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MICROBIOLOGICAL AND PARASITOLOGIC INVESTIGATIONS OF CATTLE SLURRY FERMENTED AEROBICALLY IN THERMOPHILIC CONDITIONS

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ABSTRACT

Microbiological and parasitologic investigations dealing with the influence of aeration on fecal bacteria and Ascaris suum eggs survival in cattle slurry were carried out. The results showed that already the first stage of aeration (reactor I) eliminated quickly a number of Salmonella senftenberg (10^5 times during 2.5 hours in relation to the control test) and EHEC (10^8 times during 2.5 hours in relation to the control test) respectively. The longest survival time was for D group streptococci. Inactivation of Ascaris suum eggs due to thermic conditions fluctuated between 50 and 90 minutes. The final stage of aeration (reactor II) completely destroyed pathogen germs and Ascaris suum eggs present in cattle slurry.

Key words: aerobic fermentation, cattle slurry

INTRODUCTION

Animal liquid waste is likely to contain numerous pathogenic microorganisms depending on the flock's health condition and origin. The following microorganisms may occur: Salmonella, Leptospira, Treponema, Erysopelotrix, Mycobacterium, Brucella, Bacillus, Riketsje, Chlamydie and others [2, 8, 9, 10]. Considering the bacteriological charge included in cattle slurry, some regions prohibit to use it in agriculture as a fertiliser. The prohibition of cattle slurry outflowing concerns mainly aquiferous layer regions.This problem is raised mainly in UE countries, especially on the territory of Germany [13, 14]. From 1988, according to the law, cattle slurry outflowing has been prohibited in I and II protection zone. Since these regions are inhabited by farmers breeding cattle according to the non-bedding system, a clear need was formed to manage safely the generated cattle slurry. On the basis of created pilot programmes, cattle slurry aeration systems were constructed in order to liquidate the microorganisms included cattle slurry [3]. It is a preliminary condition enabling its later utilisation as a fertiliser in these regions.

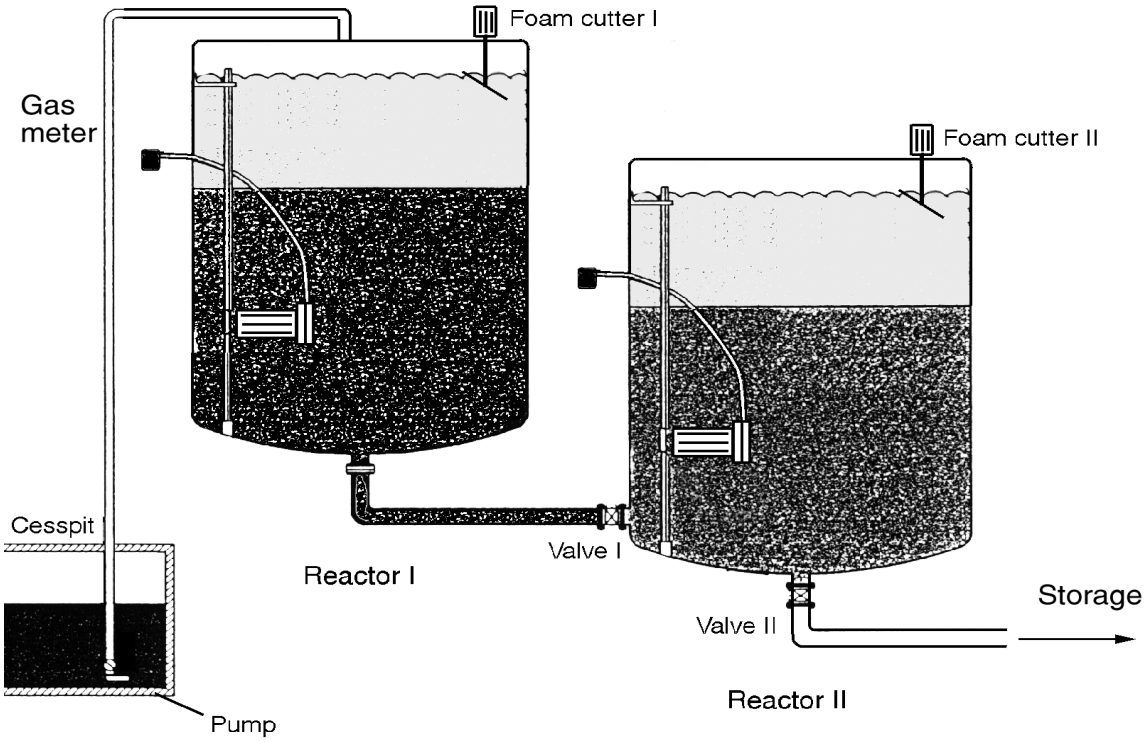
The aim of the undertaken research was to specify the sanitary-hygienic condition of cattle slurry aerated basing on selected fecal microorganisms and Ascaris suum eggs survival. On the basis of obtained results, an effectiveness assessment was conducted concerning cattle slurry desinfection systems, which is extremaly important for ecological reasons.

