation-reduction potential is defined as the ease with which the substrate loses or gains electrons and is usually expressed in millivolts. The lower the E_h, the more anaerobic the substrate. The ability of *Eisenia fetida* to grow in sludge has been related to the change in oxidation-reduction potential of the sludge when it is aged in an aerobic environment (Lee, 1985). Anaerobic sewage sludge was toxic to *Eisenia fetida* and it only grew when the sludge became more aerobic and the E_h exceeded 250 mV.

2.4.4 Water relationships/Humidity

Both excessive and insufficient moisture can adversely influence growth of earthworms. This factor is especially important for the envisaged experiments because the amounts of wash water to be added will be high (Section 2.6.4). Neuhauser, *et al* (1988) found optimal worm growth to occur between 9 % and 16 % solids (91 % and 84 % water). They remarked that in the vermistabilisation process, more liquid wastes can be applied as long as the liquids drain from the media, organic material is retained and aerobic conditions are maintained.

The influence of moisture and moisture preferences of *Eisenia fetida* were determined by Reinecke & Venter (1985, 1987). They found that the moisture of the medium directly influenced growth and reproduction. Clitellate worms showed a preference range up to 80 % moisture but juveniles and cocoons preferred the range 55 - 70 % moisture.

From the literature, a high moisture content (as expected in the proposed cascade system) can affect the reproductive behaviour of *Eisenia fetida*.

2.4.5 Light

Earthworms move away from light and perform best in the darkness, for example under a cover when converting abattoir wastes (Rieckert, 1990).

2.4.6 Electrolyte concentration and presence of toxic compounds

The cuticle of the earthworm is permeable to water and other substances. Although some control over transfer rates is achieved, earthworms are particularly sensitive to high concentrations of electrolytes in their environment (Lee, 1985). Earthworms are also sensitive to the concentration of inorganic ions, as is measured by electrolytic conductivity, in their environment. Edwards (1988) showed that sharp cut off points between toxic and non-toxic salt concentration exist for *Eisenia fetida* in the presence of sodium and calcium ions. According to Hartenstein & Bisesi (1989), problems may arise in vermicomposting slurry from intensively housed animals when the conductivity reaches 3 mS per cm. Bhawalkar (1995) mentioned that Na^+ and Cl^- are more inhibitory than K^+ or Ca^{++}.

Earthworms are also extremely sensitive to a variety of toxic substances. They are able to accumulate, often in high concentration, various environmental pollutants like heavy metals, biocides and other agricultural pollutants and their residues (Sabine, 1988). Earthworms are used as sensitive indicators of toxic substances in water and soil (cf. Ireland, 1983; Venter & Reinecke, 1988), for environmental monitoring (Rhett, Simmers & Lee, 1988) and as ecotoxicological assessment tool (Callahan, 1988). It can, therefore, be expected that they will be sensitive to toxic substances in rumen contents (including antihelminthics, feed additives, hormones and antibiotics), blood and the abattoir cleansers and sterilants in the wastewater. Some other potentially toxic substances in abattoir wastes and wastewater are the fatty acids and ammonia in rumen contents, ammonia and ions in (decomposing) blood and chemicals in cleansers and sterilants from wash water.

Chemical cleaners and sterilants that are used in the abattoir may be toxic to earthworms. The chemical composition of chemical cleaners and sterilants is not always known. They are usually strongly acidic or alkaline. The effect of pH when not sufficiently diluted may play a role in their toxicity. The cleansers and sterilants presently being used in the experimental abattoir at Irene are further discussed in the section 2.6.5.

2.5 Interactions between earthworms and micro-organisms

Earthworms have many complex interrelationships with micro-organisms (Edwards & Bohlen, 1996). Earthworms depend on micro-organisms as major source of food for earthworms, they promote (aerobic) microbial activity in decaying organic matter by fragmenting it and inoculating it with micro-organisms and they disperse micro-organisms through the substrate.

Lee (1985) summarised the evidence until that time, that earthworms mainly exist on the bacteria, filamentous fungi, yeasts, other soil organisms like protozoa in soils and waste substrates. Although it is accepted that earthworms subsist on these micro-organisms, results as to the role of specific organisms in feeding of earthworms are confusing and often contradictory. Lee (1985) also mentioned that it is not clear whether earthworms have a specific gut flora. However, Morgan (1988) stated that it is generally agreed that the alimentary tract of earthworms contain the same species of micro-organisms found in their environment and thus that they do not harbour a specific gut flora. He also criticized earlier work on the basis of faulty methodology. He found that in axenic culture, *Eisenia fetida* flourishes on only four out of 22 species of bacteria, fungi and protozoa as sole source of food. The bulk of these were isolated from soil in which they thrive. As he correctly pointed out, in the complex ecosystem of wastes, other organisms could remove harmful metabolites and thus his results do not give a true reflection of the natural ecosystem of these worms. Well-planned experimentation is therefore needed.

Pedersen & Hendriksen (1993) found evidence to indicate that the bacterial population of ingested feed material changed qualitatively and quantitatively during gut transit in *Lumbricus* spp. *Enterobacter cloacae*, and to a minor extent *Escherichia coli*, decreased in number during passage through the pharynx and /or crop. The numbers of *Enterobacter cloacae* increased in the hind gut but the number of *Escherichia coli* stayed low.

Numbers of human pathogens like salmonella are reputedly drastically reduced by the growth of *Eisenia fetida* on a substrate harbouring *Salmonella*. However, the results of Murry & Hinckley (1992) showed that after 48 hours the mean *Salmonella* counts for the medium with earthworms were only 6 % lower than that without earthworms. This statistically significant reduction means nothing in practical terms. The experiments of Brown and Mitchell (1981) are often quoted as demonstrating that *Salmonella enteritidis* ser. *typhimurium* in a soil medium are drastically reduced (by a factor of 99.9 %) by

Eisenia fetida. Such a 3 $\log_{10}$ reduction of a 6 $\log_{10}$ initial count does mean that a significant 3 $\log_{10}$ (between 1 000 and 9 999 organisms) per added ml still remained. It needs to be established whether *Eisenia fetida* would preferentially utilise *Salmonella enteritida* ser. *typhimurium* in the presence of large numbers of other organisms.

Pathogens and vermicompost

The chemical composition of vermicompost differs somewhat from that of ordinary compost (Vinceslas-Apka & Loquet, 1994). The microbiological quality may differ substantially. The temperature during vermicosmposting is low compared to ordinary composting so that organisms usually killed during composting, can readily survive in vermicompost. Edwards & Bohlen, (1996) recommended use of some form of partial sterilisation (heating between 60 °C and 80 °C for 24 hours or passing through a flame steriliser) to kill residual earthworms, their cocoons and insects and reduce potential pathogen problems. They also mention that if the waste is likely to contain human pathogens, some precomposting for 3-4 days might be advisable (page 255). The main pasteurising or partial sterilisation effect during traditional composting is heating. This heat is generated by microbial growth but growth and heating are not uniform throughout the compost. Appropriate time/temperature conditions need to be selected.

The very real possibility that human pathogens may be present in vermicompost exists. *Escherichia coli*, a common intestinal organism, was previously not considered a pathogen. However, in recent years pathogenic strains such as *Escherichia coli* O157:H7 from beef caused fatalities among children and the elderly. In the USA, *Escherichia coli* O157:H7 is recovered from about 2 % of slaughtered animals. Such animals are usually infected with this organism throughout the alimentary tract. Because the incidence is low, careful experimentation will be required to establish its presence/absence in vermicompost. Although it might seriously affect profitability, it appears that before marketing, treatment of vermicompost from abattoir wastes to eliminate human/animal/plant pathogens, will be required.

2.6 Abattoir wastes & wastewater as feeding substrates

In the light of the previously discussed reaction of earthworms to various chemical and physical conditions influencing growth, the factors which may play a role in feeding earthworms with abattoir waste are discussed below. In abattoir wastes the primary sources of food for the earthworms are rumen contents (rumen solids and rumen fluid), blood and wash water. Each of these is discussed in turn.

