РОЗДІЛ 2. Благополуччя довкілля та екологічна безпека
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SANITARY CONDITION OF PIG MANURE AND MICROCLIMATE IN PIGGERIES UNDER TREATMENT WITH THE BIODESTRUCTOR SANAERO

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Abstract. *Currently, technologies for managing unpleasant odors are actively being developed and implemented on farms. Among the existing technologies, microbial deodorization is considered the most promising method of odor control due to its safety, long-lasting deodorizing effect, and low cost. The aim of this study was to investigate changes in conditionally pathogenic (E. coli, S. aureus) and pathogenic (Salmonella) bacteria in pig manure during the filling of underfloor manure pits when using the biodestructor Sanaero. It was found that the microorganisms in the biodestructor Sanaero (Bacillus subtilis, Bacillus licheniformis, Lactobacillus plantarum, Saccharomyces cerevisiae) act antagonistically in pig manure against Escherichia coli and Staphylococcus aureus cells. As a result, the content of E. coli decreased, on average, by two orders of magnitude by the end of the production process of filling the underfloor manure pit, while S. aureus was completely inhibited. Moreover, the number of E. coli cells in the experimental manure was on average 4 – 8 times lower than in the control. This indicates the suppression of conditionally pathogenic bacterial activity by the biodestructor's microorganisms even at low ambient temperatures. The use of the biodestructor Sanaero during the filling of the underfloor manure pit with pig manure promotes the active development of probiotic microorganisms and improves the sanitary condition of pig manure. The use of the biodestructor Sanaero in pig housing facilities significantly reduces the level of microbial contamination of the air, particularly the amount of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and fungal microflora. The effect becomes noticeable as early as the fifth day of using the biological product and remains throughout the entire period of manure pit filling. Therefore, the use of the biodestructor Sanaero is an effective means of improving the sanitary condition of the air in piggeries.*

Keywords: pig manure biodestructor, antibiotic-resistant bacteria, sanitary condition of manure, piggery microclimate, microflora.

Introduction. Intensive pig farming leads to the production of large amounts of manure, which poses a serious environmental problem, as the generation and processing of manure often result in the release of pollutants not only inside pig housing facilities but also beyond their boundaries (Matusiak, 2016; Wang, 2021; Cao, 2023). In particular, gas emissions and the proliferation of pathogenic microflora significantly contaminate the environment. Therefore, it is essential to closely monitor and reduce the concentration of gaseous pollutants. Additionally, manure can spread a number of pathogenic microorganisms such as *Salmonella choleraesuis* var. *typhi*, *Mycoplasma* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus* spp., *Candida albicans*, *Cryptococcus neoformans*, among others (Gibbs, 2006; Yao, 2011; Vestergaard, 2018). This highlights the need to introduce new strategies to improve the microclimate by developing and applying bioproducts containing natural bacteria capable of neutralizing odors and disinfecting livestock facilities (Matusiak, 2016; Li, 2021). The use of microorganisms to reduce the concentrations of ammonia and hydrogen sulfide in the microclimate of livestock premises is an advanced and environmentally friendly solution that mitigates the impact of livestock production on the environment (Li, 2018; Shi, 2020; Li, 2020).

Bioproducts offer many advantages: they are safe to use, non-corrosive, reduce excessive microbial growth, and can adsorb heavy metal compounds. Unlike other deodorization methods, bioproducts are gaining popularity because they do not require special equipment for their application (Matusiak, 2016; Maslov, 2023). Importantly, microbial preparations also contribute to the hygienization of livestock facilities by reducing the number of pathogenic organisms, which are not only responsible for unpleasant odors but can also cause diseases in animals and humans (Gutarowska, 2014).

Furthermore, research findings (Bai, 2022) report that livestock farms are a significant source of antibiotic-resistant bacteria and bacterial pathogens in the air of agricultural environments, which can be transported over long distances into the surrounding atmosphere.

