WATER RESEARCH 41 (2007) 4397–4402

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Helminth eggs inactivation efficiency by faecal sludge dewatering and co-composting in tropical climates

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ARTICLE INFO

Article history:
Received 19 March 2007
Received in revised form
8 June 2007
Accepted 12 June 2007
Available online 15 June 2007

Keywords:
Ascaris
Co-composting
Drying beds
Faecal sludge
Helminth eggs
Trichuris

ABSTRACT

This study investigates helminth eggs removal and inactivation efficiency in a treatment process combining faecal sludge (FS) dewatering and subsequent co-composting with organic solid waste as a function of windrow turning frequency. Fresh public toilet sludge and septage mixed at a 1:2 ratio were dewatered on a drying bed. Biosolids with initial loads of 25–83 helminth eggs/g total solids (TS) were mixed with solid waste as bulking material for co-composting at a 1:2 volume ratio. Two replicate sets of compost heaps were mounted in parallel and turned at different frequencies during the active composting period: (i) once every 3 days and (ii) once every 10 days. Turning frequency had no effect on helminth eggs removal efficiency. In both setups, helminth eggs were reduced to <1 viable egg/g TS, thereby complying with the WHO guidelines 2006 for the safe reuse of FS.

1. Introduction

1.1. *Global impact of helminths (Ascaris and Trichuris) infections*

Intestinal dwelling nematodes, transmitted from person to person, are the most widespread parasitic infections worldwide. *Ascaris lumbricoides* (roundworm), *Necator americanus* and *Ancylostoma duodenale* (hookworms) as well as *Trichuris trichiura* (wipworm) are among the most common worms associated with excreta or wastewater reuse. *Ascaris* and *Trichuris* infections are estimated to range between 1450 and 604–800 million, thereby representing a disease burden of 1.8 and 1.6 million DALYs, respectively (Bethony et al., 2006; WHO, 2002). Helminth infections are particularly gender-sensitive. Due to their pathological effect causing blood loss, *Ascaris* and *Trichuris* reach their maximum infectious intensity among children between ages 5 and 15 years (Bundy et al., 2004).

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doi:10.1016/j.watres.2007.06.024

1.2. *Latrinisation and its influence on the spread of helminth eggs*

In low- and middle-income countries, most urban dwellers (>70%) use mainly on-site sanitation systems, such as unsewered latrines and septic tanks, for excreta and wastewater disposal. Faecal sludges (FS) collected from full installations are generally dumped indiscriminately in neighbourhoods, open canals or reused untreated in urban and peri-urban agriculture. FS contains extremely high pathogen concentrations responsible for the elevated endemic rate of excreta-related diseases, especially among children (WHO, 2006).

In areas where access to sustainable sanitation, i.e. safe storage, collection, treatment and safe disposal/reuse of faeces and urine, is insufficient or poor, parasites spread in the natural environment. This occurs when FS collected from on-site sanitation installations, such as pit latrines, septic tanks and bucket latrines, is reused untreated on farmland, discharged in lakes and streams, or disposed within the household compound. Online data of the WHO–UNICEF Joint Monitoring Programme data show that in both rural and urban areas, a total of 2.8 billion people use on-site sanitation infrastructure. Of that population, at least 40% of urban dwellers (i.e. more than 1.1 billion people) currently use on-site sanitation installations, which are unsustainably operated and maintained in most of the cases.

Most cities in low- and middle-income countries, which can be categorised as "latrine-based cities", rely on such infrastructure for excreta disposal. Ongoing latrine provision programmes, aiming at achieving the MGDs sanitation target, still lack service provision arrangement for the collection/emptying, haulage, safe disposal, reuse or treatment of FS produced by on-site sanitation infrastructures. Hence, many urban dwellers and urban farmers are at high risk of infection in poor sanitation settings. In Accra, Ghana for example, an estimated number of 280,000 urban dwellers (consumers and producers) benefit daily from urban vegetable farming (Drechsel et al., 2006), grown with polluted water, which indicates the number of people that may be at risk from excreta and wastewater reuse in urban agriculture. Despite regular deworming campaigns, the re-infection rate remains high (Gunawardena et al., 2004; Jinabhai et al., 2001).

