5. Laboratory experiments- vermicast bedding and inoculation

5.1 Introduction

Due to coconut husk being renowned for its water absorbency and being a good bedding for earthworms (Selden et al. 2005) it was used in the previous laboratory experiments. It was speculated that maximising the water holding capacity of earthworm bedding may improve the ability to use hot litter, by diluting the concentration of N. From the previous experiments it was evident that this batch flow system required regular water applications, effectively washing the litter while the earthworms were loaded in the system. The following two experiments incorporated the use of vermicast as the bedding material, opposed to coconut husk used in the previous experiments.

Coconut husk is not a commercially viable approach for mass litter utilisation due to the quantity required to maintain this system on a commercial scale, hence the use of vermicast. Therefore, the following two laboratory experiments were run using vermicast derived from previously vermi-processed litter. Vermicast would be a major output from on-site vermiculture waste management systems, and if suitable for bedding would render the system self sustaining from one cleanout of litter to the next.

With increasing interest in vermimeal production (Dynes 2003, Medina et al. 2003) it was evident that the earthworms produced after the litter had been converted into vermicast needed quantification. It was anticipated that results from experiment 4 would provide an insight into how vermimeal could play an important role in improving the economic viability of this waste management system. Vermicast bedding was also inoculated to determine if litter conversion and earthworm growth could be improved, and was the focus of the fifth and final laboratory experiment.

5.2 Methods

5.2.1 Experiment 4 (vermicast bedding)

This experiment was primarily designed to determine if combinations of earthworm bedding and method of water application (step-down or even) would affect the rate of litter conversion and the production of earthworms. There were two other sub-components, firstly to analyse TN in earthworm bedding over 24 hours on day 5; and secondly to monitor EC, pH, DO and Oxidation-reduction potential (ORP) in the leachate over 45 days. This experiment used 7 treatments and five replicates (Table 5.1) and all 35 containers were loaded and data collected as previously described in section 4.2.

Two bedding types were used and included the previously incorporated coconut husk and a more commercial approach using vermicast derived from litter. The vermicast was collected from a mature bed at the Laureldale Research Station vermiculture trial site (Section 6.3) and analysed for gravimetric water content (Rayment and Higginson 1992). Both bedding materials were also analysed for TKN, EC and pH as described in section 4.2.

Once the containers were established watering commenced using two approaches. For treatments 1, 2, 4 and 6 a step-down approach that had been previously used, provided earthworms with large water applications initially and smaller applications as the experiment progressed. While treatments 3, 5 and 7 had even amounts applied daily except for day 1 where 500 ml was used instead of 400 ml. Irrespective of which approach was used a total of 2100 ml of water was added to all treatments. Treatments 4 and 5 used 30 mm of vermicast bedding as it equated to the same mass as 60 mm of coconut husk on a dry weight basis. Treatment 2 was loaded with dry coconut husks, the same as previous experiments and used a step down watering approach (Table 5.1). This experiment used pre-wetted coconut husks since concerns were raised over previous experiments starting with dry husks. It was thought that salty leachate might have been drawn up into parts of the coconut husk bedding that had not fully saturated, exacerbating earthworm stress.

Table 5.1 Treatment variables for experiment 4

					Water (ml)					
Treatment	Bedding	Depth (mm)	Pre-wetted	Watering	Day 1	Day 2	Day 3	Day 4	Day 5	Total
1	coconut husk	60	yes	step-down	500	700	500	300	100	2100
2*	coconut husk	60	no	step-down	500	700	500	300	100	2100
3	coconut husk	60	yes	even	500	400	400	400	400	2100
4	vermicast	30	yes	step-down	500	700	500	300	100	2100
5	vermicast	30	yes	even	500	400	400	400	400	2100
6	vermicast	60	yes	step-down	500	700	500	300	100	2100
7	vermicast	60	yes	even	500	400	400	400	400	2100

*Coconut husk loaded the same as in previous experiments

On day 5 all treatments had a bedding sample taken at 6 hourly intervals as well as before and after watering over a 24 hour period. The bedding was then analysed for TKN as described in section 4.2. Samples were taken on day 5 as this was when previous earthworm discomfort was the most severe. As each treatment reached completion the EC, pH, ORP, and DO were recorded. Earthworms were then light retracted and separated from the vermicast and weighed to the nearest gram to determine the total increase in earthworm biomass (Ndegwa and Thompson 2000).

For treatments that experienced unacceptable levels of mortality (>40 deaths), due to inadequate bedding depth, 30 mm of vermicast was added in an attempt to recover the vermiculture process. In such instances bedding depth was increased to 60 mm which was the limit of the containers used.

Treatment 3, husk-60-even (Table 5.1) was chosen during the experiment to focus on how leachate changes during the conversion process. When enough leachate was available in the receptacle a probe was inserted into the liquid and the EC, pH, ORP and DO were recorded. Statistical analysis involved repeated measure AOV using Statistix 8.0™.

5.2.2 Experiment 5 (inoculated bedding)

The aim of this experiment was to evaluate the benefits of inoculating bedding and litter in terms of vermicast production and earthworm biomass. This was achieved by using three bedding types including coconut husk, sterilised vermicast and fresh vermicast, where both vermicast beddings were derived from litter. Each bedding type was then split into two treatments groups with five replicates receiving either water or a specially prepared inoculant (Table 5.2). All containers were loaded and data collected as previously described in section 4.2.1.

The same amount of coconut husk was used as in previous experiments, and started with pre-wetted husk. Vermicast was collect from the Laureldale field trial with a proportion sterilised in a dry heat oven at 200°C for three days (Trevors 1996). The sterilised vermicast was then re-wetted to equivalent volumetric water content as the fresh vermicast, and then added to the container. The volume of bedding in each container was the same irrespective of the bedding type.

Table 5.2 Treatment variables for experiment 5

Treatment	Bedding type	Water type
1	Husk	Water
2	Husk	Inoculant
3	Sterilised vermicast	Water
4	Sterilised vermicast	Inoculant
5	Vermicast	Water
6	Vermicast	Inoculant

All containers received the same volume of water or inoculant, which included 200 ml/day for the first 10 days then 100 ml/day for the following 10 days, then 100 ml/3 days to the end of the experiment. Inoculant was supplied and prepared under instructions from a vermiculturalist (The Worm Man®), which was as follows. A 20 l drum and an aquarium air pump were used to oxygenate the liquids. The drum was initially filled with water and 500 g of matured vermicast derived from litter, which supplied the microbial component. The inoculating vermicast was not analysed to determine microbial densities or the particular species involved. After each watering event the drum was refilled to the same level and 5 g of both litter and chicken pellets added as a microbial food source. All treatments were run to completion and the biomass of earthworms recorded using the same methods described in experiment 4.

5.3 Results

5.3.1 Experiment 4 (vermicast bedding)

Sample error for all tables is located in Appendix C, while detailed statistical analysis for all figures and tables are in Appendix D.

There were three hypotheses in this experiment. Firstly, by changing the loading procedure and using vermicast earthworm bedding both the conversion of litter and earthworm biomass could be increased. Secondly, the concentration of N in bedding would fluctuate over 24 hours between water applications. Thirdly, the EC, pH, DO and ORP of leachate would change during the conversion process.

Loading approach affected the mass of earthworms grown ($P<0.001$) and the rate of litter conversion ($P<0.001$, Figure 5.1). Water approach had no effect on mass of earthworms grown, but did have an effect on litter conversion rates ($P<0.01$, Figure 5.1).

