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THE INFLUENCE OF ANAEROBIC FERMENTATION ON THE SURVIVAL OF MICROORGANISMS

Zbigniew Paluszak
Department of Animal Hygiene and Village Environment,
University of Technology and Agriculture, Bydgoszcz, Poland

ABSTRACT

The subject of investigations was the evaluation of sanitary-hygienic state of slurry subject to anaerobic fermentation in bioreactor under thermophilic conditions. The influence of anaerobic fermentation on the survival of Salmonella senftenberg, EHEC and streptococci D group was specified. It turned out from investigations that after 4 hours the number of Salmonella senftenberg and EHEC bacteria decreased 10^8 times in relation to the control test. The lowest elimination rate was for streptococci D group. The percentage of invasive eggs of Ascaris suum decreased after 4 hours from 89% to 7%. The investigation proved proper run of anaerobic process in bioreactor and sufficient desinfection of cattle slurry.
Key words: cattle slurry, anaerobic fermentation, parasite bacteria

INTRODUCTION

Cattle slurry disposal in countries of intensive breeding and limited soil area is an important ecological problem [5, 7, 9, 10]. It gains a particular significance mainly in some European Union countries, where the quantity of cattle slurry and the possibility of its agricultural utilisation begins to limit the size of animal production [6, 16]. In order to reduce the sanitary-hygienic risk, resulting from the possibility of underground water contamination with fecal bacteria, there is a necessity to condition it before flooding it onto fields [11].

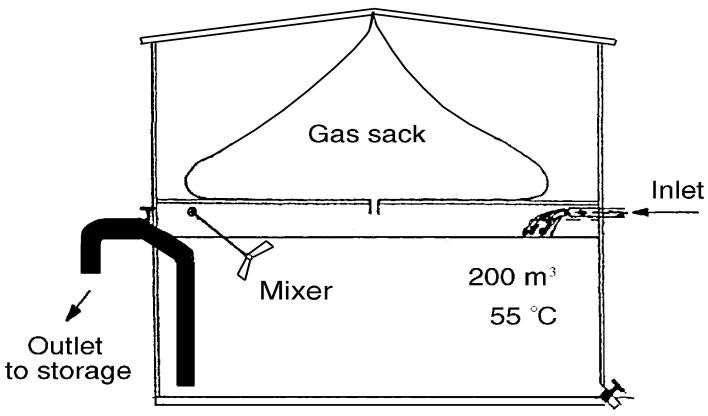
One of the alternative methods of cattle slurry desinfection is anaerobic fermentation [14]. The methanogenic bacteria, occurring in large number in anaerobic conditions and having antibiotic qualities, affect the pathogenic cattle slurry flora in a negative way [5]. For hygienic reasons, it is highly reccomendable to use anaerobic fermentation in thermophilic conditions, that is in the temp. range of 50-60°C [1]. The aim of the research was to specify the effectiveness of cattle slurry desinfection made subject to anaerobic fermentation in the bioreactor on the basis of microbiological and parasitologic control of cattle slurry. The bioreactor was formed within the pilot programme finansed by Universitat Hohenheim in Stuttgart, whose aim was to elaborate alternative possibilities of the biological method at cattle slurry conditioning and utilisation for fertilising purposes [3].

MATERIALS AND METHODS

System description

The basic biogas production apparatus was the bioreactor – a warmed steel reservuar of 200 m^3 capacity (pict. 1). The cattle slurry was submitted to anaerobic fermentation in thermophilic conditions, i.e. at 55°C. Proper thermoisolation and an automatically steered heating system guaranteed maintaining stable temperature. Every 24 hours 5 m^3 of fresh cattle slurry was added to the upper part of the bioreactor, and was mixed with formerly fermented cattle slurry. Thanks to appropriate protections one could be sure that the delivered fresh cattle slurry was going to stay in the bioreactor for at least 24 hours. The biogas formed during fermentation was accumulated in a plastic container placed above the bioreactor, and then burnt. Part of it was used to warm the biomass fermenting in anaerobic conditions, and the rest of it served as building heating. After fermentation (the period of 40 days) and the microbiological charge liquidation, the cattle slurry was supposed to serve fertilising purposes in agriculture in the areas of protected aquiferous layers.