In Kumasi, Ghana's second largest city (1 million inhabitants), an estimated 60% of the population rely on unsewered latrines and septic tanks, and 38% use unsewered public toilets. Some 500 m^3 FS are hauled daily. To increase the production rate of essential vegetables in urban and peri-urban agriculture, farmers have recently started to reuse urban waste (especially poultry manure, wastewater and FS). These practices are well known to further the endemic situation of parasitic infections (Strauss and Blumenthal, 1990).

1.3. *Factors influencing helminth eggs inactivation*

In principle, all pathogens die off upon excretion except those that multiply in intermediate hosts. Since *Ascaris* eggs are the most resistant, they are used as indicator organisms (WHO, 2006). The residual concentration of helminth eggs in biosolids is dependent on the prevalence and intensity of infection among the population from which FS or wastewater is collected, as well as on various other factors influencing parasite survival. Temperature, dryness and UV light are the main factors influencing die-off. Literature data reveal that helminth eggs can survive 10–12 months upon excretion under tropical climates (Larsen and Roepstorff, 1999; Sanguinetti et al., 2005). Where agricultural use of biosolids is common practice or aimed at, treatment or storage must be designed in such a way as to reduce helminth egg counts and viability to meet the WHO nematode guideline of $\leqslant$1 egg/l (WHO, 2006).

Composting is a low-cost and easy-to-operate potential engineering option for sludge stabilisation in low- and middle-income countries. It is a well-known and widespread process used for organic solid waste treatment and pathogen removal (Jimenez and Wang, 2006). Co-composting of FS and organic solid waste contributes to establishing health protection measures for critical FS management control points, as elucidated in the WHO guidelines (WHO, 2006).

A pilot scheme, commissioned in 2001, combined an FS dewatering treatment step in an unplanted drying bed prior to hygienisation of the dewatered solids by co-composting with municipal organic waste. Co-treatment of FS with organic solid waste appears to be a sustainable approach to recycling the organic waste generated by households and to reduce the amount of sludge and solid waste indiscriminately disposed of in the urban environment. Ghana offers a waste reuse potential, as several urban and peri-urban farmers have expressed their willingness to use recycled urban waste as organic fertiliser, provided it is available on the market at an affordable price (Danso et al., 2002).

This paper reports on the inactivation of helminth eggs and the production of hygienic biosolids from FS and organic waste in Kumasi.

2. Materials and methods

2.1. *Biosolids generation*

2.1.1. *FS drying/dewatering*

FS from public toilets and septic tanks was mixed at a 1:2 volume ratio (total volume about 15 m^3, total solid content 2–3%) and loaded onto two drying beds. The beds consisted of a concrete basin with a 25 m^2 surface area filled with 15 cm of sand (top layer), 10 cm of fine gravel (middle layer) and 15 cm of coarse gravel (drainage layer). The percolate was discharged into a stabilisation pond for further treatment. The drying process was stopped as soon as the total solids (TS) content of the sludge exceeded 20%. The dewatered sludge was subsequently separated from the sand and sent for composting. Biosolids production rate on the drying beds was estimated at 0.15 m^3/m^3 of sludge. Detailed information about the dewatering has been reported in Cofie et al. (2006).

2.1.2. *Co-composting*

The composting plant comprised a concrete platform (approx. 70 m^2) equipped with a drainage system and covered by a roof. Windrows were used to compost the organic waste and

FS. Two composting cycles were monitored between June and November 2003: cycle 1 from June to October 2003 and cycle 2 from August to November 2003. For each cycle, two compost heaps of 3 m^3 each were formed using 1 m^3 of dewatered FS and 2 m^3 of organic waste from local markets (mixing ratio 1:2). The material was thoroughly mixed and watered if necessary before the heaps were formed.

The compost was aerated by turning the heaps inside out. To measure the influence of turning frequency on composting performance, the heaps were turned at different frequencies. One heap (heap 1) was turned as a function of temperature, i.e. during the thermophilic phase (inside temperatures exceeding 55 °C) the heap was turned three times a week and later turned once a week. The other heap (heap 2) was turned every 10 days, irrespective of temperature or other factors. Heap moisture was monitored and adjusted to about 50–60% if necessary.

