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ORIGINAL ARTICLE

Possibilities of decontaminating organic waste from swine-farming complexes using anaerobic digestion

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Abstract

Anaerobic digestion with simultaneous production of biogas is one of the most common methods of recycling, processing and decontamination of organic waste to produce alternative fuels. A commonly used indicator of the sanitary safety of the substrate is eggs of *Ascaris suum* nematodes of pigs, which are characterised by extremely high resistance to environmental factors. The aim is to investigate the effect of the mesophilic mode of anaerobic digestion on the activity of pathogens, particularly *Ascaris suum* eggs. The eggs of the studied nematode species were placed in a biogas installation at a temperature of 37 °C. The digestate samples with eggs were then taken every 4 days for 28 days, followed by larvae culture to determine the viability of the eggs. The results of our research have shown that the mesophilic regime of anaerobic digestion is an effective method of controlling parasites, but at the same time, it needs to be improved, since only 7.6% of *A. suum* eggs remained alive after a 1-day stay, about 50% after the week stay and about 9% in the third week stay in the biogas reactor. Thus, further optimisation of anaerobic fermentation in the mesophilic mode can be aimed at improving the suppression of pathogenic activity.

Keywords *Ascaris suum* · Eggs · Disinfection · Anaerobic digestion · Fermentation · Biogas reactor

1 Introduction

Manure is a valuable substrate in organic plant agriculture and a fertiliser for agricultural land. However, in intensive agricultural production, manure is considered simply organic waste, a by-product of animal husbandry that contaminates the environment. This is related to the great popularity of mineral fertilisers (potassium, nitrogen and phosphorus). They are inexpensive and relatively easy to produce, compared with organic fertilisers that are problematic to decontaminate. Accumulated in animal-farming complexes, manure can be a source of many pathogens for infectious and parasitic diseases. Most commonly, ruminant faeces have oocysts of parasitic protozoans *Eimeria* sp. [1], and also eggs of trematodes, cestodes (*Dicrocoelium*

Highlights

Inactivation of nematode eggs occurred in more than 90% under mesophilic conditions.

Nine percent of A. suum eggs remained viable in the 3rd week of stay in the biogas reactor.

Regression modelling of the proportion of A. suum eggs at different stages of cell growth.

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dendriticum, *Fasciola* sp., *Paramphistomum* sp., *Moniezia* sp.) and nematodes (Trichostrongyloidea, *Haemonchus* sp., *Strongyloides* sp., *Oesophagsotomum* sp., *Dictyocaulus* sp., *Trichuris* sp. and *Toxocara vitulorum*) [2, 3], and in swine faeces, oocysts of parasitic protozoans *Eimeria* sp.; cysts of *Balantidium coli ciliates*; and eggs of helminths *Ascaris* sp., *Oesophagostomum* sp., *Strongyloides* sp., *Hyostrongylus* sp., *Trichuris* sp. (Nematoda) and *Fasciola* sp. (Trematoda) [2, 4]. Poultry manure contains the oocysts *Eimeria* sp. and *Histomonas* sp. and eggs from the helminths *Ascaridia* sp., *Heterakis* sp. and *Capillaria* sp. [2, 5]. Many parasites are very resistant to environmental impacts, thanks to adaptation that helps them to counter unfavourable conditions. The eggs of many helminths are surrounded by a dense multilayered membrane. Therefore, they are tolerant to chemical and physical factors and regular stockpiling of manure cannot lead to its decontamination [6].

There are many different methods to decontaminate manure, while producing valuable ecologically clean organic fertilisers [7, 8]. The most common physical methods include the use of high temperatures [9], electron beam treatment [10] and gamma irradiation [11], and chemical methods include using various compounds with antiparasitic properties [12, 13]. One of the common biological methods of decontamination of organic waste is vermiculture (cultivation of earthworms). Earthworms are capable of breaking down organic waste [14]. Edwards and Arancon indicate the use of worms of the species *Eisenia fetida*, *E. andrei*, *E. eugeniae*, *Lumbricus rubellus*, *Dendrobaena veneta*, *Perionyx excavatus*, *P. hawayana* and *Lampito mauritius* [15].

