VALIDATION OF MEAT BY-PRODUCTS COMPOSTING PROCESS ON THE BASIS OF THE INACTIVATION OF SELECTED MICROORGANISMS AND PARASITE EGGS

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SUMMARY

Animal by-products of category 3 can be transformed without pre-treatment in a composting plant to produce compost. In order to minimize a risk to human health and environmental hazard not only should the microbiological supervision of the final product be carried out, but the validation of composting process should be implemented as well. This allows the evaluation of a particular technology in killing pathogens introduced into raw material which undergoes the process of composting. Test organisms should be exposed in a similar matrix as treated material. In this experiment *Salmonella senftenberg* W_{775}, BPV and ECBO viruses and *Ascaris suum* eggs were applied for validation of the method. Two composting cycles with different maximal temperature were analyzed.

Keywords: animal by-product, *Salmonella, Ascaris*, BPV, ECBO, compost

INTRODUCTION

Meat waste management is a serious ecological problem in all EU member states. Although they are rich in organic matter with a high fertilizing value, at the same time they can contain numerous pathogenic microorganisms (bacteria, viruses), mainly of enteric origin, and nematodes and their ova. The microorganisms most frequently isolated from meat waste are bacteria of the genera *Salmonella*, *Escherichia*, *Clostridium*, and faecal *Streptococci* of D group. There can also occur pathogenic viruses, such as: viruses ROTA, bovine parvovirus (BPV), bovine enterovirus (ECBO), and the ova of the parasite *Ascaris suum*. For epidemiological reasons only the waste subjected to previous sanitization can be used for agricultural purposes. Meat waste can be hygienized by pasteurization, stabilization in termophilic condition, liming and composting. According to Straub (11) the temperature of 55–65^{o}C is sufficient for the elimination of pathogens contained in composted material. According to Strauch (12) unfavourable antagonistic environmental conditions have bigger influence on pathogenic microorganisms than a higher temperature itself. The notable effect of this research was the evaluation of the time and conditions needed for meat waste utilization process which guarantee the elimination of noxious microorganisms. This permitted us both to recycle vast amount of waste effectively, and to eliminate sanitary and epidemiological hazard resulting from using the products obtained in agriculture, so it will contribute greatly to the improvement of environmental safety.

MATERIAL AND METHODS

In the experiments a bio-reactor was used with a mixing drum which was loaded with the meat batch previously prepared in a device grinding and mixing meat waste (stomach contents, scraps, fats, blood) – 60% with a texturizer (sawdust) – 40%. The carriers with pathogens were placed in front, centre and back of the bioreactor. Additionally a wire baskets containing indicator pathogens were put in composting biomass, it was remained there till the end of composting process (5 days). Bone carriers included fragments of shafts and distal parts of thighbones of pigs. Bone marrow was removed from them and polycarbonate bags were placed inside, containing 10^{7-8} cfu/ml of bacteria suspension, and also filters with adsorbed viruses. Pores with a diameter of 0.01 nm in the polycarbonate foil allowed the contact of microorganisms with composted biomass. Meat carriers were particles of pork closed in cubes made of metal netting measuring 3 by 3 cm and 5 by 5 cm, which were to protect them from deformation during composting. Bacteria suspension was injected inside of the particles. Large meat carriers and bone heads, due to small recesses in the bio-reactor were placed only in a capsule inside the appliance and analyzed only at the last time of the process. In theory they were supposed to protect the bacterial suspension best from thermal conditions in the bio-reactor. The carriers were removed from the biomass in several-hour intervals and the number of streptococci was determined based on the MPN method in 3-test tube set. Two multiplying media will be used in the process of *S. senftenberg* W_{775} isolation. At the first stage weighed portions will be placed in 1% peptonic water (incubation at 37°C for 24 hours), and then 0.1 ml material will be transferred from each test tube to a line of test tubes containing 10 ml selective liquid medium following Rappaport (incubation at 43°C for 24 hours). Next the material will be sieved to selective agar medium BPLA following Kaufmann (incubation at 37°C for 24 hours). Final identification will involve using serological tests – polyvalent serum HM.