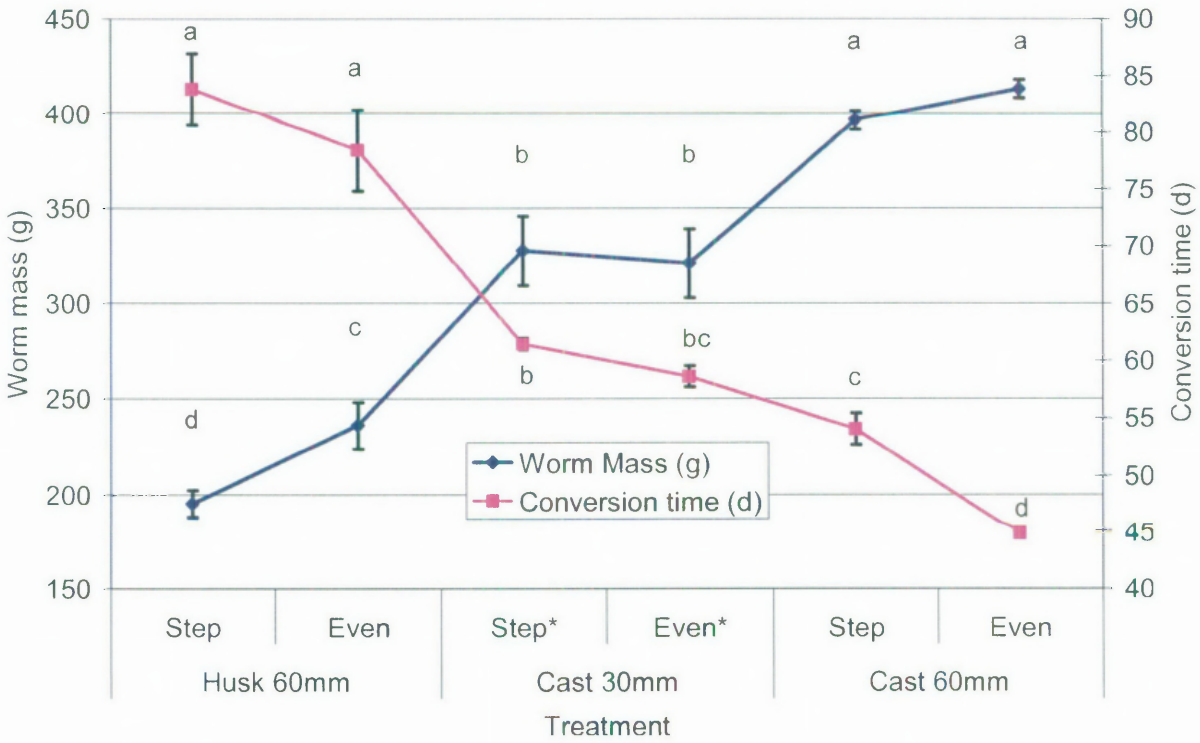

Figure 5.1 The effect of earthworm bed loading and watering approach on earthworm mass and conversion time

Different superscripts show significant differences between treatments ($\alpha=0.05$)

*Note 30 mm vermicast treatments were recovered on day 6 by the addition of 30 mm of extra vermicast.

Loading approach had an effect on total mortality with cast-30-step and cast-30-even reaching highest (P<0.001) earthworm mortality (Table 5.3). Both experimental factors (loading and water approach) had an effect on earthworm dispersion; however there was a treatment effect where using husk-60-even and husk-60-step resulted in a higher dispersion score (P<0.001) than the other treatments (Table 5.3). Both experimental factors had an effect on earthworm retraction rates, with husk-60-even and husk-60-step showing the slowest retraction (P<0.001), while faster retraction (P<0.05) occurred in cast-30-step in comparison to cast-30-even (Table 5.3). An even water approach resulted in similar volumes of leachate collected over the first five days. Cast-60-even and cast-60-step recorded the highest EC (P<0.01) in leachate at completion (Table 5.3).

Table 5.3 The effect of loading and water approach on mortality, dispersion, retraction and volume of leachate over the first 6 days and the EC of leachate at completion

Health & completion data	Husk 60 mm		Cast 30 mm		Cast 60 mm		p-value		
	Step	Even	Step	Even	Step	Even	Loading	Water	LxW
Total mortality	0.0 c	0.0 c	33.4 b	40.2 a	0.0 c	0.2 c	0.0000	NS	0.0437
Dispersion score (1-4)^	1.8 b	2.1 a	1.9 b	2.0 a	1.0 c	1.0 c	0.0000	0.0001	0.0249
Retraction (sec)	27.8 ab	30.2 a	24.4 c	27.2 b	5.4 d	5.2 d	0.0000	0.0274	NS
Mean total leachate (l)	0.630 bc	0.615 a	0.764 c	0.626 bc	0.685 bc	0.545 a	NS	0.0104	NS
EC (mS/cm)	4.75 b	4.60 b	4.44 b	4.72 b	5.00 ab	5.50 a	0.0149	NS	NS

^ Chi-square transformation

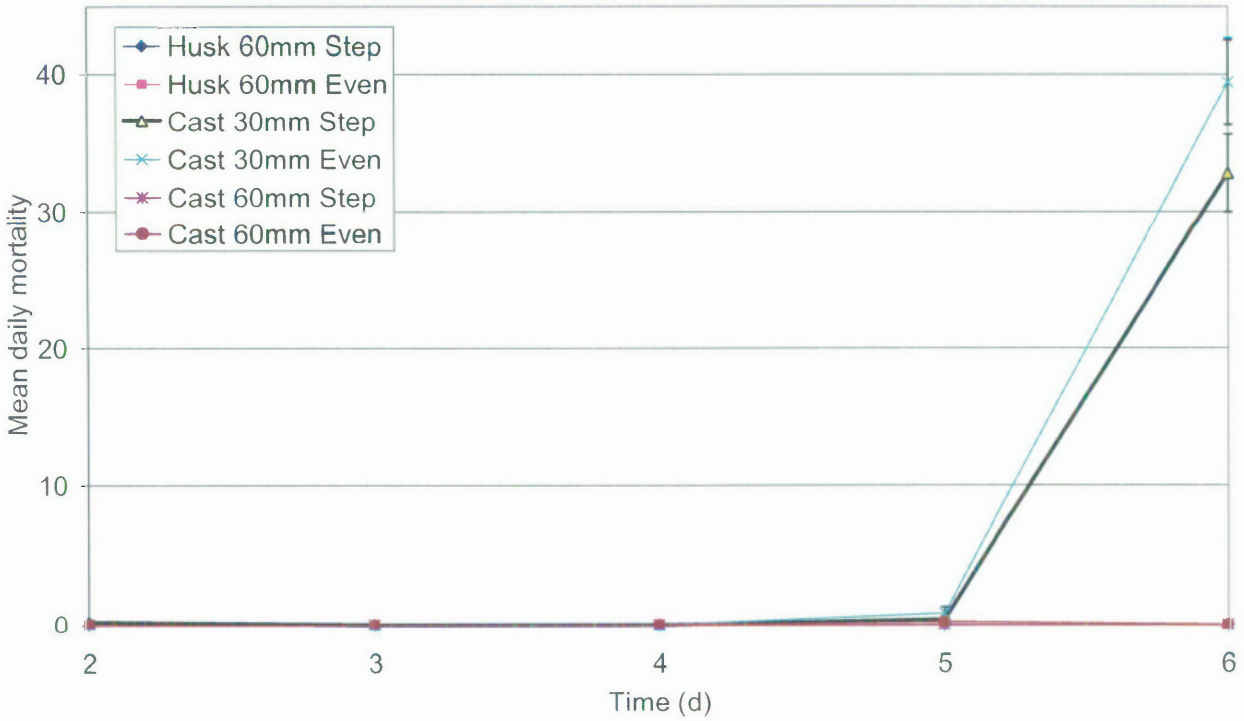

Figure 5.2 Mean daily mortality over first 6 days for all treatments (±SE)

