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Parasite Count and Survival during Fecal Waste Fermentation in a Piggery

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Abstract

To help determine the potential of piggery waste as a source of biogas, and the safety of utilizing piggery waste as organic manure, pig feces from three different piggeries were collected for investigation. One kilogram of fecal waste from each sample was utilized for biogas experiments using a locally assembled biogas set-up (*ie*, digester). Using a combination of formol-ether concentration and Stoll's techniques, the number and types of parasites in an estimated 1.0 g of feces from each digester were determined *ab initio* and every 5 days over a 75-day period. The temperature and pH inside each digester were taken on daily basis. Parasite eggs identified in the waste included *Taenia* specie, *Ascaris suum*, hookworm, trematode specie, *Trichuris suis, Oesophagostomum dentatum,* and *Hyostrongylus rubidus*. Trophozoites and cysts observed included *Balantidium*, *Entamoeba*, *Giardia,* and *Cryptosporidium*. On the whole, metazoan parasite-counts decreased between the start and end of the study (days 1 to 75), with some fluctuations in the earlier days. Conversely, protozoan parasite cysts increased with time. Implications of the observations are discussed and some recommendations are made.

Keywords: pig feces, digester, fermentation, protozoa, helminth, organic fertilizer

Introduction

Biomethanation of animal fecal wastes yields biogas and organic fertilizer. The procedure has been in existence for a significant amount of time and is popular in many countries, such as China, India, and Tanzania [1]. It has the potential to reduce the negative impact of animal waste on the environment. However, concern is always expressed about the likelihood of the potential negative side-effects of micro- and parasitic organisms.

Organic fertilizers are used to enhance agricultural yield, which in turn provides food for these countries and boosts their economies [2]. Concern is frequently expressed as to whether eggs and cysts of harmful parasites may be introduced into soil, vegetables and fruit, which could be deleterious to human health [3]. Manure, one of the organic fertilizers, is a complete fertilizer although low in nutrients. Pig feces are one of the most commonly available manures. The highest nutritional concentration is found in

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fresh manure. As it ages, becomes exposed to weather or is composted, its nutrient content drop [4]. However, organic fertilizers also have the following advantages: 1) they increase the organic content, and consequently the water-retention capacity of the soil [5]; 2) they improve the physical structure of the soil, allowing more air to get to plant roots; and 3) they increase bacterial and fungal activity in the soil. Organically derived plant nutrients are also slow to leach from the soil, making them less likely to contribute to water pollution than inorganic fertilizers [6].

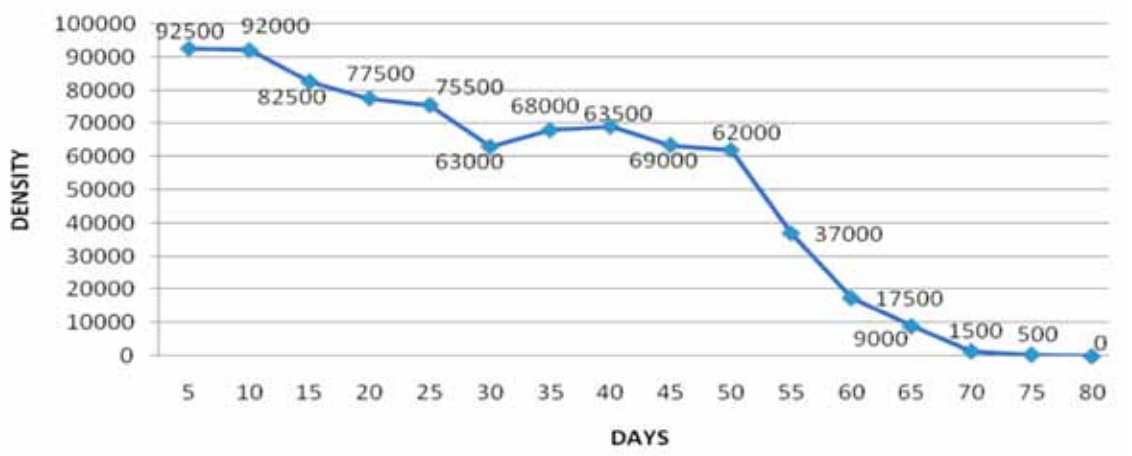
The purpose of this investigation was to assess the parasite count in the process of biomethanation during the anaerobic digestion of piggery fecal waste in the laboratory. The ultimate aim was to determine the safety of piggery fecal waste as a fertilizer.