Fig. 1



Parasitologic research

In parasitologic investigations the water slurry of Ascans suum eggs was used; it was placed in bags made of special material having the pore size 28 μm. The bags were tightly closed, and then dippred in cattle slurry subject to anaerobic fermentation. The pore size made it impossible for the eggs to get out of the bag, but at the same time it enabled their contact with the environment. After specified exposure time the bags' contents was placed on Petry plates containing a small quantity of water. After incubation (3 weeks at 29°C) the percentage of invasive eggs in particular phases of the experiment was specified.

Bacteriological research

1. Microorganisms used in the research

The following microorganisms were used in the microbiological investigations:

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

The microorganisms according to Deutschen Stammsammulung fur Mikroorganismen (DSM) numeration came from a collection belonging to Institut fur Umwelt und Tierhygiene Universitat Hohenheim in Stuttgart. The obtained microorganisms slurries(in various concentration and quantity depending on the experiment duration) were addaed to 15 ml of freshly taken cattle slurry, which was then placed in specially constructed plastic containers functioning as microorganism carriers [14]. Due to proper filters the carriers enabled liquid and gas diffusion to their inside parts, making it possible at the same time for the examined bacteria to get outside the carriers. It enabled to specify the quantity of examined microorganisms in particular experiment phases, and to establish in this way their inactivation rate under the influence of unfavourable environmental conditions.

2. Reisolation of the examined microorganisms

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

2.1 Specifying the number of salmonella

In the first phase peptone water was used (24h, 37°C), from which the material of 0.1 ml a sample taken to Rappaport liquid base (24h, 43°C). After incubation it was taken to BPL – agar stable base (24h, 37°C). Salmonella units grew in the form of pale-pink tiny colonies, and the colour of the base changed from greenish into pink.

2.2 E.coli number determination

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

2.3 Group D streptococci determination

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

RESULTS AND THEIR DISCUSSION

In the bioreactor, where the anaerobic fermentation took place in thermophilic conditions, there was stabile temperature from 50.1 to 51.8°C, and pH ranged from 7.97 to 8.11.

The survival of EHEC-coli and salmonella senftenberg microorganisms in the cattle slurry in anaerobic conditions was specified with the intervals of one hour. As can be seen in table 1 the concentration of EHEC in the carriers before the experiment began amounted approximately to 7.6 x 10^6 of microorganisms in 1g of cattle slurry. After one hour the number of bacteria decreased by 10^2 - fold, and after 2 hours as much as 10^7 – fold in relation to the central test. During the 3rd hour of experiment only in 1 out of 4 examined samples EHEC was isolated in 10g of cattle slurry, and after 4 hours there occurred their complete elimination in the examined samples.

Table 1

	Control	60 min.	120 min.	180 min.	240 min.	temp. (°C)	pH
EHEC (cfu/g)							
Sample A_1	$4.3 \cdot 10^8$	$9.3 \cdot 10^6$	$2.3 \cdot 10^1$	10g positive	nn	50.1-50.9	8.11
Sample A_2	$9.3 \cdot 10^7$	$9.3 \cdot 10^6$	$2.3 \cdot 10^1$	nn	nn		
Sample B_1	–	$2.3 \cdot 10^6$	$2.3 \cdot 10^1$	nn	nn		
Sample B_2	–	$9.3 \cdot 10^6$	$2.3 \cdot 10^1$	nn	nn		
Mean	$2.6 \cdot 10^8$	$7.6 \cdot 10^6$	$2.3 \cdot 10^1$	nn	nn		
Salmonella senftenberg (cfu/g)							
Sample A_1	$2.3 \cdot 10^8$	$9.3 \cdot 10^5$	$4.3 \cdot 10^2$	$4.3 \cdot 10^2$	nn	50.1-50.9	8.11
Sample A_2	$9.3 \cdot 10^8$	$2.1 \cdot 10^6$	$4.3 \cdot 10^2$	$4.3 \cdot 10^2$	nn		
Sample B_1	–	$2.3 \cdot 10^5$	$4.3 \cdot 10^2$	$4.3 \cdot 10^1$	nn		
Sample B_2	–	$2.3 \cdot 10^5$	$4.3 \cdot 10^2$	$1.5 \cdot 10^2$	nn		
Mean	$5.8 \cdot 10^8$	$8.7 \cdot 10^5$	$4.3 \cdot 10^2$	$2.6 \cdot 10^2$	nn		

Salmonella bacteria were eliminated at a slightly slower rate from the anaerobically fermented cattle slurry. Their reduction occurred in a far more mild way. In the 2nd and 3rd hour of investigation their number was 10^6 times smaller in comparison with the control test and ranged from 4.3 to 2.6 x 10^2 of bacteria in 1g of cattle slurry. In the 4th hour the experiment there occurred a complete Salmonella liquidation in the eramined cattle slurry (table 1).