The presence of conditionally pathogenic (*E. coli*, *S. aureus*) and pathogenic (*Salmonella*) bacteria in manure poses risks of potential environmental contamination and transmission of pathogens through the food chain. The microorganisms contained in the developed biodestructor Sanaero (*Bacillus*, *Lactobacillus*) exhibit probiotic antagonistic properties against many bacteria. In our opinion, these probiotic properties should be particularly effective in liquid pig manure during the filling of underfloor manure pits, thereby improving its sanitary quality.

The aim of this study was to determine changes in conditionally pathogenic (*E. coli*, *S. aureus*) and pathogenic (*Salmonella*) bacteria in pig manure during the filling of underfloor manure pits when applying the biodestructor Sanaero.

Materials and methods. The study was conducted at pig fattening farms in the Khmelnytskyi region. Microbiological analysis of pig manure microbiota was performed at Podillia State University. The research covered pig fattening facilities during the period from 76 to 160 days of fattening.

Preparation of the working solution of the biological product Sanaero for biodestruction of pig manure in the underfloor pit. In production conditions at the pig farm, 100 – 200 ml of the bioproduct was mixed with 10 liters of tap water heated to 30 to 37°C, along with 200 ml of molasses or sugar syrup, and left to activate for 4 – 6 hours at room temperature. After activation, 1 liter of the prepared working solution was added to the underfloor manure pit for approximately every 1 m^3 of liquid pig manure. The product was reapplied after seven days in the same proportion, and this process continued throughout the entire period of pit filling.

Sampling of manure for analysis was performed from underfloor manure pits in experimental and control facilities simultaneously – on days 2, 5, 9, 13, and 17 from the start of pit filling. Samples were transported on ice to the laboratory for determination of total viable bacterial count (TVC or mesophilic aerobic and facultative anaerobic microorganisms, MAFAnM), *E. coli*, *S. aureus*, and *Salmonella* spp.

The total viable count in manure was determined by the classical microbiological method: preparing tenfold serial dilutions, plating them in Petri dishes, and overlaying with melted and cooled meat-peptone agar (MPA). The plates were incubated for 72 hours at 30±1°C, after which the number of colony-forming units (CFU) per gram of manure was counted (DSTU ISO 4833:2006).

To quantitatively isolate *S. aureus*, tenfold dilutions of manure were plated onto Baird-Parker agar and incubated for 24 – 48 hours at 37±1°C. On this medium, *S. aureus* colonies appear golden with a black center.

E. coli was isolated similarly, but using Endo medium, where it forms characteristic pink colonies with a metallic sheen.

For isolation of *Salmonella* spp. from manure, the following scheme was used: 25 g of manure was placed in 225 ml of sterile buffered peptone water for pre-enrichment, mixed, and incubated for 18–24 hours at 37±1°C. After pre-enrichment, samples were transferred to selective Selenite Cystine broth and incubated again for 18 – 24 hours at 37 ±1°C. Then, samples were streaked onto XLD (Xylose Lysine Deoxycholate) agar and incubated for another 18 – 24 hours at 37±1°C. On XLD agar, Salmonella colonies appear as transparent or colorless colonies, sometimes with black centers due to H_2S production.

Air sampling in pig fattening units was performed using the standard sedimentation method («settling plate method» or «envelope method»). Prepared Petri dishes with selective media for different bacterial groups were placed at a height of 1 m from the floor (four dishes at the corners and one in the center). After a 30-minute exposure, the dishes were closed and placed in a cooler bag, then transported to the microbiology laboratory of the veterinary faculty within two hours for further analysis. The number of MAFAnM in the bioaerosol of pig houses was determined using meat-peptone agar (MPA); *Staphylococcus* spp. were cultured on blood agar containing 5% blood and 7% sodium chloride; Enterobacteriaceae on Endo medium; fungi on Sabouraud medium (HiMedia, India). Incubation times and temperatures were chosen according to the optimal biochemical activity of each type of microorganism (Kukhtyn & Kravchenyuk, 2023; Kukhtyn et al., 2018). Identification of isolated microbial cultures was based on microscopic morphology and metabolic activity using commercial API test kits (bioMerieux, Poland).