Materials and methods

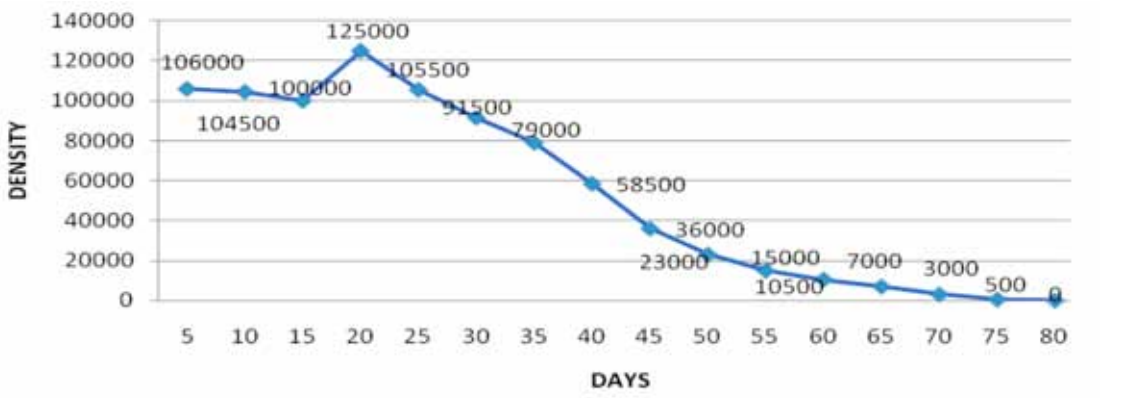
The pig feces used in the study were collected from three different sources (A, B, C). Source A was the University agricultural research farm, source B was a piggery in Modakeke, and source C was a piggery in Ile-Ife. Both fresh and dried fecal samples were collected in labeled polythene bags and brought to the laboratory within two hours. In the laboratory, 1,000 g of feces were mixed with 1,000 ml distilled water. The resulting paste was loaded into a digester, a laboratory model of a biogas plant (Fig 1). Attached to the lower rear side of the digester for parasitological sampling was a 10.0 cm long, 0.05 cm diameter flexible hose with a clip at the free end. After vigorously shaking the digester, 5 ml of the raw mixture was discharged into a test tube. 4 ml of 10% formol water was added to the mixture and was thoroughly shaken. This was followed by an additional 3-4 ml of 10% formol water. The emulsified fecal matter was sieved into a conical centrifuge tube and 3-4 ml of diethyl ether were added. Applying a glass stopper, the content was mixed for one minute. Removing the stopper, the suspension was centrifuged at 750-1,000 g for one minute. A glass rod was used to dislodge the layer of fecal debris from the side of the tube while the debris, ether and formol water were discarded. The bottom of the tube was then tapped to re-suspend the sediment with the remaining supernatant. The suspension was later poured onto a microscope slide and a cover slip was placed on the poured suspension. The whole slide was examined microscopically using a 10x

Fig 1 Complete biogas set-up (digesters with and without nylon).