Table 2 presents examination results obtained in the second experiment. The investigation samples were taken in other time intervals. The survival of EHEC and Salmonella bacteria was determined after 1, 2, 2.5 and 3 hours from the moment of inserting bacterial carriers into the environment of anaerobic fermentation bioreactor. A slightly different change dynamics was observed in comparison with experiment 1 (table 1). After 1 hour the number of EHEC

bacteria in the reactor environment did no undergo any changes. After 2 hours their concentration dropped 10^3 times, and after 150 min. – 10^5 times in relations to the control test. In the 3rd hour of investigation there occurred an insignificant number of microorganisms in 3 out of 4 investigated samples. In this experiment, as in the first one, the number of salmonella bacteria in the anaerobic environment was eliminated in a milder way. After 2 hours their concentration decreased 10^3 times, and after 3 hours – 10^6 times in relation to the control test, and had the values of 1.8 x 10^1 in 1g of cattle slurry.

Table 2

	Control	60 min.	120 min.	150 min.	180 min.	temp. (°C)	pH
EHEC (cfu/g)							
Sample A_1	$4.3 \cdot 10^8$	$9.3 \cdot 10^6$	$1.5 \cdot 10^3$	$4.3 \cdot 10^1$	10g positive	50.1-51.8	7.97-8.18
Sample A_2	$2.3 \cdot 10^7$	$9.3 \cdot 10^7$	$9.3 \cdot 10^2$	$9.3 \cdot 10^1$	nd		
Sample B_1	–	$2.3 \cdot 10^7$	$4.3 \cdot 10^2$	$2.3 \cdot 10^3$	10g positive		
Sample B_2	–	$2.3 \cdot 10^7$	–	$1.5 \cdot 10^2$	$3.6 \cdot 10^1$		
Mean	$3.3 \cdot 10^7$	$2.0 \cdot 10^7$	$1.5 \cdot 10^4$	$1.3 \cdot 10^2$			
Salmonella senftenberg (cfu/g)							
Sample A_1	$2.3 \cdot 10^7$	$9.3 \cdot 10^6$	$2.3 \cdot 10^3$	$1.5 \cdot 10^2$	$1.5 \cdot 10^1$	50.1-51.8	7.97-8.18
Sample A_2	$9.3 \cdot 10^7$	$9.3 \cdot 10^6$	$1.5 \cdot 10^3$	$4.3 \cdot 10^2$	$1.5 \cdot 10^1$		
Sample B_1	–	$2.3 \cdot 10^6$	$4.3 \cdot 10^4$	$2.3 \cdot 10^2$	$4.3 \cdot 10^1$		
Sample B_2	–	$9.3 \cdot 10^5$	$4.3 \cdot 10^3$	$2.6 \cdot 10^2$	$9.2 \cdot 10^1$		
Mean	$5.8 \cdot 10^7$	$5.5 \cdot 10^6$	$1.3 \cdot 10^4$	$2.6 \cdot 10^2$	$1.8 \cdot 10^1$		

