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PRESENCE OF MICROORGANISMS OF SELECTED ENZYME GROUPS IN VARIOUS STAGES OF POULTRY WASTE COMPOST FORMATION PART II. SUBSTRATE: PROTEIN, FAT, AND STARCH

OBECNOŚĆ MIKROORGANIZMÓW Z WYBRANYCH GRUP ENZYMATYCZNYCH W POSZCZEGÓLNYCH FAZACH POWSTAWANIA KOMPOSTU Z ODPADÓW DROBIARSKICH CZĘŚĆ II. SUBSTRAT: BIAŁKO, TŁUSZCZ I SKROBIA

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Streszczenie. Produkcja mięsa drobiowego na coraz większą skalę sprawia, że proporcjonalnie wzrasta ilość odpadów z nią związanych. Pojawiają się trudności związane z zagospodarowaniem tego typu materiałów ze względu na ich niejednorodny charakter oraz występujące zanieczyszczenia. Dlatego utylizacja materiałów odpadowych z drobiu jest bardzo kłopotliwa i wiąże się z ponoszeniem dużych nakładów finansowych. Dobrym rozwiązaniem może być wprowadzenie drobnoustrojów wykazujących wysoką aktywność enzymatyczną w celu zoptymalizowania procesu zagospodarowania uciążliwej masy odpadowej i uczynienia jej bardziej przyjazną dla środowiska. W pracy określono liczebności drobnoustrojów zdolnych do rozkładu białka, skrobi oraz tłuszczu w różnych materiałach odpadowych pochodzących z uboju drobiu i jego zagospodarowania (szlam, osad z oczyszczalni biologicznej, kompost właściwy). Próbki badawcze pochodziły z kompostowni na terenie zakładu drobiarskiego w Polsce zachodniej. Materiały zostały poddane analizie mikrobiologicznej na selektywnych podłożach, zgodnie z zaleceniami. Na podstawie otrzymanych wyników stwierdzono, że wszystkie badane produkty odpadowe były zasiedlone przez drobnoustroje enzymatyczne. Największą liczebność mikroorganizmów stwierdzono w szlamie pochodzącym z basenu odpadów ciekłych, natomiast najmniejszą w kompoście właściwym. Wyróżniającą się grupą drobnoustrojów fizjologicznych były mikroorganizmy zdolne do rozkładu tłuszczu.

Key words: microorganisms, poultry waste, enzymatic activity, composting.

Słowa kluczowe: mikroorganizmy, odpady drobiarskie, aktywność enzymatyczna, kompostowanie.

INTRODUCTION

Currently in Poland, production of poultry meat predominates over other types of meat. According to the data (Statistical Yearbook of Agriculture 2014), by about 55 thousand tons more poultry than pork meat was produced in 2013. The inevitable result is the formation of large quantities of different-form by-products. The processing poultry generates feathers, fat and meat materials, wastewater, and many others (Glatz et al. 2011; Moreki and Keaikitse 2013).

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Wastewater from the poultry slaughter plant has diverse composition, including burdensome oily substances (Beux et al. 2007; Tarntip and Sirichom 2011; Rajakumar et al. 2011). In turn, poultry droppings are abundant, among others, in protein, thus it can have a wide range of applications in agriculture, e.g. as an organic fertilizer. It effectively improves the soil properties in physical, chemical, and biological terms by providing the appropriate substrates (Ngodigha and Owen 2009; Bolan et al. 2010; Bhoi and Mishra 2012). Introducing the nitrogen to the soil in a form of poultry manure using appropriate agricultural treatments significantly affects the activity of many soil enzymes (Mankolo et al. 2012). Colonization by microorganisms with high enzymatic activity, including towards cellulose, is characteristic feature of the material (Akpomie et al. 2013). These microorganisms are crucial in many important processes associated with the waste management, in particular composting process (Prabhakaran and Manivannan 2014).

Waste materials, including wastewater, are an excellent habitat for microorganisms, including pathogens. Their development is promoted by nutrient availability (Periasamy et al. 2013). An example of beneficial bacteria isolated from the wastewater is *Rhodocyclus gelatinosus*. It is characterized by specific properties, e.g. degradation of gelatin, which is unique among microorganisms from this environment (Ponsano et al. 2002).

The aim of this study was to determine the number of microorganisms metabolizing the protein, starch, and fat in different stages of poultry waste processing into a proper compost.