The experimental data were statistically processed using Statistica 9.0 software. The mean value (m) and standard error (M ± m) were calculated. Differences between the studied values were considered statistically significant at $P \leq 0.05$.

Results. We have developed a biological manure destructor called Sanaero – a microbial product that combines selected symbiotic, saprophytic microorganisms aimed at decomposing organic matter and significantly reducing the production of harmful and unpleasant gases.

The composition of the biological product includes microorganisms of the genera: *Bacillus*, *Lactobacillus*, *Pseudomonas*, *Azotobacter*, *Cellulomonas*, and *Saccharomyces*. Through their metabolic activity, these microorganisms affect the manure microbiota, altering nitrogen transformation processes and reducing the generation of various foul odors.

Taking into account the mode of action of the Sanaero biodestructor, changes in the content of opportunistic (*E. coli, S. aureus*) and pathogenic (*Salmonella*) bacteria in pig manure during the filling of the underfloor manure pit were determined (Table 1).

Table 1

Effect of the Sanaero biodestructor on the content of *E. coli*, *S. aureus* and *Salmonella* spp. in pig manure during filling of the underfloor manure pit at an ambient temperature of +20 to +25°C, M ±m

Study duration, days	Number of microorganisms, log CFU/g					
	E. coli		*S. aureus*		*Salmonella* spp.	
	1	2	1	2	1	2
2	5.50 ±4.15	5.89 ±4.42	2.83 ±1.32	3.61 ±2.05	0	0
5	4.65 ±3.36	5.71 ±4.33	2.72 ±1.34	3.88 ±2.33	0	0
9	4.33 ±3.14	5.62 ±4.14	1.86 ±0.69	3.50 ±2.15	0	0
13	3.76 ±2.30	4.41 ±3.16	1.82 ±0.77	2.83 ±1.43	0	0
17	3.91 ±2.31	4.53 ±3.36	0	2.71 ±1.38	0	0

Note: 1 – in treated manure with biodestructor; 2 – in control manure.

According to Table 1, on the second day of the study, the content of *E. coli* in manure treated with the biodestructor was 5.50 ±4.15 log CFU/g, compared to 5.89 ±4.42 log CFU/g in the control group. Thus, in the experimental manure, the number of *E. coli* was 2.4 times lower ($P < 0.05$). The slight initial effect of the biodestructor on *E. coli* cells at this stage can be explained by the gradual adaptation of the probiotic bacteria to the manure environment and the onset of bacteriocin production.

During the first five days of filling the manure pit, the content of *E. coli* in the experimental manure remained lower compared to the control. In particular, by day five, the number of *E. coli* in the manure treated with the biodestructor decreased by nearly one order of magnitude ($P < 0.05$) to 4.65 ±3.36 log CFU/g, compared to the second day. This value was also almost one order of magnitude lower ($P < 0.05$) than in the control manure (5.71 ±4.33 log CFU/g). A similar trend continued on the ninth day of filling, although the rate of decline in *E. coli* numbers in the treated manure was less intense than between the second and fifth days. Nevertheless, compared to the control group, the difference was still more than one order of magnitude ($P < 0.05$), indicating that the microorganisms of the biodestructor became more active, suppressing *E. coli* through environmental acidification, accumulation of volatile fatty acids, bacteriocins, and fermentation products.

The lowest level of *E. coli* in the experimental manure was recorded on day 13, at the level of 3.76±2.30 log CFU/g, compared to 4.41±3.16 log CFU/g in the control. Subsequently, no further significant reduction in *E. coli* was observed in either group, suggesting stabilization of the biodestructor's microbial activity due to the establishment of anaerobic conditions in the manure pit.

Given the widespread use of antimicrobials in pig farming and the emergence of methicillin-resistant staphylococci (MRSA), it was important to evaluate the effect of the Sanaero biodestructor on the survival of pathogenic staphylococci.

Results from Table 1 showed a relatively low content of *S. aureus* in manure in both groups on the second day. However, the amount in the experimental group treated with Sanaero was, on average, one order of magnitude lower (P <0.05), measuring 2.83 ±1.32 log CFU/g compared to 3.61 ±2.05 log CFU/g in the control.

