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MICROBIOLOGICAL AND PHYSICO-CHEMICAL COMPOSITION OF SEWAGE SLUDGE DERIVED FROM THE FOOD INDUSTRY

SKŁAD MIKROBIOLOGICZNY I FIZYKOCHEMICZNY OSADÓW ŚCIEKOWYCH POCHODZĄCYCH Z PRZEMYSŁU SPOŻYWCZEGO

Abstract: The aim of the study was to analyze the microbiological and physico-chemical composition of sewage sludge from the food industry. The research material was dewatered sewage sludge with and without lime, derived from the water and drink production plant in the Opole region, obtained for testing in March 2013. Physico-chemical analysis included the determination of: pH, temperature of sludge, sedimentation properties, the dry weight, mineral substances, biogenic elements and heavy metals. Microbiological evaluation included quantitative and qualitative determination of mesophilic, psychrophilic and potentially pathogenic microorganisms. Sewage sludge with and without lime characterized similar physico-chemical parameters. However, the addition of lime to the sludge led to a change in ratios of different groups of microorganisms. In the sludge with lime, we observed reduction in the number of mesophilic bacteria and yeast, and total inhibition of psychrophilic bacteria and fungi. Knowledge of the physico-chemical parameters and microbiological composition of the sewage sludge is needed to determine the directions of their development.

Keywords: sewage sludge, microbiological determination, physico-chemical analysis, food industry

The sludge, which is created after the biological wastewater treatment, within the meaning of the Act of 27 April 2001 on waste is the waste from the Q9 category. Its chemical and biological composition varies according to the type of wastewater and pre-treatment methods [1, 2]. The produced sewage sludge is a worry for the operators of wastewater treatment plants [3]. So far it has been used mainly in agriculture and land reclamation or stored in landfills [4]. However, due to the changes in legislation [5] from January 1, 2013 there are significant restrictions on the storage of sewage sludge. The storage of hazardous waste is prohibited, in which carbon is at level of at least 5% of dry matter and the heat of combustion- at least 6 MJ/kg of dry matter [4- 6].

Sewage sludge from food industry vary widely in chemical composition, so it is difficult to find a consistent way of their development. Therefore, the use or utilization should always be preceded by a study of sludge composition and considered individually for

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each treatment plant. Knowledge of the physico-chemical parameters and microbiological composition of the sewage sludge is needed to determine the directions of their development.

Physico-chemical determination has to be performed in accordance with the Regulation of the Minister of Environment of 13 July 2010 on municipal sewage sludge. The pH and the contents of elements such as K, Ca, Mg, N and P allow to determine the possibility of use the sewage sludge as a fertilizer [7]. It is also important to assess the ability of migration of metals from sewage sludge into the soil [8]. The study of the different forms of metals in the fractions (fraction of exchangeable metal ions, fraction of metals associated with carbonates, medium and easily reducible fractions, and residual fraction) allows to evaluate not only the total metal content in the sediment, but also their availability to plants [9-11]. Maximum levels of heavy metals in sewage sludge for agricultural use as defined also in the Regulation of the Minister of Environment of 13 July 2010 on municipal sewage sludge [12].

Sewage sludge may be contaminated by pathogenic microorganisms and spores. Unfortunately, the study of sludge are carried out using traditional methods and is limited to the detection of potentially pathogenic bacteria of the *Salmonella* genus, and the eggs of intestinal parasites ATT (*Ascarislumbricoides*, *Toxocara* sp., *richuris* sp.). Less commonly performed test for coliform bacteria, *Pseudomonas aeruginosa* and *Clostridium perfringens*. In fact, there are not carried out a comprehensive microbiological testing because there is no such legal requirement [1, 12], and applied diagnostic methods are taken from various industries. Accredited laboratories perform analyzes based on their own testing procedures, which are based on the acts and regulations on: water and wastewater, soil, municipal waste, feed and food, and general rules for the microbiological testing [13]. It follows that there is a need for changes in the legislation and standards for diagnostics, especially in microbiology.

The aim of the study was to analyze the microbiological and physico-chemical composition of sewage sludge from the food industry.