A bimetal thermometer was used to measure the daily temperature at different locations in the centre and in the upper layers of the heaps. Mean values in the centre and upper layers were also calculated. The "active" composting process lasted for about 60 days. During this period, the heaps were turned and watered. The heaps were left to mature without watering or turning once maturation set in. The temperature gradually decreased to ambient temperature—an indicator of compost maturity. This phase lasted three weeks for cycle 2 and six weeks for cycle 1. At the end of the maturation period, the compost was sieved (1 cm) and bagged.

2.2. Sampling

2.2.1. Raw FS

Subsamples of raw FS were collected from eight different spots in the sludge storage tank prior to loading onto the drying beds. The samples were subsequently mixed and 2 l filled into thoroughly washed plastic bottles for laboratory analysis of TS.

2.2.2. Compost

During each composting cycle, two samples were taken while turning (one from the centre and one from the outer layers of the heaps) and thoroughly mixed with a shovel. About 2 l of this mixture was filled into a polyethylene bag for sampling. No samples were taken during the maturation period. Only the final product was analysed before and after sieving. All samples were immediately transported to the lab (about one-h drive from the plant) and stored at 4 °C prior to analysis.

2.2.3. Dewatered FS

Dewatered sludge was collected and heaped onto the composting platform. Subsamples were taken from three different locations of the heap and mixed to form a 2-l sample, which was subsequently sent to the laboratory for analysis.

All the samples were transported straight away to the lab (about one-h drive from the plant) and stored at 4 °C until analysed.

2.3. Analytical methods

2.3.1. Total solids

About 60 g of each sample were weighed in a Petri dish, dried for 24 h at 105 °C, reweighed and the TS determined in % by dividing the dried weight with the initial weight.

2.3.2. Ascaris and Trichuris eggs recovery

The literature discusses different analytical procedures to recover and detect viable and non-viable helminth eggs. In most cases, accuracy and precision are influenced by media properties or type of helminth eggs to be analysed (Bowman et al., 2003). A WHO task force has been recently assigned to develop a standard guideline.

For this study, *Ascaris* and *Trichuris* eggs were determined in compliance with the US EPA protocol (1999) modified by Schwartzbrod (2003). A QA/QC study reveals that accuracy or percentage of recovered eggs by this method amounts to 75.5% when applied to biosolids analysis (Bowman et al., 2003). In wastewater analysis, when similar flotation solution and centrifugation procedures were used as described in the following paragraphs, Maya et al. (2006) reported a minimum detection level of 1 helminth/l.

Due to high *Ascaris* and *Trichuris* prevalence in FS collected in Kumasi, Ghana, helminth eggs analysis was conducted with 4 g of biosolids. 250 ml of tap water was added to a sample (corresponding to 4 g of dry matter) and the mixture blended with a kitchen blender for 1 min at high speed. The blended sample was poured into a 1-l beaker and topped up with a phosphate-buffered H_2O solution. The mixture was left to settle for at least 3 h or overnight. The sample was subsequently poured through an 80-mesh sieve to remove coarse particles and the sieve was thoroughly rinsed with tap water. The percolate and rinsing water were collected in a 2-l bucket and left to settle for at least 3 h to allow the eggs to deposit.

After settling of the eggs, the supernatant was removed with a water jet pump and the sediment centrifuged in 150-ml tubes for 5 min at 400*g*. The supernatant was poured off and 60 ml of an $MgSO_4$ solution (specific gravity = 1.29) was added to the pellet in each tube. The pellet was resuspended by stirring carefully. On account of the lower density, *Ascaris* and *Trichuris* eggs, i.e. 1.10 and 1.15 respectively (David and Lindquist, 1982), will float in the $MgSO_4$ solution. Ten minutes after $MgSO_4$ addition, the tubes were centrifuged again for 5–10 min at 800*g* (without the use of brake), the supernatant was poured into 2 l of tap water and left to settle for at least 3 h.