By day five, there was no statistically significant difference in *S. aureus* content between experimental and control groups compared to day two. However, on days nine and 13, the content of *S. aureus* decreased more significantly in the manure with the biodestructor: on average by seven times (P <0.05) to 1.84 ±0.67 log CFU/g, whereas in the control group it decreased four times (P <0.05) to 2.83 ±1.43 log CFU/g compared to day five.

By the end of the filling period – day 17 – S*. aureus* was not detected in the treated manure samples, whereas in the control it remained at 2.71 ±1.38 log CFU/g.

Additionally, throughout the entire filling period of the manure pit, no pathogenic bacteria of the genus *Salmonella* were detected in either the experimental or control groups, indicating a good epizootic status of the farm.

Overall, the microorganisms of the Sanaero biodestructor demonstrated antagonistic effects on *E. coli* and *S. aureus* cells in pig manure. The *E. coli* count decreased, on average, by two orders of magnitude by the end of the manure pit filling process, while *S. aureus* was completely inhibited. Moreover, the *E. coli* content in the experimental manure was on average 4 – 8 times lower than in the control, highlighting the potential of Sanaero to improve the sanitary condition of pig manure.

It is worth noting that lower ambient temperatures can slow down the development of microorganisms, meaning biological processes in manure proceed more slowly. Since the action of the biodestructor depends on the viability and biochemical activity of its constituent microorganisms, it was scientifically important to study the effectiveness of Sanaero during the colder period of the year and its impact on *E. coli*, *S. aureus*, and *Salmonella* spp. content (Table 2).

From Table 2, it can be seen that in both the experimental and control pig manure samples, the content of *E. coli* gradually decreased over the 17-day filling period of the manure pit. Specifically, in manure treated with the biodestructor Sanaero, the number of *E. coli* decreased by an average of two orders of magnitude over this period, whereas in the control manure the reduction was only by one order of magnitude. This indicates that at an ambient temperature of +15 – +17°C, the microorganisms in the biodestructor produce bioactive compounds (biocins) in the manure that inhibit the development of *E. coli*.

At the same time, when comparing the effect of Sanaero on *E. coli* at two temperature ranges – lower (+15 – +17°C) and higher (+20 – +25°C) – the difference lay only in the faster activation of the biodestructor's microflora at the higher temperature. This is due to the temperature's effect on physiological processes in microbial cells, leading to a quicker production of biologically active substances that suppress *E. coli* multiplication. As a result, its growth is inhibited sooner, although by the end of the observation period (day 17), the *E. coli* content was nearly the same under both temperature conditions.

Table 2

Effect of the Sanaero biodestructor on the content of *E. coli*, *S. aureus* and *Salmonella* spp. in pig manure during filling of the underfloor manure pit at ambient temperature +15 to +17 °C, M ±m

Study duration, days	Number of microorganisms, log CFU/g					
	E. coli		S. aureus		Salmonella spp.	
	1	2	1	2	1	2
2	5.29 ±3.93	5.70 ±4.23	2.79 ±1.67	2.90 ±1.73	0	0
5	5.02 ±3.57	5.82 ±4.25	2.43 ±1.23	3.53 ±2.05	0	0
9	4.89 ±3.35	5.53 ±4.01	2.40 ±1.07	2.82 ±1.51	0	0
13	4.40 ±2.98	5.23 ±3.56	0	2.96 ±1.64	0	0
17	3.92 ±2.73	4.84 ±3.01	0	2.38 ±1.11	0	0

Note: 1 – in treated manure with biodestructor; 2 – in control manure.

The dynamics of *S. aureus* also changed in manure treated with the biodestructor Sanaero. In the experimental samples on the fifth day of pit filling, the *S. aureus* content was approximately one order of magnitude lower compared to the manure without the addition of biodestructor microorganisms: 2.43 ±1.23 vs. 3.53 ±2.05 log CFU/g.