MATERIAL AND METHODS

The study used selected waste materials from the poultry industry i.e. sludge from the reservoir for liquid waste, sludge from the sewage treatment facility, and the final processing product, so-called proper compost. Samples were collected 4 times (1st date – 05.03.2015, 2nd date – 21.05.2015), 3rd date – 25.06.2015, and 4th date – 14.01.2016) from one of poultry slaughter plants located in the Western Poland. A detailed description of the measurement points and ambient temperatures are in the work by Wrońska and Cybulska (2016).

Test materials were subjected to microbiological analysis determining the number of proteolytic, amylolytic, and lipolytic microorganisms, and the results were given as colony forming units (CFU) per 1 gram of dry matter. For this purpose, a plate method was used and the appropriate selective media were applied depending on the metabolic group: protein on Kędzia and Koniar substrate (1980), starch according to Conney and Emerson (1964), and fat according to the procedure by Kosewska (1991). Cultures were grown at 23–25°C for 3–7 days. Microbiological analysis were carried out in three replicates.

Achieved results were statistically processed using Statistica 12 software, in which the LSD test was used.

RESULTS AND DISCUSSION

The analysis of the test post-slaughter material revealed a diverse number of selected physiological groups colonizing them (Fig. 1).

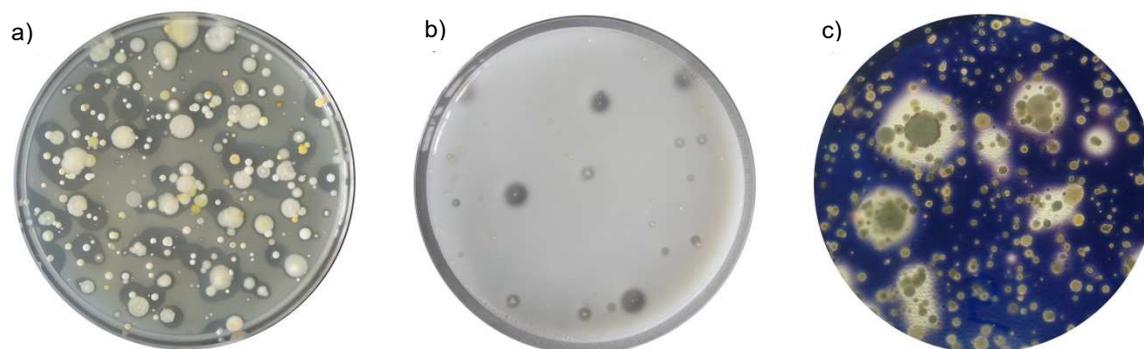


Fig. 1. The number of selected examples of enzymatic microorganisms: a) lipolytic, b) proteolytic, c) amylolytic

Ryc. 1. Wybrane przykłady liczebności drobnoustrojów fizjologicznych: a) lipolitycznych, b) proteolitycznych, c) amylolitycznych

Sludge from the reservoir for liquid waste appeared to be the test sample most numerously inhabited by enzymatic microorganisms. Predominant physiological group consisted of lipolytic microorganisms ($3.6 \cdot 10^8$ CFU \cdot g⁻¹ DM), the number of which was significantly higher than proteolytic microorganisms. In the third date of tests, when the ambient temperatures were highest (17°C), the largest number of studied microorganism groups was found ($4.8 \cdot 10^8$ CFU \cdot g⁻¹ DM) (Fig. 3).

In the case of biological sludge, it was observed that the number of microorganisms capable of degrading the protein and fat was at a similar level. The average population of proteolytic microorganisms amounted to $7.5 \cdot 10^7$ CFU \cdot g⁻¹ DM, which was a few percent higher than lipolytic and almost 2 times higher than amylolytic ones. The greatest number of enzymatic microorganisms at the level around 10^8 CFU \cdot g⁻¹ DM was detected in the 4th date of measurement, when ambient temperature was relatively low (°C). However, it should be noted that the biological sludge from centrifuge is produced at much higher temperatures of waste processing and stored temporarily on prisms for further management. Thus, the outer temperature does not have such a significant impact on the biomass within.

Relatively smaller number of tested groups microorganisms, in particular proteolytic, was found in the final product of the poultry waste processing, i.e. proper compost. The compost contained dominant group of lipolytic microorganisms ($5.2 \cdot 10^6$ CFU \cdot g⁻¹ DM), which exceeded 14 times the contribution of protein-decomposing and 5-fold starch-decomposing microorganisms, which was confirmed by substantial differences in statistical analysis (Fig. 2). Relatively larger number of tested groups of microorganisms in the composted material were recorded on the 3rd and 4th dates of measurement.