Gishkaeva and Polonkoeva [16] report the necessity of using an ecologically closed biological system in agriculture, with a high degree of resource and energy use: modern biotechnologies can process organic waste of agricultural animals to produce not only organic fertilisers but also alternative fuels and fodders. One of such modern technologies is the anaerobic digestion of organic waste with the production of a valuable fuel, biogas [17]. The production of valuable gas, methane, is not the only benefit, since manure amounts are reduced and disinfected and nutrients are recycled. The introduction of unprocessed manure into the soil can contribute to the infiltration of groundwater with pathogens and other contaminants. Therefore, from the perspective of ecological risks, it is crucial to control these pollutants [18, 19]. One of the main causes of environmental contamination and health risks to the population is the widespread use of outdated sanitation technologies [20]. New ways of inactivating pathogens can improve sanitary conditions in many regions of the world and mitigate the negative impact of waste on the environment [20–22]. However, not all current methods promote the preservation of the environment, and therefore, the search for new ecologically safe and economically effective technologies continues [23, 24].

Anaerobic digestion can substantially reduce pathogenicity, but at the same time, it is not always effective against the eggs of intestinal parasites [25]. The US Environmental Protection Agency (EPA) presented standards regarding the use and recycling of the resulting digestate and solid biological compounds. The agency mentions the density of pathogens in solid biological compounds as the number of pathogens per unit mass. Class A biosolids have the density of vital helminth eggs of helminths equalling < 1 eggs/4 g of dry solid compounds [26, 27].

Agriculture animal manure is one of the main sources of bioenergy production around the world [28, 29]. Currently, dozens of thousands of biogas reactors are in operation to recycle animal manure. There are still open questions about the high-risk pathogens in manure that pose to the health of people and animals and the environmental contamination they cause [30]. Several studies have focused on the evaluation of the thermophilic regime as the regime that most effectively inhibits the development of pathogens [31, 32]. However, the effect that mesophilic digestion has on pathogen activity should be studied more thoroughly [6, 33], because, as mentioned earlier, this regime is the most economically beneficial for temperate latitudes [34, 35].

There is enough data in this research area since 1963 [36, 37]. But, there are significant differences in the results of experiments carried out by scientists on the disinfection of pig manure, which contains Ascaris eggs [38], in particular under mesophilic anaerobic conditions [39, 40]. Therefore, it is necessary to perform an experiment that summarises the results of previous studies. At the same time, it was possible to determine the likely impact of the difference in the results of individual conditions during experiments.

The aim of this study is to determine the viability of *Ascaris suum* Goeze, 1782 eggs during treatment of pig manure in a mesophilic anaerobic digestion regime.

According to the goal, the following task was solved:

- Determination of the development stages of *A. suum* eggs cultivated under anaerobic conditions
- Experimental study of changes in the proportion of *Ascaris suum* in different stages of its development during mesophilic anaerobic digestion
- Regression processing of experimental results to estimate the proportion of *A. suum* eggs found at a certain development stage during anaerobic digestion under mesophilic conditions

2 Materials and methods

2.1 Characteristics of the substrate and inoculants

The pig manure was provided by a farm in Novomoskovsk District of Dnipropetrovsk Oblast (Ukraine). The inoculum was also provided by the anaerobic digester on the same farm. In the experiment, one type of substrate was used: pig bedding manure. The typical chemical composition of manure in Ukraine is shown in Table 1 [41]. We used digestate, 400 mL of inoculum, 400 mL of water and 50 g of pig manure, placed in a biogas reactor.

The faeces and inoculums were sampled under strict aseptic conditions to prevent contamination infiltration during treatment. Pig manure and inoculum were placed in a standard cylindrical hermetic glass vessel (2 L) and transported to the Laboratory of Parasitic Research of the Department of Parasitology and Veterinary-Sanitary Expertise of the Dnipro State Agrarian-Economic University. The inoculum was stored at + 6 °C in a sealed polystyrene container ("L box", Ukraine, 2021). During the day immediately before preparing the digestate, the inoculum was placed in a thermostat at a temperature of + 37 °C.