2.6.1 Amounts of waste generated

Abattoirs generate solid wastes and effluents like rumen contents, blood and wash water. From data prepared for the Water Research Commission (Water Research Commission, 1990) the following information was extracted :

- A D-Grade abattoir licensed to slaughter 15 cattle units per day typically generates about 76 kg of rumen contents and blood per cattle unit (total blood per head of cattle *ca.* 16 kg, total paunch content per head of cattle *ca.* 60 kg) or a total of up to 1 ton of rumen contents and blood per day.
- Total wastewater (that is including water used for cleaning the holding pens, offal cleaning and steam generation), for a D-Grade abattoir estimated at 35.7 kl per day.
- Estimated national water use per water related cattle unit (wrcu) for a D-Grade abattoir amounts to 2.38 kl.
- Water use for slaughtering and carcass dressing only, amounts to 476 l per cattle unit wrcu (i.e. 20 % of total) in D-Grade abattoirs.

2.6.2 Rumen contents as feed substrate for earthworms

Up to 10^{11} viable bacterial cells/ml are present in the rumen (Stewart & Bryant, 1988). Rumen contents also contain anaerobic fungi, protozoa and other organisms. Since many of these are strict anaerobes, they probably die off before they are fed to the earthworms. However earthworms also ingest dead bacteria thus the rumen contents present a rich and varied foodstuff to the earthworms and other organisms living in the composted material. Rumen contents also contain potentially toxic substances such as ammonia and fatty acids and in exceptional cases also antihelminthics, feed additives, hormones and antibiotics.

2.6.2.1 Ammonia

Earthworms are particularly sensitive to ammonia and will not survive in organic wastes containing large amounts of ammonia e.g. fresh poultry litter. *Eisenia fetida* rapidly dies off in the presence of between 1 and 2 mg NH_3/g of waste (Edwards, 1988). It is difficult to estimate the amount of ammonia in rumen contents since the concentration of ammonia in the rumen changes over time (Van Soest, 1994). In cattle, the ammonia concentration in the rumen peaks 2 hours after feeding at about 1200 mg/l rumen fluid and then decreases to 400 mg/l at 8 hours after feeding. Since cattle are slaughtered after starvation the possibility for the worms to be inhibited by ammonia from rumen contents is slight.

2.6.2.2 Fatty acids

Acetate, propionate, butyrate, isobutyrate, valerate and isovalerate are generated in the rumen (Kistner, A, personal communication). No data could be traced on the effects of lower fatty acids to earthworms.

2.6.3 Blood as feed substrate for earthworms

Blood is a rich medium, with cattle blood containing about 19 % dry solids. It contains 17 % protein, 0.19 % cholesterol, 0.2 % lecithin and 0.05 % fat (Gorbatov, 1988). With such a high concentration of proteins, production of considerable amounts of ammonia can be expected during deamination of the amino acids. In comparison, ammonia in circulating blood is low (1 -2 mg/l in man) (Diem & Lentner, 1970).

The sodium level of 0.36 g/100 ml blood according to Gorbatov (1988) is relatively high compared to values of up to 0.28 g/100 ml quoted by Dittmer (1961). However values vary for different breeds of cattle under different conditions. Concentration of chloride in blood is also high (0.31 g/100 ml). These electrolytes may interfere with earthworm growth in concentrated blood.

2.6.4 The potential influence of wastewater on the earthworm population

Large amounts of wastewater are potentially generated in abattoirs. Abattoir wastewaters have high pollutant loads of organic and suspended solids with typical national mean values for all grades of abattoirs of 5.5 kg chemical oxygen demand (COD) and 1.6 kg suspended solids (SS) per mean water-related cattle unit (wrcu) of 1.57 kl (Water Research Commission, 1990).

It is not known what effect the large amounts of wastewater will have on the functioning of a 3-container percolating system. Preliminary experiments showed that the quality of the composted material and especially its pH plays an important role in deodorising the liquid that percolates through it. Constant washing with wastewater may affect the earthworms by creating an excessively wet environment. Constant washing may have an important effect on the accompanying microflora and the pH of the composted material. The microflora associated with the earthworms may even be washed out of the system.

However, according to Edwards (1988) both ammonia and ionic substances can readily be washed out of wastes. The percolation of wash water may have a beneficial effect.

A system proposed by Loehr, Martin & Neuhauser (1988) where compost accumulates on top of a filter bed, holds promise as a way to rid the system of excess water.

2.6.5 Presence of cleansers and sterilants in wash water

Earthworms may be sensitive to the cleansers and sterilants used in abattoirs. A variety of cleansers and sterilants are used in abattoirs. Present data only refers to conditions in the experimental abattoir at Irene. Even here, the concentration of cleansers and sterilants is difficult to determine. Cleaning solution (Marvel supplied by Messrs Firechem) is used as 0.3 l per 40 l in a high-pressure hose. Sterilant (Stericlean supplied by Messrs Firechem) is likewise used at a concentration of 0.3 l per 40 l in a high-pressure hose. In between cleaning and sterilisation, a complete rinse (volume unknown) is made with a high-pressure hose. Although a concentration of 1.3 % of the cleaner and/or sterilant could come into contact with the earthworms, it is unlikely. These substances will usually be much diluted by the time they reach the earthworms. Among other compounds, the active ingredients in the cleaner and sterilant include sodium hydroxide and ethylene diamine tetra acetic acid (EDTA). The final pH of a 1 % solution is over pH 10 for the cleaner and pH 10 for the sterilant. The effect of these substances on the earthworm system needs investigation.

2.7 General considerations

From the literature survey it was clear that the most appropriate earthworm for the study would be *Eisenia fetida*. "Red worms" such as *Eisenia fetida* do not produce permanent burrows but collect where large quantities of foodstuffs are available and, under suitable conditions, can "work through" large amounts of waste material. *Eisenia fetida* is also the best-researched earthworm, much information on growth and other requirements are available and compared to other earthworms described in the literature it appears to be robust and can readily be cultivated. It was also the organism of choice for previous studies under South African conditions.

Eisenia fetida readily tolerates water provided adequate oxygen is available. Good drainage is a prerequisite in any system utilising *Eisenia fetida* where they are required to work through organic matter while large amounts of water pass through the system.

The literature survey, especially the South African work of Rieckert (1990) on rumen solids, also showed that the earthworms are unable to efficiently deal with thick layers of abattoir waste. Their efficiency is measured by the surface area rather than volume that they work through at one time. Overloading of the system with too thick layers of waste may lead to inefficient conversion of rumen solids, failure to reduce odours and putrefaction.

Temperature has a profound influence on the growth and performance of *Eisenia fetida*. It can tolerate temperatures between 0 °C and 35 °C with acceptable biomass production between 25 °C and 20 °C and even down to 15 °C but it is strongly reduced at 10 °C. This important fact must be kept in mind for field trials especially when dealing with small (laboratory-scale) systems that do not afford a good buffer against high or low temperatures.

From the literature it also became apparent that earthworms are particularly sensitive to the presence of ammonia and electrolytes. Blood is rich in both proteins and electrolytes. Deamination of amino-acids in blood proteins could lead to the build up of too high concentrations of ammonia for the earthworms to tolerate (they die off in the presence of between 1 and 2 mg ammonia/g of waste).

The factors referred to above were taken into account in the experiments and the design of the earthworm systems.

3. MATERIALS AND METHODS

The first objective of the project was to design and test a laboratory-scale system for the treatment of abattoir wastes with earthworms while liquid waste in addition to solid waste is put through the system. In Section 2 it was noted that no documentation could be found for the concept to use earthworms to "clean up" wastewater from abattoirs in addition to solid wastes and new methods had to be developed. Thus, especially in the beginning of the experiments, various problems were experienced with the system as planned. For example, the 3-container system in large rectangular plastic trays (0.4 m^2 surface area) was placed in an exposed position on a stand outside in the vicinity of the abattoir. During cold winter nights, the chill factor with the relatively thin layers of vermicompost (10 or 17 cm) cooled the system to such an extent that collection of experimental data became unacceptably slow. Therefore, the decision was taken to conduct the laboratory-scale studies indoors. Problems were also caused by the addition of blood to the "unadapted" system (see Section 3.3.2 below).