Among the analyzed study materials, the sludge was most numerously colonized by lipolytic microorganisms. Their number amounted to $1.4 \cdot 10^9$ CFU \cdot g⁻¹ DM, indicating a 3-fold advantage over the biological sludge and 77-fold over the proper compost. Similar tendencies in the number were showed by amylolytic microorganisms. It was found that their population was 4 times higher in the sludge than in biological sediment and 140-fold higher than in the proper compost. In turn, the proteolytic microorganisms dominated in biological sediment from centrifuge, where their numbers was at the level of $3 \cdot 10^8$ CFU \cdot g⁻¹ DM, which meant a 7-fold advantage over the sludge and 200-fold over the compost.

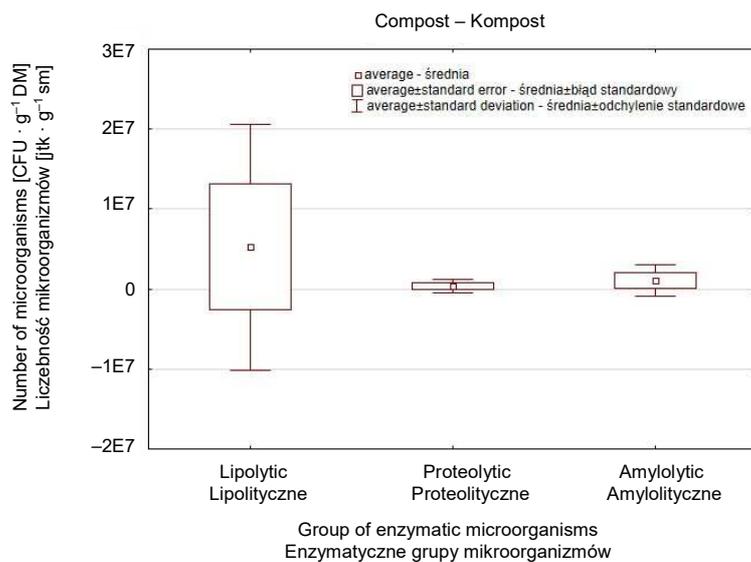
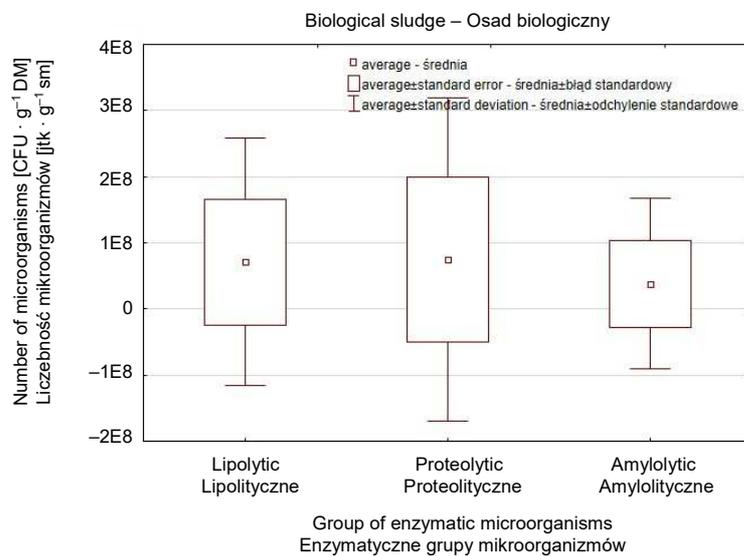
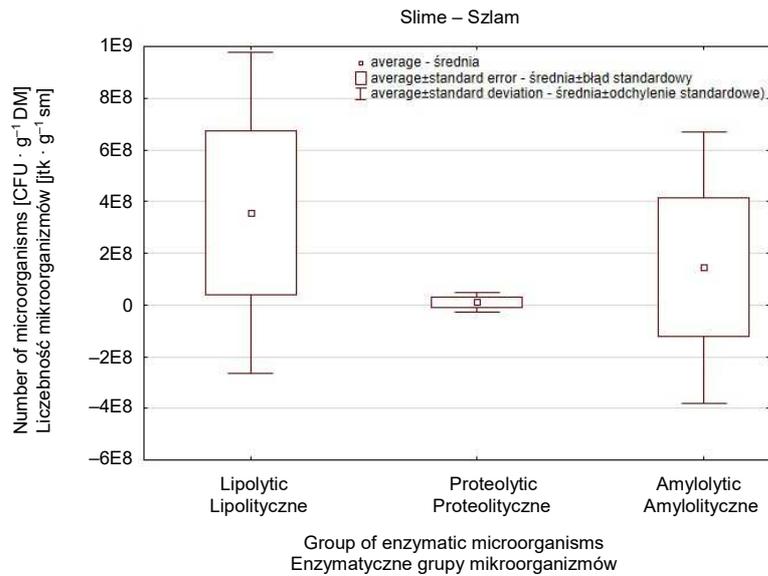
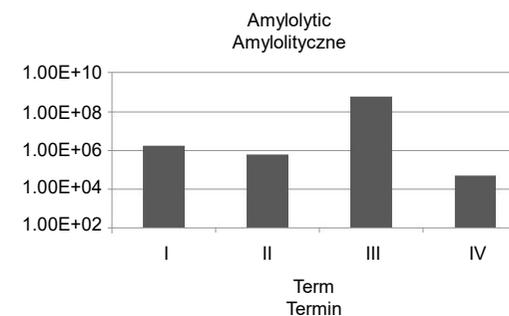
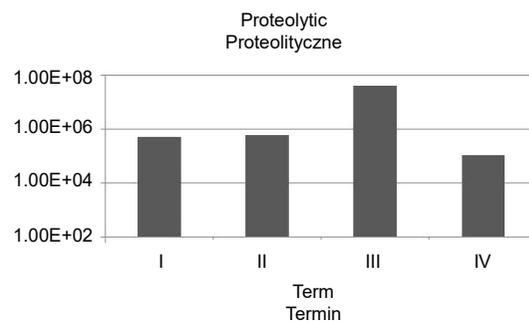
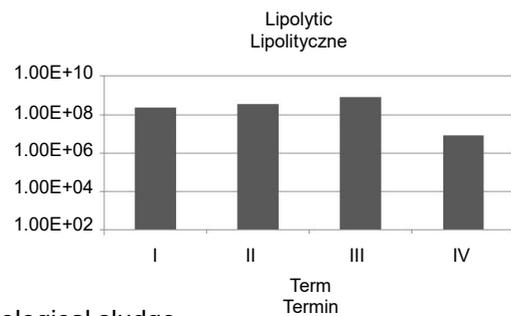
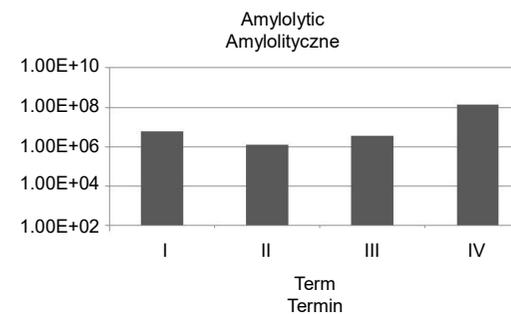
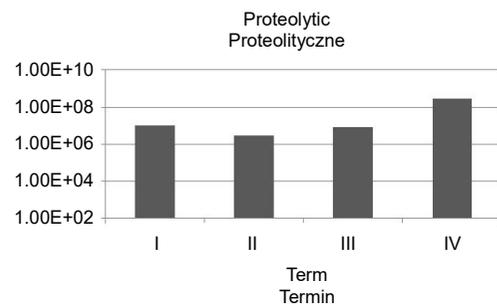
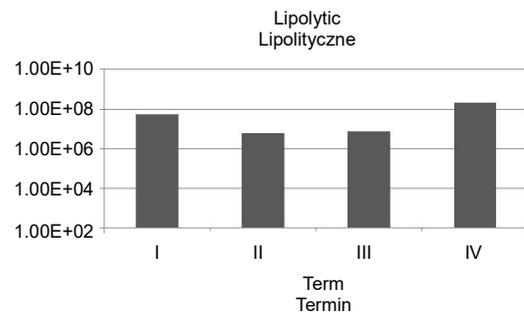


Fig. 2. Characteristics of enzymatic microorganisms number distribution in tested materials
Ryc. 2. Rozkład liczebności mikroorganizmów enzymatycznych w badanych materiałach