2.2 Experimental reactor for anaerobic digestion

Anaerobic digestion was performed at the temperature of 37 °C. There were three series of experiments. The substrate was periodically mixed mechanically. The pH was maintained at 7.0 during the digestion process. The retention time of the substrate in anaerobic digestion was 28 days.

For the biogas reactor, a 1-L hermetic polystyrol tank was selected with a hermetically sealed valve opening at the bottom for sampling the liquid phase during fermentation and an outlet for the gas phase (biogas) connected to gas collection reservoirs. The bioreactor was periodically mixed mechanically (twice a day with an intensity of 80 rpm). During the bioreactor sampling, the temperature was measured using a temperature sensor along with the pH value. The bioreactor was thermostated. The temperature in the thermostat itself was 314 K, which ensured that the temperature inside the bioreactor was maintained at 310 K. The TCO-80 "MICROmed" thermostat (Shanghai Youding International Trade Co., Ltd., Minghua Mansion Fangxie Road Shanghai, China, 2020) was used to maintain the required temperature.

2.3 Testing the development of *Ascaris suum* eggs

In parasitology, there is an etalon species of helminths that is used to study the influence of various factors on the vitality of eggs under in vitro conditions. A commonly used indicator is eggs of swine nematodes *A. suum*. This is related to the high resilience of eggs of *A. suum* in the environment over a long period [42–44]. The experiment was carried out under mesophilic conditions with the addition of the *Ascaris suum* eggs Goeze, 1782 (Nematoda, Ascaridida, Ascarididae), swine helminths. The research was carried out between August and November 2023. The pigs' faeces contained immature helminth eggs (without larvae) (average 6883 eggs/g of faeces). The biogas reactor with parasite eggs was placed on a thermostat (TCO-80 "MICROmed", Shanghai Youding International Trade Co., Ltd., Minghua Mansion Fangxie Road Shanghai, China, 2020) at a temperature of 37° for 28 days.

From the biogas reactor, we collected 10-mL samples every 4 days from the first to the 28th day. The viability of the eggs of *A. suum* was measured by periodic selection and digestate study until the end of the biogas production process (28 days) by filtration through sieves, sedimentation using a centrifuge (CM-3 M.01 "MICROmed", Shanghai Youding International Trade Co., Ltd., Minghua Mansion Fangxie Road Shanghai, China, 2021) and incubation of the eggs for 3 months. Microscopy of the samples was performed using an optical microscope (MICROmed Fusion FS-7630, Ningbo Zhanjing Optical Instruments Co., Ltd, China, 2019). The degree of development was determined according to changes in the internal structure of the eggs: no changes, cleavage, or presence of a formed larva [45, 46]. At the same time, morphometric characteristics were analysed using Live Web Cam software.

The data were analysed using the standard methods of variance statistics: we calculated the median, first and third quartiles and minimal and maximal values. To evaluate the dynamics of a studied characteristic, we used regression analysis (equation of linear regression) [47]. The significance of the regression equation was evaluated using the determination coefficient (R^2) [48].

The statistical analysis of the results was performed through a set of Statistica 8.0 (StatSoft Inc., Tulsa, OK, USA). The tables present mean value (x) ± standard deviation (SD).

Table 1 Chemical composition of pig bedding manure (mean value (x) ± standard deviation (SD), $n = 5$)

Substrate	pH	Content at natural humidity, %			C:N
		Total nitrogen	Ammoniacal nitrogen	Phosphorus	
Pig manure	7.9	0.84	0.15	0.58	13:1
Test samples	7.4 ± 0.3	0.82–0.86	0.14–0.16	0.56–0.59	(12.7 ± 0.4):1