As experimentation continued, valuable experience was gained and methods changed over time. General materials and methods are presented below. Procedures followed specifically for experiments with the 3-container systems are described in Section 4 and single-container systems in Section 5.

3.1 Choice and cultivation of suitable earthworms

As already mentioned in Section 2, *Eisenia fetida* appears to be the most suitable earthworm species for degradation of abattoir wastes. It is not only robust, readily cultivated and the best-researched earthworm, but sufficient quantities of *Eisenia fetida* for experimentation were readily available for purchase. Earthworms were purchased from two sources (Mr. PA Frylinck, Pretoria and the Springs abattoir project of WormCo). The identity of the worms was confirmed by Mrs. WJ Meyer.

In all experiments sufficient earthworms (corresponding to a density of 17 000 to 20 000 earthworms per m^2) were used to ensure that a layer 2-3 cm thick of rumen solids were worked through in 24 hours. Occasionally the number of earthworms remaining in a container was estimated (Rieckert, 1990). Since earthworms are not able to work in strong light, the top layers were kept dark by covering them with a black plastic bag.

To ensure that sufficient earthworms were available for later experimentation, earthworms were grown in bulk. For this purpose, plastic meat trays, supplied by Messrs. Henderson, Olifantsfontein, with internal dimensions 81,6 x 46,5 x 26,7 cm (0,4 m^2 surface area) were used. Five drainage holes (5-mm diameter) were drilled in the bottom along the center and more holes added as needed. These containers were those initially selected for outdoors trails with the 3-container system as described above. A versatile stand that consists of stackable components made of angle iron (1-inch angle, 5 mm thick) was built to support the containers. A system with racks and square containers for the growth of earthworms was also borrowed from the University of Potchefstroom.

These were fed with a 2-3 cm layer of rumen contents twice weekly and covered with a black plastic bag to keep them moist and dark. In this way, the earthworms have been successfully grown for 3 years.

3.2 The influence of temperature

As already mentioned, the temperature of the system has a profound influence on the activity of the earthworms and thus their efficiency. Early attempts with (unprotected) smaller containers and subsequent indoor experimentation were described. However, breeding of earthworms in more protected, thicker layers in a large plastic container (1.4 x 1.4 x 1.4 m^3) outdoors proceeded successfully for 3 years. They also survived the 1998 winter and summer in the untested pilot-plant (see Appendix) near the abattoir. According to figures supplied by the SA Weather Bureau (private communication, 1999), during the 1998 winter the average minimum monthly temperatures from May to August ranged between 5.8 °C and 6.9 °C falling below 0 °C only once. The maximum temperature is also important for earthworm growth and the average maximum daily temperature ranging from 24.7 °C to 27.8 °C for the remaining months of the year. Maximum daily temperatures between 30 °C and 33 °C were recorded for 8 consecutive days during February 1998 but is still within the tolerance range of the earthworms. Ambient temperature of course does not reflect the temperature of the earthworm systems.

3.3 Feeding materials and the handling of blood

3.3.1 Rumen contents and water

Preliminary experiments showed the ratio of rumen solids to rumen fluid to be roughly 3:2. Feed mixtures were made up and added in the ratio 3:2 liters of rumen solids: rumen fluid. Especially in early experiments, tap water was added in manageable quantities. Addition of feeding material was made manually except with the prototype apparatus where the liquid mixture was irrigated onto the surface layer. Before measurements started, the system was usually run for two weeks to bring it into a steady state.

3.3.2 Blood

Initially the addition of blood to the system created problems. At the start of experimentation, blood was added to the 3-container system (in rectangular plastic trays) at a rate of about 1 % of the total volume of liquids passing through the system. This caused the earthworms to die 2-3 days after addition. Irrigating the system with large volumes of tap water when signs of stress (earthworms crawling out, development of putrid odour) were observed usually prevented damage to the system. However, when large amounts of water were poured into the containers, the system often became waterlogged and slurries that prevented further drainage, developed. Slurries were manually removed before anaerobiosis set in.

In the well-draining single-container systems, adaptation of the earthworm ecological system to tolerate blood by addition of incremental amounts of blood was attempted. The experiment started with the addition of only rumen solids, rumen fluid and tap water (3:2:6 liters respectively). Afterwards blood was incrementally added starting at 0.01 % of total volume (or 11 ml per 11 l added) till the amount of blood reached about 0.25 % of the total liquid added per day. The ecosystem in the containers apparently changed (e.g. red mites in the system increased substantially) and could better tolerate added blood.

In the prototype apparatus (see below) addition of blood on a daily basis to form about 0.7 % of the effluent flowing into the adapted system, was well tolerated by the earthworms. The expected concentration of blood in the effluent is 0.7 % provided all liquid effluent from the abattoir is mixed before being passed through the earthworm system.

3.4 Analytical procedures

The following analytical methods were used:

- Total and suspended solids as described by Frederickson & Knight (1988)

- Measurements of pH according to the standard methods for compost and water as used by the ARC-Soil, Climate and Water Institute (ARC-SCWI personal communication, 1996)

- COD with a HACH DR/2000 spectrophotometer (HACH, 1990)

4. STUDIES WITH STACKED 3-CONTAINER SYSTEMS

The original concept was to use a system with 3 stacked containers each with earthworms in vermicompost and to percolate the abattoir effluent through the containers. The concept was to allow sufficient contact time to deodorise the effluent. Solid rumen contents accumulate and form a layer on top of the compost in the topmost container. The liquid portion, rich in bacteria, percolates through and may then serve to nourish the earthworms in the lower containers. Outdoor trials in plastic trays were mentioned in Section 3. The present section deals with indoor laboratory-scale studies. The stacked 3-container concept was explored in small containers (stacked flower pots) and the main conclusions confirmed with medium size containers (domestic buckets) since it was expected that results may not be directly transferable from the small containers to a pilot-scale plant.

4.1 Small scale experiments in stacked container systems

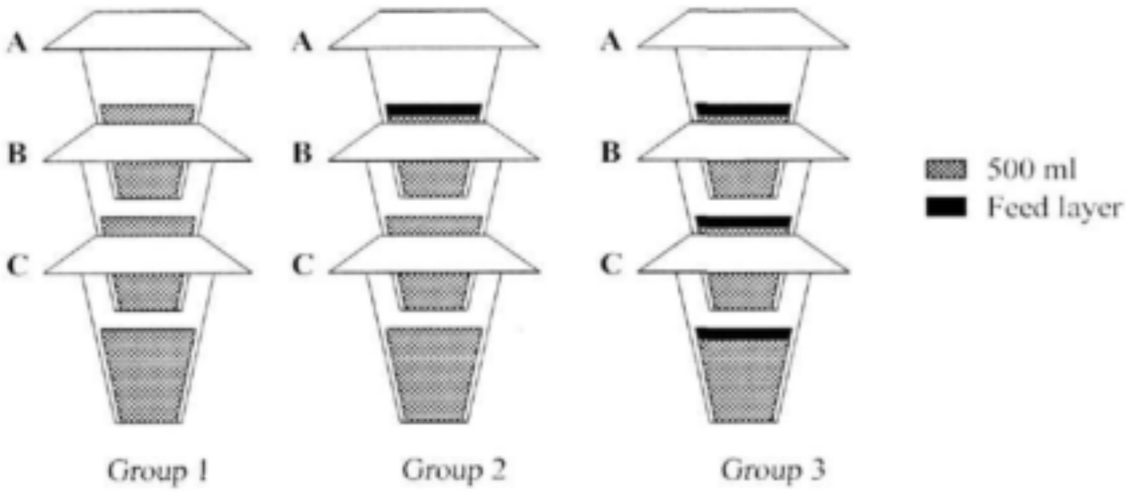
The small stacked 3-container systems consisted of 3 flowerpots mounted one above the other. The pots (conical, 13 cm high, with diameter from 10 to 16 cm) had five 7 mm holes drilled in the bottom. The pots were suspended one above the next by resting the pot in a 12 cm hole cut in it's drip tray as is illustrated in Figure 4.1. The containers had 500 ml of vermicomposted rumen contents and 100 earthworms were added to each.