From day 13 and until day 17 of the manure pit filling period, *S. aureus* was absent in the treated manure samples, while in the control samples, although its concentration was low, it still remained at 2.96 ±1.64 log CFU/g. This demonstrates the inhibitory effect of the biodestructor’s microorganisms on *S. aureus* even under lower ambient temperatures.

Regarding the effect of Sanaero on *Salmonella* spp. cells under in vivo conditions, no effect could be evaluated because pathogenic *Salmonella* bacteria were not detected in either the experimental or control manure samples.

Thus, based on the obtained results, it was established that the use of the biodestructor Sanaero at an ambient temperature of +15 – +17°C positively influences the microbiological condition of the manure substrate. In particular, a more intensive decrease in *E. coli* numbers was observed in the treated samples – on average by two orders of magnitude over 17 days, which is twice as effective compared to the control samples. Significant suppression of *S. aureus* was also recorded: from day 13, the bacteria were completely absent in the treated samples, while in the control they remained in low concentrations. This indicates the antagonistic activity of the biodestructor’s microorganisms, even under reduced temperature conditions. As for *Salmonella*, the pathogen was not detected in either the treated or control samples, which does not allow conclusions about the biodestructor’s effectiveness against this pathogen under the studied conditions.

In general, the Sanaero biodestructor demonstrated its antimicrobial activity and can be recommended for improving the sanitary and epidemiological state of manure under low-temperature conditions.

Since the addition of the Sanaero biodestructor to the manure pit improves the manure’s microbiota, it was reasonable to investigate whether this treatment could also influence the microbiological indicators of the air in pig housing facilities. The results of quantitative measurements of yeast and mesophilic microflora in the air of pig houses during the warm season are presented in Table 3.

From Table 3, a gradual reduction in the microbial contamination of air in the pig houses where the Sanaero biodestructor was added to the underfloor manure pit was observed. Already by the fifth day of filling the manure pit, the number of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in the air of the experimental pig houses was on average three times lower ($P < 0.05$) compared to the second day ($2.1 \pm 0.1 \times 10^4$ CFU/m³) and approximately one order of magnitude lower than in the air of the control pig houses ($1.1 \pm 0.2 \times 10^5$ CFU/m³).

From the fifth to the ninth day of filling the manure pit, the MAFAnM content in the air of the experimental pig houses remained relatively stable, while by the 17th day it decreased on average another threefold (P <0.05) compared to the fifth day. This indicates that the Sanaero biodestructor improves the sanitary condition of the air and remains effective throughout the entire manure pit filling period.

Table 3

Microbiological indicators of the microclimate in fattening pig pens when using the Sanaero biodestructor at ambient temperature +20 – +25°C, M ±m

Study duration, days	Parameters			
	MAFAnM, CFU/m³		Fungi, CFU/m³	
	1	2	1	2
2	$6.3 \pm 0.3 \times 10^4$	$9.1 \pm 0.3 \times 10^4$	$5.3 \pm 0.2 \times 10^2$	$8.4 \pm 0,3 \times 10^2$
5	$2.1 \pm 0.1 \times 10^{4*}$	$1.1 \pm 0.2 \times 10^5$	$3.1 \pm 0.2 \times 10^{2*}$	$8.9 \pm 0.3 \times 10^2$
9	$1.0 \pm 0.1 \times 10^{4*}$	$9.8 \pm 0.3 \times 10^4$	$1.4 \pm 0.1 \times 10^{2*}$	$3.7 \pm 0.2 \times 10^3$
13	$8.5 \pm 0.3 \times 10^{3*}$	$1.3 \pm 0.2 \times 10^5$	$1.5 \pm 0.1 \times 10^{2*}$	$7.8 \pm 0.2 \times 10^2$
17	$7.1 \pm 0.3 \times 10^{3*}$	$1.0 \pm 0.1 \times 10^5$	$1.4 \pm 0.1 \times 10^{2*}$	$8.9 \pm 0.2 \times 10^2$

Notes: 1 – in treated manure with biodestructor; 2 – in control manure; *P <0.05 – compared to control samples

In the control air samples, starting from the 13th day, the MAFAnM content was approximately one and a half orders of magnitude higher than in the experimental samples, reaching $1.0 \pm 0.1 \times 10^5$ CFU/m³. Notably, in the experimental pig houses, the air MAFAnM levels did not exceed the indicative normative value of 1.0×10^5 CFU/m³, whereas in the control samples, starting from the 13th day, the MAFAnM content slightly exceeded this threshold.