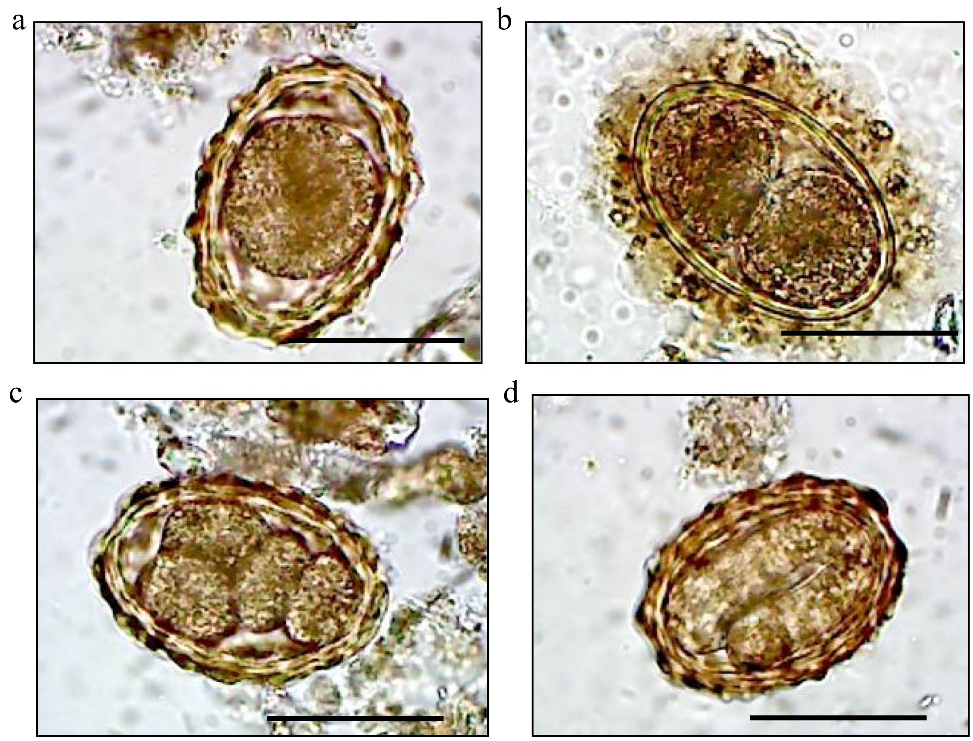

Fig. 1 Stages of embryonic development of eggs of *A. suum*: **a** one-cell stage; **b** two-cell stage; **c** four-cell stage; **d** formation of larva. Length of black bar—50 µm

3 Results

Figure 1 shows the developmental stages of *Ascaris suum* eggs that were cultured after anaerobic conditions in a biogas plant: one-cell stage (Fig. 1a), two-cell stage (Fig. 1b), four-cell stage (Fig. 1c), formation of the larva (Fig. 1d). In the first 24 h of the experiment, more than 90% of the eggs of *A. suum* in the biogas reactors survived (Fig. 2a). This was accompanied by the development of larvae from these eggs in the following 3 months. At the same time, larvae in 7.6% of the eggs could not achieve the invasive stage. The embryos in 3.2% of the total number of eggs were in the initial stages of cleavage almost 3 months since the experiment started (Fig. 2b).

Similar results were also observed for eggs collected from the biogas reactors on the fourth day of the experiment: over 90% of the eggs of *A. suum* reached the invasive stage of development after 3 months. The absence of larva formation was observed only in 6% of the eggs collected on the fourth day of the experiment. Only 2% of the eggs were at the initial cleavage stages (Fig. 2b).

A rapid decrease in the number of vital eggs was observed after 8 days of the experiment. In the samples taken from the biogas reactors on the eighth day of the experiment, we found that around 50% of the eggs were vital 3 months later. At the same time, embryos in almost 20% of the eggs were in the initial stages of cleavage, but did not reach the larval stage (Fig. 2b). A total of 25% of the larvae did not start to develop (Fig. 2c).

Twelve days after the experiment, the share of vital eggs in the samples was about 33% (Fig. 2a). Despite such a long period under anaerobic conditions, 3 months later, 27% of *A. suum* eggs had embryos in different stages of cleavage (Fig. 2b). Only around 40% of the eggs were killed in 12-day retention under anaerobic conditions (Fig. 2c).

In the samples collected on the 16th experimental day, the eggs had a 3.5 times lower number of larvae than the samples collected on the 12th day (Fig. 2a). At the same time, 3 months later, 90.6% of the eggs showed no development of the larvae. Cleavage started in 28.2% of the cases, although no developed larvae were found 3 months later (Fig. 2b).