After settling, the supernatant was extracted by a water jet pump and the sediment was collected in several 15-ml tubes and centrifuged for 5 min at 800*g*. The supernatant was subsequently poured off and 7 ml of H_2SO_4–ethylalcohol and 3 ml of ethyl acetate were added to each tube. The tubes were shaken several times and centrifuged again for 5 min at 660*g*. The two supernatant layers were carefully removed by a pipette.

2.3.3. Viability test and eggs count

Viability of *Ascaris* and *Trichuris* eggs was determined using the Safranine dying method developed by de Victorica and Galvan (2003). Following the last centrifugation (660*g*) and

supernatant removal, the sample was stained by adding two to three drops of Safranine O (2.5% in H_2O) to the sediment. After 10 min, the tubes were filled with water and centrifuged for 5 min at 800*g*. The supernatant was poured off, the pellet resuspended with water and the tubes centrifuged again. This process was repeated three times. The sediment was then diluted with 0.1 N H_2SO_4 and the total eggs were counted in a Sedgwick–Rafter cell under an at least 100× magnified microscope. If the dye had penetrated the eggs, they were counted as non-viable.

3. Results

3.1. *Prevalence of Ascaris and Trichuris eggs in random FS samples in Kumasi*

Table 1 shows a high prevalence of *Ascaris* and *Trichuris* eggs in raw FS collected in Kumasi, with a viability amounting to 58%. *Ascaris* eggs outnumbered (13–94 eggs/g TS) *Trichuris* eggs (2–24 eggs/g TS). There is no apparent connection between type of sample source (public toilet or septic tank) and degree of contamination. Furthermore, according to Amoah et al. (2006), vegetables irrigated with urban wastewater contain higher concentrations of *Ascaris* eggs (55–60%) than *Trichuris* eggs or schistosoma (2–3%). The predominance of *Ascaris* in FS and the environment can be explained by its higher egg production and capacity to survive. Indeed, the female *Ascaris lumbricoides* worms produce 200,000 eggs per day, compared to the 2000–10,000 eggs produced by *T. trichiura* (Feachem et al., 1983).

3.2. *Ascaris and Trichuris removal and viability reduction efficiency*

As shown in Fig. 1, the FS dewatering process on the drying beds was not efficient enough to inactivate all the helminth eggs. A total count of up to 22–38 helminth (*Ascaris* and *Trichuris*) eggs was recovered after dewatering, of which 25–50% were viables. The co-composting process allows one to drastically reduce *Ascaris* eggs concentration to a safety level in all cycles in both operational conditions. Total helminth eggs (HE) counts in final biosolids ranged between 0.2 and 1.7 HE g TS^{-1} (Fig. 1) during the composting process, with a similar pattern for both operational conditions and replicate heaps (Fig. 2). Total helminth count was reduced to less than 20, 10 and 5 HE/g TS after 30, 60 and 80 composting days, respectively.

Table 1 – Prevalence of *Ascaris* and *Trichuris* eggs in Kumasi's (Ghana) raw faecal sludge

	Ascaris		*Trichuris*		Total	
Public toilet sludge						
Sample 1	13[a]	(38)[b]	2	(13)	16	(34)
Sample 2			9	(52)	9	(52)
Septage						
Sample 3	3	(23)	2	(0)	5	(13)
Sample 4	94	(53)	24	(58)	118	(54)
Sample 5	29	(37)	15	(25)	44	(32)

[a] Number of eggs/g TS.
[b] Percentage (%) of viable eggs in parentheses.

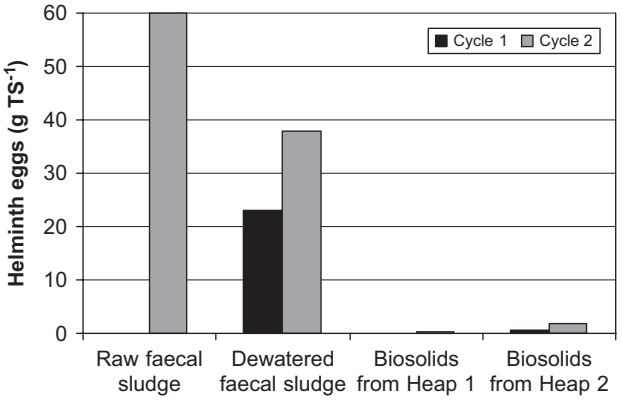

Fig. 1 – Helminth eggs (*Ascaris* and *Trichuris*) removal efficiency in a combined process of faecal sludge dewatering and subsequent composting with organic solid waste.

