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EFFECT OF THE INTENSIVE AEROBIC BIOSTABILIZATION PHASE ON SELECTED MICROBIOLOGICAL AND PHYSICOCHEMICAL PARAMETERS OF WASTES

Katarzyna Wolny-Koładka[1], Mateusz Malinowski[1], Agnieszka Sikora[1], Kamil Szymonik[1], Grzegorz Pelczar[2], Katarzyna Wawrzyniak-Turek[2]

[1]University of Agriculture in Krakow, [2]EKO-BIOMASA Sp. z o.o.

Abstract

One of the most frequently applied methods of mixed municipal solid waste biological treatment in mechanical-biological treatment installations (MBT) is their disposal in aerobic biostabilization process. The process comprises an intensive phase and maturation phase. The intensive phase relies on waste heating in result of organic matter breakdown conducted by microorganisms settling the wastes. Microorganisms living in wastes have optimal conditions for development, i.e. a considerable space volume, optimal material fragmentation and organic matter availability. The aim of the aerobic biostabilization process is stabilizing and hygienization of wastes, so that they become a valuable raw material for other recovery processes (e.g. RDF production, Refuse-Derived Fuel) or their disposal is safe for the environment and do not pose any epidemiological hazard for people employed in waste treatment plants.

Analyses presented in the paper aimed to determine the number and species composition of vegetative and endospore bacteria, mold fungi, actinomycetes and pathogenic microorganisms, i.e. *Staphylococcus* spp., *E. coli, Salmonella* spp., *Shigella* spp., *E. faecalis*, *C. perfringens*, settling municipal wastes prior to and after aerobic stabilization process. The aerobic stabilization process (intensive phase) was conducted in a laboratory BKB 100 bioreactor using the wastes, characterized by low share of biodegradable wastes (<40 %), obtained from MBT installation during the period from December 2015 to February 2016. The temperature, loss on ignition, waste density, ash content, moisture and pH were monitored dur-

ing the analyses. The research was conducted on the undersize and oversize fraction separated from mixed municipal wastes on MBT installation.

The maximal temperature reached in the bioreactor, between c.a. 40 and 55°C, persisting for many hours is insufficient for efficient elimination of the determined microorganism groups. Paradoxically, the conditions created in the bioreactor proved convenient for the microorganisms, therefore an increase in their number was observed. Only in one case, when the share of biodegradable wastes exceed the value of 45 % it was observed, that the aerobic stabilization process of these wastes was going correctly and caused among others a decrease in loss on ignition.

Keywords: waste, bacteria, fungi, mechanical-biological treatment, biodegradable waste,biostabilization process

INTRODUCTION

Aerobic biostabilization process involves the oxygen supply to wastes placed in special bioreactors or to the wastes formed into aerated windrows (Dziedzic *et al*. 2015). Supply of oxygen (fresh air) is a crucial element of the process, necessary for development of waste settling microorganisms. Microorganisms are responsible for the increase of waste temperature in result of processing organic matter contained in them. Increase of the temperature over 60-65°C persisting for the several hours guarantees hygienization, i.e. elimination of pathogenic microorganisms, whereas over a longer period of time – the waste stabilization, i.e. prevention of a renewed increase of the temperature, e.g. in the storage wastes (Jędrczak 2008).

The process is technologically similar to green waste composting, however not a compost (fertilizer) but a stabilized waste is obtained. Aerobic stabilization is used as a method of biological disposal of waste from MBT process containing at least 40 % d.m. of organic matter (Szpadt, Jędrczak 2008). During the intensive phase of this process (conducted in bioreactors) the waste temperature grows even to 70°C and persists for several days, resulting in initial hygienization and stabilization of waste stream, preventing its renewed heating. After 14 days of the process wastes are formed into windrows and the maturation phase starts, which lasts between 6 and 10 weeks.

Aerobic stabilization should result in a total breakdown of biodegradable fraction contained in the waste (diminishing loss on ignition and organic carbon contents, as well as increase in the ash content). The process is conducted also to reduce the mass of wastes deposited on the landfill and limit greenhouse gas emission by stabilized wastes deposited there (Sungi *et al*., 2005; Baran *et al*., 2016). The aerobic stabilization process was described in the papers of Adani

et al. (2002), Adani *et al*. (2004), Dziedzic *et al*. (2015), Sugni *et al*. (2005) and Titta *et al*. (2007).