With an increase in the duration of egg retention in the biogas reactors of up to 20 days, the share of dead eggs increased to 98.3%. At the same time, 25.6% of the eggs contained embryos consisting of several cells, but we did not observe larva formation 3 months later (Fig. 2b).

In the samples taken from the biogas reactors on the 24th and 28th days after the cultivation had started, we found no larva-containing eggs (Fig. 2a). In 10.6% of the eggs collected after 24 days of the experiment, we saw initial cleavage stages at the end of the cultivation. In the samples collected after 28 days of the experiment, we also observed cleavage in 3.8% of the eggs after 3 months of cultivation.

The number of embryos at the stage of larva formation, and also the one-cell stage, changed linearly (Table 2), and at the cleavage stage, it changed according to the square equation (Table 2).

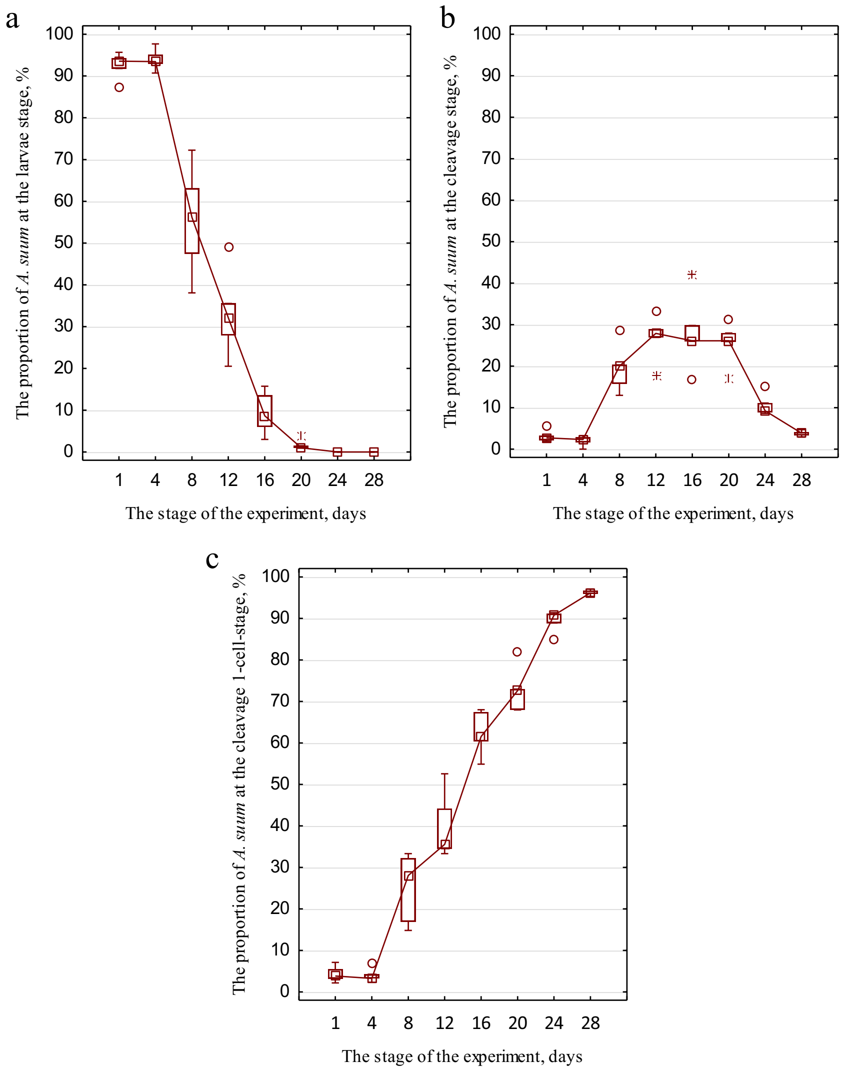

Fig. 2 Change in the proportion of *Ascaris suum* at the stages: of the formed larvae (**a**), cleavage (**b**) and the one-cell stage (**c**) during the experiment ($n=5$)

Table 2 Regression equation for the proportion of *A. suum* eggs found at a certain development stage (%), depending on the stage of the experiment (x, days)