The method of viruses' determination was based on the Filter-Sandwich principle. 1 ml of virus suspension (BPV – bovine parvovirus and ECBO – enteric cytopathogen bovine orphan virus) with a titre of 10^6 – 10^8 $TCID_{50}$/ml in phosphate buffer with pH 6.5 was placed on a Virosorb-Zetapor membrane. Membranes containing the carriers were placed in polycarbonate bags with pores of 0.01-μm diameter, that prevented virus particles from penetrating beyond the carrier, and then they were placed in appropriately prepared bone and meat carriers – in bones the filters were surrounded with bone marrow, and a meat carrier was minced meat placed inside of a plexiglass container. The carriers were placed in the bio-reactor and then removed at definite intervals and the titres of viruses that did not undergo inactivation during composting were determined. Virological tests were also carried out on the base of inactivation of the suspension of viruses occurring in Eppendorf test tubes. The test tubes were placed in a composting facility. Examination of BPV and ECBO viruses' titres was conducted on cell lines MDBK with the microplate method. Strain suspensions in elution medium were diluted in the Eagle minimal essential medium in logarithmic progression (10^{-6}–10^{-8}). 0.05 ml of appropriately diluted virus suspension, 0.05 ml of the minimal essential medium and 0.1 ml of a cell line was added to each of four wells in microplates. The microplates were incubated in a thermostat at 37°C. Infected cultures were observed on day 4 and 5 under the microscope. The titres were calculated with the method of Karber and presented as $TCID_{50}$/ml.

Infective eggs were prepared from adult *A. suum* worms collected from pigs at slaughter. The eggs were obtained from the proximal 2 cm of uterus by dissection of female worms. Thereafter nylon bags with eggs (20 μm pore size) were placed into biomass. Determination of *Ascaris suum* eggs survival - eggs after removing from the bags were placed in a Petri dish with sterile distilled

water and incubated during 30 days at 30°C. After this time the percentage of live eggs from which larvae developed was counted under the microscope.

RESULTS

The research comprised two cycles of composting. The temperature of the biomass was monitored continuously. In cycle I it ranged from 50 to 60°C and was similar at all points where the carriers were placed, while in cycle II it reached the highest values at the back part of the appliance, yet not exceeding 50°C (Fig. 1).

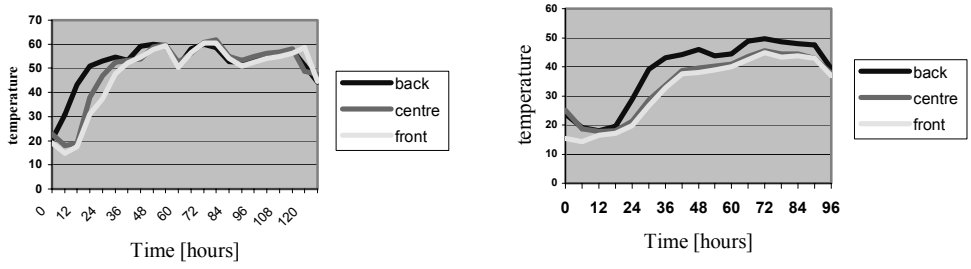


Figure 1. Temperature of composted biomass during cycle I (A) and II (B)

The high temperature of the biomass in the cycle resulted in the fast elimination of the bacteria. They were not detected in the bone carrier in the 48th hour, and in the meat carrier in the 72nd hour of the process (Fig. 2).

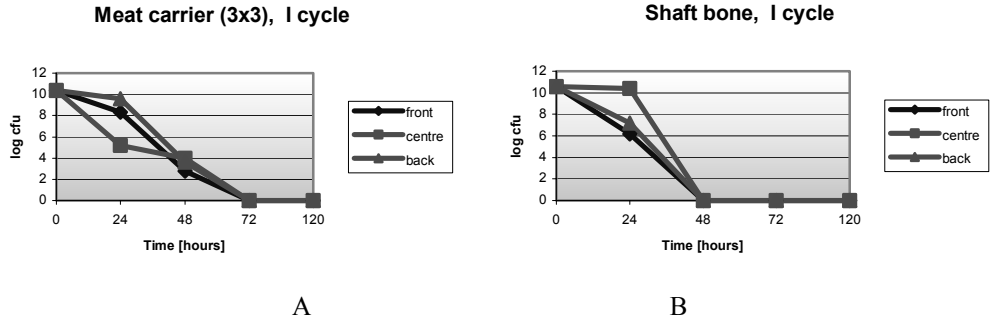
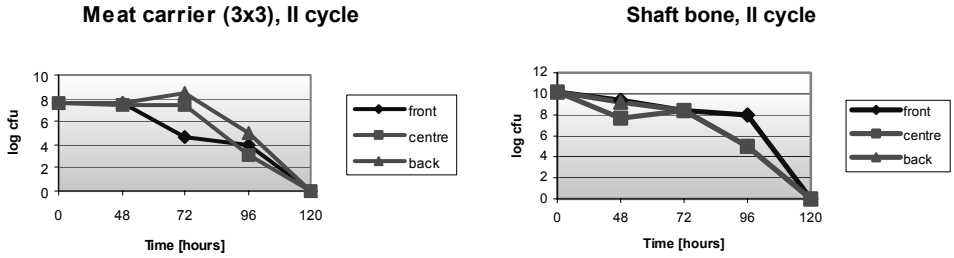