Presented research was conducted to assess the possible application of aerobic stabilization as a process contributing to hygienization of wastes (the undersize and oversize fractions separated from MBT installation) with low share of biodegradable fraction (<40 %). The temperature, calcination losses, waste density, ash content, moisture content and pH were monitored during the analyses. Moreover, it was determined whether aerobic stabilization would efficiently reduce the occurrence or totally eliminate microorganisms settling raw materials used for refuse-derived fuels (RDF) production. Subsequently, the effect of aerobic stabilization on microbiological parameters was assessed, because it was supposed to contribute to elimination of particularly pathogenic microorganisms posing epidemiological hazard.

The analyses described in the article constitute a part of the project with an objective to create stable and safe fuel from wastes ("ekoRDF") for the commercial power industry. The research objective resulted from the necessity to find waste stabilization and hygienization method, so they would no longer pose epidemiological hazard or contribute to fire danger. Yasuhura *et al*. (2010) and Tambore *et al*. (2011) also point to an increase in safety of life and health of people working in the waste disposal buildings and installations where post-MBT waste is deposited. The above mentioned hazards result from the fact, that e.g. raw materials used for RDF production are a mixture of many kinds of waste, including organic wastes (green wastes, kitchen wastes, paper, cardboard, etc.). Some parts of organic wastes, irrespective of the precise sorting process, penetrate to RDF and worsen its fuel (energy) qualities, but at the same favor multiplication of often pathogenic microorganisms, threatening human health and life. Unfortunately, both the raw materials for RDF production and the refuse derived fuel itself may pose a microbiological hazard to people engaged in its manufacturing. Therefore, from the cognitive point of view, the analysis of the number and species composition of microorganisms settling the wastes before and after stabilization process, preceding ekoRDF production, conducted in the presented paper is interesting.

MATERIAL AND METHODS

The research involved aerobic stabilization (intensive phase) was conducted using laboratory BKB 100 bioreactor on the wastes collected from MBP installation of MIKI Recycling Ltd in Krakow. The wastes were collected from December 2015 to February 2016, so that they would reveal a possibly low share of organic matter and biodegradable fraction (<40 %). Samples for the analyses were prepared according to the methodology recommended by the European

Committee for Standardization, 2006, Characterization of Waste – Sampling Waste Materials – Framework for the Preparation and Application of a Sampling Plan (EN 2006, 14899).

The wastes were placed in the bioreactor for 14 days. The weight of a single input product was about 20 – 40 kg depending on the analyzed fraction (oversize fraction – lower weight, undersize fraction – higher weight of waste). Two replications were conducted for each fraction. Air supply to the bioreactor chamber (116 dm^3) was maintained on the level between 0.5 and 1.5 m^3 per 1 kg of dry organic matter per 24 hours. The intensity of aeration was regulated following the Schultz rule, which states that oxygen demand depends on the process temperature, as follows (Jedrczak 2008):

$$W = 0,1 \cdot 1,067^{t}$$

where: W – oxygen demand [mg O_2 $\cdot$(g d.m. $\cdot$h)$^{-1}$],
t – temperature in 20-70°C range.

The following laboratory tests were conducted for the analyzed wastes, before and after the stabilization process, in order to determine the collected waste susceptibility to biological treatment process and aerobic stabilization result:

a) determining the morphological composition (only before the process) and the share of biodegradable wastes, as the total of: 100 % organics, 100 % paper and cardboard, 50 % wood, 50 % textiles, 40 % multi-material waste and 30 % fine fraction, i.e. <10mm. The share of these morphological groups in the waste intended for biological treatment is very important because it directly influences the parameters and the course of the process. The analysis of morphological composition was conducted in 3 replications on samples weighing ± 1000 g for the oversize fraction and ± 2000 g for the undersize fraction,
b) determining the waste density (by measuring the weight of the input to the bioreactor and the volume occupied by the waste in the bioreactor), loss of weight and volume of treated waste and monitoring of the leachate volume (the container was placed under the bottom aerating the bioreactor),
c) pH measurement by pH meter,
d) establishing the moisture content (water content in relation to the initial weight of the dried sample) by means of PN-EN 14774-3:2010 method. The mass of each waste sample intended for drying was c.a. ± 1000 g,
e) determining loss on ignition and ash content according to PN-EN 14775:2010 through sample calcination in a muffle furnace; the weight loss, which occurred in result of a sample calcination informs about organic substance content in wastes,

f) monitoring of the waste temperature changes during the process (recording every 30 seconds).