Sample A



Sample B



Sample C

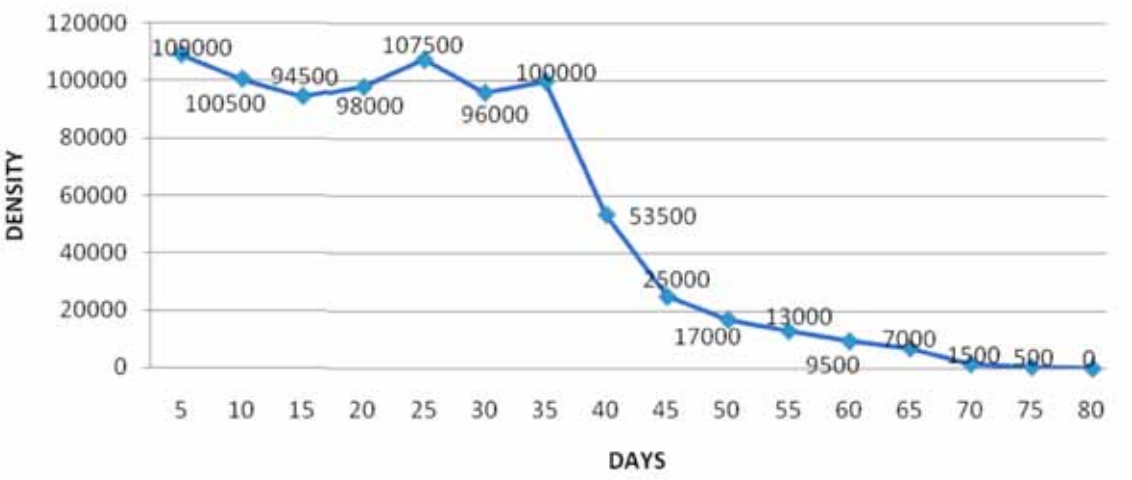


Fig 2 Total parasite population decline of samples with days.

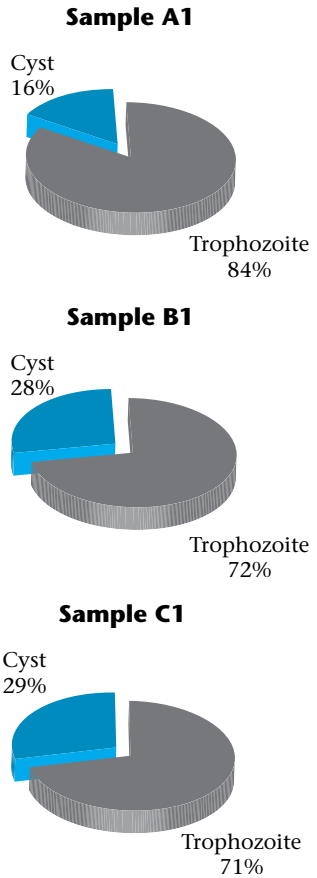


Fig 3 Protozoan population density in the samples.

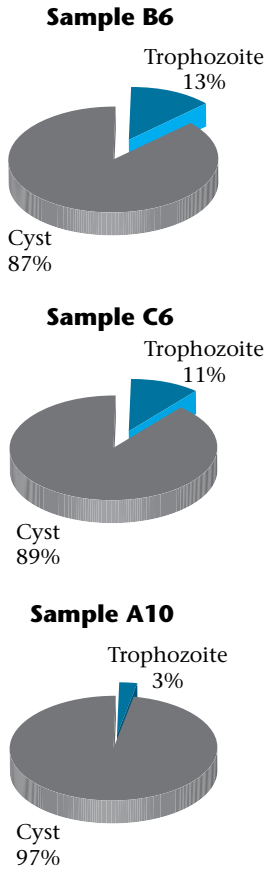


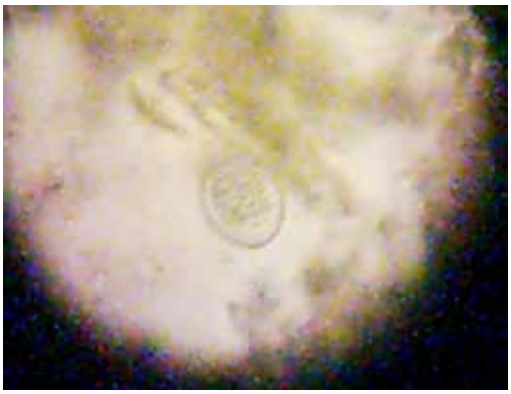
Fig 4 Protozoan population density in the samples.

objective lens for metazoa and a 100x objective lens for protozoa. Alongside the formol-ether technique, 3 g of feces were added to 42 ml of formol water to yield a 1 : 15 dilution of feces. The mixture was thoroughly shaken while 0.15 ml of the suspension was transferred onto a slide and covered with a cover slip. Slides were examined under a microscope. All eggs lying outside the edges of the cover slip were counted, since they were part of the 0.15 ml sample. The number of eggs was multiplied by 100 to give the number of eggs per gram of feces, and then by 5, being fluid specimens [7]. The temperature and pH of each of the slurries was taken daily. The whole process was repeated every 5 days over the course of the 75-day trial.