MATERIALS AND METHODS

System description

The system comprised appliances consisting of two cattle slurry aeration bioreactors = initial aeration bioreactor (I) and final aeration bioreactor (II). The volume of each of them was 15 m^3 (picture 1). The cattle slurry was aerated and mixed by the use of appliances with two 3 kW – engines. The oxygen, forced in together with atmospheric air, caused the expansion of microflora, which decomposed the organic matter included in the cattle slurry. It was an exothermic process, in result of which the temperature of the bioreactor's cattle slurry rose to over 60° C. Within 24 hours a 5 m^3 release of already aerated cattle slurry was performed from bioreactor II to an external reservuar. Simultaneously, the same quantity of initially aerated cattle slurry was moved from bioreactor I to bioreactor II, and also raw cattle slurry was moved from cowhouse to bioreactor I. The process was automatically steered and controlled. According to the designer, the oxygenated cattle slurry, after moving through the two reactors, should be completely desinfected.

Fig. 1.



Parasitologic investigations

Ascaris suum eggs were used in parasitologic investigations, since they are particularly resistant to the activity of unfavourable environmental conditions. Ascaris egg water slurry was inserted into Perlon bags made of a special material with pores no larger than 28 μ m. The bags were closed with a clip and then dipped into aerated cattle slurry. The pore size made it impossible for the eggs to get out, enabling at the same time their contact with the surroundings. After a specified time of exposure in the cattle slurry, the bags with eggs were taken out, and their content was shifted to Petry plates including with a small amount of running water. The plates were incubated for 3 weeks in the incubator at 29° C. Then, 300 eggs were differentiated under the microscope into those containing larvas and degenerated stages. Accepting the control test as 100% (the percentage of larval eggs after the direct slurry incubation) the percentage of invasive eggs in particular experiment phases was specified.

Bacteriological investigations

1. Microorganisms used in the investigations

The following microorganisms were used in the microbiological research:

Salmonella senftenberg	DSM No 10062
Eschericha coli (EHEC)	DSM No 4779
Streptococcus faccium	DSM No 2146

The microorganisms, according to Deutschen Stammsammulung fur Mikroorganismen (DSM) numeration, came from the collection belonging to Institut fur Umwelt und Tierhygiene Universitat Hohenheim in Stuttgart. Clean colonies were taken to a nutritions bouillon, which was incubated for 24 hours at 37° C. The microorganism slurries gained in this way (in various concentration and quantity depending on the experiment duration) were added to 15 ml of freshly taken cattle slurry, which was then placed in specially designed plastic bags functioning as microorganism carriers. Thanks to proper filters they enabled liquid and gas diffusion to their internal parts, making it at the same time impossible for the examined bacteria to get out of the carriers' reach [11]. It permitted the quantity specification of the examined microorganisms in particular experiment phases, and also the specification of their inactivation rate under the influence of unfavourable environmental conditions.