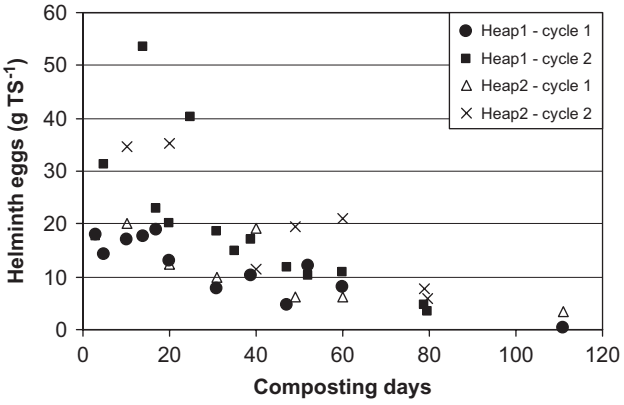

Fig. 2 – Helminth eggs (*Ascaris* and *Trichuris*) removal dynamics during co-composting of faecal sludge and organic solid waste.

As shown in Fig. 3, the temperature at the centre of the compost heaps was maintained at >45 °C (lethal temperature for *Ascaris*) for 40 days, thus indicating the positive effect of temperature on the inactivation of *Ascaris* eggs. In both setups, viability of *Ascaris* eggs was reduced from 58% to less than 20% and 10% within 40 and 60 days, respectively (Fig. 4).

3.3. *Thermal effect during co-composting*

The temperature pattern was similar in both experimental setups irrespective of turning frequency (Fig. 3). The inner temperature of the compost heaps decreased from 68 to 65 °C at the beginning of the process and reached about 60 °C 20 days later. In both cases, the inner temperature was equal to or higher than 45 °C during 40 days of composting. The outer temperature exhibited less variation, i.e. from 50 to 53 °C at the beginning of the composting process and 45 °C 20 days

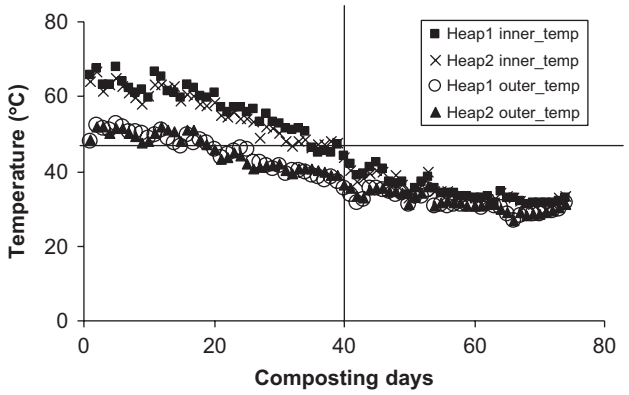

Fig. 3 – Temperature pattern during faecal sludge and organic solids waste co-composting.

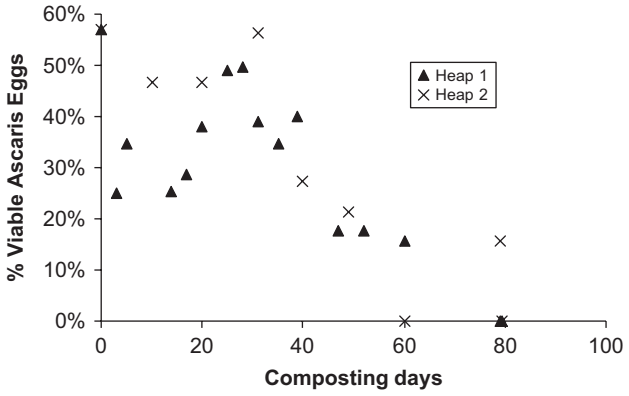

Fig. 4 – Dynamics of *Ascaris* eggs viability reduction during co-composting of faecal sludge and organic solids waste.

later. In both cases, the inner temperature of the heaps equalled the ambient temperature after 50 days.