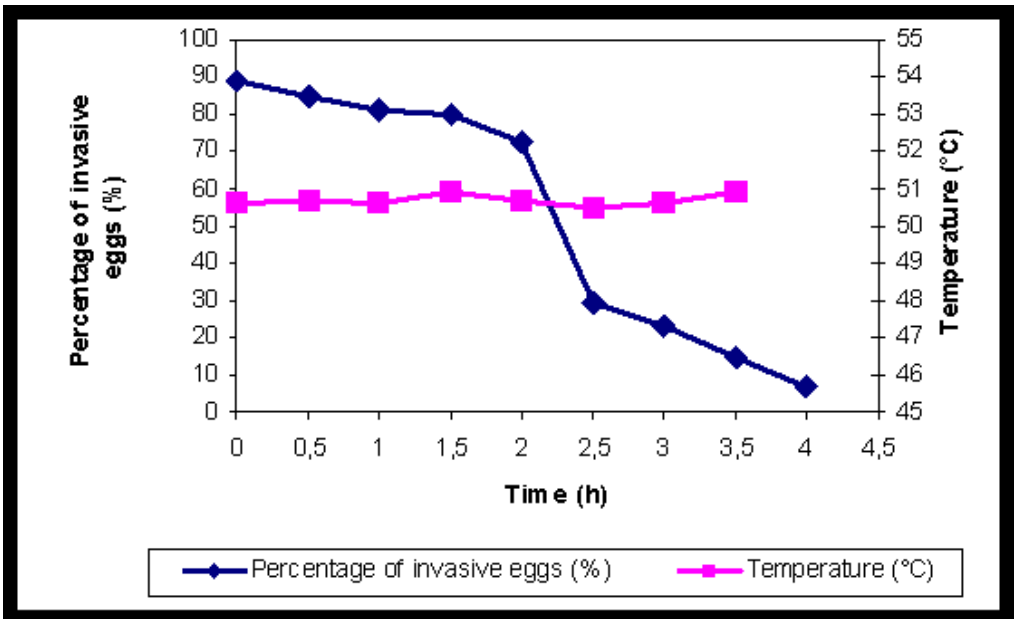
Fecal streptococci turned out most resistant to the anaerobic environment activity (table 3). Their number decreased far more slowly than in both remaining kinds of microorganisms. After 20 hours their concentration dropped 105 times in comparison with the control test (3.3 x 10^9 of microorganisms in 1g). In the next 4 hours their reduction was not observed, since their number varied between 6.3 and 3.6 x 10^3 of bacteria in 1g of cattle slurry.

Table 3

	Control	20 hours	22 hours	24 hours	temp. (°C)
Sample A_1	$4.3 \cdot 10^9$	$9.3 \cdot 10^3$	$4.3 \cdot 10^3$	$2.3 \cdot 10^3$	50.15-51.80
Sample A_2	$2.3 \cdot 10^9$	$2.3 \cdot 10^4$	$2.3 \cdot 10^3$	$7.5 \cdot 10^3$	
Sample B_1		$4.3 \cdot 10^4$	$9.3 \cdot 10^3$	$2.3 \cdot 10^3$	
Sample B_2		$1.5 \cdot 10^4$	$9.3 \cdot 10^3$	$2.3 \cdot 10^3$	
Mean	$3.3 \cdot 10^9$	$2.3 \cdot 10^4$	$6.3 \cdot 10^3$	$3.6 \cdot 10^3$	

The parasitologic examination results are presented in picture 2. The control test, performed on eggs prepared from the uterns of pig ascarids, proved the occurrence of 89 per cent of larval eggs (active eggs) after 30 – day incubation at 28°C. Under the conditions present in the anaerobic bioreactor there occurred a fast invasiveness rate drop of Ascaris eggs. After 1 hour in anaerobic conditions in the cattle slurry, the percentage of invasive eggs decreased to 81.3 %, and after 4 hours – to 7 %. In spite of not conducting further control for technical reasons, one can assume on the basis of egg invasiveness drop rate that practically there is no possibility of their survival in the 24-hour anaerobic fermentation bioreactor.

Fig. 2



DISCUSSION

Anaerobic fermentation, next to aeration, belongs to effective methods of biological cattle slurry desinfection. The advantage of this method in comparison with aeration are the good sides resulting from: lower energy input necessary to conduct the process, smaller quality of bacterial biomass formation and biogas creation, which can be used for heating purposes [14]. Moreover, this method also reduces the intensity of cattle slurry smell, improves the fertilising value and its fluidity.