Group 1 stacks received vermicompost and earthworms at the start of the experiment and afterwards, for the duration of the experiment, they were watered with 500 ml water every working day. In the Group 2 stacks, only the top container (A) was fed every working day with 250 ml rumen solids, 166 ml rumen fluid and 500 ml water. In Group 3 stacks the top container (A) was fed every working day with 250 ml rumen solids, 166 ml rumen fluid and 500 ml water and containers B and C fortnightly with 250 ml solids (see Figure 4.1 Groups 1-3).

The findings were confirmed in a 3-container stack using medium sized containers. Three 15-liter domestic buckets (conical, 30 cm high, with diameter from 20 cm to 26 cm) with five 7-mm holes drilled in the bottom, served as containers (Figure 4.1, Group 4). These were supported one above the other on wooden slats. The containers were filled with 5 l vermicomposted rumen contents and 1000 earthworms were added to each container. The top container (A) was fed every working day with 1.8 l rumen solids, 1.2 l rumen fluid and 24 l tap water and containers B and C fortnightly with 1.8 l rumen solids (see Figure 4.1 Group 4).

Figure 4.1 Schematic drawings of stacked 3-container systems

Experiments in stacked small containers



Feeding substrate (Group 1)

* 500 ml water

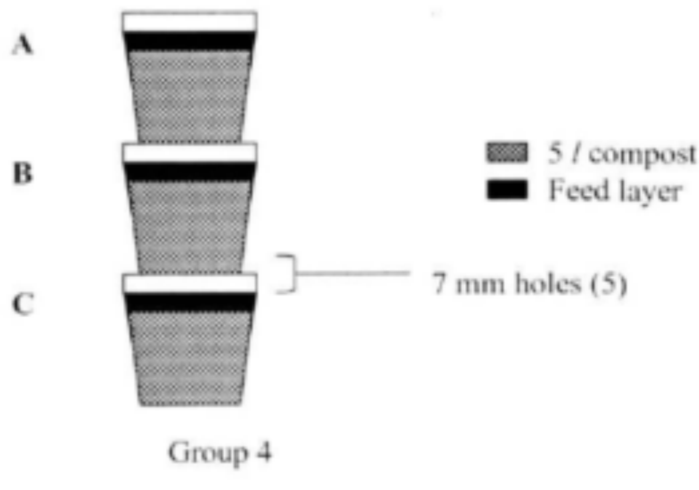
Feeding substrate (Group 2)

* 500 ml water
* 250 ml rumen solids
* 166 ml rumen fluid

Feeding substrate (Group 3)

* 500 ml water
* 250 ml rumen solids
* 166 ml rumen fluid

Experiments in medium containers



Feeding substrate

* 24 *l* water
* 1.8 *l* rumen solids
* 1.2 *l* rumen fluid

4.2 Results

Main results were as follows:

4.2.1 Deodorisation of the effluent

Personal observation showed that the liquid that had percolated through the system became deodorised. It was also found that the liquid after passing through the top container was sufficiently deodorised and did not appear to require further percolation through the system for the purpose of deodorisation.

4.2.2 Chemical Oxygen Demand (COD) values

The results for the COD values and the change in COD values appear in Table 4.1.

Table 4.1 COD (mg/l) and % change in COD (effluent over original feed material) at different stages of the filtering process (Figure 4.1, Group 3)

Days after start	Pot no.	Feed	After A	% change	After B	% change	After C	% change
21	6	15000	1100	-93	1750	-88	1500	-90
	10	15000	1100	-93	1100	-93	1800	-88
	15	15000	1000	-93	1000	-93	1400	-93
	16	15000	1100	-93	1250	-92	800	-95
	20	15000	1200	-92	1400	-91	1500	-90
Mean & SD (day 21)				-92.8 ± 0.5		-91.4 ± 2.0		-91.2 ± 2.8
28	6	11500	1250	-89	1250	-89	1500	-87
	10	11500	1500	-87	1000	-91	1400	-88
	15	11500	1500	-87	1750	-85	2500	-78
	16	11500	2000	-83	1250	-89	1750	-85
	20	11500	1100	-93	1250	-89	1000	-91
Mean & SD (day 28)				-87.8 ± 3.6		-88.6 ± 2.2		-85.8 ± 4.9
Overall mean change from feed				**-90**		**-90**		**-88**
Overall SD				**± 3.6**		**±2.5**		**±4.7**

These results show that percolating the effluent through the system decreased the COD of the effluent. COD reduction mostly occurred in the top container. Typically, a 90 % reduction in COD was obtained after the feed filtered through the top container (A) with on the average no further decrease in COD after filtering through the middle (B) and bottom (C) container of a stack.

4.2.3 Suspended solids (SS)

Table 4.2 shows that the concentration of SS decreased when the effluent filtered through the system. Decrease in SS mostly occurred in the top container and increased slightly when the feed percolated through the remaining containers of the stack. For filtered feed, typically 82 % of suspended solids were removed after percolating through the top container of a stack which remained substantially unchanged with 77 % and 72 % removal after having passed through the middle and bottom containers of a stack respectively (Table 4.2).

Table 4.2 Suspended solids (mg/l) and % change in suspended solids (effluent over original feed material) at different stages of the filtering process (Figure 4.1, Group 3)

Days after start	Pot no.	Feed	After A	% change	After B	% change	After C	% change
21	6	1190	160	-87	190	-84	270	-77
	10	1190	160	-87	250	-79	360	-70
	15	1190	170	-86	220	-82	270	-77
	16	1190	180	-85	220	-82	250	-79
	20	1190	170	-86	250	-79	320	-73
Mean & SD (day 21)				-86 ± 0.8		-81 ± 2.2		-75 ± 3.6
28	6	760	180	-76	190	-75	230	-70
	10	760	180	-76	200	-74	230	-70
	15	760	180	-76	230	-70	270	-64
	16	760	190	-75	220	-71	260	-66
	20	760	--	--	220	-71	220	-71
Mean & SD (day 28)				-76 ± 0.5		-72 ± 2.2		-68 ± 3.0
Overall mean change from feed				**-82**		**-77**		**-72**
Overall SD				**± 5.5**		**± 5.2**		**± 4.9**

4.2.4 Changes in pH

Changes in the pH during the experiment were monitored and results appear in Table 4.3 below.

The trend was for the pH of the effluent to increase slightly on passing through the 3 containers. The pH stayed well within the range tolerated by earthworms (pH 4 to pH 9).

Table 4.3 pH of the feed material and effluent at different stages of the filtering process (Figure 4.1, Group 3)

Days after start	Pot no.	Feed	After A	After B	After C
21	6	6.3	7.3	7.5	7.8
	10	6.3	7.5	7.7	7.8
	15	6.3	7.4	7.6	7.7
	16	6.3	7.3	7.4	7.6
	20	6.3	7.0	7.3	7.6
Mean & SD (day 21)			7.3 ± 1.9	7.5 ± 1.6	7.7 ± 1.0
28	6	7.0	7.5	7.8	8.0
	10	7.0	7.4	7.7	7.9
	15	7.0	7.4	7.7	7.9
	16	7.0	7.4	7.7	7.8
	20	7.0	7.4	7.7	7.8
Mean & SD (day 28)			7.4 ± 0.5	7.7 ± 0.4	7.9 ± 0.8
Overall mean			**7.4**	**7.7**	**7.8**
Overall SD			**± 0.7**	**± 0.4**	**± 1.4**

4.2.5 Further observations

- Some of the earthworms in small containers from Group 1 (Figure 4.1) that received only tap water for the duration of the experiment, crawled out. The remainder of the worms did not appear to be particularly stressed and readily fed on rumen contents when offered to them at the conclusion of the experiment. Many of the earthworms in containers B and C of Group 2 that were not directly fed, migrated to container A that was regularly fed. Fewer earthworms from containers B and C in Group 3 migrated upwards to the regularly fed container A and thus encouraged a more stable distribution of earthworms between the containers in the stacks.
- The earthworms in the middle and lower containers survived but did not remain active unless they were occasionally fed with rumen solids.
- At times slurries were produced that effectively blocked drainage. This was an important drawback to the 3-container system because when the system was flooded, anaerobic conditions rapidly developed and the earthworms either crawled out or died. The system failed unless the water was drained in some other way.