Stage	Regression equation	R^2
Larva formation	$y=-15.50x+105.48$	0.8840
Cleavage	$y=-2.08x^2+19.55x-19.86$	0.8357
One-cell stage	$y=14.70x-16.89$	0.9794

4 Discussion

For the experiment, several-hour-old excrement from a premise for swine was collected. The eggs of the parasites were in aerobic conditions before being placed in the bioreactor. That is, some of them started to develop at the cleavage stage. Therefore, in Fig. 2b, some of the dead eggs contained blastomeres. Exactly the same process will occur in the eggs placed in biogas reactors under industrial conditions: a small part of the manure will be 2–4 days old and contain several dividing cells. Thus, our model describes the actual parameters of the *A. suum* eggs in industrial conditions. Therefore, it is very important to monitor how the embryos behave in the future at the cleavage stage, as well as embryos that did not start to divide.

In the study [6], it was reported inactivation of *A. suum* eggs at a temperature below 45 °C, contrary to the current guidelines. Those authors placed the *A. suum* eggs at the temperature of 34–45 °C, also in anaerobic conditions. The results of [6], as well as our research, revealed that the mesophilic anaerobic regime can be used for the treatment of organic waste to inactivate pathogens and provide access to safe food and water.

During anaerobic digestion in the study [49], the main factors for the inactivation of eggs, other than temperature, were the concentration of ammonia and the moisture content [49]. In addition, a crucial factor in the survival of pathogens was the duration of their subjection to anaerobic conditions. The research conducted by Moretti et al. [50] evaluated the potential of using swine effluents from the anaerobic digestion system in agricultural farming. Swine faeces were placed in an anaerobic digester with a retention time of 30, 100, 130, 180 and 210 days. After 100 days, elimination of faecal *Escherichia coli* and also *Salmonella* spp. was observed. At the same time, the growth of other species of bacteria was seen. The inactivation of pathogens depending on the temperature and time of exposure was also described by Espinosa et al. [33]. The objective of their research was to identify "safe zones" to reduce the number of vital pathogens in four groups (bacteria, viruses, protozoan cysts and eggs of helminths) during thermal treatment, using regression analysis to determine correlations of time, temperature and vitality of pathogens. These authors conducted a large systematic review of data in the literature and determined that, at high temperatures, the correlation curves between temperature and time were controlled by thermally stable viruses. The temperature required to decrease the vitality of the pathogens in all groups per log10 unit turned out to be higher than previously reported, and the time required to do so was longer. Espinosa et al. [33] also stated the insufficiency of data regarding protozoans and also the poor research on how low temperatures affect all groups of pathogens. Analysis of the results from our studies also highlights the importance of the duration of anaerobic conditions during the mesophilic regime in influencing the development of *A. suum* eggs. Highly efficient inactivation (51.4%) was recorded for wastewater sediments using the thermophilic regime of anaerobic digestion with exposure at 60 days [9]. Thus, this digestion regime can be an effective stage of pretreatment in order to reduce the vitality level of eggs of helminths (*Ascaris*, *Trichuris*, *Hymenolepis*, *Toxocara*).

The research conducted by Seruga et al. [31] studied how long the eggs of *A. suum* survived and the rates at which they were inactivated during thermophilic anaerobic digestion under laboratory conditions. The swine ascariasis pathogen was found to be removed in 10 h. These results confirm that anaerobic digestion in the thermophilic regime produces high sanitary effectiveness. According to [32], anaerobic thermophilic digestion led to an increased concentration of readily available forms of nitrogen in wastewater. At the same time, this process produced ecologically safe samples of organic fertilisers, decontaminated from viable eggs and larvae of helminths. Patil and Mutnuri [51] investigated the effect of anaerobic conditions on the inactivation of *A. suum* eggs and found, using correlation analysis, a positive relationship between the number of nonviable eggs and pH. In the study [52], it was found that compounding effects on eggs with *A. suum* of alkaline pH (≥ 10.5) were observed at 35° C.