Figure 2. Survival of the bacteria in a small meat particle (A) and shaft bone (B) during cycle I

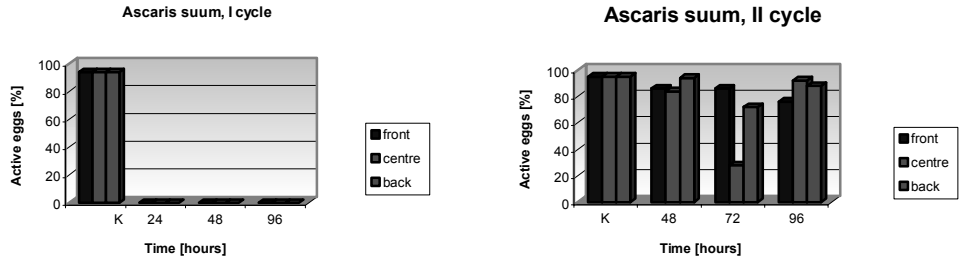
In cycle II, the elimination rate of the microorganisms was remarkably slower than in cycle I. Both in the meat carrier and bone carrier the inactivation of rods proceeded only in the fifth day of composting. Besides, a minor multiplication of the bacteria occurred during the process (Fig. 3).



A B

Figure 3. Survival of the bacteria in a small meat particle (A) and shaft bone (B) during cycle II

In respect of the survival of *A. suum* eggs only cycle I proceeded effectively. Viable eggs were not isolated as early as after 24 hours of composting, while in cycle II, almost the identical percentage of invasive eggs as in the control was still noted in the 96th hour (Fig. 4).



A B

Figure 4. Inactivation of *Ascaris suum* eggs in cycle I (A) and II (B) in different parts of the composting device

S. senftenberg were not isolated from the largest carriers placed inside the biomass after 120 hours. Thus theoretically, the best protected bacteria, placed in the largest carriers, undergo equally efficient elimination in both cycles of composting (Fig. 5).

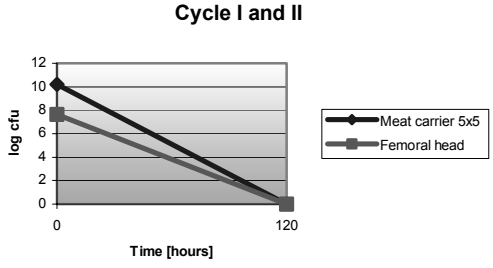


Figure 5. Survival of the bacteria in a big meat carrier 5x5 and a head of thighbone placed in the big carrier during both of cycles.

The thermoresistant virus BPV was still detected in the carriers in the 72 hour of composting. It was subject to the fastest reduction in the bone carrier (a decrease in titre by 2 units already in the 48th hour), while in the eppendorf tubes its titre practically remained at the same level (Tab. 1). The ECBO virus studied in the cycle with a lower temperature underwent a weakest inactivation in the meat carrier, while in bone and eppendorf a decrease in titre by almost 3 log was observed.

Table 1. Titre of the virus BPV in three different times of cycle I

Carrier	Time (hours)			
	0	24	48	72
	Virus titres [$TCID_{50}$/ml]			
meat (filters)	5.30	5.20	4.80	3.81
bones(filters)	5.30	4.55	<1.55	<1.92
Eppendorfs	6.15	6.42	6.17	5.55

Table 2. Titre of the virus ECBO in four different times of cycle II

Carrier	Time (hours)				
	0	48	72	96	120
	Virus titres [$TCID_{50}$/ml]				
meat (filters)	4.40	4.05	3.80	3.55	-
bones(filters)	4.40	3.80	<1.55	<1.55	-
eppendorfs	5.70	5.70	4.80	4.42	2.80

– carrier damaged

DISCUSSION

Of the applied methods for meat waste utilization, composting is gaining an increasing popularity. A properly handled composting process results in an effective pathogen elimination (effectively eliminates pathogens as a result of) due to the self-heating of composting biomass (4, 14). When the development of the thermophilic stage cannot be obtained, the composted material may pose a risk of spreading microorganisms in the environment. For a proper hygienization of composted material, the process should proceed for at least 3–4 weeks, and in addition for 1 week the temperature in the composted biomass should amount at least 65^{o}C (13). In the present studies in cycle 1 the temperature ranged between 50 and 60^{o}C, while in cycle II it did not exceed 50^{o}C, which was reflected in the differences in bacteria and parasite egg survival. Namely, after 72 hours in cycle I bacteria elimination in both the meat and bone carriers was obtained, while at the same time in cycle II the number of *Salmonella* sp. was almost identical in comparison with the control. Due to the environmental hazard, the inactivation of rods is one of the basic measures of utilization efficiency of waste intended for agricultural use. This sort of experiments was conducted in Germany, where *Salmonella senftenberg* W_{775} was the indicator organism (3, 15). This serotype rarely causes infections in humans, while is characterized by a remarkable resistance to high temperatures. In the experiment by Jepsen et al. (5) the number of *Salmonella*, which amounted to 3600 cells in 100 g of raw sewage sludge, in a sample of ready compost of the same weight was reduced to 2 cells. The microorganism elimination rate in the composted material depends mainly on a temperature and the time of its action, though an increasing role is