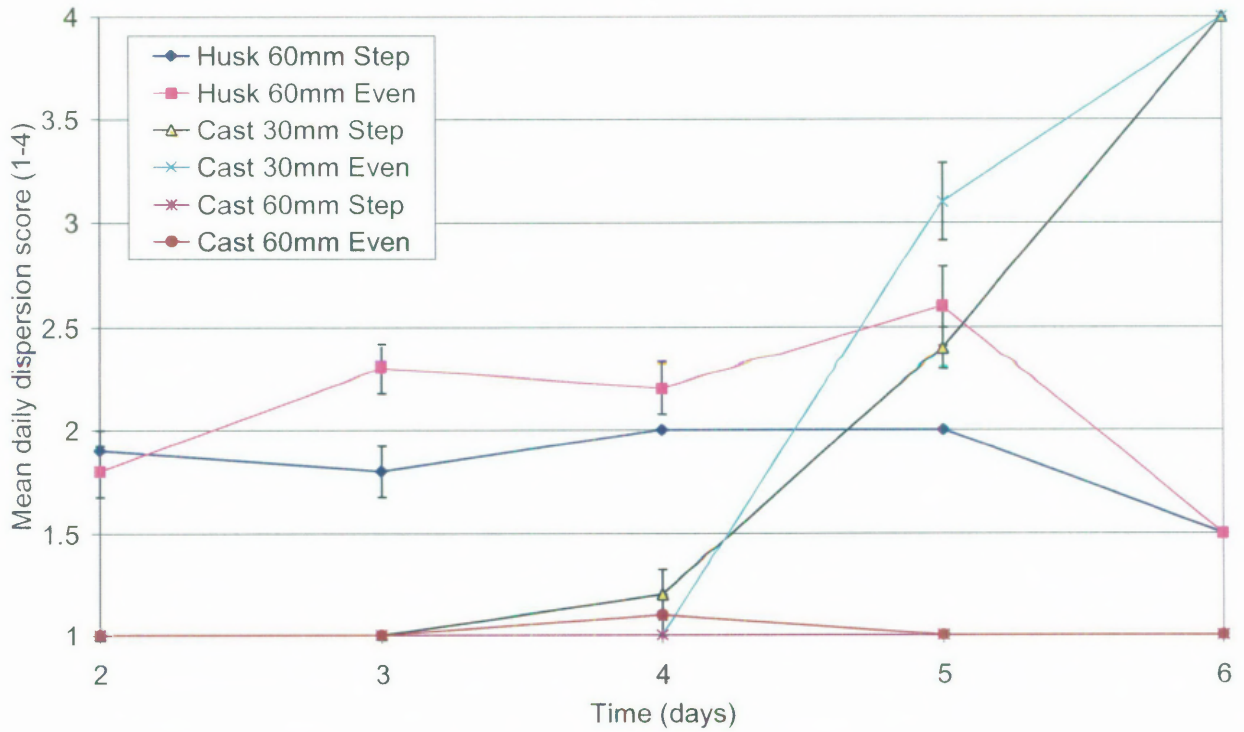

Figure 5.3 Mean daily dispersion over first 6 days for all treatments (±SE)

The time bedding was sampled had an effect on TN concentrations, with the largest decreases ($P<0.001$) occurring 12 hours after watering (Figure 5.5). There was a treatment effect, where cast-60-even resulted in a lower TN fluctuations ($P<0.05$, Figure 5.6).

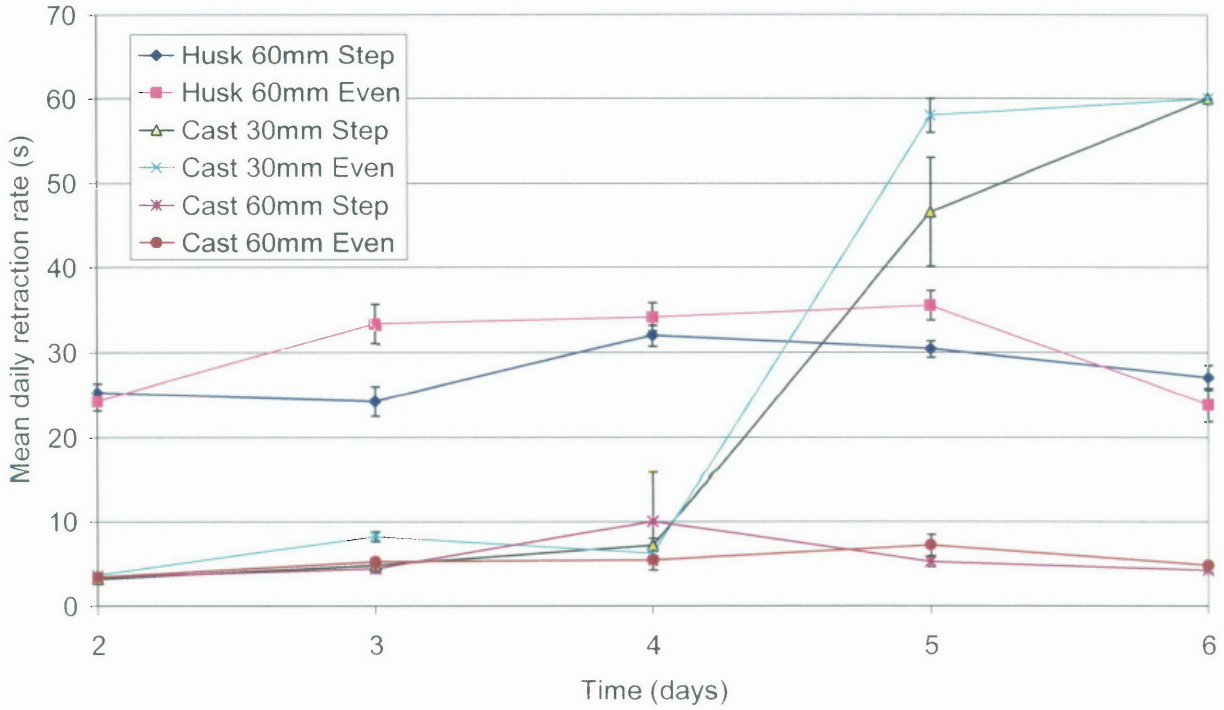

Figure 5.4 Mean daily retraction rate over first 6 days for all treatments (±SE)