After each replication (2 replications for the undersize and 2 for the oversize fraction) waste samples with 1000cm^3 volume were prepared and subjected to microbiological tests. Microbiological tests were also conducted on control samples (the undersize and oversize fractions before the aerobic stabilization process). 10g of the analyzed material was weighed from each sample to isolate microorganisms. The isolation was conducted by serial dilutions technique after Koch on microbiological media. The following groups of microorganisms were determined: total vegetative and endospore bacteria (agar MPA, BTL, cultured at 37°C for 24h), mold fungi (maltose agar MEA, BTL, cultured at 28°C for 5 days) and actinomycetes (Pochon agar, BTL, cultured at 28°C for 7days). The number of vegetative and endospore bacteria evidences the analyzed raw material abundance in nutrients, easily available to microorganisms. A numerous presence of bacteria, mold fungi and actinomycetes testifies also to favourable conditions (the temperature, pH and moisture content) for the growth and development of microorganisms. Investigated was also the occurrence of potential pathogenic microorganisms: *Staphylococcus* spp. (Chapman agar, BTL, cultured at 37°C for 24h), *Escherichia coli* (TBX agar, BTL, cultured at 44°C for 24h), *Salmonella* spp. and *Shigella* spp. (agar SS, BTL, cultured at 37°C, for 24h), *Enterococcus faecalis* (Slanetz Bartley medium, BTL, cultured at 37°C, for 48h), *Clostridium perfringens* (agar with sulphate and cycloserine SC, BTL, cultured at 37°C, for 24h). Presence of pathogenic microorganisms (*Staphylococcus* spp., *E. coli*, *Salmonella* spp., *E. faecalis*, *C. perfringens*) which may pose epidemiological hazard is an important signal informing about a potential microbiological contamination.

Analysis of the serial dilutions was conducted in three replications. The number of colony forming units (CFU) of the microorganisms was determined using culture dilutions, converting the result per one gram of the analyzed waste.

Bacteriological preparations stained using Gram method and intravital preparations in Lugol's iodine were made for an initial identification of the microorganisms isolated from the obtained wastes.

Statistical analysis of the obtained results was conducted using Statistica v. 12.5 (StatSoft) software. Mean number of microorganisms in the analyzed samples was computed, as well as the correlation between pH of the analyzed substrates and the number of isolated microorganism groups.

RESULTS AND DISCUSSION

Among all samples collected for the laboratory analysis from MBP installation, the share of biodegradable waste exceeded 40 % only in one case.

Because of the period in which the samples were collected, the undersize fraction revealed a low share of organics and a high share of fine fraction, with grain size <10mm (Tab.1). On the other hand, the oversize fraction was characterized by a considerable share of biodegradable waste in comparison to the results reported by other authors, among others Malinowski and Sikora (2014) or Malinowski, Wolny-Koładka (in print).

Table 1. Morphological composition of the undersize and oversize fractions – control sample (before the process)

Waste group	Undersize fraction		Oversize fraction	
	Control before I session	Control before II session	Control before I session	Control before II session
	share [%]		share [%]	
Fine fraction < 10mm	48.4 ± 4.9	36.6 ± 4.3	3.2 ± 0.6	7.0 ± 1.2
Organics	15.4 ± 5.2	10.9 ± 4.1	7.3 ± 3.3	4.1 ± 1.9
Paper and cardboard	2.6 ± 0.8	18.4 ± 3.3	12.2 ± 3.2	8.6 ± 0.9
Plastics	9.8 ± 0.9	11.3 ± 2.6	49.5 ± 4.7	60.6 ± 4.5
Metal	2.0 ± 0.6	0.4 ± 0.2	1.1 ± 0.7	0.9 ± 0.2
Glass	7.6 ± 0.6	10.5 ± 1.9	4.1 ± 3.7	0.3 ± 0.1
Textiles and clothing	4.4 ± 0.7	0.8 ± 0.4	5.5 ± 1.9	6.0 ± 1.1
Personal hygiene products	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
Wood	0.3 ± 0.1	3.1 ± 1.1	7.7 ± 1.2	4.0 ± 0.8
Multimaterial waste	4.7 ± 1.3	5.3 ± 0.7	1.0 ± 0.7	2.6 ± 1.6
Hazardous waste	0.0 ± 0.0	0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Inert and other categories	4.6 ± 1.6	2.6 ± 0.2	8.4 ± 1.2	5.9 ± 1.2
Biodegradable waste – total	**37.4** ± 4.2	**46.4** ± 3.7	**31.4** ± 3.7	**23.1** ± 2.2