The increased bioreactor's temperature and a large concentration of methanogenic bacteria, acts bactericidally on pathogenic microorganisms included in the cattle slurry [1]. It should be stressed, moreover, that anaerobic fermentation occurring in mesophilic conditions does not guarantee complete liquidation of cattle slurry bacteriological input [4,19]. In order to gain full cattle slurry desinfection it is necessary to ferment it anaerobically in thermophilic conditions [15]. In one's own investigations the bioreactor's temperature reached the value from 50.1 to 51.8°C and was exceptionally stabile. The EHEC microorganisms in anaerobically fermented cattle slurry were eliminated in the fastest way, since as early as in the second hour of investigation their number decreased 10^7 times in relation to the control test in experiment I, 10^3 times in experiment II. After 4 hours of investigation there was a complete elimination of EHEC bacteria in anaerobically fermented cattle slurry. Also Salmonella senftenberg microorganisms, even though they underwent a slightly slower reduction, after 4 hours they decayed completely. The highest resistance to the bioreactor's conditions was proved by fecal streptococci, but even in this case their number decreased 10^6 times after 24 hours of investigation. These observations show an exceptionally high resistance of the examined microorganisms to high temperature.

The conducted parasitologic investigations showed a clear invasiveness rate drop of Ascaris suum eggs in the cattle slurry subject to anaerobic fermentation. In relation to 89% of invasive eggs inserted to fresh cattle slurry, after 4 hours their drop to 7% was observed in anaerobic conditions. This radical decrease of invasive eggs percentage in such a short time shows that they have no chance of surviving a 24-hour stay in the bioreactor.

Even though it is difficult to compare one's own investigation results with data included in the literature of the subject due to different input concentrations of the eramined microorganisms and different biomass content in the bioreactor, it should all the same be highlighted that also other authors confirm strong bactericidal qualities of anaerobic fermentation. Lund [8] noticed that in order to gain a 100-fold reduction of salmonella typhimurium number in anaerobic conditions it is necessary to use the temperature of 50°C for 30 minutes. Plym-Forschel [12] gained complete elimination of S.senftenberg, S.typhimurium and Ascaris eggs after anaerobic fermentation of cattle slurry in the period of 24 hours in the temperature of 55°C. Dizer and co-authors [2] think that it is sufficient to use the temperature of 50°C for 5.5 hours for Salmonella elimination in sludges, while Pohling-Schmitt [13] states that the temperature of 55°C must affect them for 13.5 hours. Since the bioreactor is a closed system, ammonia concentration takes place in it, and then the quantity increase of ammonian compounds occurs.

It should be underlined that the microorganism concentration in the carriers was much higher than one could expect in terrain conditions. In the light of conducted investigations it can be stated that obtaining such a significant number reduction of pathogenic bacteria and Ascaris suum eggs in anaerobically fermented cattle slurry indicates a high usability of the examined systems to animal sewage desinfection.

The number of places used to conduct cattle slurry anaerobic fermentation grows in EU countries, esp. in Germany. Recently, the cattle slurry co-fermentation method has been used successfully, consisting in fermenting cattle slurry together with various industry waste, e.g. fats, mass nutrition residues and others. Apart from the desinfection of inserted products, far more biogas is obtained. No matter if the cattle slurry is anaerobically fermented or co-fermented, it should be subject to the activity of at least 55°C temperature for 24 hours, the hydraulic retention time being at least 20 days. For protective reasons, in case of co-fermentation in thermophilic conditions, it is reccommendable to warm up the material to the temp. of 70°C within 1 hour before or after the process is finished [17]. It seems that in case of proper soil ratios, the cattle slurry fermented anaerobically in thermophilic conditions can be safely used in agricultural areas forming no microbiological hazard for the water-bearing layers. In order to obtain a thorough assessment of cattle slurry anaerobic fermentation systems it is also necessary to perform efficiency examinations in low-temperature periods.

CONCLUSIONS

1. The achieved results confirmed the usability of cattle slurry desinfection systems in the period of conducting the research.
2. In cattle slurry fermented anaerobically in thermophilic conditions there occurred a complete microorganism elimination of salmonella and EHEC kinds.
3. A radical drop of fecal streptococci and active Ascaris suum eggs was observed in anaerobic conditions.
4. After 24-hour period of cattle slurry anaerobic fermentation in thermophilic conditions it can be safely used as a fertilisers.

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Zbigniew Paluszak
Department of Animal Hygiene and Village Environment,
University of Technology and Agriculture
28 Mazowiecka St., 85-084 Bydgoszcz, Poland
e-mail: paluszak@zootech.atr.bydgoszcz.pl