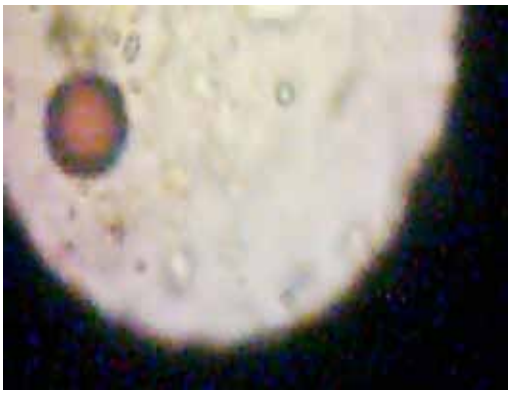
Results

The parasite density of the various three samples (A, B, and C) reduced gradually as the piggery waste fermented in the digesters (Fig 2). Even though the population densities of the three samples varied *ab initio*, they ended up being the same (500 per gram) on the 15th sampling, *ie* on day 75, and parasite-free after 75 days.

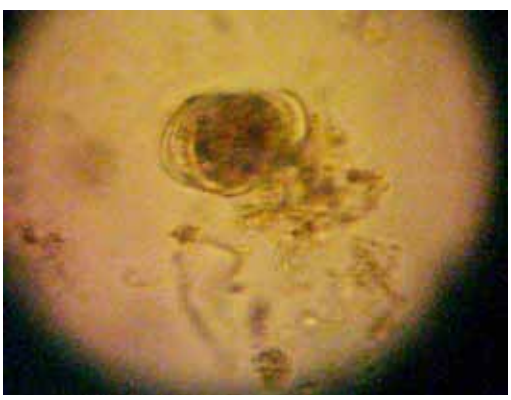
The protozoan organisms observed in the slurries included *Entamoeba* sp, *Giardia* sp, *Balantidium* sp, and *Cryptosporidium* sp. Although the prevalence of the various organisms in the three samples varied, the total percentage of cysts in the 1st sampling (*ie* on day 5) was smaller than the total percentage of trophozoites (Fig 3). By the 6th sampling for samples B and C (*ie* day 30) and



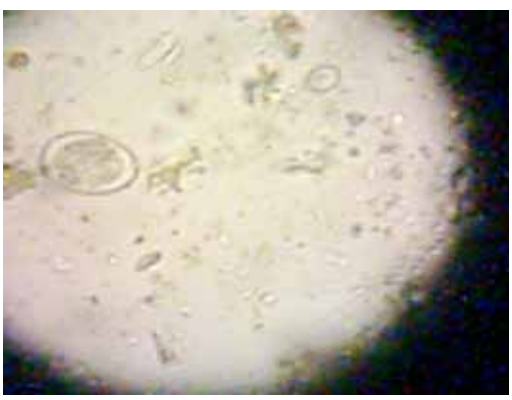
Hystrongylus rubidus



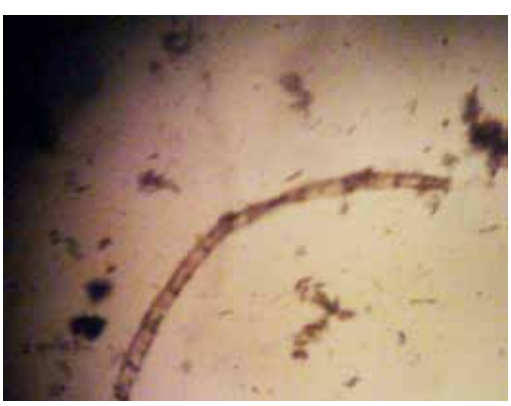
Ascaris suum



Oesophagostomum dentatum



Hookworm egg



Tapeworm segments

Strongyloides larva

Fig 5 Some eggs, detached segment of a cestode and *Strongyloides* larva observed in the piggery fecal samples.

10th sampling for sample A (*ie* day 50) the reverse occurred in respect of the population of cysts and trophozoites, culminating in higher prevalence of cysts (Fig 4).

