Supplementary Information (SI)

SI1. Standard Operating Protocol (SOP) for the Multiple-Straining Method

The SOP for the multiple-straining method used to analyse and count *Ascaris* eggs from the biodigesters and the control systems. The following steps provide a detailed description of the procedures illustrated in **Fig. 3**:

1. Wear lab coats, face masks, and gloves, and prepare all materials required for the multiple-straining method according to a checklist.
2. Place a graduated plastic cup on a digital weighing scale, tare to zero, and weigh 10 g of the vermicompost sample using a steel spatula.
3. Add tap water to the 80 mL mark and mix thoroughly using a steel spatula.
4. Assemble the Flukefinder sieve set in order of decreasing sieve size (#1 = 189 µm, #2 = 104 µm, #3 = 59 µm) from top to bottom.
5. Position the Flukefinder sieve set slightly slanted over a 3 L graduated plastic jug, ensuring the top of the sieve is away from personnel, and the holes face upwards.
6. Gradually transfer the well-mixed vermicompost sample from the graduated cup onto the top of the Flukefinder sieve set, gently shaking the sieves by hand or against the side of the jug to facilitate passage. Care should be taken to prevent overflow.
7. Rinse the graduated plastic cup three to five times with tap water and repeat the transfer procedure.
8. Sequentially rinse each sieve (#1 to #3) with tap water until the effluent passing into the 3 L jug appears visually clear.
9. Carefully pour the collected material from the jug onto a 20 µm sieve, holding the sieve by hand and gently shaking to distribute the sample.

10. Rinse the jug three times, pouring the rinse over the 20 µm sieve as described in step 9.

11. Collect the material retained on the 20 µm sieve and transfer it to a plastic cup by rinsing with tap water.

12. Transfer the combined sample from the plastic cup into 50 mL plastic conical tubes.

13. Centrifuge the tubes at 5,000 revolutions per minute (rpm) for 5 minutes.

14. Carefully decant the supernatant until the remaining sediment volume is less than 5 mL. If the sediment exceeds 5 mL, repeat centrifugation (step 13) and decantation as necessary.

15. Mix the sediment thoroughly and adjust the volume to 5 mL if required.

16. Take a 50 µL aliquot of the sediment, place it on a microscope slide, and cover with a coverslip.

17. Examine the prepared smear under a microscope and enumerate *Ascaris* eggs.

18. Please note that, similarly, 10 g of sample was used for processing the coir and pig faeces layers. However, for the gravel layer, 1,000 g of sample was processed following the same SOP (steps 1–17), as the total gravel mass exceeded 15 kg in both the biodigesters and control units. The sediment volume (step 14) was kept at 10 mL to facilitate slide preparation and microscopic examination, since reducing it to 5 mL resulted in excessive solids that made slide reading difficult.

SI2. Separation of vermicompost within the coir layer

Post feeding, TWT biodigesters were left undisturbed for an additional time (six weeks for the first experiment; 6, 10, and 14 weeks for the second experiment) to allow the tiger worms to decompose the remaining pig faeces and facilitate vermicompost formation. It was observed that vermicompost was formed in the coconut coir layer, with some of it dispersed into the gravel layer. To separate vermicompost from the coconut coir layer, the following steps were performed:

1. At the end of the resting period, the coir layer from each biodigester was collected separately in a 10 L bucket and labelled accordingly (e.g., B1 bucket, B2 bucket). This layer contained vermicompost, tiger worms, and their cocoons.
2. Tiger worms and cocoons were manually separated from each bucket and transferred into labelled 2 L containers (e.g., B1 tiger worms, B2 tiger worms) containing 250 g of new coir to provide a similar environment for the tiger worms. The labels corresponded to their original buckets to ensure that the tiger worms could be returned to the same biodigester after the vermicompost was separated from the corresponding layer.
3. Now the coir from each bucket was free of tiger worms and cocoons; it was mixed thoroughly and homogenised. Afterwards, fresh coir samples were taken for processing and *Ascaris* eggs counting, while the remaining sand was placed in fibre trays (40 × 28 × 8 cm), which were labelled accordingly (e.g., B1 coir layer, B2 coir layer, etc.).
4. The trays, now containing vermicompost and coir, were kept air-drying at 25 ± 1 °C for five days (**Fig. SI2.1**). This drying step helped distinguish the coir (which will remain fibrous) from the finer vermicompost.

