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Krystyna CYBULSKA[1], Natalia SUCHECKA[1], Ilona WROŃSKA[1]
and Sanaa MAHDI-ORAIBI[1]

THE NUMBER OF *E. coli* AND *C. perfringens* BACTERIA IN POULTRY WASTE AND SUBSEQUENT PHASES OF COMPOSTING

LICZEBNOŚĆ BAKTERII *E. coli* I *C. perfringens* W ODPADACH DROBIARSKICH ORAZ KOLEJNYCH FAZACH ICH KOMPOSTOWANIA

Abstract: Rapidly growing commercial poultry production generates large amounts of waste. Waste that accumulates during the poultry slaughter process often remains unprocessed, becoming a serious threat to people's health and the natural environment. Poultry production waste constitutes problems odour threat and dangerous sanitary threat. The aim of this study was to determine the population size of *Escherichia coli* and *Clostridium perfringens* in poultry waste and in successive stages of waste composting. Research material consisted of raw feathers collected immediately after the slaughter, samples of biological sludge from the centrifuge, mixture of straw, feathers and lime and processed compost. Microbiological analyses were conducted with the use of spread plate count method and the substrate was used in accordance with research standards. The size of population of microorganisms in the samples analyzed corresponded to the waste processing stage and group of microbes. The presence of *C. perfringens* strains was ascertained in all samples, whereas *E. coli* strains were identified only in raw feathers and centrifuge sludge, being most numerous in unprocessed material. A reverse trend was observed in case of *C. perfringens* with the highest population density in centrifuge sludge and least density in raw feathers.

Keywords: *E. coli*, *C. perfringens*, poultry waste, composting

Introduction

Within the last years a continuous development of poultry industry has been observed, which causes the increase of produced wastes. It depends mostly on the size of the farms and the meat processing plants and the applied production technologies. According to the Regulation (EC) No 1069/2009 of the European Parliament and of the Council of October 21, 2009, which came into force on March 04, 2011, the wastes are included in category 3 as inedible and by-products from animals subject to slaughter in a slaughterhouse [1]. The poultry slaughter by-products may become a material hazardous for both the natural environment and human health. Next to the high number of organic components, the wastes are full of pathogenic microorganisms, mainly from intestines and parasites' eggs [2, 3]. In the processing plants bacteria hazardous to human health are observed, *ie Salmonella enteritidis*, *Listeria monocytogenes*, *Yersinia enterocolitica*, and pathogenic strains *E. coli* and *C. perfringens* [4-6]. Thus the wastes should immediately undergo the treatments stopping undesired changes and should be processed into environmentally safe products. However the management process of large amounts of such wastes is relatively difficult. Sharpening the restrictive regulations of the European Union concerning the usage of slaughter wastes as additions to feeds even increased the problem of the wastes

[1] Department of Microbiology and Biotechnology of Environment, West Pomeranian University of Technology in Szczecin, ul. J. Słowackiego 17, 71-434 Szczecin, Poland, phone +48 91 449 64 24, email: krystyna.cybulska@zut.edu.pl
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management [7, 8]. Thus it is so important to improve the existing methods of rendering the wastes harmless, as well as searching for and implementing new methods [9]. The most efficient methods of the slaughter wastes utilization include composting [10]. Compliance with appropriate treatment criteria may significantly force the pace of processing the wastes into non-hazardous, harmless and useful products as well as significantly reduce the processing costs [11-13]. Properly conducted the composting process can reduce the number of pathogenic microorganisms in poultry waste [14].

The conducted researches have been aimed at the determination of quantity of *E. coli* and *C. perfringens* in the poultry wastes and successive stages of the composting.

Materials and methods

The researches focused on the raw feathers from the poultry slaughter and the samples from the successive stages of their composting *ie* biological sludge from a centrifuge, mixture of straw, feathers and lime and the processed compost, received in the composting plant of a poultry processing plant (Fig. 1). The samples have been subject to a series of dilutions that have been used to perform microbiological inoculation on selective media, with the use of plate method. The scope of the conducted researches included determination of the quantity of: *E. coli* on a ready ENDO Agar medium (Scharlau company) and *C. perfringens* on Wilson-Blair medium used for the cultivation of anaerobic spore bacteria, reducing sulphates. The cultures were incubated in the temperature of 37°C for 48 hours. The quantity of the microorganisms has been provided in colony forming units (cfu) to 1 gramme of fresh samples mass.