There are many reports about improving filtrate decontamination under conditions of anaerobic digestion by adding disinfecting compounds to biogas reactors. Cui et al. [53] used potassium ferrate, potassium peroxymonosulfate and ferrate combined with peroxymonosulfate for preliminary and combined treatment to control pathogenic microorganisms in human waste. The best results were produced by all the pretreatments. Under such conditions, pathogenic bacteria and eggs from helminths were inactivated. Pretreatment with potassium ferrate was the most efficient way of inhibiting pathogenic micrograms, decreasing the overall number of coliforms by 3.5 log (N/N_0). After using sodium dichloroisocyanurate (disinfecting agent), a synergic increase of the acidogenic process of sludge and inactivation of pathogens was observed [54].

According to [55] sediment used for irrigation contained eggs and larvae of helminths *Strongyloides stercoralis*, *Ancylostoma duodenale*, *Necator americanus*, *Trichuris trichiuria*, *Hymenolepis nana* and *Ascaris* spp. On average, the vitality of their eggs was 57.7–74.5%. Treatment with activated carbon caused the adsorption of eggs of these parasites by 95 to 100%. Activated carbon can be used to treat the sediment.

Many of the added compounds can not only inhibit the vitality of pathogens but can also negatively impact the anaerobic bacterial process in bioreactors. The addition of phenol disinfectant inhibited the growth of the archaea of the *Methanosaeta* and *Methanobacterium* genera. The high concentration of this compound was able to inhibit the processes of oxidation and methane formation during anaerobic digestion [56].

The research conducted by Yang et al. [57] evaluated the effects of a quaternary ammonium-based disinfectant on the anaerobic digestion of wastewater sediments. Analysis of the microbial community revealed that the changes in the archaea and bacteria communities depended mainly on the doses of this compound. This disinfecting compound inhibited methanogenesis due to the accumulation of volatile fatty acids and the sensitivity of methanogens to it [57]. Shao et al. [58] also studied the effects of chlorine-containing disinfecting compounds on the structure of the microbial community and the effectiveness of anaerobic digestion of swine manure. They reported inhibiting the action toward methanogenesis in the initial stage during the mesophilic regime. However, under thermophilic conditions, the inhibition process decreased significantly at the initial stage. The disinfecting compound with residual chlorine under such conditions led to an increase in the number of chlorine-tolerant bacteria and archaea of the *Methanosarcina* genus.

Therefore, our studies and also the analysis of sources from the literature revealed that the use of mesophilic conditions (+37 °C) of waste recycling is a sufficiently widespread ecologically and economically beneficial method of recycling organic waste from animal farm complexes and other enterprises. However, the *A. suum* eggs were not inactivated completely. Such experiments with filtrate decontamination under anaerobic mesophilic conditions were also carried out by [59]. Their research revealed that anaerobic digestion reduced the amount of vital parasite eggs by only 37%. The best results were achieved by combined methods of batch sequencing treatment, anaerobic biological treatment and intensive aeration treatment: there was a significant decrease in the quantity of vital forms of helminths—nematodes (*Capillaria* sp., *Toxocara* sp.) and cestodes (*Hymenolepis nana* and *Taenia* sp.). However, given that the eggs of this nematode are among the most resilient to environmental factors and that their inactivation, according to our results, occurred in more than 90% of the cases, we may assume that the eggs of the remaining species of nematodes will be completely exterminated under such conditions.

5 Conclusion

Anaerobic digestion under mesophilic conditions requires improvement, since only 7.6% of *A. suum* eggs remained viable during the first 24 h of stay in the tank, approximately 50% of eggs stopped their further development during 8 days and about 9% in the biogas reactor remained viable even in the third week of our experiment. Thus, in this study, 100% mortality of the most resistant eggs among all helminth eggs was not achieved before the biogas extraction was completed.

It is proposed to recommend further optimisation of anaerobic mesophilic digestion settings with substrate pretreatment to suppress helminth egg viability. It is probably necessary to further adapt existing methods of organic waste decontamination (low absorbed radiation dose, high temperature in the biogas reactor, the addition of calcium hydroxide and other regulators of the medium acidity etc.).

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Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate N/A

Consent to publication N/A

Competing interests The authors declare no competing interests.

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