The toxicity of ammonia/ammonium to the vermifiltration wastewater treatment process

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ABSTRACT

This study was undertaken to assess the toxicity of ammonia/ammonium to key species within the vermifiltration process. The key species, the earthworm Eisenia fetida, was subjected to a series of tests in solid phase mesocosms and full-scale units. The solid phase tests showed a relatively low toxicity to ammonium with ammonium chloride having an LC50 for ammonium of 1.49 g/kg. Ammonium sulfate did not show an effect on mortality at 2 g/kg ammonium. The full-scale units showed that ammonia hydroxide can change the pH and concentration of ammonia in wastewater and while it caused some mortality to the worms its overall effect on system functioning was minimal with no significant difference in terms of worm survival found between treatments. The affect on nitrifying bacteria was also minimal with no linear trend shown with ammonia concentration.

Key words | ammonia, inhibition, toxicity, vermifiltration, wastewater

INTRODUCTION

The toxicity of particular waste components (e.g. pH, ammonia and salt content, heavy metal concentration etc) has been found to limit the effectiveness of vermicomposting, because they can kill the key worm species; however, in regards to vermifiltration systems, the inhibition and toxicity of common wastewater chemicals is largely unknown.

One of the key constituents of wastewater and the principal form of nitrogen in influent is ammonia. Wastewater ammonia has three principal sources i.e. household cleaners and disinfectants, food wastes and urine. The majority of domestic influent ammonia (70–90%) comes from urine, whilst the final 20% comes from cleaning agents, disinfectants and food wastes (Henze 1997). In a cluster or onsite context, where there are fluctuations in wastewater chemistry and mass loading (Crites & Tchobanoglous 1998), it is likely that the discharged ammonia concentration increases with toilet usage and decreases with the shower/bath and/or laundry use. Cleaner use may also contribute to toxicity, especially where there is very little dilution, or the form of ammonia in the cleaner is toxic i.e. un-ionised ammonia (NH₃).

The usage patterns of households may raise the concentration of household influent to above 500 mg/L. For example, fresh urine contains about 580 mg/L of ammonia (Udert et al. 2003b), but increases in ammonia concentration (from urea hydrolysis) at a rate of about 400 mg/L/hr (Udert et al. 2003a). Given these factors, ammonia concentrations in household discharge may potentially reach over 500 mg/L, especially where water conservation strategies such as waterless urinals, urine diverting toilets, low or micro toilets are used. An example of this was found in a residential area of Sweden, where greywater diversion caused the wastewater ammonia level to rise due to a lack of dilution (Palmquist & Hanaeus 2005). The authors noting that the high ammonia concentration was due to the presence of urine in the wastewater.

The toxicity of ammonia from domestic influent to vermifiltration systems is largely unknown but may pose problems to domestic systems. The key species in vermifiltration
systems, the worm species *Eisenia fetida*, is susceptible to ammonia levels present in manure (poultry) around 500 mg/kg (Edwards & Arancon 2004). This species is responsible for converting the organic waste in the vermi-filtration process into a suitable matrix filter; hence, if toxic shock occurs the filter may clog due to the formation of an impermeable sludge barrier.

This study was undertaken to assess the toxicity of ammonia to the key vermifiltration species and to assess the risks from ammonia/ammonium in a variety of forms. Ammonium chloride and sulfate were chosen as the chemicals for the solid phase tests, because they can show the differences in toxicity from interfering ions such as chloride. The inorganic forms were chosen because wastewater largely contains ammonium in an inorganic form unlike manures, which may have more forms of organic ammonium e.g. proteins. Finally, the full-scale test was undertaken with ammonia hydroxide, a common cleaning agent, representative of those on the market. The product was also the most likely to convert the ammonium in the wastewater into a toxic form (e.g. unionized ammonia), which would represent a worse case scenario.

**MATERIALS AND METHODS**

**Solid phase tests**

The tests followed the OECD guidelines for acute toxicity tests (Spurgen et al. 2003) and took place over two weeks in 2L polyethylene containers. Each container received manure and vermicompost inoculate to a combined wet weight of 500g (80% moisture content). At the start of the test 10 worms of the species, *Eisenia fetida*, were placed into each container. The concentration of each ammonium salt added to the mesocosms is detailed below (Table 1). There were three replicates of each salt concentration and the control.