5. Once dried, the contents of each tray were sieved using a round sieve set (30 cm in diameter) with mesh sizes of 12 mm, 10 mm, 8 mm, 5 mm, 3 mm, and 1 mm (**Figs. SI2.2** and **SI2.3**).

Figure SI2.1: Trays with coir and vermicompost kept for drying (left), and dried coir and vermicompost after five days (right).

Figure SI2.2: Separation of coir and vermicompost using different sieves

Figure SI2.3: Distinct visible materials in a tray: vermicompost (left) and coconut coir (right)

6. The sieved vermicompost from each tray (**Fig. SI2.4**) was collected, weighed, and stored in airtight bags for use in the validation experiment.

Figure SI2.4: Vermicompost separated from each biodigester

The vermicompost samples were examined under a light microscope at 100× magnification to assess texture and particle size. The vermicompost resembled dark brown to black soil in colour and texture, with small coir fragments present. The samples were also compared to a reference vermicompost obtained from the earthworm supplier. Both samples showed similar physical characteristics, although the experimental vermicompost contained a slightly higher proportion of fibrous coir.

SI3. Protocol for feeding tiger worms with faeces spiked with viable *Ascaris* eggs

The protocol for spiking pig faeces and feeding tiger worms in the present study was based on a study that assessed STH contamination in soil samples in Ethiopia[49]. In that study, *Ascaris* and *Trichuris* eggs were spiked into soil, followed by the addition of water and thorough homogenisation to validate and optimise the diagnostic method. The following step-by-step procedure is adapted from that methodology.

1. Wear a lab coat and gloves before starting the experiment to ensure safety and prevent contamination.
2. Prepare a 50 mL stock solution of viable *Ascaris* eggs by mixing raw concentrated *Ascaris* eggs with potassium dichromate at a 1:6 dilution ratio. The stock solution was freshly prepared every Monday.
3. Take a 20 µL aliquot from the prepared stock solution and count the number of viable eggs under a 25x magnification. Perform 8 replicates, along with 2 additional replicates by each of two laboratory technicians to assess reproducibility, for a total of 12 replicates. Calculate the mean of all replicates to determine the egg concentration per microliter.
4. Take a 2 mL tube and prepare a 1 mL suspension by adding the appropriate volume of stock solution containing 8,000 eggs (equivalent to 80 eggs per gram[44]). Add tap water to bring the total volume to 1 mL.
5. Weigh 100 g of fresh pig faeces using a 200 mL disposable plastic beaker and a scale, handling the sample with a wooden stick.
6. Mix the egg suspension in the 2 mL tube by aspirating and dispensing three times with a 1 mL pipette to ensure uniform distribution.

7. Evenly spike the egg suspension over the entire surface of the weighed faeces to ensure full coverage.
8. Add 1 mL of water along the inner surface of the 2 mL tube to rinse any remaining eggs and pour over the faeces. Repeat this rinsing and spiking step three times.
9. Mix the spiked faeces thoroughly using a metal spatula by pushing downward 10 times, followed by further mixing to ensure even distribution of eggs.
10. Open the lid of the biodigester and the control systems, spread the spiked pig faeces evenly across the entire surface, covering all sides and corners.
11. Add 20 mL of water to the 200 mL beaker, rinse and mix with the metal spatula, then pour the liquid uniformly over the faeces in the container. Repeat this rinsing step three times to ensure complete transfer of all faeces particles.
12. Wash the metal spatula with detergent and a brush. Discard the beaker, tube, and wooden stick in the hazardous waste bin. Clean the work surface with a sponge and spray disinfectant over the entire area where the experiment was performed.

SI4. Viability protocol: Vital Dye Staining of *Ascaris* eggs

1. A staining solution was prepared by mixing 9 mL of 1× phosphate-buffered saline (PBS) with 1 mL of propidium iodide (1 mg/mL in 0.1× PBS, pH 7.2), resulting in a total volume of 10 mL.
2. From the prepared staining solution, 1 mL was added to the washed *Ascaris* eggs sample previously processed using the multiple-straining method.
3. The sample was incubated on an orbital shaker at 150 rpm for 30 minutes at room temperature, protected from light to prevent photobleaching of the fluorescent dye.
4. Following incubation, 1× PBS was added to the tube to reach a total volume of 50 mL.
5. The sample was centrifuged at 800 x g for 5 minutes to pellet the eggs. The supernatant was then carefully decanted, leaving approximately 5 mL of sediment.
6. A 50 µL aliquot from the prepared sample was pipetted onto a clean glass microscope slide, covered with a coverslip, and examined under a fluorescence microscope.
7. Propidium iodide intercalates into DNA but only penetrates cells or eggs with compromised membranes. Therefore, eggs that fluoresce red under the microscope are considered **non-viable**, while those that do not take up the dye and remain unstained are classified as **viable**.