Material and methods

The research material was dewatered sewage sludge with and without the addition of burned lime, derived from the food industry in Opole region. Lime was added in an amount of 1.5 kg/Mg of sludge. The designations were made of samples averaged. The research included:

I. Physical analysis determination of sediment temperature [°C], the properties of the sediment by the volume-sedimentation method (PN-EN 14702-1:2008) and humidity by the gravimetric method (PN-EN 12880:2004) [13].
II. Chemical analysis - determination of pH (PN- EN 12176:2004), mineral content-loss on ignition (PN-EN 12879:2004), nitrogen (total) by the Kjeldahl method (PN-EN 16169:2012), ammonia nitrogen (PN-EN 14671:2007), total phosphorus (PN-EN 13346:2002), phosphorus as P_2O_5 (PN-EN ISO 11885:2009), the content of elements: Ca, Mg, Zn, Pb, Cd, Cr, Cu, Ni (PN-EN 13657:20068) and mercury (PN-EN 13346:200) [14, 15].
III. Microbiological analysis - quantitative and qualitative determination of mesophilic, psychrophilic and potentially pathogenic microorganisms by the culture method (serial

dilutions) on the media: nutrient agar - mesophilic and psychrophilic bacteria; YPD - yeast; Čapek - filamentous fungi; and selective media: Hektoen-bacteria of the *Enterobacteriaceae* family; Parker - *Staphylococcus spp*.

Cultures were incubated 24-48 h at 35°C - mesophilic and potentially pathogenic bacteria (*Enterobacteriaceae* family, *Staphylococcus* and *Streptococcus* genus), 48-72 h at 20°C - psychrophilic bacteria, at 30°C - yeast, and 7 days incubation at 25°C - fungi. After incubation, the colonies were counted and the result given in 1 g CFU/g d.m. of sludge. Additionally the bacteria were differentiated by the Gram stain and divided morphologically into groups.

Results and discussion

Results of the physico-chemical analysis of sludge with and without lime content, used test standards and their ranges are shown in Table 1.

Table 1

Physico-chemical analysis of sewage sludge

Analysis	The unit of measure	The sewage sludge after drinks production without lime	The sewage sludge after drinks production with lime	Research method	Current standards	Range of standards
Chemical analysis:						
pH		6.9	7.1	Potentiometric (Electrometry)	PN-EN 12176:2004	1-13.5
Dry weight 105°C	[%]	10.9	10.7	Thermogravimetric (Weight)	PN-EN 12880:2004	0.50-99.5
Mineral and organic substances - loss on ignition 600°C	[% d.m.]	83.1	83.2	Weight	PN-EN 12879:2004	0.50-99.5
Nitrogen (total) Kjeldahl	[% d.m.]	6.32	6.92	Titration	PN-EN 16169:2012	0.003-8.00
Ammonia nitrogen	[% d.m.]	< 0.010	< 0.010	Titration	PN-EN 14671:2007	0.003-2.00
Phosphorus	[% d.m.]	1.2	1.2	ASA	PN-EN 13346:2002	0.0005-10.00
Phosphorus P_2O_5	[% d.m.]	2.74	2.74	Optical emission spectrometry with inductively activated plasma	PN-EN ISO 11885:2009	0.0005-10.00
Ca	[% d.m.]	1.01	1.18	ASA	PN-EN 13657:2006	0.0001-15.0
Mg	[% d.m.]	0.29	0.3			0.0001-2.00
Zn	[mg/kg d.m.]	150	145			0.50-10000
Pb	[mg/kg d.m.]	2.25	1.61			1.00-3000
Cd	[mg/kg d.m.]	4.34	4.45			0.05-200
Cr	[mg/kg d.m.]	36.2	35.6			0.30-1000
Cu	[mg/kg d.m.]	26	27.4			0.40-5000

Analysis	The unit of measure	The sewage sludge after drinks production without lime	The sewage sludge after drinks production with lime	Research method	Current standards	Range of standards
Ni	[mg/kg d.m.]	10.7	10.5			0.40-1000
Hg	[mg/kg d.m.]	0.36	0.33	Atomic Spectrometry with upconcentration on amalgamator	PN-EN 13346:2002	0.05-25.00
Physical analysis:						
Temperature	[°C]	16.5	-	-	-	-
Sedimentation properties	[cm^3/dm^3]	293.75	-	Volume-sedimentation method	PN-EN 14702-1:2008	100-1000
Determination of humidity	[%]	-	99.23	Thermogravimetric (Weight)	PN-EN 12880:2004	0.50-99.5

The results found that liming had no effect on studied physico-chemical parameters. In the sludge with lime, compared with the other one, there was a slight increase in pH (from 6.9 to 7.1), total nitrogen, cadmium, copper and calcium. The concentrations of most metals were slightly (irrelevant) decreased, and the concentration of phosphorus and ammonia nitrogen were unaffected. The average temperature of sludge was 16.5°C, the average value of the sedimentation properties was 293.75 cm^3/dm^3 and the average dry weight of limed sediment was 99.23%.

Microbiological analysis showed that liming had no effect on the number of mesophilic bacteria and yeast but inhibited growth of psychrophilic bacteria and fungi (Figs. 1 and 2).