Regarding the fungal microbiota in the air of the pig houses treated with the Sanaero biodestructor, it also gradually decreased, although less intensively than the bacterial population. Specifically, in the experimental samples from the 2nd to the 17th day, the fungal content in the air decreased by 3.8 times (P <0.05) and amounted to $1.4 \pm 0.1 \times 10^2$ CFU/m³. By contrast, in the control air samples on the 17th day, the number of fungi was $8.9 \pm 0.2 \times 10^2$ CFU/m³, which was 6.3 times higher than in the experimental air samples.

Overall, these data indicate that using the Sanaero biodestructor is an effective measure to improve the microclimate in pig fattening houses, which directly affects animal health, productivity, and the sanitary condition of the facilities.

The results of quantitative analysis of yeast and mesophilic microflora in the air of pig houses during the cold period of the year are presented in Table 4.

Analysis of the data in Table 4 revealed a similar dynamic regarding the effect of the Sanaero biodestructor on the air microflora in pig houses. Specifically, a gradual statistically significant decrease (P <0.05) in the content of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in the air was observed during the filling of the manure pit with the application of the Sanaero biodestructor, while the control facilities showed practically stable values.

Thus, summarizing the results, it can be concluded that the use of the Sanaero biodestructor in pig houses contributes to a significant reduction in the microbial contamination of the air, particularly in the quantity of MAFAnM and fungal microbiota. The effect becomes noticeable as early as the fifth day of applying the biological preparation and persists throughout the entire manure pit filling period.

In the experimental pig houses, the level of microbial contamination in the air did not exceed the indicative threshold (100.000 CFU/m³), unlike the control houses, where the norm was exceeded. Therefore, the use of the Sanaero biodestructor is an effective means of improving the sanitary condition of air in pig housing facilities.

Discussion. With the development of large-scale and intensive livestock farms, emissions of unpleasant odors from these agricultural facilities have attracted significant attention in recent years (Sklyar, 2020; Wang, 2023; Song, 2023). Consequently, technologies for managing unpleasant odors on farms are actively being developed and implemented (Matusiak, 2016; Maslov, 2023; Tian, 2023). Among existing methods, microbial deodorization is considered the most promising odor purification technique due to its safety, prolonged deodorizing effect, minimal use of biopreparations, and low cost (Thomas, 2017).

Table 4

Microbiological indicators of the microclimate in pig fattening pens when using the Sanaero biodestructor at an ambient temperature of +15 – +17°C, M ±m

Study duration, days	Parameters			
	MAFAnM, CFU/m³		Fungi, CFU/m³	
	1	2	1	2
2	2.4 ±0.2×10^5	3.1 ±0.1×10^5	8.1±0.3×10^2	8.9±0.3×10^2
5	1.8 ±0.1×10^5*	4.5±0.2×10^5	6.5±0.2×10^2	8.1±0.3×10^2
9	8.9 ±0.3×10^4*	3.3±0.3×10^5	4.3±0.2×10^2*	2.6±0.1×10^3
13	8.2 ±0.3×10^4*	1.3±0.2×10^5	3.8±0.1×10^2*	2.7±0.3×10^3
17	8.5 ±0..3×10^4*	1.7±0.1×10^5	4.2±0.2×10^2*	1.2±0.3×10^3

Notes: 1 – in treated manure with biodestructor; 2 – in control manure; *P <0.05 – compared to control samples

In this context, we investigated the changes in conditional pathogenic (*E. coli, S. aureus*) and pathogenic (*Salmonella*) bacteria in pig manure during the filling of the underfloor manure pit when applying the developed biological preparation Sanaero.