Figure 1 shows the changes of the wastes temperature, subjected to the aerobic stabilization process. The maximum waste temperature reached for the undersize fraction during the 1st replication was 53°C (Fig.1). High temperature at the beginning of the process resulted from the waste heating already during transport from RIPOK installation to the laboratory. The thermophylic phase started on the 3rd day of the process and lasted until day 8. The maximum temperature reached in the 2nd replication was 56°C. Thermophylic phase started very soon in comparison to e.g. results obtained by Baran *et al*. (2016), i.e. on the first day of the process and lasted until the 7th day. The maximum obtained temperature of the process persisted on the level of over 50°C for several days. Results

of analysis of the temperature changes during aerobic stabilization of the oversize fraction were different from the experiments conducted so far (Malinowski, Wolny-Koładka, in print).

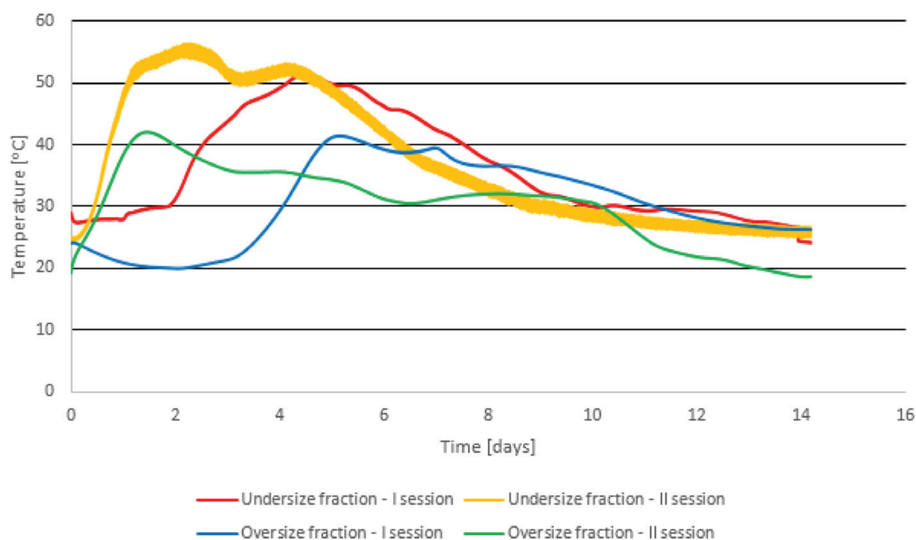

Figure 1. Temperature changes during aerobic stabilization process

Table 2 and 3 present selected parameters of stabilized wastes. Water loss in the input weight (over 10 %) and in consequence the waste weight loss (including 400g of leachates), occurred during the 2nd replication of the undersize fraction aerobic stabilization. A decrease in calcination loss on ignition by 17 % also indicated the correct course of the process. No significant loss of moisture content in the input weight or any marked loss in the stabilized waste mass was registered during aerobic stabilization of the oversize fraction. No leachates were observed, either. Organic matter content (loss on ignition) increased slightly, whereas ash content decreased slightly (Tab. 3). From the Authors' own experience and basing on literature reports it should be stated that in an adequately large bioreactor (with working volume between 20 and 30 m^3) wastes become initially stabilized, whereas the obtained result as mass and volume reduction is markedly greater (Malinowski, Wolny-Koładka, in print).

Presence of a numerous microorganism population was observed in the analyzed substrate samples, including pathogenic microorganisms (*Staphylococcus* spp., *E. coli*, *Salmonella* spp., *C. perfringens*). The analyzed material revealed a considerable microbial biodiversity, in which worthy of note is the presence of pathogenic microorganisms posing a potential epidemiological

hazard (Tab. 4). On the basis of the analysis of data in Table 4 referring to the assessment of microbiological parameters of the samples subjected to aerobic stabilization process, numerous presence of both vegetative and endospore bacteria was registered (Fig. 2 and 3).