2. Reisolation of examined microorganisms.

The number of all examined microorganisms was calculated according to NPL method.

2.1 Determination of Salmonella particle number

In the first phase peptone water was used (24h, 37°C), from which 0.1 ml of sample at a time was taken to a liquid Rappaport base (24 h, 43°C). After incubation the material was sifted onto a stable BPL-agar base (24h, 37°C). Salmonella particles grew in the form of tiny pale-pink colonies, accompanied by a change in the base colouring.

2.2 E.coli number determination

In the first phase cattle slurry portions were added to Mac Concey liquid base (43°C for 24h). Then, the material was sifted onto agar with tergitol, together with 1% of 2, 3, 5 – TTC solution (24h, 37°C). E.coli grew in the form of yellow colonies, around which the base colour cleared up.

2.3 Group D streptococci determination

As a liquid base for selective fecal streptococci growth glucose and azide bouillon was used. After 48 hours of incubation at 37°C the material from positive samples was moved onto a stable base, e.g. agar with canamicine and esculine (37°C for 48 hours). Fecal streptococci grew in the form of characteristic colonies, around which a darkly-coloured base appeared.

RESULTS AND THEIR DISCUSSION

The investigations results concerning the examined microorganism survival in bioreactor I (initial aeration) are presented in table 1, 2 and 3.

The content of E.coli (EHEC) inside the carriers ranged from 2.3×10^7 to 4.3×10^8, 1.9×10^8 of microorganisms in 1g of cattle slurry (control test). The carriers were placed in the bioreactor's environment, where the oxygenated cattle slurry reached the temperature from 51.0 to 51.5°C, and its pH varied between 7.73 – 7.78. As can be seen in table 1, the number of E.coli (EHEC) particles decreased gradually under the influence of the bioreactor's environment activity. E.coli drop was insignificant (10-fold) in the first hour of the experiment, but in the second hour the number of bacteria decreased 10^6 – fold on average, in

relation to the control test. Complete EHEC elimination was observed after 150 minutes from the beginning of the experiment.

Table 1

	Control	30 min.	60 min.	90 min.	120 min.	150 min.	temp. (°C)	pH
EHEC (cfu/g)								
Sample A_1	$2.3 \cdot 10^8$	$2.3 \cdot 10^7$	$4.3 \cdot 10^6$	$4.3 \cdot 10^5$	$2.3 \cdot 10^2$	nn	51.0-51.5	7.73-7.78
Sample A_2	$2.3 \cdot 10^7$	$2.3 \cdot 10^7$	$9.3 \cdot 10^6$	$1.5 \cdot 10^5$	$4.3 \cdot 10^2$	nn		
Sample B_1	$4.3 \cdot 10^8$	$2.3 \cdot 10^7$	$2.3 \cdot 10^7$	$2.3 \cdot 10^5$	$9.3 \cdot 10^2$	nn		
Sample B_2	$9.3 \cdot 10^7$	$2.3 \cdot 10^7$	$9.3 \cdot 10^6$	$7.5 \cdot 10^4$	$2.3 \cdot 10^2$	nn		
Mean	$1.9 \cdot 10^8$	$2.3 \cdot 10^7$	$1.1 \cdot 10^7$	$2.2 \cdot 10^5$	$2.5 \cdot 10^2$	nn		

Salmonella senftenberg particles were eliminated at a considerably slower space in the bioreactor. After 30 minutes their number did not undergo any change, and later a mean 10 – fold drop of bacteria number was observed every 30 minutes. After 150 minutes of experiment duration the mean value of microorganism concentration was 2.5 x 10^2 of microorganisms in 1g, which was a 10^5 – fold drop in relation to the control test (table 2).