**Mesocosm monitoring**

The mesocosms were monitored for moisture content (MC), pH, electrical conductivity (Ec) and ammonium. Survival was measured by counting the worms and a logistic regression model for the dose–response relationship was produced using the Probit statistic in SPSS. Biodegradation was assessed by approximating the amount of biodegraded material compared to degraded material. The technique involved placing the vermicompost on an open surface and counting the percent of pebbles (~2mm), representing vermicompost, compared to manure.

**Full-scale tests**

The full-scale test was conducted at the Biolytix research site, Queensland, Australia. The units were loaded with the use of a program logic controlled dosing program and received 260L of influent in the morning and afternoon, from the Maleny sewage treatment plant (STP). The STP influent provided a suitable background source of ammonium/ammonia. Additional ammonia was added through the test chemical, cloudy ammonia (20g/L NH₄OH), which represents most ammonia based cleaning agents, besides surfactant based ammonia compounds, on the market. The test employed one control and three dosed units. The units were dosed once at the start of the experiment and monitored over a one month period. The doses were:

1. the recommended dosage (100ml),
2. three times the recommended dosage (300ml),
3. the maximum possible dose i.e. a full container of the product (1,000ml).

The influent and effluent of each unit was measured for pH, dissolved oxygen (DO), electrical conductivity (Ec), chemical oxygen demand (COD), nitrate and ammonia. The recovery time of the system and major mortality events, representing shock loads, were recorded.

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**Table 1** | The ammonium concentrations, number of replicates and worms and time of the tests

<table>
<thead>
<tr>
<th>Salt</th>
<th>Nominal concentration (g/kg)</th>
<th>Replicates</th>
<th>Worms added (10/replicate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>0.1, 0.2, 0.5, 1 and 2</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>NH₄Cl</td>
<td>0.1, 0.2, 0.5, 1 and 2</td>
<td>3</td>
<td>50</td>
</tr>
</tbody>
</table>
RESULTS AND DISCUSSION

Solid phase

The MC between all mesocosms during the test was 72.3% (SD 4.6). The pH values were 7.43 (SD 0.4) and 7.49 (SD 0.2) for ammonia sulfate and chloride, respectively. Both the MC and pH were suitable for worm survival (Hughes et al. 2007). The Ec was also suitable but generally increased from the lower to the higher concentrations as shown in Figure 1, below. In the majority of concentrations, the Ec increased from the start to the end of the experiment. This is most pronounced in the 1 and 2 g/kg ammonium sulphate and the 0.5 g/kg ammonium chloride concentrations. The increase in Ec is largely the result of an increase in ions in the measured Ec extract, which is largely attributed to the increase in ions from the added ammonium salt. The Ec increase at the end of the test would be attributed to the increase in mineralisation from vermicomposting which causes greater ion solubility (Kaviraj & Sharma 2003).

The actual ammonium concentration was lower at the start of the test than the nominal (added concentration) concentration (Figure 2). At the end of the test, the ammonium concentration had reduced to below 0.025 g/kg in all the mesocosms. The conversion of ammonium through nitrification typically occurs over a period of two weeks or less (Broadbent & Reisenauer 1985); hence, in this test it is likely that there was little biological inhibition to nitrifying bacteria, because the ammonium was not present within the expected nitrification time. Some volatilisation may have also occurred.

The logistic regression of mortality and concentration was only completed for ammonium chloride, as there was no mortality observed in the mesocosms containing ammonium sulfate. Figure 3 below shows the ammonium chloride LC50 value at 1.49 g/kg. Surprisingly, both the nominal and actual concentrations show a LC50 of close to 1.49 g/kg, because the mortality observed occurred during the first 24 h when the actual ammonia concentration was highest.

The biodegradation of manure shows a steady decline from the control to the highest concentration (Figure 4). The decline is highly correlated with ammonium sulfate and
chloride having $R^2$ values of 0.99 and 0.86, respectively. The ammonium chloride concentrations tend to show a lower level of biodegradation at the same concentration, when compared to the ammonium sulfate concentrations. This may be due to the additional effect chloride has on earthworm feeding and uptake of food when compared to sulfate. Research on earthworms has shown chloride is readily absorbed through uptake sites to increase internal osmotic pressures and reduce water loss (Edwards & Bohlen 1996). Edwards & Bohlen (1996) noting that the mechanism is particularly important to a terrestrial invertebrate vulnerable to water stress. It is unlikely that sulfate is important for reducing water stress and it may not be accumulated to the same extent; thus, explaining the difference in biodegradation at the same concentration.