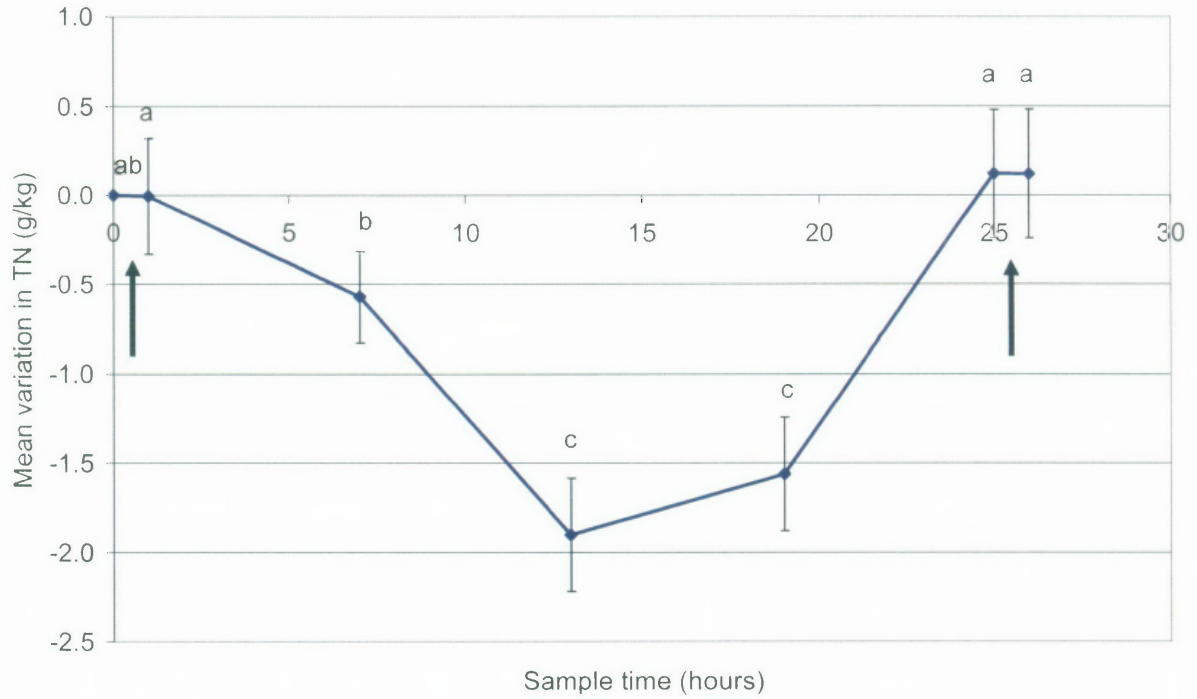

Figure 5.5 Change in TN concentration of earthworm bedding measured over 24 hours averaged for all treatments on day 5, normalised for S1 (±SE)

Different superscripts show significant differences between treatments (α=0.05), arrows indicate when water was applied

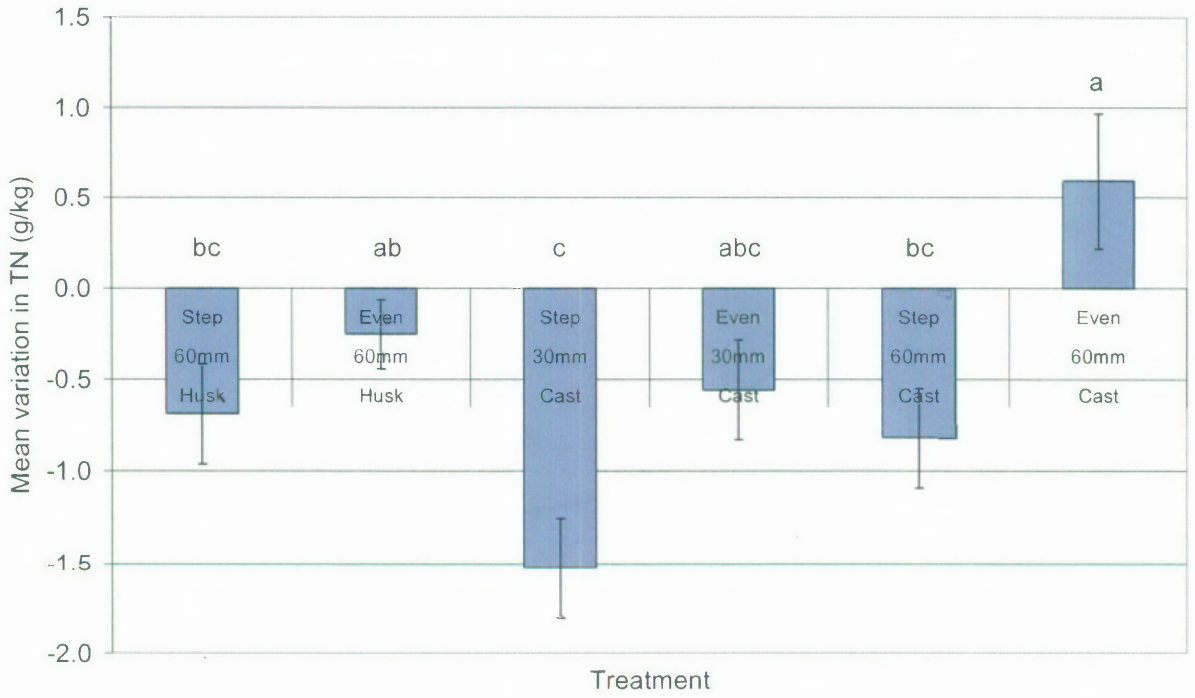

Figure 5.6 Mean change in TN concentration of earthworm bedding measured over 24 hours on day 5, normalised for S1 (±SE)

Different superscripts show significant differences between treatments (α=0.05)

As the conversion process matured the EC of the leachate from cast-60-even increased initially to reach a maximum on day 10 then decreased and remained constant from day 22 onwards ($P<0.001$, Figure 5.7). In contrast, the pH initially fell and then increased from day 14 onwards ($P<0.001$, Figure 5.8). Dissolved oxygen (DO) remained very low during the experiment with some minor fluctuations apparent ($P<0.001$, Figure 5.9), while the ORP of the leachate generally moved towards a more oxidising state ($P<0.001$, Figure 5.10).

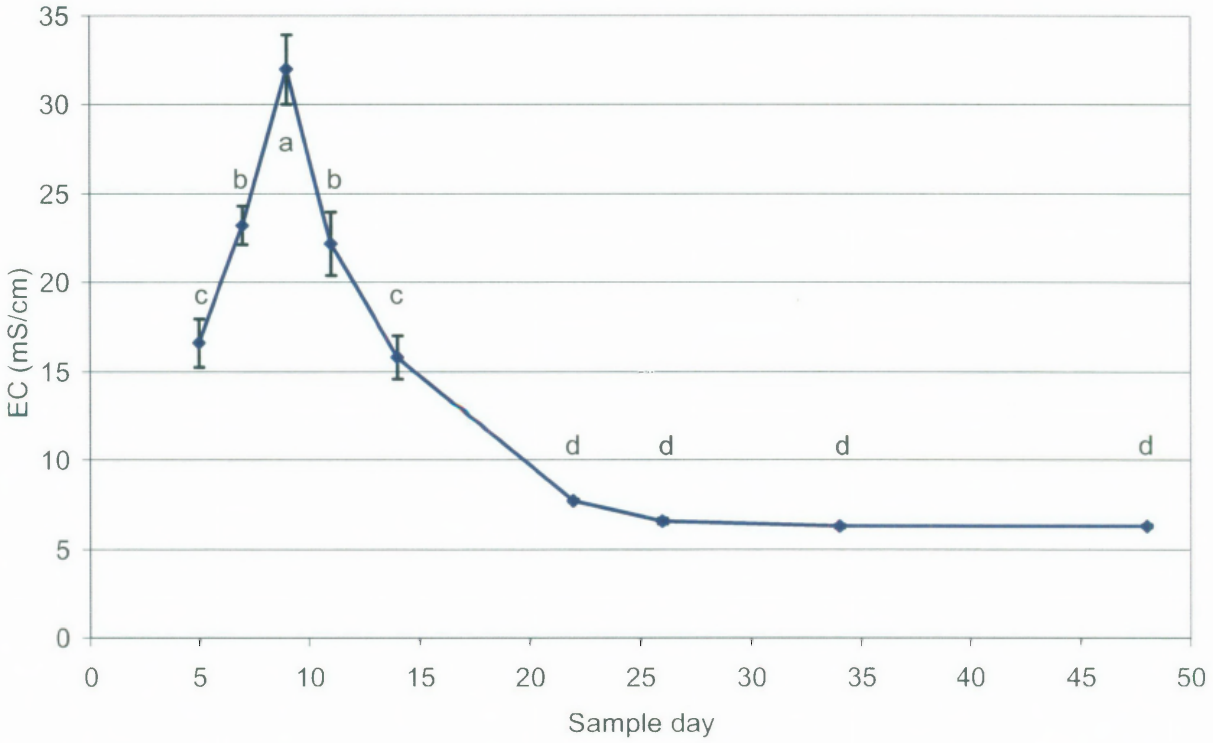

Figure 5.7 Change in EC of leachate from Treatment 7 during the conversion process (±SE)