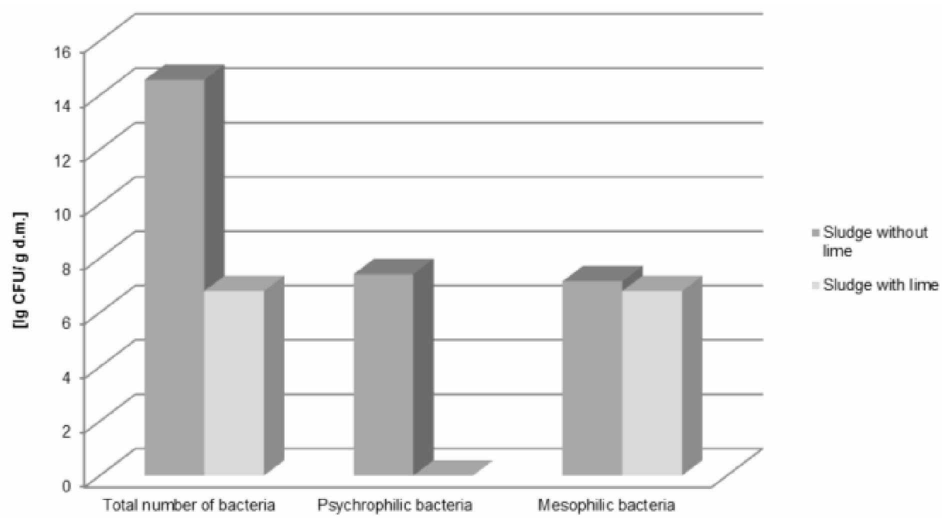

Fig. 1. The total number of mesophilic and psychrophilic bacteria [lg CFU/g d.m.] in sewage sludge with and without lime

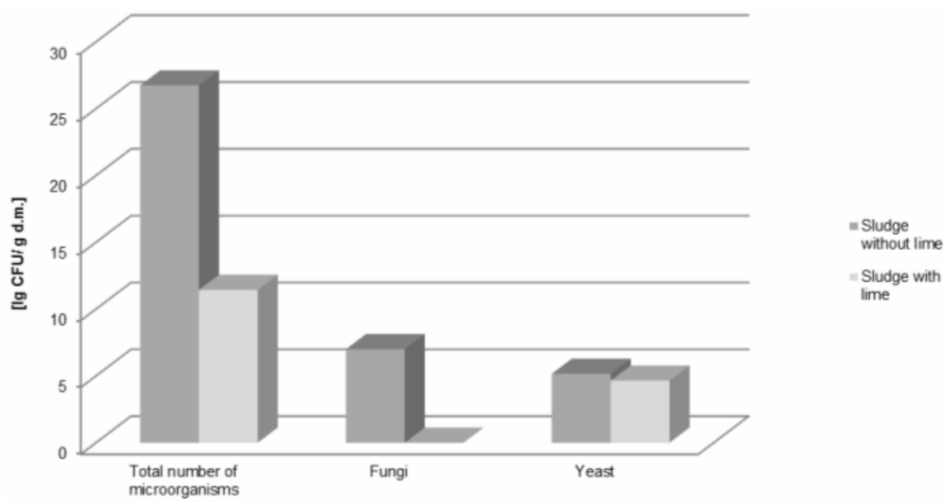

Fig. 2. The total number of microorganisms including yeast and fungi [lg CFU/g d.m.] in sewage sludge with and without lime

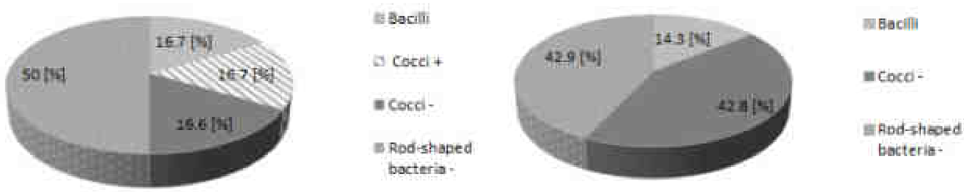

Fig. 3. The composition of mesophilic bacteria population [%] in the limed sediment without and with the addition of lime

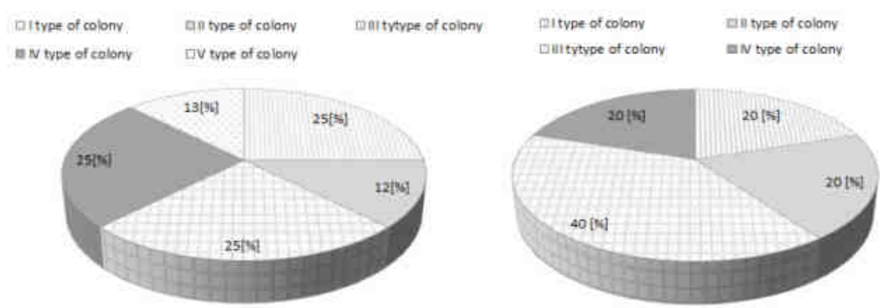

Fig. 4. The composition of yeast population [%] in the limed sediment without and with the addition of lime