Table 2. Selected properties of the undersize fraction before and after the process

Parameter	Unit	Undersize fraction I		Undersize fraction II	
		Before process	After process	Before process	After process
Water content (moisture)	%	35.1 ± 2.2	32.2 ± 1.3	38.6 ± 2.4	26.9 ± 2.1
pH		7.4	8.1	7.1	7.7
Density	$kg{\cdot}m^{-3}$	544.2	597.9	520.9	551.4
Ash content	% d.m.	49.3 ± 3.3	54.5 ± 1.2	39.7 ± 1.2	57.3 ± 1.1
Loss on ignition	**% d.m.**	**51.1** ± 3.2	**45.5** ± 1.2	**60.3** ± 1.2	**42.7** ± 1.1
The weight loss	%	1.8		6.2	
Volume loss	%	13.5		11.4	

Table 3. Selected properties of the oversize fraction before and after the process

Parameter	Unit	Oversize fraction I		Oversize fraction II	
		Before process	After process	Before proces	After process
Water content (moisture)	%	29.2 ± 4.1	25.7 ± 3.3	24.1 ± 6.1	22.6 ± 5.2
pH		7.4	7.5	7.3	7.3
Density	$kg{\cdot}m^{-3}$	216.4	224.3	186.4	190.9
Ash content	% d.m.	17.1 ± 1.6	17.0 ± 1.3	21.3 ± 1.4	20.9 ± 2.1
Loss on ignition	**% d.m.**	**82.9** ± 1.7	**83.0** ± 1.3	**78.7** ± 1.4	**79.1** ± 2.2
The weight loss	%	1.5		0.9	
Volume loss	%	4.7		3.6	

Observed phenomenon evidences favourable conditions (availability of organic substances, moisture content and raw material pH) for the growth and development of these microorganisms. Moreover, the number of vegetative bacteria, both in the samples subjected to aerobic stabilization and control ones, considerably exceeded the number of endospore bacteria, which also demonstrates that raw materials used for refuse derived fuel production are perfect medium for bacteria development. Endospore forms (coatings, integuments or spores)

are developed by bacteria under stress (lack of nutrients, low moisture content or extreme pH). Obtained results indicate the fact that the material tested for bacteria presence was their optimal environment where they were able to develop and reach high numbers. The factor, which may significantly affect the number of both bacteria and fungi, is pH. However, on the basis of conducted measurements no marked changes of pH and therefore its influence on the number of both endospore and vegetative bacteria were observed in the substrate used for RDF production.

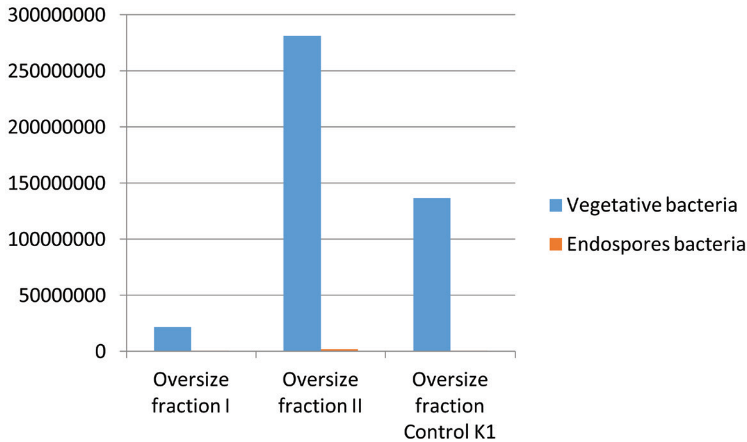

Figure 2. Mean number (CFU ·g^{-1} RDF) of vegetative and endospore bacteria in the analyzed material

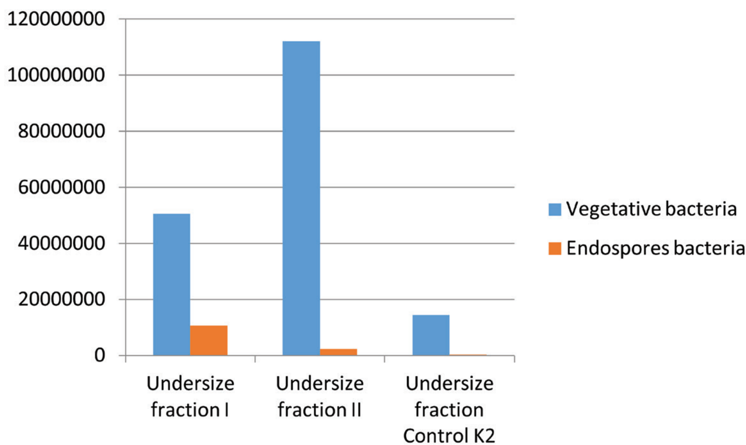

Figure 3. Mean number (CFU · g^{-1} RDF) of vegetative and endospore bacteria in the analyzed material