Table SI5. The analytical performance of the multiple-straining method on the vermicompost

Nº of eggs	Nº of replicates	Nº of slides with a positive test result	Percent of slides with a positive test (95% CI)	Median recovery percentage for positive test (min, max)
0	8	0	0 (0–32.4)	0 (0–0)
50	8	5	62.5 (30.6–86.3)	50.0 (0–150)
100	8	8	100 (67.6–100)	62.5 (50–125)
200	8	8	100 (67.6–100)	81.3 (75–125)
400	8	8	100 (67.6–100)	84.4 (75–125)
Recovery across spiking levels (50,100,200,400)	32	29	90.60%	72%

Table SI6. The analytical performance of the multiple-straining method on pig faeces

Nº of eggs	Nº of replicates	Nº of slides with a positive test result	Percent of slides with a positive test (95% CI)	Median recovery percentage for positive test (min, max)
0	8	0	0 (0)	0(0)
50	8	6	75 (40.9–92.9)	75 (0–200)
100	8	8	100 (67.6–100)	75 (75–150)
200	8	8	100 (67.6–100)	81.3 (75–113)
400	8	8	100 (67.6–100)	84.4 (75–100)
Recovery across spiking levels (50,100, 200, 400)	32	30	93.78%	78%

176 **Table SI7. Total recovered *Ascaris* eggs with (95% CI) in each layer of the**

177 **biodigesters and the control units**

Containers	Coir	Gravel	Effluent	Vermicompost	Pig faeces
B1	133,993 (73,052–194,934)	9,510 (4,460–14,560)	302 (76–528)	4,380 (3,093–5,667)	Not Applicable
B2	121,946 (51,957–191,935)	15,949 (8,279–23,619)	368 (272–464)	4,103 (2,504–5,702)	Not Applicable
B3	117,784 (89,125–146,443)	22,225 (8,896–35,554)	653 (485–821)	3,563 (2,055–5,071)	Not Applicable
B4	122,061 (76,312–167,810)	17,503 (6,092–28,914)	1,150 (267–2,033)	3,961 (1,104–6,818)	Not Applicable
B5	121,360 (67,760–175,200)	22,821 (10,005–35,637)	300 (76–524)	3,171 (1,397–4,945)	Not Applicable
B6	92020 (16,320–167,720)	39,366 (26,797–51,935)	1,079 (897–1,261)	3,300 (2,253–4,347)	Not Applicable
C1	3,575 (0–9,144)	0 (0–0)	0 (0–0)	Not Applicable	148,511 (83,468–213,554)
C2	5,233 (0–10,784)	0 (0–0)	0 (0–0)	Not Applicable	142,137 (89,805–194,469)
C3	8,259 (0–30,265)	0 (0–0)	0 (0–0)	Not Applicable	146,025 (76,238–215,812)

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Table SI8. Pairwise layer combinations for Wilcoxon tests on the biodigesters and the control units

Containers	Layer–combination 1	Layer–combination 2	Median difference (eggs)	p-value
Biodigesters (B1–6)	Coir	Gravel	101,548.50	0.03
	Coir	Effluent	120,985.50	0.03
	Coir	Vermicompost	117,971.50	0.03
	Gravel	Effluent	18,962.50	0.03
	Gravel	Vermicompost	16,102.00	0.03
	Effluent	Vermicompost	2,890.50	0.03
Control units (C1–3)	Coir	Gravel	5,233.13	0.18
	Coir	Effluent	5,233.13	0.18
	Coir	Pig faeces	137,765.62	0.18
	Gravel	Effluent	0.00	0.18
	Gravel	Pig faeces	146,025.0	0.18
	Effluent	Pig faeces	146,025.0	0.18