Our study demonstrated the efficacy of the Sanaero biodestructor in reducing the content of conditional pathogenic microorganisms in manure. By the second day after application, a statistically significant (P <0.05) reduction in *E. coli* counts was observed compared to the control. On the fifth and ninth days, bacterial growth inhibition was even more pronounced. The lowest *E. coli* level was recorded on day 13, indicating an activation of the biodestructor microorganisms' activity due to the accumulation of metabolic products, pH reduction, and creation of an unfavorable environment for pathogens. A similar trend was observed for *S. aureus*. Its presence in the treated manure decreased significantly faster than in the control, and by day 17, Staphylococcus aureus was no longer detected. This suggests an antagonistic effect of the biodestructor on pathogenic microflora.

The relevance of implementing such technology to improve the sanitary condition of manure and the environment in pig farms is supported by studies (Looft et al., 2012) showing that pig farms are significant biotopes for the formation of microbiocenoses with antibiotic-resistant bacteria. Indeed, the widespread emergence and dissemination of antibiotic-resistant bacteria have been documented in pig feces in many countries (Yan et al., 2024; Scicchitano, 2024). For example, Zhu et al. (2021) identified 149 unique antibiotic-resistant pathogens in pig feces and compost samples. On livestock farms, antibiotic-resistant microbial strains in feces can not only enter the environment via agricultural fertilizers (Chen, 2019) but also spread through bioaerosols and volatilization of feces into the air (McEachran, 2015). This allows us to conclude that the use of the Sanaero biodestructor is an effective tool for biological control of manure microflora, particularly in reducing *E. coli* and *S. aureus* populations, which is especially relevant in the context of combating antimicrobial resistance.

To control the quality of the microclimate in livestock facilities, it is important to study the contribution of fecal bacteria transmission via bioaerosols. Since most airborne pathogens are bacteria resistant to multiple drugs that can directly cause infections through the respiratory tract and skin contact (Wang, 2020; Gao, 2023), we examined the impact of the biological preparation Sanaero on quantitative microbiological indicators of air in pig fattening facilities. It was found that microorganisms in the developed biodestructor, when applied to underfloor manure pits in pig barns, positively influenced the sanitary condition of the air, contributing to the reduction of bacterial and fungal contamination. By the fifth day after the preparation's application, the amount of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) in the air decreased threefold (P <0.05) compared to the second day and was significantly lower than in the control rooms. Throughout the manure pit filling period (up to day 17), MAFAnM levels in the experimental pig houses remained consistently below the regulatory limit, whereas in the control rooms, levels exceeded permissible values starting from day 13. This indicates the sustained antimicrobial effect of the Sanaero biodestructor.

A reduction in fungal microflora in the air of experimental pig barns was also recorded – 3.8 times lower (P <0.05) over the study period, providing additional confirmation of improved microbiological air quality. In the control group, fungal counts on day 17 were 6.3 times higher than in the experimental group, underscoring the biodestructor's effectiveness not only against bacteria but also against microscopic fungi.

Overall, the application of the Sanaero biodestructor significantly improves the microbiological quality of air in livestock facilities, reducing health risks for animals and farm workers. Furthermore, research (Bai, 2022) reports that livestock farms are important sources of antibiotic-resistant bacteria and bacterial pathogens in agricultural air environments, which can be transported over long distances in the surrounding atmosphere.

It is also noteworthy that manure treatment with the biodestructor affects the fungal microbiota of pig barn air, as fungi are an important component of the microbial population in bioaerosols on pig farms (Sowiak, 2011). Most studies confirm the presence of genera such as Aspergillus, Scopulariopsis, Penicillium, and Cladosporium in the air (Viegas, 2013). Overall, respirable fungi can constitute up to 70% of the total fungal load in the air, posing a serious health threat to farm personnel (Viegas, 2013).