4. Discussion

The analytical method used for *Ascaris* and *Trichuris* eggs identification was tested to have obtained an average accuracy of 75.5% (Bowman et al., 2003). The high variability observed in the decay of helminth eggs concentration at the beginning of the composting process is attributed to the random distribution in the compost heap when mixed with organic solid waste containing coarse material. However, when the material was digested and particles became finer, variability dropped due to sample homogeneity.

The high concentration of helminth eggs contained in the raw FS could not be inactivated by dewatering on drying beds. The beds were unloaded after 7–10 days, when moisture content reached 80%. Literature data showed that *Ascaris* eggs can survive with such a moisture content in biosolids stored in the environment (Sanguinetti et al., 2005; Stromberg, 1997; Wharton, 1979).

The excellent removal efficiency of *Ascaris* and *Trichuris* eggs observed in this study can be attributed to the good temperature pattern. The high temperature observed in this experiment indicates that dewatered FS and municipal organic solid waste are very good complementary materials for co-composting. Exposure to temperatures over 45 °C for at least 5 days is known to inactivate *Ascaris* eggs. Higher temperatures speed up the desiccation rate of *Ascaris* cells and destroy the cells' ability to slow down desiccation (Cappizzi-Banas et al., 2004; Feachem et al., 1983; Gaspard and Schwartzbrod, 2003).

In this study, an optimum composting period of at least 2 months was necessary to produce biosolids complying with the WHO guidelines of 1 *Ascaris* egg/g TS. *Ascaris* viability was reduced to <10% with a maximum count of <5 eggs/g TS in the final product.

Aside from temperature, a decrease in moisture content in the living environment (i.e. compost heap) reduces helminth larvae's mobility and movement, thus affecting their decay (Sanguinetti et al., 2005; Stromberg, 1997; Wharton, 1979). Basic pH also influences helminth eggs decay in biosolids (Cappizzi-Banas et al., 2004; Gaspard and Schwartzbrod, 2003). In the experiments, moisture content was kept constant at 60% during the active composting period (2 months). However, when the heaps were no longer watered, humidity dropped to 40–50% in the maturation phase (1 month).

The results obtained reveal that the combination of unplanted drying beds and co-composting of subsequently dewatered sludge produce hygienic biosolids safe for agricultural reuse. Hence, to reduce the monitoring costs of co-composting plants in Ghana, only samples displaying more than 10 helminth eggs/g TS in the final product after co-composting are recommended to be processed for the viability test.

5. Conclusions

The following conclusions can be drawn:

- Turning frequency during the active composting period can be reduced since it does not influence helminth eggs removal efficiency but lowers operational costs. In this study, the compost heap turned every 10 days produced the same hygienic compost as the one turned frequently during the thermophilic phase.
- An optimum composting period of at least 2 months was necessary to produce biosolids complying with the WHO guidelines of 1 *Ascaris* egg/g TS, irrespective of turning frequency. *Ascaris* eggs viability was reduced from 40–60% in the raw sludge to less than 10% in the biosolids with a total count of <5 *Ascaris* eggs/g TS (1–1.5 log unit reduction).
- High *Ascaris* removal efficiency (90–100%) was reached after 80 days due to heat generation during the composting process, thus exposing the helminth eggs for more than 1 month to temperatures over 45 °C.

The optimum composting period (60+30 days) is long enough to reduce all helminth eggs. However, when planning

regular use of material for farm application, it is necessary to find a way to reduce the required composting time.

Acknowledgements

The authors express their grateful appreciation to the French Development Cooperation for funding the pilot plant setup, to the Swiss National Centre of Competence in Research North–South for its generous research support, to the Swiss Agency for Development and Cooperation for funding the analytical study, to the Water Supply and Environmental Sanitation Programmes of Kwame NKrumah University of Science and Technology, Kumasi Ghana for their laboratory facilities and, last but not least, to the Waste Management Department of Kumasi Metropolitan Assembly for supplying the solid and liquid wastes.

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