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TESTING THE EFFECT OF DIFFERENT CONCENTRATIONS OF FORMALIN FOR DECONTAMINATION OF CATTLE MANURE

T. P. POPOVA[1], B. D. BAYKOV[2] and V. K. KIROV[1]
[1] *University of Forestry, Faculty of Veterinary Medicine, BG – 1756 Sofia, Bulgaria*
[2] *New Bulgarian University, BG – 1618 Sofia, Bulgaria*

Abstract

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Comparative studies of the use of formalin in concentrations of 2% and 4% were carried out for the processing of fresh and aged manure litter from dairy cows with a view to decontamination. For this purpose, changes in the quantities of contained in fertilizer materials microorganisms were tracked, as well as of pathogenic test strains of *Proteus vulgaris*, *Pseudomonas aeruginosa* и *Staphylococcus epidermidis*, differing in resistance to gentamicin and tetracycline antibiotics. Test bacteria were imported in fertilizer materials in quantities of 10^5 CFU / g of their total contents. It was found that treatment with formalin in the two tested concentrations of 2% and 4% ensures inactivation of the pathogenic test microorganisms and of *Clostridium perfringens*, contained in the tested samples of fresh and aged manure, even after 24 h. Inactivation of *Esherichia coli*, found in both manure samples, processing with 2% formalin, was achieved in 48 h, and at a concentration of 4% - only in 24 h. In fresh manure after treatment with 4% formalin all microorganisms were killed after 96 h, and in aged manure - even after 72 h. Administration of 2% formalin ensures destruction of all microorganisms in fresh and aged cattle manure for a period of 120 h= The use of formalin in a final concentration of 4% does not give much better results than double the smallest concentration of 2%, which is recommended for use.

Key words: cattle manure, formalin, test bacteria, decontamination

Introduction

The content of pathogenic microorganisms in organic wastes from livestock prevents their direct use as soil fertilizer without pretreatment of securing them. This is one of the main reasons why the European Union legislation also prohibited that (Böhm, 2005; Pawelczyk, 2005). To avoid the accumulation of large quantities of such wastes their processing must provide fast and safe decontamination, but also to be economical, convenient and environmentally safe. The search for means and methods for this purpose is a topical issue. By the traditional versions of composting achieved good results in this direction, but the full decontamination of fertilizers thus take a long time (Popova et al., 2009). Another disadvantage of this method of treatment is to create better conditions in composted without chemical treatment organic materials for breeding of crop pests like snails, mole crickets, etc. While an important part of the problem is the obtained as a fertilizer material not to be toxic to soil or crops.

Formaldehyde is a chemical compound with significant activity against microorganisms and parasites (Francis-Floyd, 1996). When administered in high concentrations its bactericidal action is fast and sure. Since in this case it has corrosive and toxic properties (Neochim, 2010), when used in appropriate dosing trough, but to have an antimicrobial effect without negative impact on plants and soil properties. Haas et al. (1995) recommended use 20-40 liters of 35-37% formalin/ton of fertilizer for inactivation of viruses at temperatures above 20°C as are needed for at least four days to achieve inactivation. Other authors like Herniman et al. (1973) suggested the use of significantly higher final concentration of 10% formalin. Even so, however the safe decontamination treated material is toxic to the environment and can be used for fertilizing only in very limited quantities

The aim of these studies was tracking the survival of pathogenic test microorganisms imported in fresh and aged bovine manure bedding after treatment with formalin in different relatively not high concentrations in order to assess

E-mail: dr_tpopova@abv.bg

opportunities to achieve fast and efficient decontamination while receiving epizootiologicaly safe end products.

Materials and Methods

Cattle manure. Fresh and aged manures from dairy cows were examined.

Chemical compound. The effect of formalin (37% formaldehyde), administered at final concentrations 2 and 4% was rested.

Microorganisms. Pure cultures of three pathogenic bacterial test-strains were used in the investigations: *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis***.** They were isolated from animals with chronic infections and were selected by their poly-resistance *in vitro* to Gentamicin and tetracyclines (Tetracycline, Doxycycline and Oxytetracycline). An additional cultivation of these strains on nutrient media with antibiotics from these groups was performed to isolate and use in studies of branches, the most thriving in the presence of high concentrations of these antibiotics.

Nutrient media (Antisel, Sharlau Chemie S. A., Spain). Selective nutrient media with added together doxycycline (50 μg/ml) and Gentamicin (16 μg/ml) were used. Eosin Methylene Blue agar for *Proteus vulgaris*, Cetrimide agar for *P. aeruginosa* and Chapman Stone agar for *S. epidermidis* were selected. On these media, other bacteria were not grown, except the test strains selected by the resistance to the antibiotics pointed out. The total number of microorganisms in the studied materials was reported on Mueller Hinton agar without antibiotics. The contents and quantities of *Clostridium perfringens* on selective agar (Biolab Zrt., Budapest), as well as of *E. coli* and *Salmonella enterica* on Eosin Methylene Blue and Salmonella-Shigella agar were also tracked.