Table 2

	Control	30 min.	60 min.	90 min.	120 min.	150 min.	temp. (°C)	pH
Salmonella senftenberg (cfu/g)								
Sample A_1	$4.3 \cdot 10^7$	$2.1 \cdot 10^7$	$2.3 \cdot 10^6$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$2.3 \cdot 10^2$	50.4-52.1	7.67-7.77
Sample A_2	$4.3 \cdot 10^7$	$4.3 \cdot 10^7$	$2.3 \cdot 10^6$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$2.3 \cdot 10^2$		
Sample B_1	–	$9.3 \cdot 10^7$	$2.3 \cdot 10^6$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$2.3 \cdot 10^2$		
Sample B_2	–	$4.3 \cdot 10^7$	$4.3 \cdot 10^6$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$2.3 \cdot 10^2$		
Mean	$4.3 \cdot 10^7$	$5.0 \cdot 10^7$	$2.8 \cdot 10^6$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$2.3 \cdot 10^2$		

Group D streptococci behaved differently. Carriers containing the cattle slurry with microorganism slurry was placed in bioreactor I after adding 5 m^3 of raw cattle slurry to it. As a result the cattle slurry temp. initially dropped to about 10° C and as late as after several hours it returned to the norm range. Fecal streptococci survival is illustrated by table 3. In both experiments the number of fecal streptococci decreased very slowly. After 17 hours it dropped from 10^1 to 10^2 times, and after 24 hours as many as 2.3 x 10^4 of bacteria was observed in 1g of cattle slurry.

Table 3

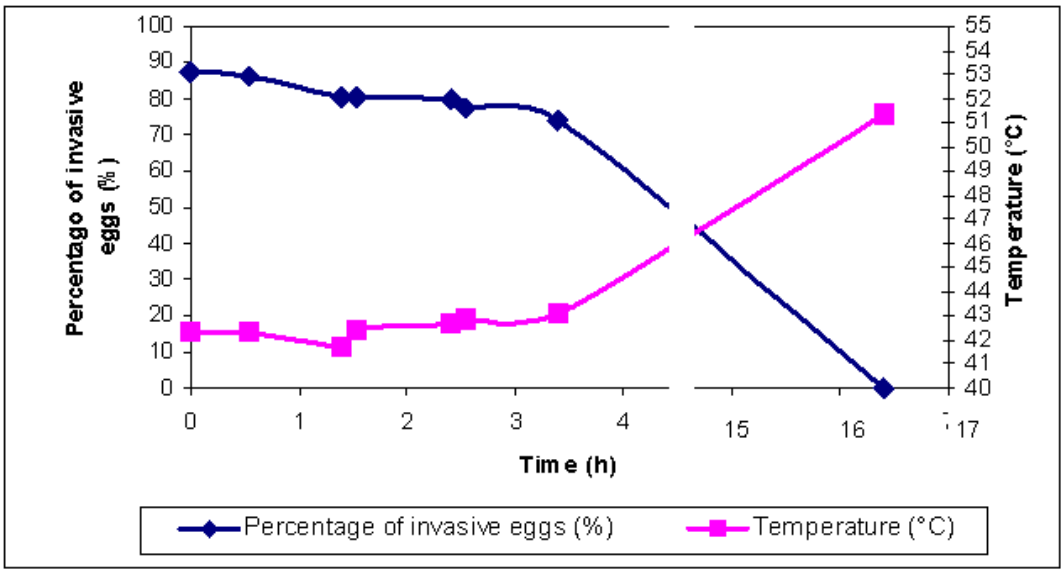
	Control	17 hours	24 hours	temp. (°C)	pH
Experiment I					
Sample A_1	$7.5 \cdot 10^6$	$9.3 \cdot 10^3$	$2.3 \cdot 10^4$	41.7-51.5	5.76-7.80
Sample A_2	$9.3 \cdot 10^6$	$9.3 \cdot 10^4$	$2.3 \cdot 10^4$		
Sample B_1	–	$4.3 \cdot 10^4$	$2.3 \cdot 10^4$		
Sample B_2	–	$4.3 \cdot 10^4$	$2.3 \cdot 10^4$		
Mean	$8.4 \cdot 10^6$	$4.7 \cdot 10^4$	$2.3 \cdot 10^4$		