also attributed to the activity of native microorganisms. Their particular importance consists in the ability to inhibit the process of *Salmonella* re-multiplication in biomass (10).

Also *Ascaris suum* eggs are commonly applied to studies on evaluation of organic waste hygienization efficiency, as they are easily available, as well as highly resistant to environmental factors (17). Veerannan (16) indicated that in low temperatures sewage sludge are not free of invasive eggs until after 3 years of storage. Only in thermophilic conditions the full elimination of the eggs takes place (7, 8). Composting method ensures the full waste hygienization and parasite eggs inactivation on condition that all the biomass is subject to the temperature of 55^{o}C for 2 weeks or 65^{o}C for one week (14). Similar results were obtained in the present studies. The inactivation of *Ascaris suum* eggs proceeded more effectively in cycle I. The presence of viable eggs were not already recorded after 24 hours, while in cycle II, after 96 hours they still made almost 100% of all the population placed in the carrier (like in the control).

Viruses are determined in organic wastes definitely less frequently than other pathogens due to the lack of simple methods for their isolating in contaminated samples. The current state of the research suggests that the influence of the environment on virus inactivation is multiform. Apart from the temperature, an increased content of ammonia has also an effect on their elimination in waste (18), heavy metals (1) as well as the proteases and nucleases of microorganisms. The effect of temperature on the elimination rate of enteroviruses was studied by numerous authors. Within the range 70-80^{o}C they were inactivated after 1-2 minutes and at 70^{o}C - after 10 minutes (9). Enteroviruses are inactivated at a temperature of 60^{o}C already after 15 minutes, and at 54^{o}C - after 45 minutes (8). Rehmnan (9) reported that a temperature of 50^{o}C is not sufficient to inactivate the virus ECBO, though it significantly decreases its titre In the present studies, the virus ECBO placed in the biomass of the cycle with a lower temperature underwent the weakest inactivation in the meat carrier (0.85 log), while in the bone and eppendorf we observed a decrease in titre by nearly 3 log. The virus BPV is characterized by termostability in the moist heat of 75^{o} to 90^{o} (2). Due to its extraordinary resistance against moist heat, parvoviruses proved to be well-suited for checking chemo-thermal and thermal disinfection procedures. Monteith and Shannon (6) studied the inactivation of enteric viruses by composting cattle faeces. The parvovirus was seeded into the solid fraction of cattle manure. The operating temperatures reported in this study were 30° C on day 1, 45° C on day 2 and 60° C for the rest of the experiment. The results showed that the parvovirus did not survive composting for 28 days. In the present study physical and chemical parameters were not sufficient for the total elimination of the virus BPV. It underwent the fastest reduction in the bone carrier (a decrease in titre by 2 units as early as in the 48th hour), while in the eppendorf its titre practically remained at the same level.

CONCLUSIONS

1. Mistakes are made during waste utilization process, resulting in an improper hygienization of the biomass. The effect of this is that large quantities of parasite eggs may penetrate into the environment, posing a hazard for human and animal health. The application of the indirect control of the process based on introduction of indicator bacteria, parasite eggs and viruses into the biomass seems to be necessary for ensuring the environmental biosafety.
2. Varied temperatures in the bio-reactor in two analyzed cycles caused the difference in the elimination rate of *S. senftenberg* W_{775} and *A. suum* eggs in the carriers tested.

3. During a properly conducted composting process, the elimination of the bacteria and *A. suum* eggs occurred irrespective of the size of carriers used.
4. Thermo-resistant viruses (BPV) underwent reduction only in the bone carriers. The inactivation rate in the other carriers did not exceed 1.4 log. The ECBO virus underwent a weakest inactivation in the meat carrier, while in bone and eppendorf a decrease in titre by almost 3 log was observed.
5. The study indicates that the elimination of *Salmonella* and *A. suum* eggs is possible in the case of temperature reduction from 70^{o}C to 55-60^{o}C, on condition that it has a long-term effect on biomass.

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