COMPARATIVE HYGIENE ASSESSMENT OF TECHNOLOGIES FOR ORGANIC MANURE UTILIZATION WITH HIGH CONTENT OF DRY MATTER 1. REDUCTION OF PATHOGENIC MICROORGANISMS IN A CONTINUOUS MESOPHILIC PROCESS OF ANAEROBIC DEGRADATION

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SUMMARY

The total plate count changes of bacteria (after the introduction of marked strains of *Esherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) in a bioreactor, with continuous mesophilic regime of anaerobic degradation of litter substrate from broiler production, is tracked down. Their quantity decrease in the bioreactor is determined, as on the 3rd day it is approximately 1 log, on the 5th day – 2–4 log, on the 10th day – 5–6 log lower compared to the initial quantity. At the end of the process P. *Aeruginosa* is eliminated completely and the other strains remain in low quantities.

Keywords: anaerobic degradation, mesophilic regime, marked bacteria

INTRODUCTION

Searching for efficient methods for decontamination of animal manure and their transformation from waste to useful products is an important ecological task. One of the economically paying possibilities in this respect is methane fermentation with continuous regime. Until now it is still not clear if safe decontamination of the final product is reached after processing of litter from broiler production – afterwards introduced in soil. According to some authors, during manure methane fermentation, a satisfactory decontamination of the incoming animal manure is reached (Baykov et al., 2005). When analyzing the efficiency of the methane fermentation by decontamination of liquid manure, Tivchev et al. (1986) determine considerable decrease of microorganisms' quantity in the final product during mesophile fermentation at a periodic regime. Research of other collectives points that some pathogenic microorganisms remain at a certain extend in the final compost (Petkov and Baykov, 1988; Philipp et al., 2005).

The aim of this research is tracking down the quantitative changes of marked pathogenic microorganisms, introduced into a bioreactor at a continuous mesophilic regime of anaerobic degradation, with a view to assessing the possibilities for decontamination of litter from broiler production in this type of processing and obtaining epizootologically safe final product.

MATERIAL AND METHODS

Microorganisms. Pure cultures of three pathogenic bacterial strains are used in the research: two Gram negative – *Esherichia coli* O6-21C and *Pseudomonas aeruginosa* 33, and one Gram positive – *Staphylococcus aureus* 230. *In vitro* microorganisms show poly-resistance to different antibiotics, mainly to amphenicol antibiotics (Chloramphenicol and Thiamphenicol) and tetracyclines (Tetracycline, Doxycycline and Oxytetracycline).

Quantitative determination of microorganisms is carried out using the classical method in serial 10 times increasing dilutions of the analyzed materials in a sterile physiological solution. Cultures are prepared from these dilutions on selected media with and without antibiotics, three for each media and dilution. After incubation at 37^{o}C for 24–48 hours, the average count of the cultivated colonies is determined and the CFU is calculated in 1 ml of the diluted solution.

Experimental equipment. The experiments are conducted at a continuous anaerobic degradation process of organic substances in a laboratory bioreactor – with capacity of 2l and working volume 1 l (Simeonov et al., 2006). The anaerobic fermentation is carried out for 22 days at a mesophilic temperature regime –34 ± 0,5^{o}C. The bioreactor is charged daily with liquid manure of broiler litter suspension (7% dry matter and pH 6.2); at the same time the same amount of compost is taken out (4.4% dry matter and pH 7.2).

After preliminary determination of bacterial quantity of *E. coli*, *Pseudomonas spp*. and *Staphylococcus spp*. and the total plate count of the incoming and the outgoing material, the three marked microbial strains are introduced in the bioreactor each in concentration 10^7 CFU/ml from its total quantity. Samples from the outlet (the ready compost) are taken on the 3rd, 5th, 7th, 10th, 14th and the 21st day for determination of the quantity of marked and unmarked microorganisms.

The statistical analysis of the results is done using one-way analysis of variance (ANOVA) and Dunnett post-hoc test.

RESULTS AND DISCUSSION

The results from the analysis are presented in table1.

As it is seen from the summarized data, during the first week after the introduction of marked bacteria, a gradual growth of the total plate count of microorganisms in the bioreactor is observed., which is statistically reliable on the 7th day($P<0.05$). This probably is due to the introduction of these bacteria into the system, as well as the every day addition of liquid manure, which contains microorganisms, too. From the 10th day on, a gradual and reliable decrease of the total plate count of bacteria is determined with more than 1 log compared to the initial quantity and to the previous periods of analysis ($P<0.05$). These results correspond to the obtained by Petkov and Baykov (1988) at similar conditions of fermentation.

Analogical results are observed with the total plate count of *E. coli, Pseudomonas* bacteria and staphylococcus bacteria. After reliable increase on the 3rd day, due to the introduction of marked microorganisms ($P<0.001$), a gradual decrease of total plate count of the corresponding bacteria as well as of the marked strains begins, which can be observed in table 1. This decrease is expressed the highest on the 5th day – with approximately 2–4 log, and in the middle of the experiment (on the 10th day), the quantity of microorganisms reaches its minimal values. Factors, further this, could be the every day taking out of compost (together with bacteria), the unfavorable conditions in the bioreactor and the competition among microorganisms.