The average worm weight increased in most of the replicates from the start to the end of the experiment (Figure 5). The highest ammonium sulfate concentration of 2 g/kg had worms which decreased in weight, whilst the second highest ammonium sulfate concentration of 1 g/kg had a minor weight gain. The worms in the control gained less than half the weight of the worms in the other concentrations, indicating that the majority of worms may have increased in weight due to other factors besides normal growth. In this case, the additional weight-gain shown by the worms, maybe due to the accumulation of chloride and subsequent increase in water uptake.

Full scale

Monitoring

During the test, the COD was the least affected by the ammonia dosing and had a reduction of approximately 70% (SD 15). The Ec increased linearly ($R^2 = 0.99$), from 830 $\mu$S/cm at the control to 1,010 $\mu$S/cm at the highest concentration. The TDS also increased linearly ($R^2 = 0.98$), from 410 mg/L at the control to 500 mg/L in the highest concentration.

The influent concentration of ammonia generally fluctuated over the 4 weeks of the test, averaging 92.1 mg/L NH$_3$-N (SD 23). The concentration of ammonia was highest during the dosing and the following week, with both values above 100 mg/L NH$_3$-N. During the dosing of the cloudy ammonia, the ammonia concentration continued to fluctuate, although the highest concentration receiving 1,000 ml of cloudy ammonia increased above any of the recorded influent concentrations (Figure 6). The average concentration of ammonia in the effluent throughout the experiment was 24.4 mg/L NH$_3$-N (SD 22). The relatively high concentration of ammonia in the effluent and corresponding high SD was attributed to the poor function of the unit receiving the 100 ml dose. This system had received urine from the toilet onsite and therefore had a substantially higher ammonia load; hence, the monitoring of this unit led to erroneous results. Without the data from monitoring the unit
with the 1st dosage, there was an overall concentration of ammonia in the effluent of 10.1 mg/L NH₃-N (SD 9.1), indicating the three other systems had less variation. An analysis of variance on all the units showed that dosage 2 and 3 were not significantly different.

The nitrate concentrations in the effluent of the units receiving dosages 2 and 3 were twice as low as the control. However, given that the overall removal of ammonia was similar for the control, second and third dosage, this may indicate there was more denitrification in the second and third dosages. Vermifiltration systems have been found to nitrify and denitrify ammonia within the same unit, as they provide a heterogeneous environment with many micro-environments, some anoxic (Taylor et al. 2003). Since the dissolved oxygen in the effluent of the control was 1–2 mg/L higher than the dosage 2 and 3 units, this may indicate denitrification was more effectual in the dosage 2 and 3 unit whilst nitrification occurred at a similar level in all three units.

The influent pH showed an increase from the lowest to the highest concentration during the dosing of the units (Figure 7); before and after dosing the influent pH was approximately 7.1 (SD 0.23). During most of the experiment, the effluent of the control had an average pH of 4.5 (SD 0.3), whilst the three other units had pH values of approximately 7.

Worm mortality was only observed in the highest dosage and some of the worms in this unit had skin lesions two weeks after the dosing. Nevertheless, there was no evidence of a large reduction in population numbers, although a slight decline may have occurred, and the population tended to fluctuate throughout the experimental period. To support this, an analysis of variance between the units was carried out (Table 2) and showed that the populations were not significantly different; hence, the system seems to be able to buffer the toxicity in terms of worm numbers from the ammonia entering the system. This may indicate that the systems have areas unaffected by toxic shock, which can supply worms to replenish effected areas, once the toxic shock has passed through the system.

### CONCLUSIONS

There was a low level of toxicity shown by ammonium chloride and sulfate in the solid phase tests; due to this, only one LC50 value of 1.49 g/kg could be calculated for ammonium chloride. The toxicity was largely attributed to the synergistic action of chloride and ammonium. The inhibition of ammonium on the biodegradation of test media was relatively high compared to the toxicity. However, because the ammonium was converted within a period of two weeks in the tests, the inhibition on biodegradation was probably due to other factors such as chloride accumulation. Chloride accumulation was attributed to weight gain in most of the worms.

The full-scale tests have indicated that ammonia based cleaning agents can change the chemistry of wastewater and increase its pH and ammonia concentration; however, the effects on the system were quite minimal. The toxicity of the product to the worms did not effect the functioning of the system. There was also no evidence that the product caused inhibition to nitrifying bacteria. Therefore, in this case, ammonia based cleaning agents do not seem to cause substantial toxic shock, beyond some minor changes.

### REFERENCES