Eggs of different species of helminths were observed in the slurries. These included trematode sp, *Ascaris suum*, *Taenia* sp, *Trichuris suis*, hookworm sp, *Oesophagostomum dentatum* and *Hyostrongylus rubidus* [8] (Fig 5). Detached segments of a cestode were also seen in one of the samples. Samples of eggs and the detached segments are shown in Fig 5. On comparing the

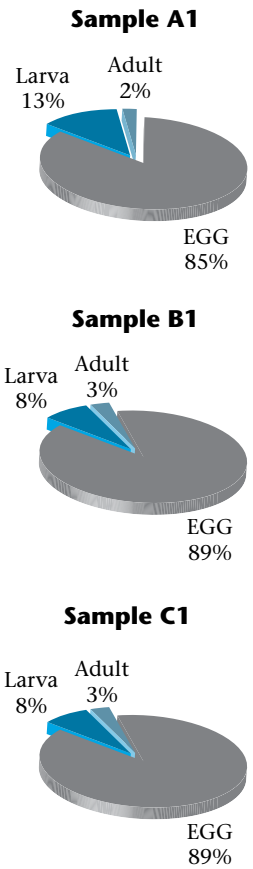


Fig 6 Helminth population density in the samples.

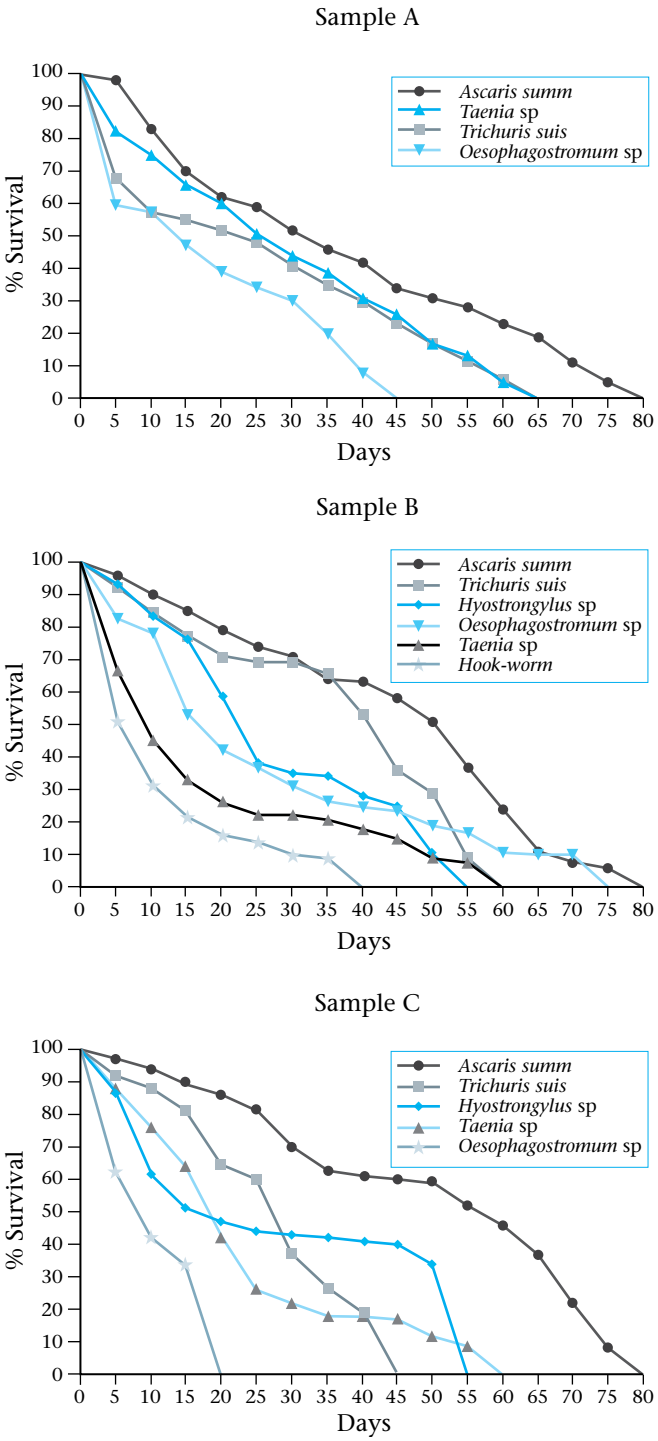


Fig 7 Decrease in population of helminth eggs in piggery slurries.

egg, larva and adult developmental stages, eggs predominated in the three different samples *ab initio* (Fig 6). This trend was maintained, such that by the 10th sampling (day 50) no larva or adult stages were found in sample A. Similarly, by the 6th sampling (day 30), there were no adults in sample C. The developmental stages present in this sample were eggs and a reduced population of larvae.