4.3 Conclusions

- The overall conclusion from these experiments was that the system showed promise in that it effectively deodorised the effluent and that the earthworms actively worked through the material.
- Characteristics of the 3-container system were that most of the reduction in odour, COD and SS took place in the top container and that the two lower containers did not contribute substantially to cleaning of the effluent. Activity of the earthworms in the lower containers was also much reduced unless they were occasionally fed with rumen solids.
- The use of a single container that allows for improved drainage of the water needs to be explored (see Section 5).

5. STUDIES WITH WELL-DRAINING SINGLE CONTAINERS

As an alternative to the stacked 3-container system and to obviate problems caused by passing large volumes of effluent through the system, the use of specially designed single containers was explored.

5.1 Experiments in medium size and deep containers with filter systems

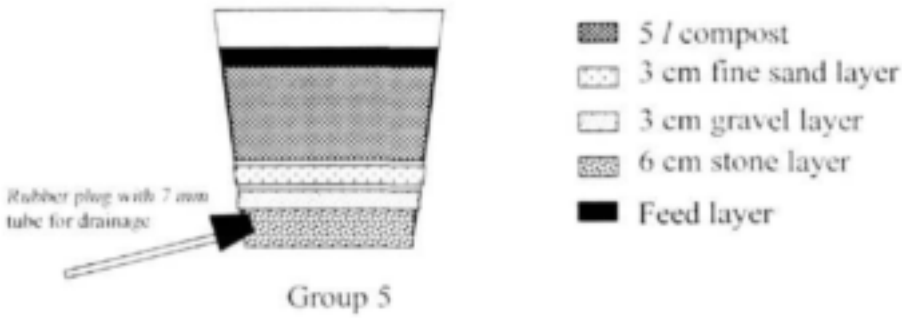
The system of Loehr *et al* (1988) where a sand and gravel filter was used to retain solid wastes in liquid municipal sludge was adopted for the first experiments (Figures 5.1 and 5.2). The medium size container was, as previously (Section 4.1), a domestic bucket. A drainage tube was let into its side (Figure 5.1). It was designed to handle 1.8 l rumen solids with 1.2 l rumen fluid in 24 l tap water per day. Occasionally crust formation on the surface of the material prevented the liquid from draining away when the liquid was manually poured into the container. As remedial measure and to allow rapid drainage, a column of stones in mesh was inserted on top of the sand. However the bucket soon filled up and the experiment had to be restarted.

Deep containers (dustbins) were used for further experiments. These were 32 cm by 32 cm and 52 cm high. The different layers consisted of a 6 cm layer of stones, a 3 cm layer of gravel and a 3 cm layer of sand used for swimming pool filters. Different types of *drainage columns consisting of a 50 mm diameter PVC pipe with large holes drilled into* them and filled to various heights with gravel, were placed at different positions inside the deep container as illustrated in Figure 5.2. These were tested using the same amount of feed (1.8 l rumen solids with 1.2 l rumen fluid in 24 l tap water per day) as in the previous experiment.

The results for the COD and SS in both the medium and deep containers were slightly lower but similar to that obtained for the stacked 3-container system. Results for the COD and SS that were obtained with the different columns were substantially the same and drainage was considerably improved.

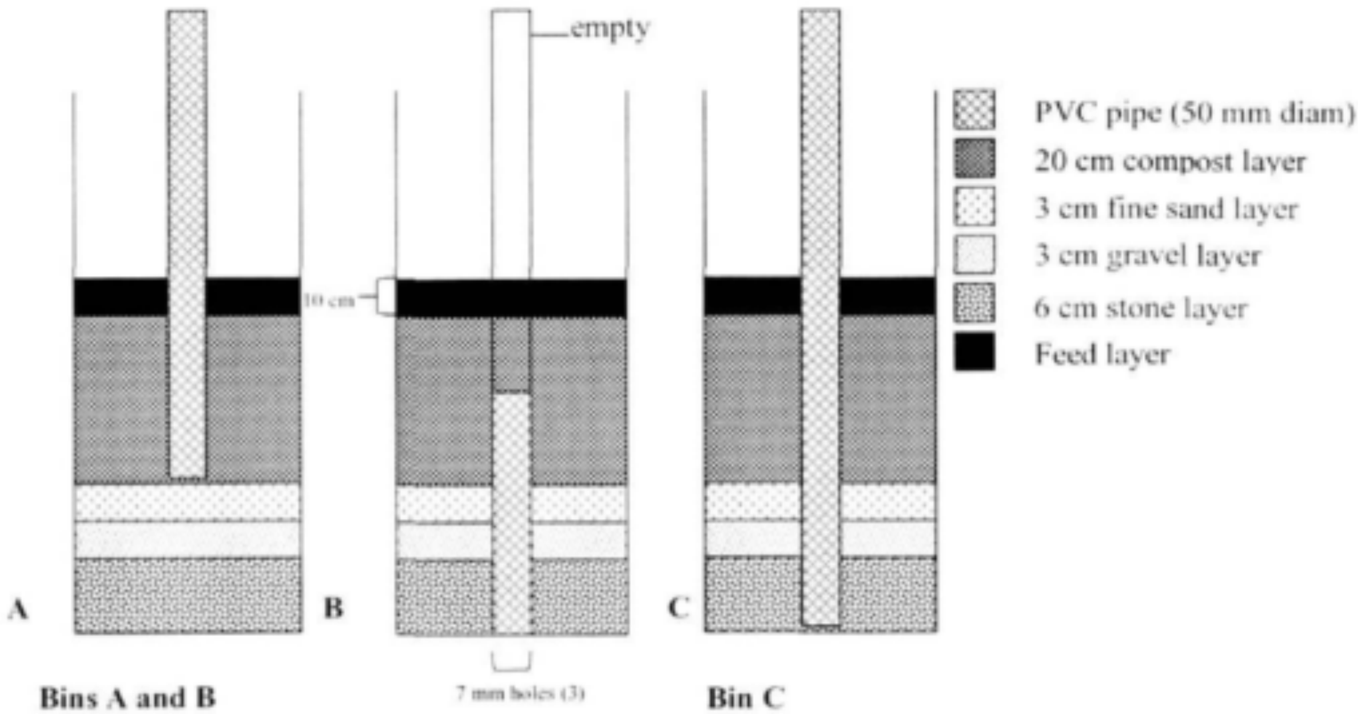
The conclusion was reached that removal with a single container was sufficiently similar to that obtained with the 3-container system for the use of a single container system to be further explored. Both the use of drainage columns and harvesting of the vermicompost were to be addressed in a prototype laboratory scale apparatus.

Figure 5.1 Medium size container with sand filter



Feeding substrate
* 24 *l* water
* 1.8 *l* rumen solids
* 1.2 *l* rumen fluid

Figure 5.2 Deep container fitted with sand filters and different columns



Bins A and B
* 24 *l* water
* 1.8 *l* rumen solids
* 1.2 *l* rumen fluid

Bin C
* 24 *l* water
* 1.8 *l* rumen solids
* 1.2 *l* rumen fluid
* 5 ml blood [additional 5 ml blood every 2nd feeding day up to 75 ml per day]

5.2 Laboratory-scale prototype apparatus

From the previous results it was concluded that a single container was as efficient as the 3-container stack. Thus, a laboratory-scale prototype apparatus that also allowed for the harvesting of vermicompost and that was much nearer to a continuous process was further studied.