Different superscripts show significant differences between treatments (α=0.05)

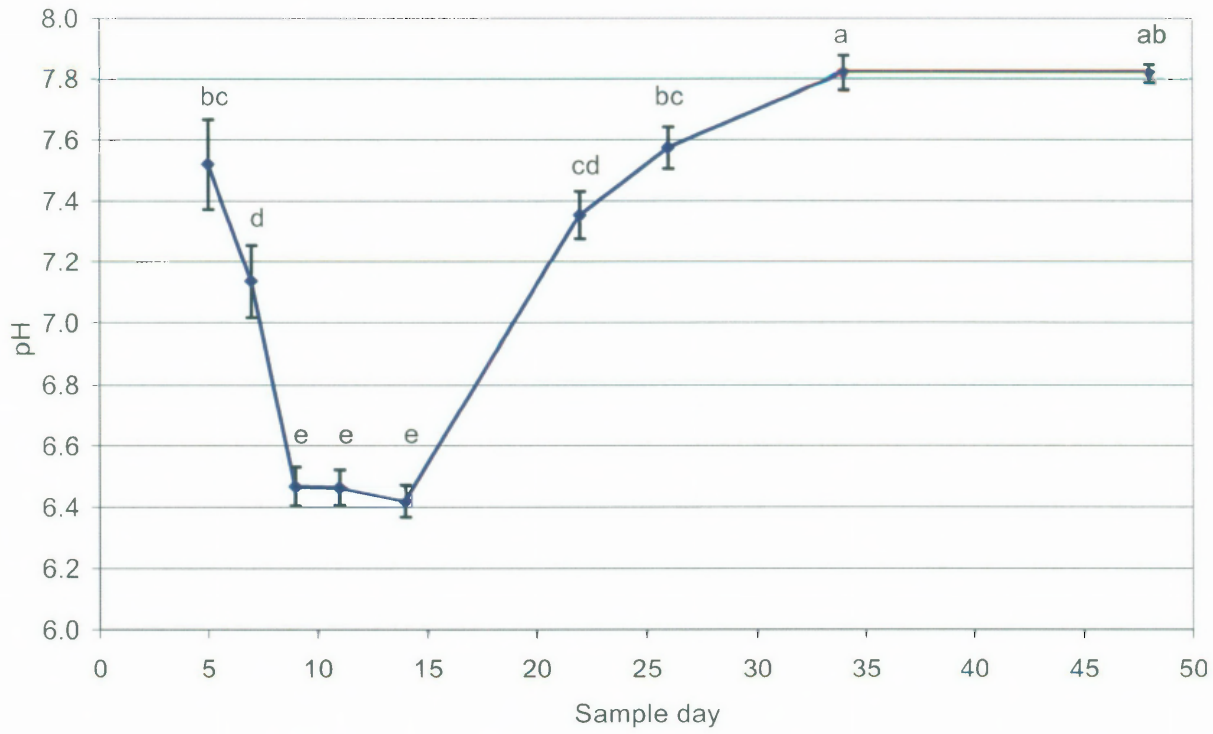

Figure 5.8 Change in pH of leachate from Treatment 7 during the conversion process (±SE)

Different superscripts show significant differences between treatments (α=0.05)

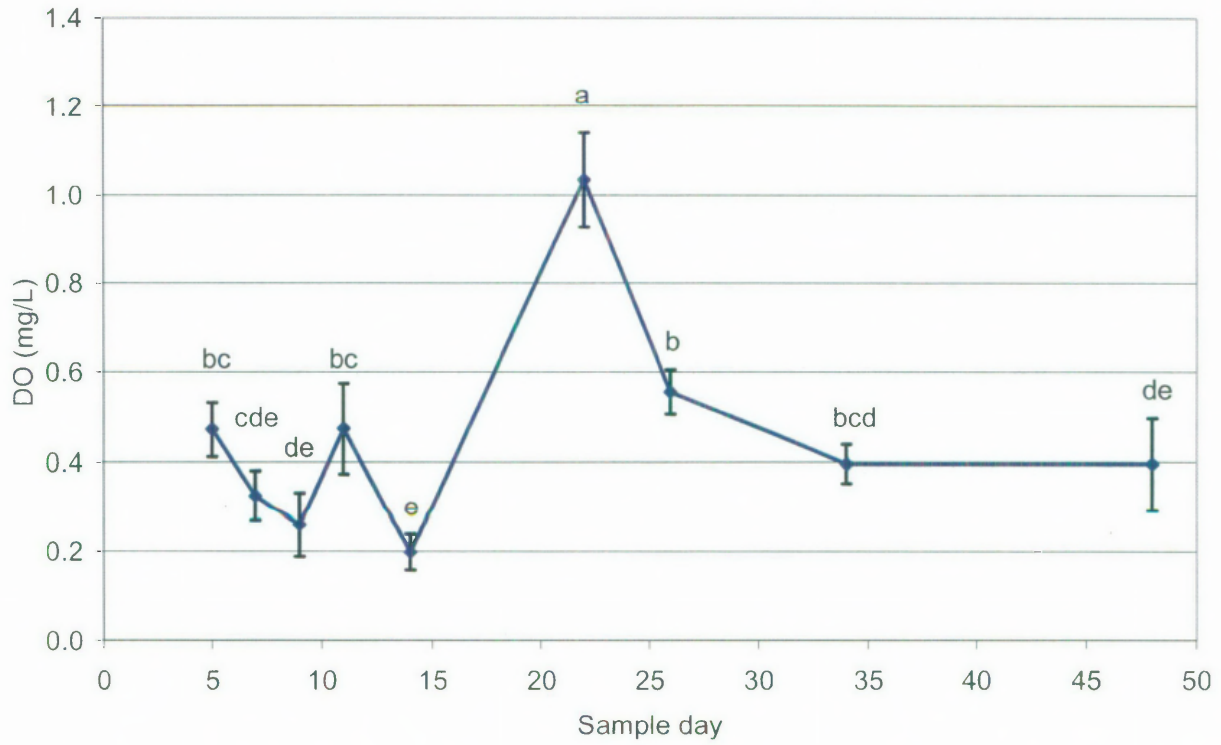

Figure 5.9 Change in DO concentration in leachate from Treatment 7 during the conversion process (±SE)

Different superscripts show significant differences between treatments (α=0.05)