Table 1. Dynamics of microorganisms in a bioreactor at a continuous process

Sample	Total plate count bacteria	*E. coli* – total plate count	*Pseudomonas spp.* – total plate count	*Staphylococcus spp.*– total plate count	*E. coli* marked	*P.aeruginosa*- marked	*S. aureus* marked
Inlet before addition of marked bacteria	$3{,}4.10^9$* ±0,38**	$8{,}0.10^4$ ±0,37	$1{,}7.10^4$ ±0,38	$2{,}7.10^5$ ±0,87	–	–	–
Outlet before addition of marked bacteria	$3{,}3.10^9$ ±0,35	$2{,}1.10^4$ ±0,54	$1{,}3.10^4$ ±0,26	$2{,}2.10^5$ ±0,73	–	–	–
3rd day	$4{,}8.10^9$ ±0,9	$3{,}6.10^6$ ±0,71	$1{,}4.10^6$ ±0,58	$1{,}5.10^6$ ±0,88	$3{,}1.10^6$ ±0,62	$1{,}2.10^6$ ±0,39	$1{,}4.10^7$ ±0,58
5th day	$5{,}6.10^9$ ±0,78	$1{,}2.10^6$ ±0,45	$7{,}9.10^3$ ±0,87	$2{,}6.10^5$ ±0,87	$1{,}2.10^3$ ±0,55	$3{,}2.10^3$ ±0,43	$3{,}6.10^3$ ±0,49
7th day	$6{,}9.10^9$ ±0,79	$1{,}8.10^6$ ±0,52	$3{,}9.10^3$ ±0,65	$2{,}1.10^4$ ±0,89	$1{,}5.10^3$ ±0,68	$0{,}96.10^2$ ±0,33	$1{,}3.10^2$ ±0,46
10th day	$3{,}9.10^9$ ±0,52	$7{,}7.10^5$ ±0,77	$1{,}6.10^3$ ±0,56	$4{,}9.10^3$ ±0,98	$0{,}5.10^3$ ±0,18	$0{,}27.10^2$ ±0,06	$0{,}12.10^2$ ±0,003
14th day	$2{,}1.10^9$ ±0,76	$1{,}7.10^5$ ±0,68	$6{,}4.10^4$ ±0,98	$4{,}6.10^4$ ±0,79	$2{,}7.10^3$ ±0,48	$2{,}4.10^3$ ±0,88	$6{,}0.10^2$ ±0,38
21st day	$1{,}2.10^8$ ±0,32	$7{,}6.10^4$ ±0,78	$5{,}0.10^4$ ±0,53	$3{,}5.10^4$ ±0,86	$5{,}9.10^3$ ±0,78	–	$1{,}0.10^3$ ±0,45

* *Average.* ** *Standard deviation*

However after that an increase begins, probably due to growth in the bioreactor, which is not statistically reliable towards the two previous measurements ($P>0.05$). This increase is possible at the corresponding conditions, due to the fact that the analyzed microorganisms are facultative anaerobes; they also are characterized with high resistance to unfavorable physical and chemical factors. It is interesting that on the 10th day only the total plate count of *E. coli* is increased in comparison with all others($P<0.001$), after that day it starts to decrease while for the others a slight increase is observed. Obviously at this stage the bacterial balance in the system is disturbed in favour of E. *coli* which dominates, suppressing other bacteria – this probably is due to the antibiotics that this microorganism excretes – colicines. Its dominance to other tracked species is kept till the end of the experiment.

The behavior of the marked strain of *P. aeruginosa* is interesting. Its quantity oscillates during the second week, on the 10th day it rapidly decreases and after a slight increase on the 14th day, at the end of the experiment it is eliminated completely. At the same time the left saprophytes, *Pseudomonas spp*., normally present in liquid manure, are in relatively high amount. Maybe it has something to do with interspecies antagonism in the genus, probably through bacteriocines. It is less likely to be a display of sensitivity to colicines, excreted by the dominating specie *E. coli* in the system, because obviously the other species from the *Pseudomonas* are not influenced much from their activity. On the other hand the slight increase of *S. aureus* at the end could be related to vanishing of *P. aeruginosa*.

There is data that at termophilic anaerobic fermentation, the decrease of pathogenic microorganisms is considerable in comparison to that at mesophilic regime (Philipp et al., 2005;

Sahlström et al., 2005). After termination of the termophilic process the authors do not determine presence of pathogenic microorganisms.

From the obtained results it is obvious that the quantity of the introduced pathogenic microorganisms considerably decreases and reaches minimal values, but they are not eliminated completely except for P. *aeruginosa*. Even though that their quantity in the end of the continuous mesophilic methane fermentation is very low, there is certain risk transferring these microorganisms to the environment through compost. When mixing them with soil however, their quantity per unit area will be neglectfully low, so the risk for animal infection would be minimal.

CONCLUSIONS

At continuous mesophilic regime of anaerobic degradation, for 21 days, a high level of microbial decontamination is reached regarding pathogenic microorganisms. Using this type of fermentation does not provide complete decontamination of manure.

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