Slime – Szlam



Biological sludge Osad biologiczny



Compost – Kompost

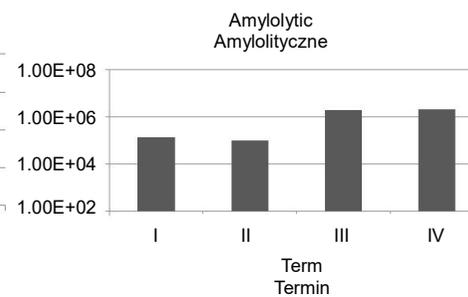
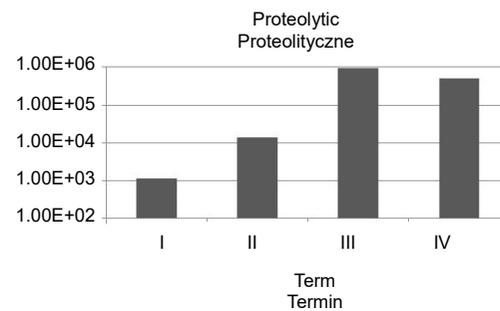
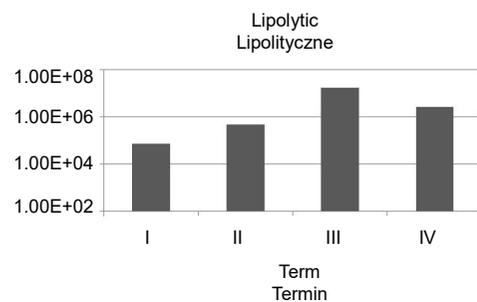


Fig. 3. The average number of enzymatic microorganisms [CFU · g⁻¹ DM] in tested materials
Ryc. 3. Średnia liczebność mikroorganizmów enzymatycznych [jtk · g⁻¹ sm] w badanych materiałach

Biodiversity of microorganisms included in the tested enzymatic groups was as follows: the largest share was shown by lipid-decomposing microorganisms 62%, then microorganisms secreting polysaccharides in the form of starch 26%, and the lowest contribution the microorganisms able to degrade protein 12%.

A significantly higher number of lipolytic microorganisms in the analyzed test materials, i.e. sludge from the reservoir for liquid waste, biological sediment from the centrifuge, and final product – proper compost, can be explained by the availability of fats necessary for their appropriate development, which was confirmed by numerous authors (Martínez et al. 2011; Worwąg et al. 2011). Fats accumulated in wastewater from the slaughterhouses are responsible for 67% of chemical oxygen demand in this waste (Silva et al. 2013; Kempka et al. 2013). Studies by Dors et al. (2013) revealed that applying lipase originating from pork pancreas during the methane fermentation process can have very positive impact on fats removal and in consequence the decrease in COD in wastewater generated in the poultry industry. Cirne et al. (2007) reported the possibility to fast accumulation of intermediate products from fat decomposition due to exogenous lipases, which may reduce the biogas production. Enzymatic activity, including lipolytic, on the example of *Fusarium* genus fungi depends, among others, on accumulated fats in the substrate (Oliveira and Lima 2014).

CONCLUSIONS

1. Poultry waste materials were numerously colonized by enzymatic microorganisms. Their numbers was affected by the date of sampling and the stage of their processing.
2. Sludge was material the most numerously colonized by microorganisms, which were characterized by a high substrate bio-degradation potential. Subsequently, it was a biological sediment and proper compost was the least colonized by microorganisms of enzymatic properties.
3. Lipolytic microorganisms were a prevailing enzymatic group, particularly in the sludge. In the case of biological sediment, the numbers of lipolytic and proteolytic microorganisms were at similar levels. The proper compost contained a dominant enzymatic group of microorganisms capable of fat degradation, but their number was smaller as compared to other test materials.

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Abstract. The increasing production of poultry meat makes the amount of associated waste proportionately increases. The difficulty arises with the management of this material type, because of its heterogeneous nature and present contaminants. Therefore, the utilization of poultry waste materials is very troublesome and is associated with large financial inputs. A good solution might be the introduction of microorganisms having high enzymatic activity in order to optimize the process of management of arduous waste mass and making it more environmentally friendly. The aim of the study was to determine the number of microorganisms capable of degrading the protein, starch and fat in a variety of waste materials derived from the poultry slaughter and its management (sludge, sediment from biological sewage treatment plant, proper compost). Test samples came from the composting facility within the poultry processing plant in Western Poland. The materials have been subjected to microbiological analysis on selective media, according to recommendations. Based on achieved results, it was found that all of the tested waste products were colonized by enzymatic microorganisms. The predominant number of microorganisms was found in sludge from the reservoir for liquid waste, while the smallest population was detected in the proper compost. An outstanding group of physiological microorganisms were microorganisms capable of fat degrading.