The prototype laboratory-scale apparatus, able to deal with about a fifth of the rumen solids from a cattle unit per day (0.25 m^2 surface area), was designed and built (Figure 5.3 below). Previous problems with sufficiently rapid drainage of the liquid, crust and slurry formation and harvesting of the vermicompost were addressed. The unit consists of a main container (51 x 51 cm by 1.2 m high) fitted with 3 drainage columns on each of the 2 sides (6 columns in all). The main container has a sand filter and is fitted with a movable scraper capable of removing a 2.5 cm layer of vermicompost when passed from front to back of the apparatus directly over the sand filter. In this apparatus the system was irrigated with the feed liquid by means of a peristaltic pump that allowed more even distribution of the effluent and control of the rate of effluent addition. The peristaltic pump cannot pump large particles and solids are added separately.

A bottom container catches the water and any solid particles such as compost that wash through the main compartment. It also contains a sand filter and a layer of vermicompost with earthworms through which the effluent is filtered for a second time before drainage. In previous 3-container experiments the second and third containers with vermicompost did not contribute to cleaning of the effluent. Here the bottom container with vermicompost could thus serve as a check for the efficiency of the prototype apparatus.

The prototype apparatus was fed with 6.6 liters rumen solids 4.4 liters rumen liquid and 300 liters of tap water. Solids were placed in the main container and the liquid feed delivered by irrigation over a period of about 3 hours. Harvesting of vermicompost was usually conducted once a week.

After running the apparatus for some time, "blood-adapted" compost was added to the prototype apparatus (see Section 3.3.2). For this part of the experiment, 2.2 l blood was added to the material being irrigated onto the surface. The blood formed about 0.7 % of the liquid passing through the system.

5.3 Results for COD measurements

The results for COD as determined for the apparatus are given in Tables 5.1. The difference between the COD of the feed and effluents is graphically represented in Figure 5.4.

Figure 5.3 Schematic drawings of prototype apparatus

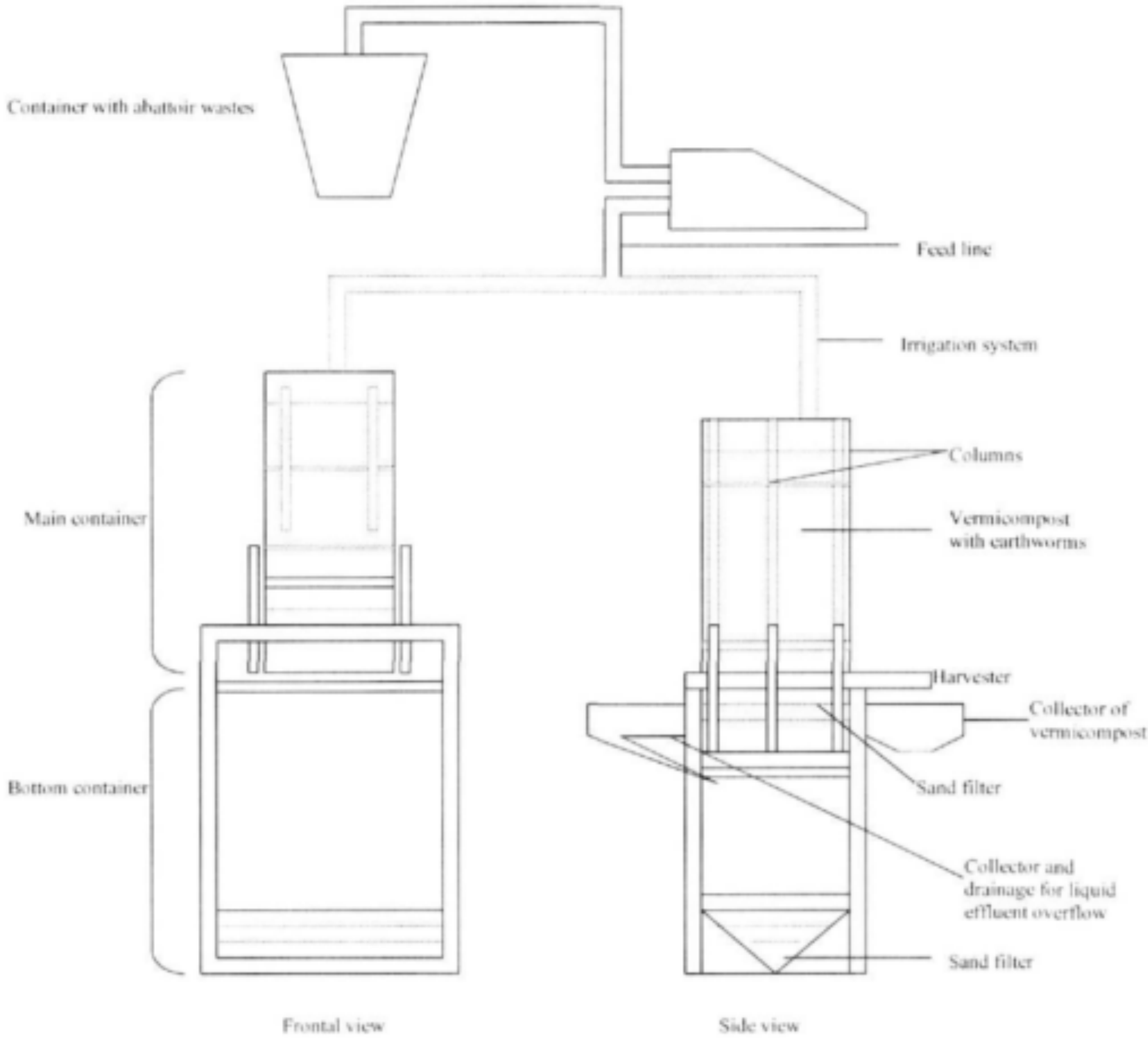


Table 5.1 Prototype apparatus main and bottom containers - COD

COD and % change in COD values of feed without blood * and with blood # after draining through main (effluent 1, first filtration) and bottom containers (effluent 2, second filtration) of prototype apparatus (Figure 5.3)