Experiment II					
Sample A_1	$4.3 \cdot 10^6$	$9.3 \cdot 10^5$	$2.3 \cdot 10^5$	42.2-50.5	7.58-7.67
Sample A_2	$4.3 \cdot 10^6$	$9.3 \cdot 10^5$	$4.3 \cdot 10^5$		
Sample B_1	–	$4.3 \cdot 10^5$	$9.3 \cdot 10^4$		
Sample B_2	–	$2.3 \cdot 10^5$	$9.3 \cdot 10^4$		
Mean	$4.3 \cdot 10^6$	$6.3 \cdot 10^5$	$2.3 \cdot 10^4$		

The conducted investigations confirm a high resistance of fecal streptococci to the influence of environmental factors.

The parasitologic investigation results conducted in bioreactor I are presented in pictures 1 and 2. The cattle slurry aeration within the temperature range of 41.4 – 43.1°C caused an insignificant drop of Ascaris suum egg invasiveness (picture 1). After 3 hours the bioreactor's environment caused a minimal drop of invasive egg percentage from 87.17 to 73.8%. Whlie the cattle slurry temperature increased, the invasive egg quantity decreased gradually. After 16 hours of experiment duration, when the temp. in the bioreactor reached the value of over 51°C, none of 300 examined eggs proved the presence of invasive larvas.

Fig. 2



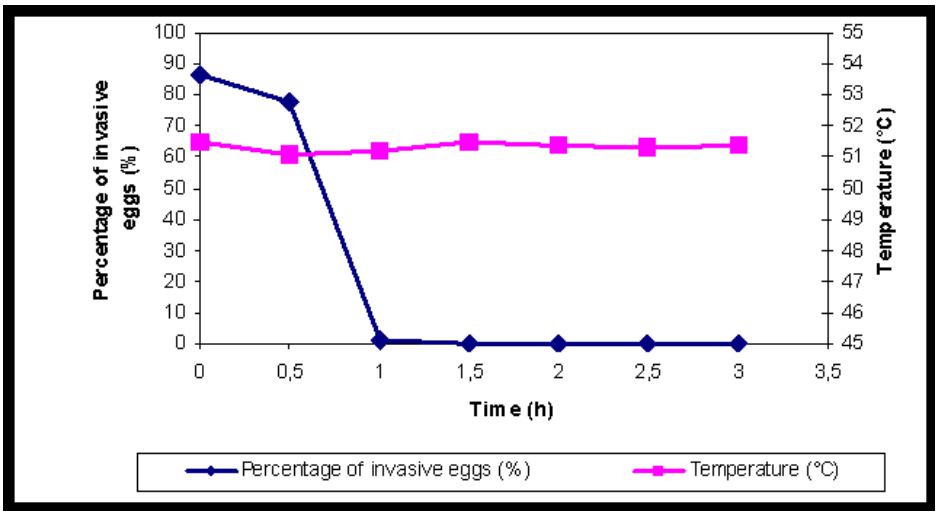
Picture 2 illustrates the drop of invasive egg percentage in bioreactor I in the temperature range of 51.4 – 51.5°C. After 1 hour of aeration, the invasive egg percentage decraesed from 86.5 to 1.16%, and after 90 minutes no invasive eggs were present in the aerated cattle slurry. In bioreactor II (final aeration) the influence of existing conditions on fecal streptococci survival and Ascaris suum behaviour was specified.