Quantitative determination of microorganisms was carried out using the classical method in serial (10 times) increasing dilutions of the examined materials in a sterile physiological solution. Cultures on the selected media with and without antibiotics are prepared from these dilutions, three for each medium and dilution. After incubation at 37°C for 24–72 h under aerobic and anaerobic conditions (with anaerobe pack with palladium catalyst – $H_2 + CO_2$ – BUL BIO NCIPD Ltd. – Sofia), the mean arithmetical number of the developed colonies was calculated and the colony forming units (CFU) in 1 g of the initial material were determined.

Microscopic studies of microorganisms were carried out under immersion at 1000 x magnification after staining by various classical methods (Gram, Klett for capsules and Möeler for spores) of materials from different cultures on the nutrient media.

Experimental equipments. After a preliminary determination of the total number of microorganisms and those of the examined groups in the fresh and aged cattle manure, in each were imported test strains, each in quantity 10^5 CFU / g of total fertilizer material. Each of them (fresh and aged) was divided into two groups: treated with 2% formalin and treated with 4% formalin. Studied fertilizers were distributed in glass containers of 200 g, were added by 100 ml of formalin solution and the mixtures were well homogenized. Samples for quantification of microorganisms were taken at 24 hour intervals in a week's worth.

Statistical analysis of results is made using the classic method of Student-Fisher.

Results

The results of the quantitative changes of microorganisms in fresh cattle manure after treatment with formalin at final concentrations 2% and 4% are presented in Table 1.

The table shows also the quantitation found in it of *E. coli* and *C. perfringens* before and after chemical effects. It is not found to contain *Salmonella enterica*.

The results show that treatment with formalin in applied concentrations ensures inactivation of pathogenic microorganisms in the test fresh manure from dairy cows even after 24 h. *Clostridium perfringens* also died within 24 h even after treatment with formalin at a low concentration of 2%, while for *E. coli* at the processing of 2% formalin, this is achieved after 48. Within 96 h all the microorganisms in fresh cattle manure after treatment with 4% formalin were killed, and using the double smaller concentration of 2% - up to 120 h. After 48 hours only remain viable bacteria of the genus *Bacillus*, which is established because of cultural and microscopic examinations.

From Table 2 can see the results of monitoring of quantitative changes of microorganisms in mature cattle manure after treatment with formalin in final concentrations of 2% and 4%. The pooled data shows that, as in the fresh manure, the treatment with formalin in applied concentrations ensures inactivation of pathogenic microorganisms in the test aged manure from dairy cows even after 24. The identified in the material *C. perfringens* also died within 24 h even after treatment with formalin at a low concentration of 2%, whereas inactivation of *E. coli* in the processing of 2% formalin needed 48 h. After treatment with 4% formalin, all microorganisms died in 72 h, and when administered to a concentration of 2% complete decontamination is achieved up to 120 h. The microorganisms established after 48 hours were only from genus *Bacillus*, which was shown in the result of the cultural and microscopic examinations.

Table 1
Inactivation of test organisms in fresh cattle manure after treatment with formalin in a final concentration of 2% and 4%

Sample		Total number	*E. coli*	*Clostridium perfringens*	*P. vulgaris* test strain	*P. aeruginosa* test strain	*S. epidermidis* test strain
0 h		$3.2.10^{8}$* ±1.7**	$5.0.10^{4}$±1.6	$3.8.10^{6}$±3.2	1.10^{5}	1.10^{5}	1.10^{5}
24 h	2%	$1.5.10^{6}$ ±0.5	$2.0.10^{4}$	0.00	0.00	0.00	0.00
	4%	$1.3.10^{6}$ ±3.3	0.00	0.00	0.00	0.00	0.00
48 h	2%	$1.9.10^{5}$ ±2.6	0.00	0.00	0.00	0.00	0.00
	4%	$6.8.10^{4}$ ±3.5	0.00	0.00	0.00	0.00	0.00
72 h	2%	$1.10.10^{4}$ ±0.6	0.00	0.00	0.00	0.00	0.00
	4%	$7.6.10^{3}$ ±3.6	0.00	0.00	0.00	0.00	0.00
96 h	2%	$0.9.10^{2}$ ±0.1	0.00	0.00	0.00	0.00	0.00
	4%	0.00	0.00	0.00	0.00	0.00	0.00
120 h	2%	0.00	0.00	0.00	0.00	0.00	0.00
	4%	0.00	0.00	0.00	0.00	0.00	0.00