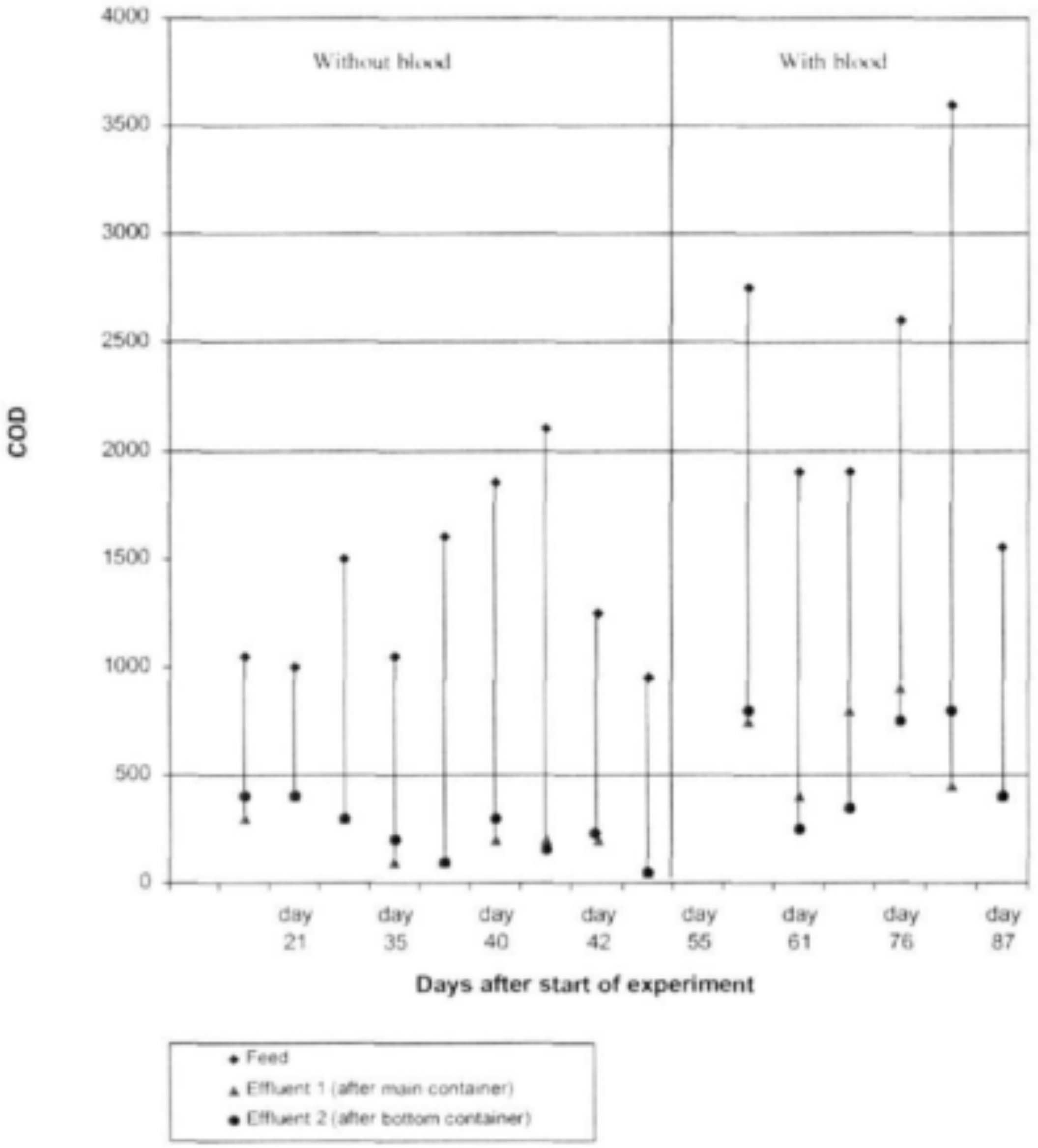
Days after start	Feed COD	Effluent 1	% Change	Effluent 2	% Change
14*	900	450	-50	--	--
17*	1050	300	-71	400	-62
24*	1000	400	-60	400	-60
27*	1500	300	-80	300	-80
35*	1050	100	-90	200	-81
38*	1600	100	-94	100	-94
40*	1850	200	-89	300	-84
41*	2100	200	-90	150	-93
42*	1250	200	-84	200	-84
49*	950	50	-95	50	-95
Mean	**1325**	**230**	**-80**	**233**	**81**
SD	**±417**	**±132**	**±15**	**±125**	**±13**
Blood adapted compost added	**Blood adapted compost added**	**Blood adapted compost added**	**Blood adapted compost added**	**Blood adapted compost added**	**Blood adapted compost added**
59#	2750	750	-73	800	-71
61#	1900	400	-79	250	-87
69#	1900	800	-58	350	-82
76#	2600	900	-65	750	-71
83#	3600	450	-87	800	-78
87#	1550	400	-74	400	-74
Mean	**2383**	**617**	**73**	**558**	**-77**
SD	**±751**	**±225**	**±10**	**±252**	**±6**

* 6.6 liters rumen solids & 4.4 liters rumen fluid & 300 liters tap water added
\# 6.6 liters rumen solids & 4.4 l rumen fluid & 300 l tap water & 2.2 l blood added

Figure 5.4 Experiments in prototype apparatus – comparison of COD measurements

COD values (mg/l) of added diluted rumen contents* # as feed before and after draining through main (first filtration) and bottom containers (second filtration) of prototype apparatus. (X-axis intervals not according to scale)

*Without blood: 6.6 liters rumen solids & 4.4 liters rumen fluid & 300 liters tap water added
#With blood: 6.6 liters rumen solids & 4.4 liters rumen fluid & 300 liters tap water & 2.2 liters blood added



5.4 Further observations

- Suspended solids were slightly reduced (typically from 130 mg/l to 120 mg/l). On standing hardly any solids settles out from the effluent. The low levels of suspended solids are advantageous for subsequent treatment of the effluent.
- The pH of the original material as well as the pH of the filtrates collected 1, 2 and 3 hours after the start of irrigation were measured. The pH of the effluent varied between pH 6 and pH 8 and as before in the 3-container system did not appear to be restrictive for earthworm activity.
- The system can tolerate addition of blood provided it is not too concentrated. Although COD values of the effluent were higher, (up to 900 mg/l), the reduction of COD values was on average 73 ±10 %.
- A second filtration through the bottom container did not reduce the COD further. A second filtration through a layer of vermicompost with earthworms is, therefore, unnecessary.
- The prototype apparatus functioned well and allowed efficient drainage of the effluent and maintenance of a good rate of composting.
- The tests showed that the prototype functioned well and that the concepts are suitable to be incorporated into a pilot-plant.

6. GENERAL RESULTS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Results

- Typically from a feed with a COD of 1325 mg/l an 80 % (±10 %) reduction was achieved.
- The earthworm systems effectively removed the suspended solids. On standing hardly any solids settled out from the effluent. The low level of suspended solids is advantageous for subsequent treatment of the effluent.
- The solids collecting on the surface of the container were efficiently deodorised. The effluent was also deodorised.
- The original concept of using 3 stacked containers did not prove have any advantage for the removal of solid effluents and deodorisation of the effluent. After passing through the top container, no further improvement in the COD values or removal of suspended solids were observed when the effluent percolated through the middle or bottom container of a stack. This trend was observed for small and medium containers (also for large trays).
- A laboratory-scale prototype apparatus was designed in which solutions to previously experienced problems were incorporated. This apparatus was able to deal with about a fifth of the rumen solids from a cattle unit per day (0,25 m^2 surface area). Previous problems with sufficiently rapid drainage of the liquid, crust and slurry formation, harvesting of the vermicompost and distribution of the liquid effluent were successfully addressed. Solid wastes were manually placed into the apparatus.
- The earthworm ecosystem could be adapted to tolerate addition of blood provided it was not too concentrated (blood 0,7 % of the feed liquid). COD values of the effluent with blood were higher than without blood (COD values up to 900 mg/l and 450 mg/l respectively). The reduction of COD with added blood was on average 73 ± 10 %.
- Provided the water could drain away within hours (about 3 hours in the present series of experiments), the earthworms were able to maintain a good speed of composting (10-15 cm per week) even when large volumes of water (similar to the amount of effluent from an abattoir) passed through the system.

6.2 General conclusions and recommendations

- In general it can be stated that the use of earthworms for cleaning up abattoir wastes is technically feasible. It is an attractive system, especially for odour removal and prevention of putrefaction at small (D-Grade and smaller) abattoirs.
- The system works well provided the layer of added solids does not exceed 2-3 cm per day; the liquid drains away fairly fast and aerobic conditions are maintained. The system is simple but adequate supervision to ensure a well maintained system is required.
- The present process opens up the possibility to rid abattoir effluent of solids and to make the resultant liquid effluent more amenable to further treatment with existing

systems. The effluent from the earthworm plant is not yet sufficiently clean to be released into the environment without further cleaning and polishing.

- Successful application in abattoirs will only become a sustainable proposition once it also becomes financially rewarding for the abattoirs to sell the vermicompost and/or to avoid financial penalization for pollution of the environment.
- It can be concluded from the the study that the following aspects still need attention:
  - Testing of the system on a larger (pilot-plant) scale.
  - Determination of sustainable conversion rates by the earthworms, including effect of population density (harvesting).
  - Determining the chemical composition of the effluent from the prototype apparatus. The effluent is brown (even without blood in the feed).
  - Factors that play a role regarding utilisation of blood by the earthworm ecosystem. These could include the maximal amount of blood the system can tolerate and the influence of congealed blood on the system.
  - The chemical composition of the vermicompost. It is foreseen that the large volumes of water passing through the system may leach components out of the vermicompost and that the composition may differ from typical vermicompost from solid abattoir wastes.
  - Biological quality of the vermicompost. The vermicompost may occasionally contain plant/animal/human pathogens or viable plant seeds.
  - Cost-effective ways of pasteurizing (partially sterilising) the vermicompost need to be explored.