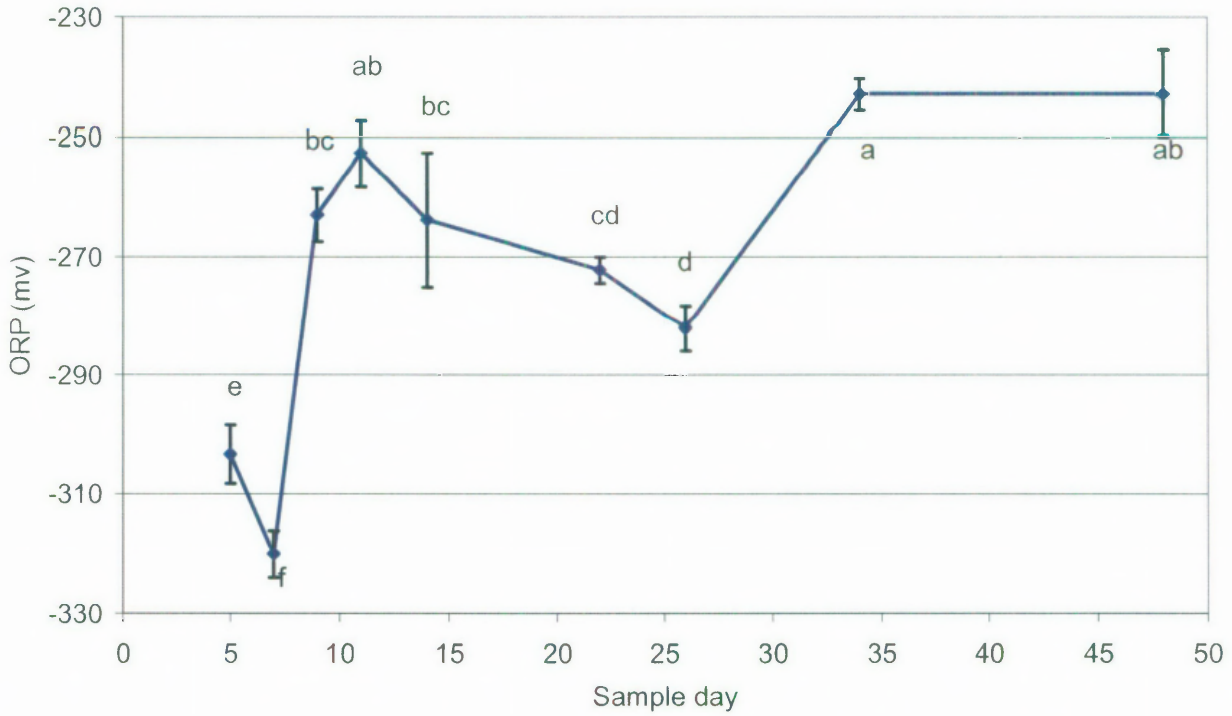

Figure 5.10 Change in ORP of leachate from Treatment 7 during the conversion process (±SE)

Different superscripts show significant differences between treatments (α=0.05)

Pre-wet or dry coconut husk bedding

Starting with pre-wet coconut husk did not improve earthworm biomass ($P<0.05$) at completion, or the conversion rate of litter ($P<0.01$) into vermicast (Appendix D, experiment 4, page liii).

5.3.2 Experiment 5 (inoculated bedding)

The hypothesis of this experiment was that inoculants applied to the earthworm substrate would increase earthworm biomass and improve the conversion rate of litter. As with previous experiments, the completion time was faster ($P<0.001$) and the earthworm mass higher ($P<0.001$) in vermicast bedding as opposed to husk, while the use of inoculant had no significant effect. Sterilised vermicast bedding had not reached completion by day 96, therefore was destructively sampled to determine the percentage of litter converted. This treatment group had achieved 79% conversion on day 96 and again inoculation had no affect.

Mortality and dispersion of earthworms were affected by bedding type ($P<0.001$), while the addition of inoculant had no affect (Table 5.4). Mean retraction rate was lowest in vermicast bedding, and inoculated sterilised vermicast resulted in higher retraction rates than non-inoculated (Figure 5.11). Mean daily dispersion and retraction rates of vermicast were initially high, however compared to other bedding types these parameters were lower (Figure 5.12 and Figure 5.13).

Table 5.4 The effect of bedding and inoculation on mortality, dispersion, completion time and earthworm mass of earthworms

Parameters	Husk		Sterilized cast		Cast		Significance		
							Bed.	Inoc.	Bed. x Inoc.
Completion time (days)	88.7	a	>96		57.6	b	0.0000	NS	NS
Worm mass (g)	166.6	b	NA		366.4	a	0.0000	NS	NS
Mortality (%)	12.3	b	17.2	a	6.3	c	0.0000	NS	NS
Dispersion (score)^	1.9	a	1.9	a	1.6	b	0.0000	NS	NS

^Chi-square transformation

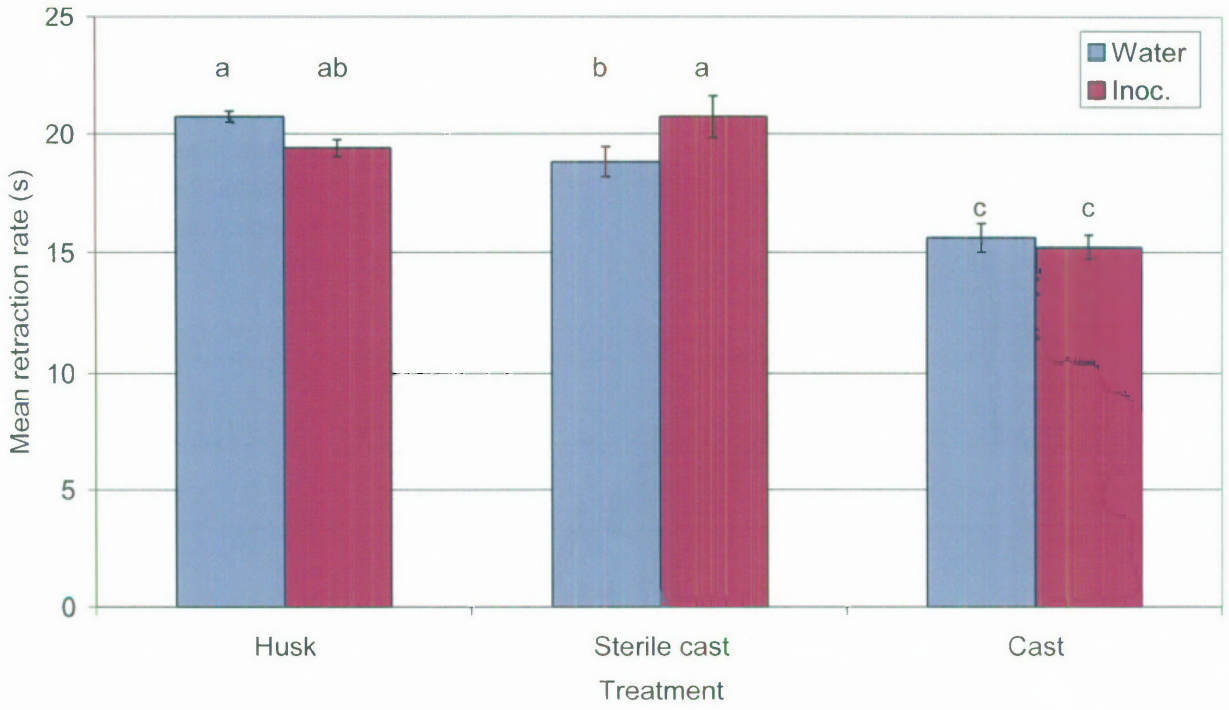

Figure 5.11 The effect of bedding type and inoculation on mean retraction rate of earthworms (±SE)

Different superscripts show significant differences between treatments (α=0.05)