Table 2
Inactivation of test organisms in matured cattle manure after treatment with formalin in a final concentration of 2% and 4%

Sample		Total number	*E. coli*	*Clostridium perfringens*	*P. vulgaris* test strain	*P.aeruginosa* test strain	*S.epidermidis* test strain
0 h		$3.4.10^{9}$* ±3.5**	$9.2.10^{5}$ ±7.3	$4.3.10^{6}$ ±3.8	1.10^{5}	1.10^{5}	1.10^{5}
24 h	2%	$9.5.10^{5}$ ±0.4	$4.0.10^{4}$	0.00	0.00	0.00	0.00
	4%	$5.0.10^{5}$ ±0.3	0.00	0.00	0.00	0.00	0.00
48 h	2%	$8.5.10^{4}$ ±5.0	0.00	0.00	0.00	0.00	0.00
	4%	$4.5.10^{4}$ ±4.6	0.00	0.00	0.00	0.00	0.00
72 h	2%	$1.3.10^{3}$ ±0.3	0.00	0.00	0.00	0.00	0.00
	4%	0.00	0.00	0.00	0.00	0.00	0.00
96 h	2%	$0.4.10^{2}$ ±0.2	0.00	0.00	0.00	0.00	0.00
	4%	0.00	0.00	0.00	0.00	0.00	0.00
120 h	2%	0.00	0.00	0.00	0.00	0.00	0.00
	4%	0.00	0.00	0.00	0.00	0.00	0.00

The results presented in the tables also show that the total microbial content in aged manure is higher than in fresh. This concerns also the quantities of the tracked bacterial species *E. coli* and *C. perfringens*. Differences between the total number of microorganisms in the two materials were statistically significant ($P < 0.001$), and those between the amount of *E. coli* ($P < 0.001$).

From the data presented is seen that fast and efficient decontamination of bovine manure is achieved after treatment with formalin as in concentration of 4% and in 2%. These results from the application of both test concentrations were similar in both types of cattle manure. Pathogenic organisms were killed within 24 h after administration of the higher concentration. When using double lower concentration of 2% only *E. coli* requiring 48-hour period to complete inactivation. After this period only remain viable spore-forming bacilli. They represent the normal soil micro flora and are not epizootiologically dangerous with the exception of *Bacillus anthracis*. This allows the use of treated materials as fertilizer after two more days.

Although in older manure content of microorganisms, including *E. coli*, is significantly higher, its decontamination with 4% formalin is achieved in just 3 days. For fresh manure for this purpose are required 4 days. At 2% concentration complete inactivation of all microorganisms occurs within 5 days in both types of fertilizer.

Discussion

Our results match those of Haas et al. (1995) who found that decontamination of fertilizer materials and inactivation of viruses using 2% - 4% 35-37% formalin solution is achieved for a period of 4 days at temperatures above 20°C. Herniman et al. (1973) recommend a significantly higher final concentration of 10% formalin. It is undisputed that at this dose decontamination will be very fast and sure. But the toxic properties of the resulting product will be significant and will limit its use as fertilizer. Our research shows that even at a concentration of 2% formalin is able to destroy microorganisms in bovine manure within 5 days.

Obviously, the lower concentration of 2% formalin should be preferred when used for decontamination of litter manure. This is more economical and less toxic to the environment option. There is no need to aim for fast processing of fresh manure, because after it aging also achieves a very fast and reliable results even when using lower concentration of 2% formalin. The advantage of the processing of fresh manure is the possibility for more easily and completes homogenization of the material with formaldehyde solution.

For testing the effect of even lower concentrations of formaldehyde in its application for decontamination of fertilizer materials further research are required. If they were sufficiently effective, they would reveal possibilities for sure, environmentally safe and relatively quick recovery of fertilizer waste.

Conclusions

Microbial content in aged cattle manure is higher by about 1 lg than those in the fresh manure. This refers also to *Escherichia coli*, while quantities of *Clostridium perfringens* in both studied materials are similar. The use of formalin in a final concentration of 4% does not give much better results than the double smallest concentration of 2%. Both versions provide inactivation of pathogenic test microorganisms and of *C. perfringens* in fresh and aged manure from dairy cows even after 24 h. For inactivation of *E. coli* in fresh and aged cattle manure at processing with 2% formalin 48 h are needed, and at a concentration of 4% - only 24 h. The use of formalin in the concentration of 4% ensures death of all microorganisms in aged cattle manure for 3 days and in fresh - for 4 days. When using 2% formalin all microorganisms in the fresh and aged cattle manures are destroyed over 5 days.

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