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APPENDIX

PILOT-PLANT DESIGN

A pilot-plant was designed and built by ARC-Institute for Agricultural Engineering using the prototype apparatus as model. The pilot-plant is able to deal with 1wrcu per day. It consist of a main container (1m x 1m x 60 cm) with drainage columns and a sand filter, a storage tank for the untreated effluent, a reticulation system and pump for distribution of this feed material onto the compost in the main compartment, a container for the collection of the effluent that had passed through the plant and a harvesting system for the vermicompost. The main features of the design appear in Figures 6.1 and 6.2.

Figure 6.1 Schematic drawing of pilot-plant - View from the side

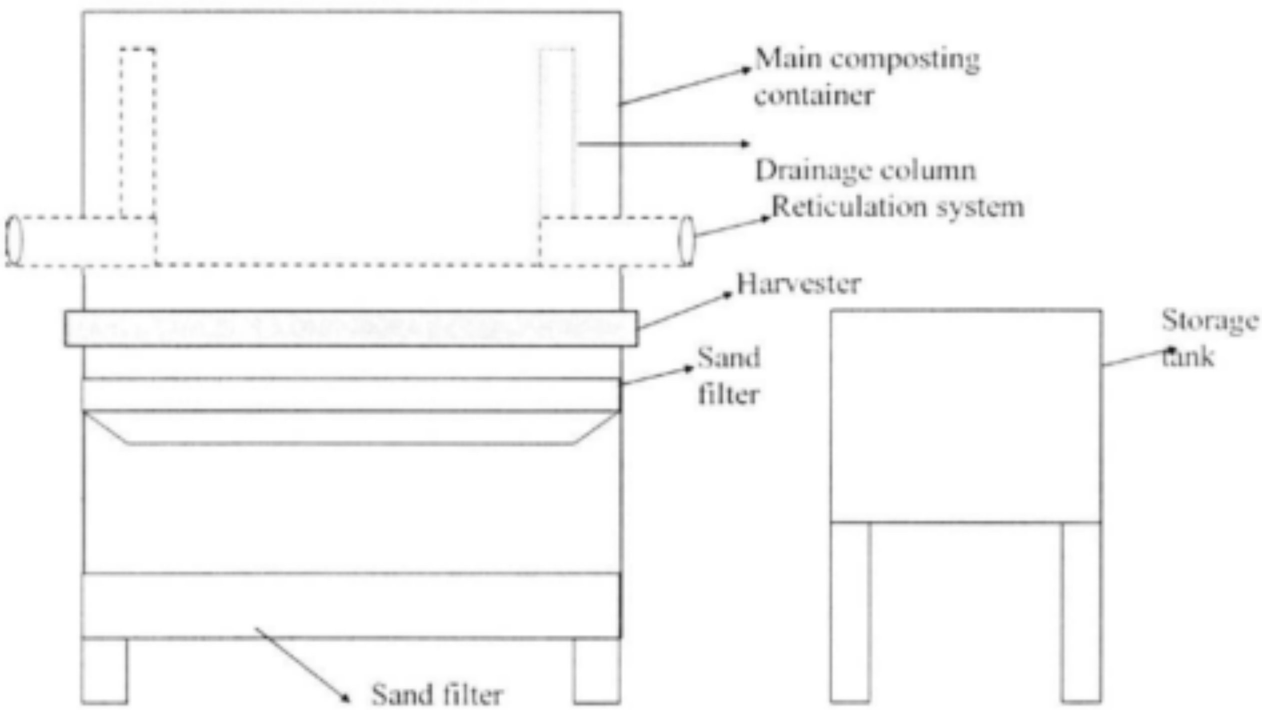
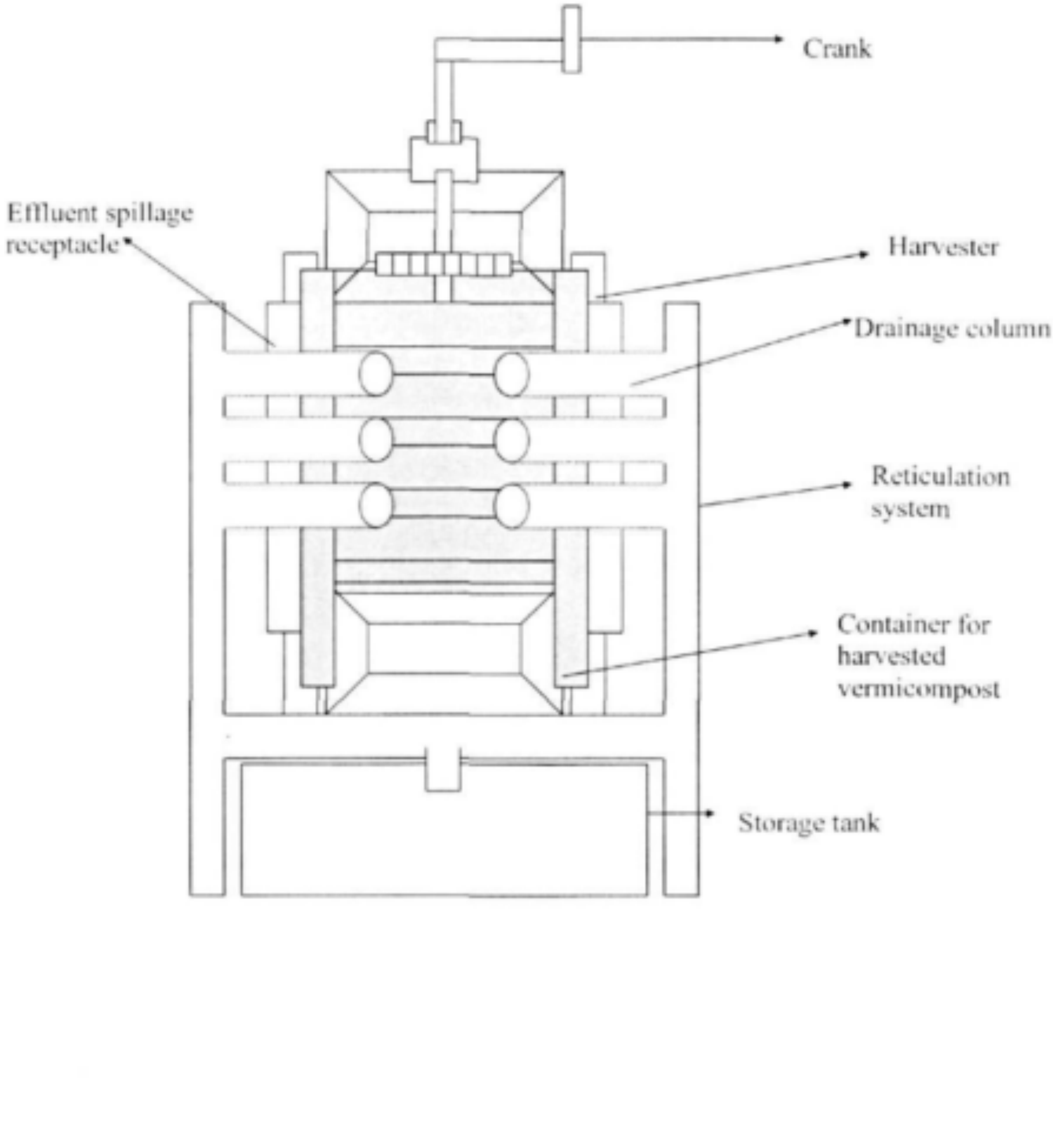


Figure 6.2 Schematic drawing of pilot-plant - View from the top



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