Table 4. Results of the number of microorganisms (CFU g^{-1} · RDF) and the pH of the tested samples of the raw material subjected to the process of aerobic stabilization

Sample	**Vegetative bacteria**	**Endospores bacteria**	**Mold fungi**	**Actinomycetes**	**Staphylococci**	***E. coli***	***Salmonella* spp.**	***Shigella* spp.**	***E. faecalis***	***C. perfringens***	**pH**
Oversize fraction I	21150000	71000	4900000	0	10654189	400	6700	0	0	170	8.03
	21470855	69310	5022389	0	11350000	380	6589	0	0	167	8.1
	22003330	70335	4700248	0	10357000	397	6600	0	0	179	8.21
Mean	**21541395**	**70215**	**4874212**	**0**	**10787063**	**392.3**	**6629.7**	**0**	**0**	**172**	**8.11**
Oversize fraction II	298000000	1810000	6300000	0	11100000	447433	11000248	0	0	60	8.25
	300250800	1728000	6187002	0	10289600	418900	10245800	0	0	54	8.27
	244896127	1658700	6289900	0	10388500	425800	10335000	0	0	58	8.23
Mean	**281048976**	**1732233**	**6258967**	**0**	**10592700**	**430711**	**10527016**	**0**	**0**	**57.3**	**8.25**
Oversize fraction K1	147226703	568073	429446	0	105998	1950	201	0	0	3336	8.34
	16051889	408132	52715	0	192264	23551	16049	0	0	79	6.41
	246985111	445150	531103	0	115195	1858	191	0	0	3032	8.08
Mean	**136754568**	**473785**	**337754.7**	**0**	**137819**	**9119.7**	**5480.3**	**0**	**0**	**2149**	**7.61**
Undersize fraction I	51385000	10375800	287000	0	9930000	13200	400	0	0	7215	8.29
	50384470	11350000	258000	0	9862000	12874	389	0	0	7548	8.23
	50038850	10258900	267950	0	9700358	13688	380	0	0	7002	8.1
Mean	**50602773**	**10661567**	**270983.3**	**0**	**9830786**	**13254**	**389. 7**	**0**	**0**	**7255**	**8.20**

Sample	**Vegetative bacteria**	**Endospores bacteria**	**Mold fungi**	**Actinomy-cetes**	**Staphylo-cocci**	***E. coli***	***Salmonella* spp.**	***Shigella* spp.**	***E. faecalis***	***C. perfrin-gens***	**pH**
Undersize fraction II	111134000	2560000	1475000	0	11214000	417200	10874255	0	0	200	8.2
	122587000	2407000	1380000	0	10385000	400248	12478000	0	0	204	8.1
	102508000	2399547	1399700	0	11288000	402899	11452800	0	0	187	8.3
Mean	**112076333**	**2455516**	**1418233**	**0**	**10962333**	**406782.3**	**11601685**	**0**	**0**	**197**	**8.20**
Undersize fraction K2	6170303	79541	770	0	375760	236	2249	0	0	358	8.1
	31774100	537049	204017	0	1659741	620143	19318	0	0	63	6.9
	5258533	604116	7673	0	324500	219	2309	0	0	514	7.72
Mean	**14400979**	**406902**	**70820**	**0**	**786667**	**206866**	**7958. 7**	**0**	**0**	**311. 7**	**7.57**

Neither the presence of *Shigella* spp., responsible for food poisonings in people was detected in the analyzed material, nor *E. faecalis* bacteria evidencing a fresh faecal contamination of the material, nor actinomycetes causing allergies, was spotted in the studied material. Malinowski and Wolny-Koładka (unpublished) state, that no presence of actinomycetes of *Azotobacter* spp. was found after the completed process of the waste biological treatment conducted in the bioreactor for 8 days at the temperature between 64 and 65°C.