The temperature of aerated cattle slurry was higher than in the initial aeration bioreactor and was in the range of 54.5 – 56.8°C (table 4). After placing fecal streptococci carriers in it, one could observe a rapid drop in the number of examined microorganisms in cattle slurry. As early as after 3 hours their number decreased from 1.3×10^8 to 4.6×10^1 of microorganisms in 1g of cattle slurry. During the 4^{th} and 5^{th} hour of investigations only an insignificant number of them was detected, since it was not possible to isolate them in 1g of cattle slurry samples, but only in 1out of four examined 10g of samples.

Table 4

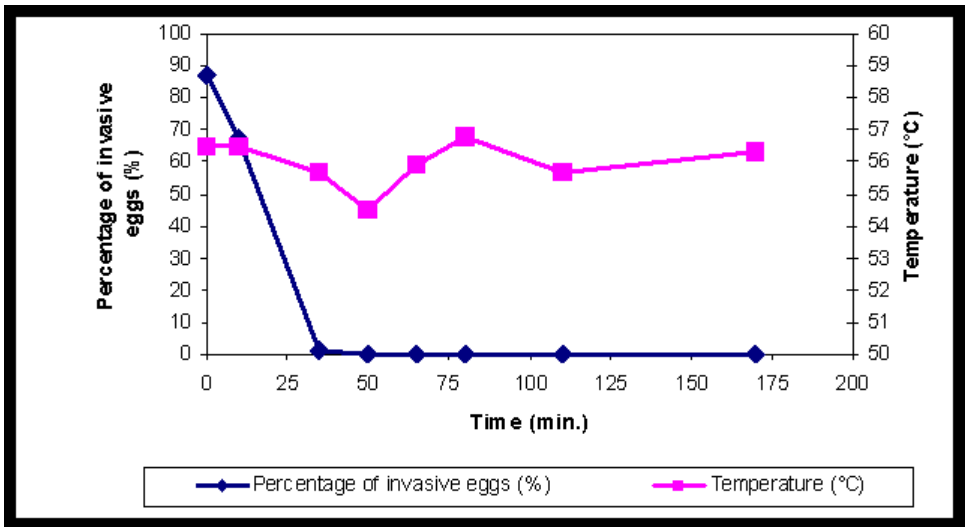
	Control	3 hours	4 hours	5 hours	temp. (°C)	pH
Sample 1	$2.3 \cdot 18^{8}$	$2.3 \cdot 10^{1}$	10g positive	10g positive	54.5-56.8	7.92-8.12
Sample 2	$4.3 \cdot 10^{7}$	$4.3 \cdot 10^{1}$	10g positive	10g positive		
Sample 1		$2.3 \cdot 10^{1}$	10g positive	nd		
Sample 2		$9.3 \cdot 10^{1}$				
Mean	$1.3 \cdot 10^{8}$	$4.6 \cdot 10^{1}$				

Fig. 3



The negative influence of bioreactor II environment on Ascaris suum egg behaviour was also observed. As early as after 35 minutes the invasive egg percentage decreased from 87.2 to 1%, and after 50 minutes no invasive eggs were present on it (picture 4). The research proved high effectiveness of aeration process activity on the environment of both bioreactors in the realm of cattle slurry desinfection.

Fig. 4



DISCUSSION

The hazards resulting from cattle slurry utilisation in agriculture depends mainly on the number and kind of pathogenic microorganisms incubated in it. In case of animal illnesses fought against as a rule, on the basis of legal regulations functioning in particular countries, the cattle slurry is subject mainly to rigour chemical desinfection. A particular hazard is created by asymptomatic illnesses. The cattle slurry does not undergo any utilisation processes then, and when flowed out in fields, is likely to create health hazard for people and animals [11,16]. To minimalise this risk new improvement methods of sanitary-hygienic condition of cattle slurry are being looked for, which would guarantee its safe utilisation in agriculture. Among biological utilisation methods, aeration deserves a special attention. Apart from the hygienic condition improvement, the aeration process causes the increase of cattle slurry fertilising value, decreases its volume and smell intensity.