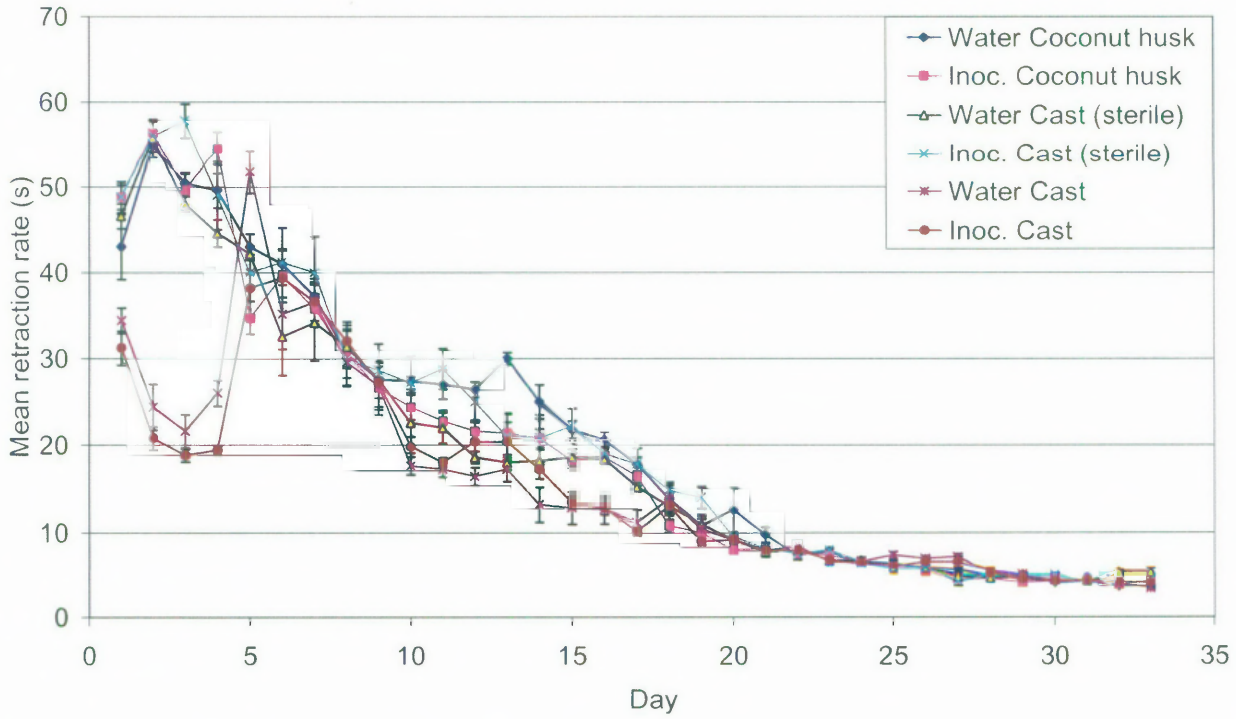

Figure 5.12 Mean daily retraction of earthworms (±SE)

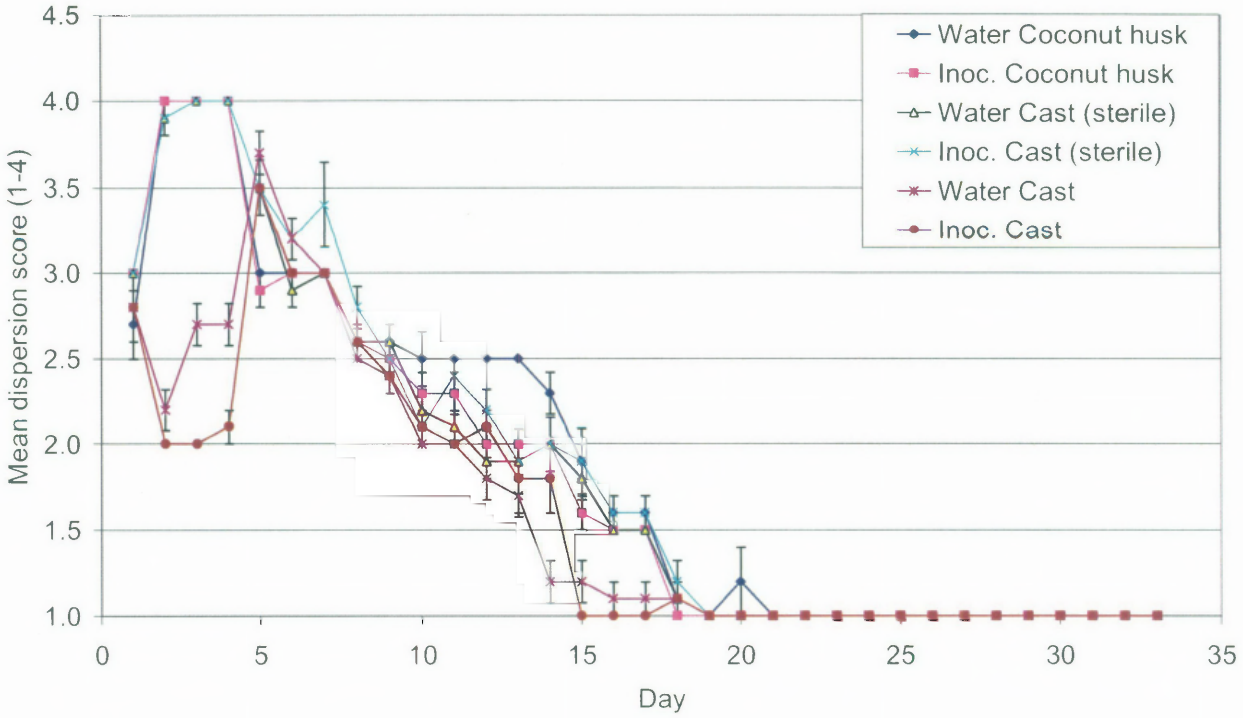

Figure 5.13 Mean daily dispersion of earthworms (±SE)

5.4 Discussion

5.4.1 Experiment 4 (vermicast bedding)

Using vermicast compared to coconut husk for earthworm bedding improved both the biomass of worms harvested and the litter conversion rate, supporting hypothesis 1. The second hypothesis was also supported with the concentration of N in bedding fluctuating over 24 hours between water applications. Finally, the EC, pH, DO and ORP of leachate changed during the conversion process, supporting hypothesis 3.

From a waste management viewpoint, maximising conversion rates of litter into vermicast is the primary objective. Second to this is the potential to harvest earthworms producing a protein rich value-added product from litter. In this experiment when litter conversion rates were maximised, earthworm biomass was also maximised (cast-60, Figure 5.1). This was also supported by the health data, where these treatments resulted in the lowest mortality, dispersion scores and retraction rates (Table 5.3).

In other vermiculture experiments, environmental conditions for earthworms have been improved by either using composted products as earthworm bedding (Garg et al. 2006), or substrates stabilised before adding earthworms (Garg et al. 2005). Therefore stabilised vermicast was chosen as an alternative bedding source to replace the coconut husk. If suitable it would provide both an alternative bedding material and reduce the need to bring other organic products onto a broiler farm.

By viewing daily earthworm health data the sensitivity of this system was revealed. When comparing husk-60 and cast-30, mean earthworm dispersion and retraction scores were similar; however this did not result in the high total mortality for husk-60 (Table 5.3). Daily dispersion and retraction scores indicated there was a problem with the system on day 5 (Figure 5.2, Figure 5.3 and Figure 5.4). The time between when earthworm health parameters responded negatively and the resultant mortality was only 1 day; therefore a commercial operator would have to respond quickly. These results are likely to have been exacerbated due to using small experimental containers which led to problems associated with leachate not draining properly. However, the field trial which was well drained did not show the same sensitivities as the small replicated laboratory experiments (see chapter 6).

Previously in experiment 2, treatments that received water beyond daily applications resulted in high mortality (Figure 4.7). This suggested that an earthworm maybe sensitive to extended

periods of elevated N concentrations in bedding and that daily watering may alleviate this concern. The results from the current experiment confirmed that TN concentrations in bedding fluctuated between daily watering events (Figure 5.5), suggesting that more frequent watering may be a convenient way to minimise salts moving into bedding and protect earthworms during initial critical stages. In the current experiment the cast-60-even treatment provided the lowest TN fluctuations (Figure 5.6), which was interesting considering that this treatment was the first to reach completion and produced a large biomass of earthworms (Figure 5.1).

Both the cast-30-even and cast-30-step treatments experienced high mortality on day 6 (Table 5.3 & Figure 5.2) and instead of terminating these treatments an additional 30 mm of vermicast was added in an attempt to recover the system. This approach was successful as both treatments went on to reach conversion faster and produce more earthworms than either of the coconut husk treatments (Figure 5.1). This was surprising as the husk treatments did not experience initial earthworm mortality (Table 5.3) and this potentially highlights the value of using microbially enriched vermicast for bedding as opposed to sterile husk.