Mold fungi constituted a numerous group of determined microorganisms. Particular attention should be paid to the presence of potentially toxic fungi, which are producers of secondary metabolites called mycotoxins. It was found that mold fungi were much more numerous in the oversize fraction. Conducted mycological analysis revealed the presence of mold fungi, including the toxinogenic *Aspergillus* spp., *Penicillium* spp., *Mucor* spp., *Cladosporium* spp., *Rhizopus* spp. and *Alternaria* spp.. They may pose a hazard to the health of persons who are in contact with raw materials for RDF production, since the fungi produce mycotoxins and cause allergic reactions (Fig. 4).

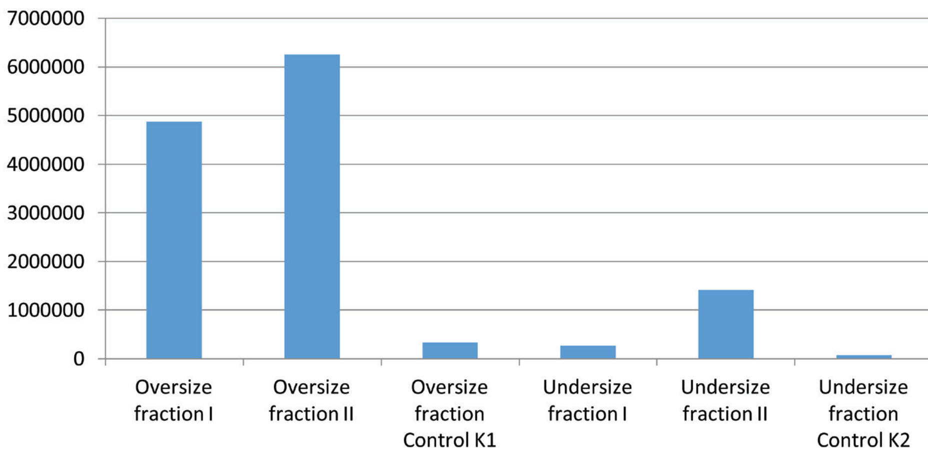

Figure 4. Mean number ($CFU \cdot g^{-1}$RDF) of mold fungi in the analyzed material

Presence of pathogenic bacteria (*Staphylococcus* spp., *E. coli, Salmonella* spp., *C. perfringens*) in the analyzed material gives cause for concern. Each of the determined bacteria poses a real hazard for the health and life of persons who are in touch with the studied material (Fig.5 and 6).

Results of the number of selected microorganism groups originating from the analyzed control samples were compared with the data obtained as an aerobic stabilization effect on individual fractions of the raw material. The assessment of microbiological parameters in the raw material after aerobic stabilization pro-

cess was based on it. It was stated that the number of vegetative and endospore bacteria and mold fungi not only remained on a high level after aerobic stabilization process application, but even increased. Applied conditions of the material hygienization did not affect a decrease in these microorganism group numbers. However, no presence of actinomycetes, *Shigella* spp. or *E. faecalis* was observed either in the analyzed material subjected to the aerobic stabilization process or in the control samples.

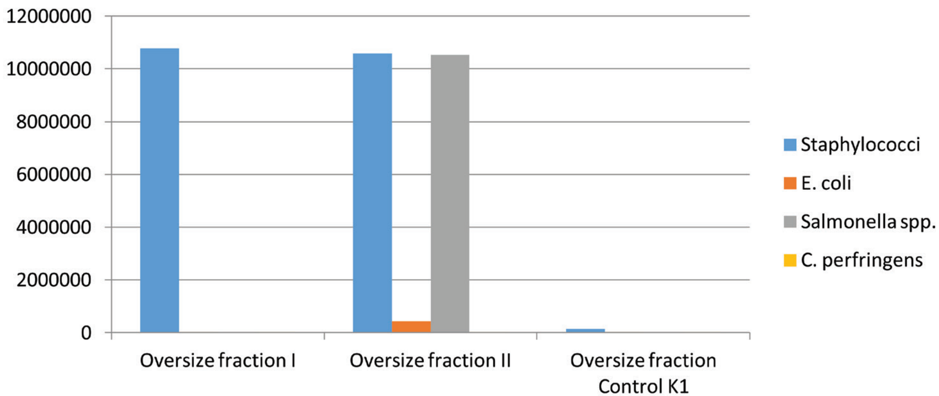

Figure 5. Mean number (CFU · g^{-1} RDF) of pathogenic bacteria in the analyzed material