Inserting air in the form of tiny bubbles into deeper cattle slurry layers guarantees the development of microflora, which causes the decomposition of organic matter included in it. This process is accompanied by the release of a large warmth quantity, as a result of which the cattle slurry warms up. In the temperature of about 40°C, the nitrifying microorganism activity is hampered, and in the temp. range of 45-55°C the mesophilic bacteria population appears [6]. The process of aerobic cattle slurry stabilisation, described as a method of animal (liquid) waste slurry composting, is commonly regarded as an effective way of its biological desinfection [9, 11, 12]. The process of constant aeration of cattle slurry introduced into the bioreactor, used in one's own research, prevented the cattle slurry from delamination, which guaranteed an even temperature distribution. In the final aeration bioreactor the temp. reached the value to 56.8°C, which was the reason why the invasive eggs were not present in cattle slurry after 50 minutes, and as for fecal streptococci content it dropped 10^7 times within 3 hours. Satisfactory results were gained as early as in the initial aeration phase. The temp. range of 50.4 – 51.5°C with pH 7.67 – 7.78 caused the liquidation of EHEC microorganisms added to the cattle slurry, EHEC having the concentration of 1.9 x 10^8/g as early as 150 min., and the number of Salmonella senftenberg particles decreased 10^5 – fold during this time. Only in the preliminary phase, when the temp. dropped to 41.7 and 42.2°C, was the fecal streptococci elimination insignificant (100 – fold within 24h). The drop of the ascarid invasive egg percentage was very inconsiderable during this time.

The effective influence of aeration on the elimination of pathogenic microorganisms included in it is also confirmed by ther authors' works. Feachem and co-authors [7] state that the survival of microorganisms of Vibrio, Salmonella and Shigella kind, and also Ascaris and Taenia eggs decreases significantly in the aerated cattle slurry. The temp. value and interaction time were the factors determining the effectiveness of aeration processes. According to the authors, in order to eliminate the undesirable microorganisms and parasite ova it is sufficient to increase the cattle slurry temp. to 62°C for 1 hour; to 50°C – for 1 day, and at 46°C the time necessary to liquidate them is one week. Also Doyle and Nouve's research [4] indicates a clear reduction of pathogenic bacteria number in the aerated pig slurry. As results from Wassen and co-authors' experiences [16] the cattle slurry aeration should last 48 hours (pH 8.7, temp. 45°C) or at least 2 hours when it's possible to reach the temp. of 50°C and pH 9.0. Errebo Larsen and Munch' investigations [5] prove that in order to reduce the Salmonella typhimurium number 10^5 – fold in the aerated cattle slurry in winter conditions, the period of 8 weeks is necessary, and in the anaerated cattle slurry – up to 30 weeks.

In the oxygenated cattle slurry in boreactors as a result of its temp. increase the release of ammonia takes place. It is one of the main factors acting viruscidally in the cattle slurry [17].

It is necessary to point out that the pathogenic microorganism concentration used in one's own research was very high. Eliminating the concentration of such a large number of microorganisms in the aerated cattle slurry proves high efficiency of the tested systems, and the sufficient rate of cattle slurry desinfection in the summer-autumn period. In case of proper mineral ratios in soils the cattle slurry can be used as a fertiliser after the aeration process, being no hazard for the protected aquiferous layers. In order to fully evaluate the cattle slurry aeation systems, it is also necessary to examine their efficiency in the low-temp. period.

CONCLUSIONS

1. During the cattle slurry aeration in bioreactors, its temp. rises to over 55.8°C.
2. In the aerobically fermented cattle slurry in thrmophilic conditions there is a fast inactivation of pathogenic microorganisms and Ascaris suum parasite ova.
3. After 24 hours of aeration an effective cattle slurry desinfection took place in the period of conducted investigations. It enables using it as a fertiliser in the regions of protected water-bearing layers.

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