The use of vermicast and stabilised organic residues for earthworm bedding is not uncommon. For example, vermicast was used as earthworm bedding in vermiculture experiments using cattle, pig and supermarket wastes (Gunadi and Edwards 2003). Other vermiculture experiments have required 7-15 days of substrate stabilisation before adding earthworms, to initiate microbial activity and promote organic matter degradation (Biradar and Amoji 2003, Garg et al. 2005). Stabilisation of vermicast probably would not be required due to the already established microbial populations, which the earthworms would harvest. Whereas the sterile husk may have needed to go through a stabilising phase, and during that time the earthworms would have been without a food source.

In the previous chapter two questions arose from experiment 2. Firstly, how does leachate chemistry change during the conversion process, secondly, would a change in leachate composition be useful as an indicator for when the system was reaching stabilisation. The cast-60-even treatment was selected to analyse leachate over the duration of the experiment, and investigated the EC, pH, DO and ORP. The EC of leachate increased in the first 10 days (Figure 5.7) and then dropped to plateau on day 20, which was why the system was possibly more vulnerable during initial stages. Once salts were mobilised, there would have been an opportunity for their movement upward into the earthworm bedding which could be exacerbated if drainage from the containers is poor. Although NH_4^+ was not measured in the leachate it would be expected that salts of N were also being mobilised which would explain early earthworm mortality. The pH of leachate changed during the process (Figure 5.8), however as it remained within the acceptable range for earthworms, it could not explain the initial sensitivity

of the system. Other reports suggest that pH of substrate will tend to become more acidic as the vermiculture process matures (Garg and Kaushik 2005), however this response may not be as apparent when analysing the leachate from vermiculture substrates.

It was expected that the microbial demand for oxygen would be high in such systems and this was indicated by the very low DO in the leachate for the duration of the experiment (Figure 5.9). However, the DO concentration was the same at the beginning and end of the experiment and could not be attributed to initial earthworm mortality. That being said, improving the oxygen availability within the system may improve the conversion process and earthworm health by improving aerobic microbial development.

The ORP of leachate moved to a more oxidising state on day 10 (Figure 5.10) and may indicate that the system was stabilising around this time. *Eisenia fetida* have been shown to survive in anaerobically digested sewage sludge once the ORP is greater than -100 mV (Mitchell et al. 1980). It was possible that microbial development could be responsible for the increase in ORP around day 10. Increasing microbial populations could partially account for earthworm health improving from this time on, possibly due to greater transitions of NH_4^+ to NO_3^-. This threshold was supported by a change in odour to a more biological (yeasty) malodour in leachate around this time.

The same control treatment (husk-60-even) was used for all four experiments and it was considered important to determine if a seasonal effect was apparent due to temperature. Experiments 3 and 4 were undertaken in summer and winter, and the controls reached completion in 35 and 84 days, respectively. The slower decomposition of litter during the cooler months could be attributed to slower microbial growth and longer retention of organic material in the earthworm gut. This temperature affect is supported by Jager et al. (2003) who found that a decrease in temperature by 10°C doubled gut retention times for *E. andrei*. While in India an experiment using green wastes showed slower decomposition during winter months (Sinha et al. 2002). For these reasons controlled experiments using *E. andrei* have maintained temperatures at 21°C (Belfroid and Sijm 1998).

Not only could cooler temperatures cause an increase in conversion time, but also the loading rate of earthworms. In an experiment using winery wastes loaded with 20 g of *E. andrei* (10%), the conversion of 200 g (dry wt) took between 4 and 8 months at 25°C (Nogales et al. 2005). This is significantly longer than the time it took to vermi-process litter in experiments 2, 3 and 4, and could be attributed in part to the higher loading rate of earthworms (19%) used in these experiments.

The EC of the leachate was measured on the day of completion and it was evident that it was similar for all treatments with the exception of cast-60-even. As this treatment was the first to reach completion it was possible that fewer salts had time to be leached from the system (Table 5.3). This would be an important consideration in vermicast consistency as conversion time would influence vermicast analysis. Therefore, maintaining a similar conversion time for each batch may reduce the chemical variability in vermicast between litter cleanouts. Some evidence suggests that adding inoculating agents to the substrate may further improve conversion rates by improving microbial population growth and accelerating stabilisation. Inoculation was investigated in the following experiment.

5.4.2 Experiment 5 (inoculated bedding)

The hypothesis for this experiment was not supported, as there was no improvement in either mass of earthworms harvested or the conversion rate of litter in inoculated treatments. The use of inoculants by vermiculturalists to improve the vermiculture process is not uncommon (Maboeta and van Rensburg 2003). Often inoculants are oxygenated in water for a period of time (24h) and then applied to the earthworm substrate or food (Dynes 2003). However, this laboratory experiment showed no improvement in litter vermi-processing by using inoculants. Trends were also similar to previous experiments where fresh vermicast bedding again resulted in one third faster litter conversion, and finished with twice the mass of earthworms. Similarly to experiment 4, the vermicast bedding showed the lowest mean mortality, dispersion scores and retraction rates (Table 5.4 and Figure 5.11).

Results indicated that inoculants used in this experiment had no affect on the conversion rate of litter into vermicast or earthworm production (Table 5.4). The sterilised vermicast bedding treatments did not reach completion within the experimental timeline, and on day 96 when the last of the husk treatments had completed, only an average of 79% of the litter was processed. It was possible that bacteria spores may have survived the dry heat sterilisation process (Trevors 1996) and that the bedding might have become overpopulated with such species once it was re-wet. Also, changes in bedding chemical properties due to the sterilisation process may have led to earthworm discomfort, however this is only speculative. The sterilised vermicast was also the only treatment to show an effect due to inoculation, evident by higher retraction rates and more stressed earthworms (Figure 5.11). Further microbiological and chemical investigations are required to explain this response.

It was expected that vermicast bedding with inoculation would improve the conversion process; however this was not the case. This may have been due to the vermicast already containing adequate background microbial activity, rendering the inoculants unnecessary. Therefore it was logical to assume that the coconut husk would show a difference when inoculated, as it is

effectively a sterile media and could require the addition of microbial populations. However, the application of inoculants in the husk bedding showed no advantages. Therefore, microbes in the earthworm's gut and excreted cast (Arancon et al. 2003) may have provided sufficient inoculation in this system. Therefore future experiments to evaluate the impact of inoculants should purge the earthworm's gut to eliminate this possible affect. These results suggest that this inoculation approach was of little benefit for this vermiculture system, and there were other more important management issues to consider (water application and drainage).

The sensitivity of this system has been shown to be most critical during the first 10 days and it was possible that earthworm stress encountered at this time strongly impacted on the efficiency of the system. It was evident that the vermicast bedding initially had lower earthworm stress both in terms of retraction rates and dispersion scores (Figure 5.12 and Figure 5.13). This possibly explains why fresh vermicast bedding outperformed the other materials. Previous experiments indicated that vermi-processing of litter using cast as bedding could be achieved within 45 days; however in this experiment completion was reached in 58 days. This was possibly due to a technical error on day 4 where leachate did not drain adequately, causing unnecessary earthworm stress.

Worm health behavioural data for the sterilised vermicast suggested that they were performing similarly to the other treatments over 35 days. However, the slow conversion rates for these treatments indicated that earthworm health data may not always be useful in predicting litter conversion efficiency. In conclusion, both dispersion scores and retraction rates were most valuable during the stabilisation of earthworms in the substrate, when the highest mortality was likely. The extent to which these behavioural characteristics could be used in the field and more importantly the likelihood this vermiculture system could operate at a commercial scale, has yet to be determined, and was the focus of the following chapter.