Individual analysis of samples A, B and C led to the discovery of a substantial number (four) of helminth eggs in sample A. These were *A. suum*, *Taenia* sp, *T. suis,* and *O. dentatum*. Six helminth eggs were observed in sample B–*A. suum*, *T. suis*, *H. rubidus*, *O. dentatum*, *Taenia* sp, and hookworm sp. Five helminth eggs (*A. suum*, *T. suis*, *H. rubidus*, *Taenia* sp, and *O. dentatum*) were observed in sample C. Eggs of *A. suum* were present longest

in the three different slurries. In samples A and C, eggs of *O. dentatum* diminished most rapidly, while in sample B, hookworm eggs diminished most rapidly. There was no clear consistency in the diminishing pattern of helminth eggs in the three samples (Fig 7).

Discussion

The laboratory analysis for this investigation was carried out at the Obafemi Awolowo University. Samples A, B, and C were collected approximately 1.5 km, 5 km, and 2 km from the university, respectively. Whatever might have been responsible for the extermination of the parasites under the anaerobic conditions was common to the three samples. This is to the extent that there remained an equal density of parasites (500 per gram) at the end of the investigation (day 75). With increase in days of fermentation there was a reduction in the quantity of available oxygen dissolved in the water. This, in addition to an increasing pressure that the parasites were subjected to, is thought to have resulted in massive trophozoite encystment by the protozoan species, leading to higher cyst densities (Fig 4). As the dissolved oxygen was depleted, the cyst population reduced correspondingly [9].

With respect to helminthic parasites, a few detached segments of a cestode were observed in one of the samples. The scolex was not found, hence the difficulty in identifying the tapeworm. Similarly, a small number of nematode larvae were observed. Such are very likely recovered from fresh or incompletely dried fecal samples. As stated, the egg stage of helminths predominated in all samples. This was expected, given their protective exoskeletal covering. Hays [10] noted that the effectiveness of anaerobic digestion in destroying cysts and eggs is dependent on time and temperature. In this investigation, the highest temperature reached between days 1 and 75 in sample A was 30.9 °C, and in samples B and C, 33.4 °C. If not for the number of days' exposure, these temperatures would have been regarded as too low to be lethal to helminth eggs. The site of sample A was the University research farm. Since better care of animals and environmental cleanliness in such a site is expected, this might be the reason for the fewer helminth eggs (four) being found in the piggery fecal waste. Sites B and C were local commercial piggery farms in the respective towns, where the environmental sanitation within the farms is expected to be of lower quality. As a result, the various helminth eggs detected among samples A, B, and C differed. On the whole, *A. suum* eggs were detected even on the 15th sampling (day 75), although at very low percentages. *O. dentatum* eggs were the first to be diminished in samples A and C (Fig 7). There was no definite pattern in the depletion period of the various eggs. It is conjectured that this is a function of their initial densities. Noteworthy is the fact that the three samples were parasite free after the 15th sampling on day 75. Incidentally, the role of exerted pressure consequent to the biogas produced is not easily quantifiable. Anaerobic digestion is equally purported to be capable of retarding or inactivating egg development due to lack of oxygen, exposure time, volatile solids, mixture content, carbon/nitrogen ratio, pH, and temperature [11]. The interactions of some of these parameters on biogas production and consequent effects on parasites are expected to be the subject of a subsequent write-up. The pH ranges in this investigation for samples A, B, and C were between 6.12 and 6.89. It can therefore be deduced that, after anaerobic digestion for more than 75 days, pigs' fecal samples should be free of parasitic organisms that are potential health hazards. In developing countries with limited technology, meager foreign exchange, with a host of competing demands (among which is the importation of inorganic fertilizers), the development of this kind of organic fertilizer from abundant, readily available sources, should be given serious consideration.

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