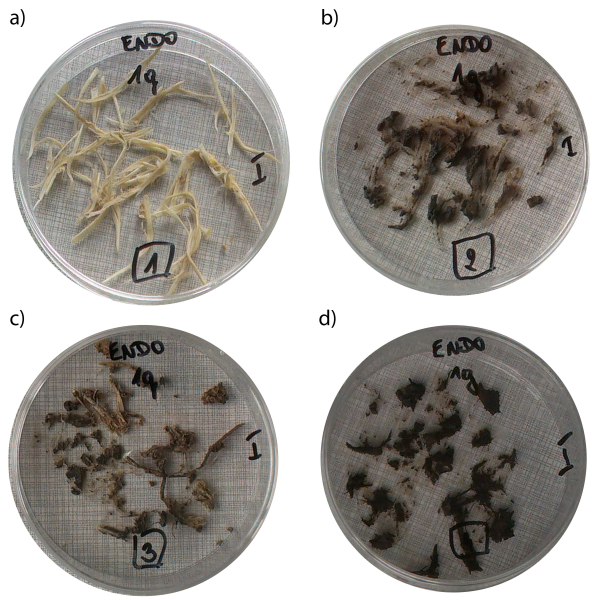

Fig. 1. The research material: a) raw feathers, b) biological sludge from the centrifuge, c) mixture of straw, feathers and lime, d) processed compost

Results and discussion

The conducted microbiological analysis of the researched samples revealed varied quantity of *E. coli* and *C. perfringens* bacteria. It has been proved that *E. coli* bacteria were the most numerous in raw feathers before the processing (Fig. 2). Their quantity was 240 cfu · g^{-1} of fresh mass and there were more of them (60%) than in the sludge from the centrifuge.

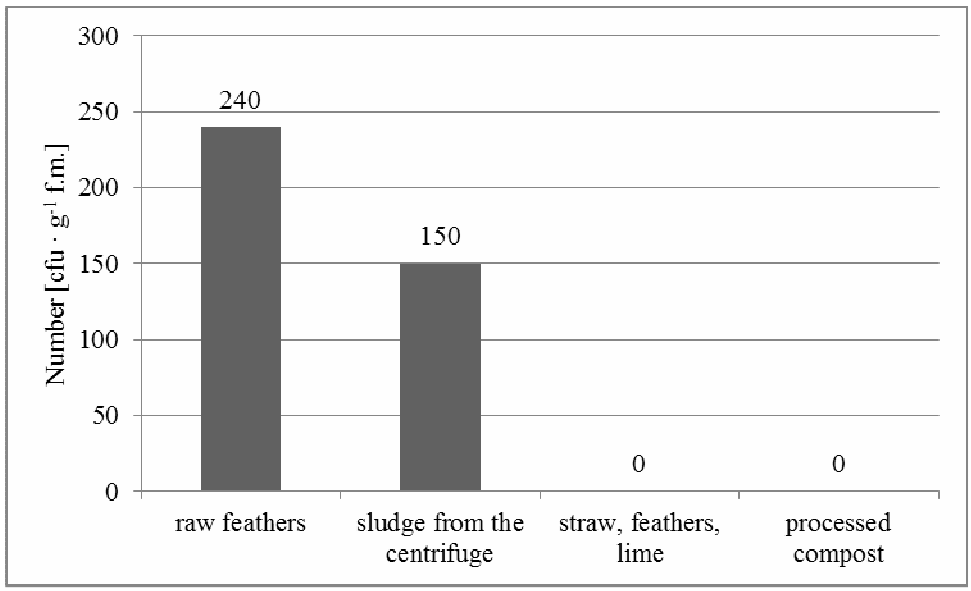

Fig. 2. The number of *E. coli* in research materials

The remaining samples *ie* the mixture of straw, feathers and the processed compost were free from this bacterium species. It has been stated that all the researched samples have complied with the standards binding in the regulation of the Minister of Agriculture and Rural Development for organic fertilizers, according to which the number of bacteria from *Enterobacteriaceae* family, specified on the basis of the number of aerobic bacteria, should be smaller than 1,000 colony forming units (cfu) per a gramme of the fertilizer [15]. The received results prove decreased number, or even elimination of *E. coli* bacteria in the successive stages of composting. The sterilization treatment has completely eliminated *E. coli* bacteria from the mixture of straw, lime and feathers. The conditions present in the composting container make it impossible for the bacteria to restore its numbers in the compost. This is proved in the researches of Wolna-Maruwka and her colleagues [16], who have stated that after 42.5 hours of the composting process the process of *Enterobacteriaceae* cultivation decreased in all the composted materials, which happened due to the increase of the temperature observed in the thermophilic stage. The same conclusions have been formed by Hassen et al [17]. According to Epstein [18] the number of *E. coli* and *Enterococcus faecalis* is reduced in temperatures 55-65°C. The author states

that in such conditions the final material is free from pathogens after 14 days of composting. Chandna et al [19] and Singh et al [20] have also stated that the survival rate of the pathogens in the composted material depends on the content of the moisture, C/N ratio, pH, etc.

The analysis of the results of *C. perfringens* bacteria quantity, presented in the chart (Fig. 3), reveals their presence in all the analyzed samples. They were distinctly more numerous in the sludge from the centrifuge with the quantity of 520 cfu · g^{-1} of fresh mass. The lowest number was observed in the feathers prior to processing 5 cfu · g^{-1} of fresh mass.