The addition of lime to the sludge lead to a change in the quantitative ratios of the various groups of microorganisms. In the sludge without lime dominated gramnegative rod-shaped bacteria (50%), and in limed sediment was similar quantities of gramnegative rod-shaped bacteria and cocci, but there was no presence of grampositive cocci (Fig. 3). In the sludge were essentially the same types of yeast, but the quantitative ratios have changed (Fig. 4). In the sludge without lime four types of filamentous fungi were selected.

Microbiological analysis for potentially pathogenic microorganisms showed that liming significantly affect the incidence of potentially pathogenic bacteria; in sludge with lime clearly increased their numbers, especially staphylococci and streptococci. However, liming did not affect the number of microorganisms of the *Enterobacteriaceae* family (Fig. 5).

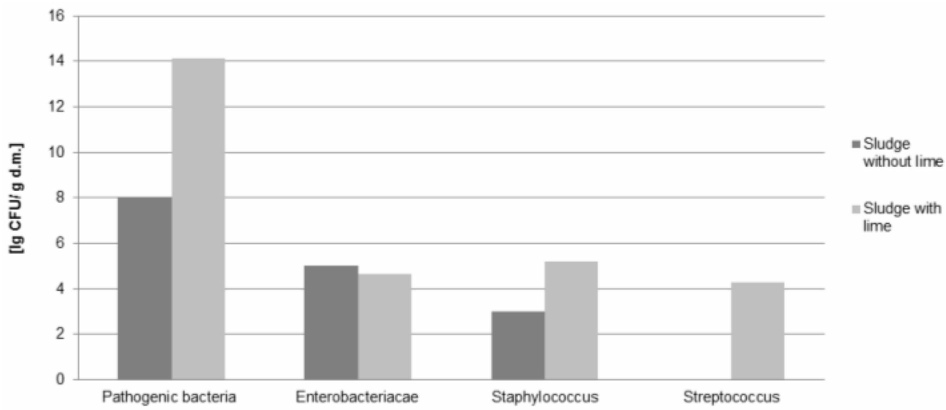

Fig. 5. The total number of potentially pathogenic bacteria [lg CFU/g d.m.] in sewage sludge with and without lime content

Conclusions

1. Sewage sludge with and without lime characterized similar physico-chemical parameters.
2. In the limed sediment, compared with the other one we observed reduction in the number of mesophilic bacteria and yeast, a total inhibition of psychrophilic bacteria and fungi and increase the number of potentially pathogenic bacteria.
3. Due to the diversity of sewage sludge, determination of their development directions should always be preceded by a study of physico-chemical and microbiological composition.

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SKŁAD MIKROBIOLOGICZNY I FIZYKOCHEMICZNY OSADÓW ŚCIEKOWYCH POCHODZĄCYCH Z PRZEMYSŁU SPOŻYWCZEGO

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Abstrakt: Celem badań była analiza składu mikrobiologicznego oraz fizykochemicznego osadów ściekowych pochodzących z przemysłu spożywczego. Materiał badawczy stanowił odwodniony osad ściekowy wapnowany i niewapnowany pochodzący z zakładu produkcji wód i napojów na terenie Opolszczyzny. Analiza fizykochemiczna obejmowała oznaczenia: pH, temperatury osadu, właściwości sedymentacyjnych, zawartości suchej masy, substancji mineralnych, pierwiastków biogennych oraz metali ciężkich. Ocena mikrobiologiczna obejmowała oznaczenia ilościowo-jakościowe mikroorganizmów mezofilnych i psychrofilnych oraz potencjalnie chorobotwórczych. Osad ściekowy zarówno wapnowany, jak i niewapnowany cechowały podobne parametry fizykochemiczne. Jednak dodatek wapna do osadu ściekowego prowadził do zmiany stosunków ilościowych poszczególnych grup mikroorganizmów. W osadzie wapnowanym, w porównaniu do niewapnowanego, obserwowano obniżenie liczby bakterii mezofilnych i drożdży oraz całkowite zahamowanie rozwoju bakterii psychrofilnych i grzybów. Znajomość parametrów fizykochemicznych oraz składu mikrobiologicznego osadów ściekowych niezbędna jest do wytyczenia kierunków ich zagospodarowania.

Słowa kluczowe: osady ściekowe, ocena mikrobiologiczna, analiza fizykochemiczna, przemysł spożywczy