Thus, the application of the developed Sanaero biodestructor, which contains probiotic microorganisms (*Bacillus subtilis, Bacillus licheniformis, Lactobacillus plantarum, Saccharomyces cerevisiae*), substantially activates biological processes in pig manure during the filling of underfloor manure pits. It promotes the intensive development of beneficial microbiota and suppresses the growth of conditional pathogenic bacteria (*E. coli, S. aureus*) and technically harmful clostridia. This is accompanied by reduced gas formation (notably hydrogen sulfide and ammonia), diminished unpleasant odors (Grigorash, 2024), and improved microbial composition and microclimate in pig barns. The introduction of this biopreparation into manure utilization technology is a promising solution for the ecological improvement of livestock enterprises.

Conclusions. It was proven that the microorganisms of the biodestructor Sanaero (*Bacillus subtilis, Bacillus licheniformis, Lactobacillus plantarum, Saccharomyces cerevisiae*) exert antagonistic effects against *E. coli* and *S. aureus* cells in pig manure. The *E. coli* content decreased on average by two orders of magnitude by the end of the production process involving the filling of the underfloor manure pit, while *S. aureus* was completely inhibited. Moreover, the amount of *E. coli* in the treated manure was on average 4 to 8 times lower than in the control. This indicates suppression of conditional pathogenic bacterial activity by the biodestructor microorganisms even under low ambient temperatures. The application of the Sanaero biodestructor during the filling of the underfloor manure pit with pig manure promotes the active development of probiotic microorganisms and improves the sanitary condition of the manure.

The use of the Sanaero biodestructor in pig barns significantly reduces microbial contamination of the air, particularly the counts of mesophilic aerobic and facultative anaerobic microorganisms (MAFAnM) and fungal microflora. The effect becomes noticeable as early as the fifth day after application of the biological preparation and persists throughout the entire manure pit filling period. Therefore, the use of the Sanaero biodestructor is an effective measure to improve the sanitary condition of air in pig barns.

Ethical consideration

Plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy have been checked by the authors.

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САНІТАРНИЙ СТАН СВИНЯЧОГО ГНОЮ ТА МІКРОКЛІМАТУ У СВИНАРНИКАХ ЗА ОБРОБЛЕННЯ ЙОГО БІОДЕСТРУКТОРОМ САНАЕРО

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Резюме. *Нині активно розробляються й запроваджуються на фермах технології з управління неприємними запахами. Серед існуючих технологій мікробна дезодорація є найперспективнішим методом очищення запахів завдяки своїй безпеці, тривалому ефекту дезодорації та низькій ціні. Метою даного дослідження було дослідити зміни умовно-патогенних (E. coli, S. aureus) і патогенних (Salmonella) бактерій у свинячому гної під час наповнення підпідлогової ванни за застосування біодеструктора Санаеро. Встановлено, що мікроорганізми біодеструктора Санаеро (Bacillus subtilis, Bacillus lichenifomis, Lactobacillus plantarum, Saccharomyces cerevisiae) діють антагоністично у свинячому гної щодо клітин кишкової палички й золотистого стафілококу. При цьому вміст кишкової палички зменшувався, в середньому на два порядки на закінчення виробничого процесу із заповнення підпідлогової гноєвої ванни, а золотистий стафілокок повністю інгібувався. До того ж кількість кишкової палички у гної у досліді була, в середньому в 4 – 8 разів менша, ніж у контролі. Це засвідчує на пригнічення активності умовно-патогенних бактерій мікроорганізмами біодеструктора навіть за низької температури навколишнього середовища. Застосування біодеструктора Санаеро під час наповнення підпідлогової гноєвої ванни свинячим гноєм сприяє активному розвитку пробіотичних мікроорганізмів та здатність покращувати санітарний стан свинячого гною. Застосування біодеструктора Санаеро у свинарниках сприяє істотному зниженню рівня мікробного обсіяння повітря, зокрема кількості МАФАнМ та грибкової мікробіоти. Ефект помітний уже на п’яту добу застосування біологічного препарату та зберігається протягом усього періоду наповнення гноєвої ванни. Отже, використання біодеструктора Санаеро є ефективним засобом покращення санітарного стану повітря у свинарниках.*

Ключові слова: біодеструктор свинячого гною, антибіотикорезистентні бактерії, санітарний стан гною, мікроклімат свинарників, мікрофлора.

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