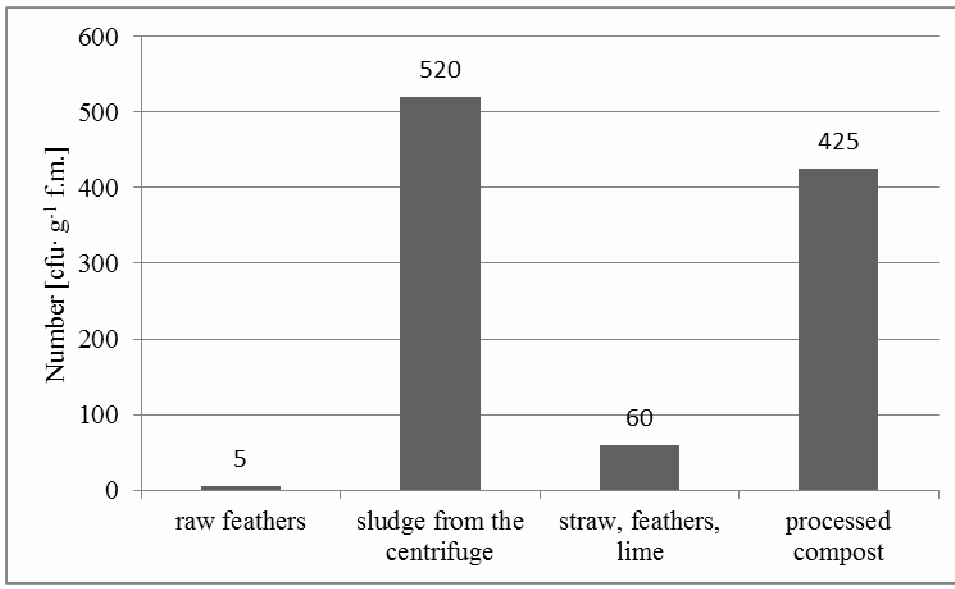

Fig. 3. The number of *C. perfringens* in research materials

In the next stage of composting the poultry waste, which was the mixture of feathers, straw and lime, a decrease of *C. perfringens* quantity was observed by about 88% in comparison to the quantity observed in the sludge from the centrifuge. The application of lime for the sterilization purposes could result in the decreased pathogen development, which is confirmed by numerous authors [21, 22]. The same phenomenon was also observed in relation to *E. coli*. However the ability of *C. perfringens* to form spores enabled them to multiply when the thermophilic conditions stopped, which was observed in case of the processed compost (425 cfu · g^{-1} of fresh mass). According to Payment [23] *C. perfringens* belongs to mesophilic microorganisms, extremely resistant to the environment changes and especially to the changes of temperature. Moreover, the researches of Raju et al [24] reveal that the spores of *C. perfringens* are able to survive in the temperature of 100°C for over 90 minutes. The specific properties of the species enabled recultivation of the bacteria after the sterilization.

Conclusions

1. The presence of *C. perfringens* bacteria was observed in all the samples, whereas *E. coli* bacteria were only observed in raw feathers and the sludge from the centrifuge. Their number depended on the stage of waste processing.
2. *E. coli* bacteria were the most numerous in the processed material, and *C. perfringens* in the sludge from the centrifuge.
3. During the advancing composting process a significant decrease of the quantity of the temperature sensitive *E. coli* bacteria was observed. While the quantity of *C. perfringens* bacteria was increased in individual stages of waste processing and in the compost.

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Zakład Mikrobiologii i Biotechnologii Środowiska
Zachodniopomorski Uniwersytet Technologiczny w Szczecinie

Abstrakt: Intensywnie rozwijająca się produkcja drobiarska wiąże się z generowaniem dużej ilości odpadów. Powstające produkty uboczne podczas uboju pozostawione w stanie surowym stają się poważnym zagrożeniem dla środowiska naturalnego i zdrowia ludzi. Mogą stwarzać problemy odorotwórcze oraz istotnie groźne zanieczyszczenie sanitarne. Celem przeprowadzonych badań było określenie liczebności *E. coli* i *C. perfringens* w odpadach drobiarskich oraz kolejnych etapach ich kompostowania. Materiał badawczy stanowiły świeże pióra bezpośrednio po uboju, osad biologiczny z wirówki, mieszanina słomy, pierza i wapna oraz kompost przerobiony. Analizy mikrobiologiczne wykonano metodą płytkową, wykorzystując wybiórcze podłoża zgodne z normami. Liczebność mikroorganizmów w badanych próbach zależała od fazy przerobu odpadu i analizowanej grupy drobnoustrojów. Obecność bakterii *C. perfringens* stwierdzono we wszystkich próbach, natomiast bakterie *E. coli* tylko w piórach surowych i osadzie z wirówki, przy czym najliczniej występowały w materiale nieprzetworzonym. Odwrotną tendencję zaobserwowano w przypadku *C. perfringens*, które najliczniej zasiedlały osad z wirówki, natomiast ich mniejszą liczebność stwierdzono w piórach surowych.

Słowa kluczowe: *E. coli*, *C. perfringens*, odpady drobiarskie, kompostowanie