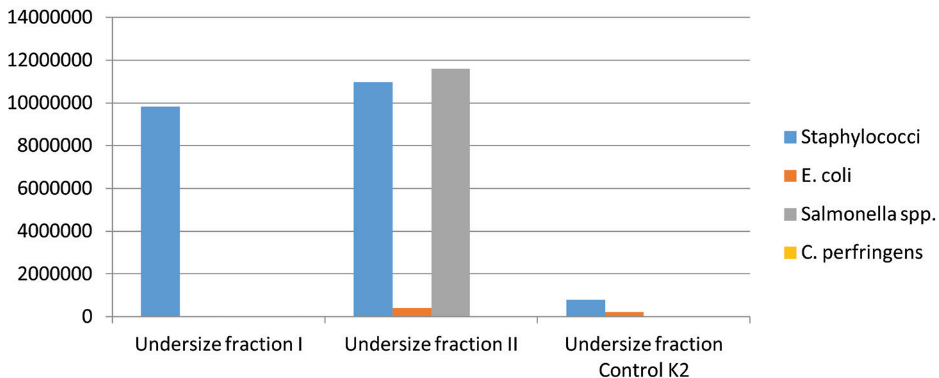

Figure 6. Mean number (CFU · g^{-1} RDF) of pathogenic bacteria in the analyzed material

Pathogenic bacteria (*Staphylococcus* spp., *E. coli, Salmonella* spp., *C. perfringens)* were numerous in the analyzed material. Especially worthy of note is a very high number of *Staphylococcus* spp., particularly after the application

of the aerobic stabilization process and *Salmonella* spp. In the oversize II and undersize I fractions. Comparing the results of bacteria numbers in the samples subjected to aerobic stabilization and in the control samples, it should be stated that applied parameters of the process contributed to increased numbers of the microorganisms. Therefore, the effect opposite of the expected was achieved, which allows to suppose that the aerobic stabilization process parameters proved favorable for the development of determined microorganisms. Moreover, presence of spore forming bacteria (*C. perfringens*) was spotted in the analyzed material, which suggests that only the temperature exceeding 100°C might prove adequate for the samples hygienization. Considering a mixed character of the microorganism population settling the raw materials for RDF production, a higher temperature, adjusted to various microorganism groups with a wide tolerance range to extreme factor, should be applied for a proper hygienization of the analyzed raw material (Macura 2008, Szewczyk 2007).

Statistical analysis of the dependence of mean microorganism number in the analyzed samples on mean pH value of the collected raw material confirmed the existence of a weak or average correlation between these values ($p<0.05$), (Table 5).

Table 5. Results of ANOVA regarding the value of Pearson correlation coefficient r for the process of aerobic stabilization

Microorganisms	**Correlation coefficient (p<0,05)**
	pH
Vegetative bacteria	0.31
Endospores bacteria	-0.16
Fungi	-0.13
Staphylococcus spp.	0.33
E. coli	-0.36
Salmonella spp.	-0.15
C. perfringens	-0.12

CONCLUSIONS

The temperature reached in the bioreactor, ranging from c.a. 40 to 55°C and persisting even for many hours proved inadequate to efficiently eliminate determined microorganism groups. Paradoxically, conditions which developed

in the bioreactor proved favorable for microorganisms, therefore a growth in their numbers was observed.

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Corresponding author:
Katarzyna Wolny-Koładka PhD. Eng.
University of Agriculture in Krakow
Department of Microbiology
Mickiewicza Ave 24/28. 30-059 Krakow
e-mail: k.wolny@ur.krakow.pl

Mateusz Malinowski PhD. Eng.
University of Agriculture in Krakow
Institute of Agricultural Engineering and Computer Science
Ul. Balicka 116b. 30-149 Krakow
Tel: +48 (12) 662 46 60
e-mail: Mateusz.malinowski@ur.krakow.pl

Agnieszka Sikora
Kamil Szymonik
University of Agriculture in Krakow

mgr Grzegorz Pelczar
dr Katarzyna Wawrzyniak-Turek
EKO-BIOMASA Sp. z o.o.
Biechów 27, 28-133 Pacanów,
e-mail: biuro@ekordf.pl

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