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MICROBIAL DIVERSITY OF SEWAGE SLUDGE*

RÓŻNORODNOŚĆ MIKROBIOLOGICZNA OSADÓW ŚCIEKOWYCH

Abstract: Sewage sludge may be a cluster of different groups of microorganisms, including potential pathogenic, therefore processing of sludge should be conducted in a manner that protects workers and the environment from the sanitary risks. The aim of the study was to assess quantitatively and qualitatively the microbial diversity of the municipal sewage sludge, from the food industry and from the coke after the treatment, with the addition of flocculant and/or lime. Research material consisted of samples taken directly from the wastewater treatment plant. Quantitative and qualitative assessment of mesophilic and psychrophilic bacteria, yeasts, filamentous fungi and potentially pathogenic microorganisms was performed by a culture method. Techniques for treatment sewage sludge towards their hygenisation and reduce the amount of sediment by supporting flocculants and/or liming affect the quantitative and qualitative microorganisms composition. In the studied sewage sludge dewatered using a flocculant, with the exception of sediments from the food industry, there was the highest total number of microorganisms, both meso- and psychrophilic bacteria, yeast, filamentous fungi and potentially pathogenic bacteria. However, in the sediments of the food industry increase in the number of bacteria was observed only after simultaneous application of liming. Among the isolated microorganisms, depending on the origin of the sludge there was a domination of gram negative rods, with the exception of coke sediments without a flocculant. The municipal and from the food industry sediments were dominated by fungi: *Aspergillus niger* and *Cladosporium sp.,* in coke sediments *Aspergillus amstelodami*, *Paecilomyces javanicus* and *Acremonium sp*. In these latter was also observed the greatest diversity of yeasts.

Keywords: sewage sludge, microbiological determination, microbial diversity

Sewage sludge is a complex ecosystem of microorganisms that are involved in the treatment process and are supplied to the wastewater treatment plant with flowing sludge. The microbiological composition varies according to the type of treatment and pre-treatment methods [1]. They may be a cluster of different groups of microorganisms, including pathogens and their spores [2, 3]. Sludge should be conducted in a manner that protects workers and the environment from the sanitary risks [4]. In order to reduce the amount of sludge, dewatering with chemicals called flocculants is commonly used [5]. A way to reduce an epidemiological risk is hygienisation sludge by using *eg* lime. These activities are designed to prepare an environmentally safe waste before export from a wastewater treatment plant.

Aim of the study

The aim of the study was to assess quantitatively and qualitatively the microbial diversity of the municipal sewage sludge, from the food industry and from the coke after the treatment, with the addition of flocculant and/or lime.

Material and methods

Material consisted of samples taken directly from the wastewater treatment plant:

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- dewatered sewage sludge from the food industry and municipal sewage treatment plant without/with the addition of lime: 1.5 kg/Mg d.m. of sludge and 200 kg/Mg d.m. of sludge, respectively;
- nondewatered and dewatered sludge from coke sewage treatment plants.

In order to dewater sewage sludge the cationic flocculant was added (acrylamide copolymer) in an amount of 2-3 kg/Mg d.m. The dry matter content in the nondewatered sediment was about 5%, whereas in dewatered sludge about 20%.

Quantitative and qualitative assessment of mesophilic and psychrophilic bacteria, yeasts, filamentous fungi and potentially pathogenic microorganisms was performed by culture method (Koch's decimal dilutions method) on media:

- Nutrient agar - mesophilic and psychrophilic bacteria,
- YPD - yeasts,
- Czapek - filamentous fungi,
- Hektoen, Endo - bacteria of the Enterobacteriaceae family,
- Parker - *Staphylococcus ssp.,*
- Enterococcus agar - *Streptococcus ssp.* (fecal streptococci).

Mesophilic and potentially pathogenic bacteria (Enterobacteriaceae family, *Staphylococcus* and *Streptococcus* genus) were incubated 24-48 h at 35°C, psychrophilic bacteria - 48-72 h at 20°C and yeast at a temperature of 30°C. The filamentous fungi were incubated for 7 days at 25°C. After incubation, the colonies which grew were counted and the result was given in [lg CFU/g d.m.]. Simultaneously, the bacteria were differentiated by the Gram stain and morphologically divided into groups. Filamentous fungi were identified on the basis of morphological features according to the identification keys [6-8].

Discussion of the results

In wastewater treatment plants are carried out different treatment techniques towards their hygienisation and reduce the amount of sediment. Frequently, dewatering with chemicals (flocculants) and/or liming are used.

In the case of sediments from the coke wastewater treatment plant the addition of flocculant to the raw waste affects the growth of the total number of bacteria. The municipal waste and sediment samples from the food industry were initially dewatered (with flocculant) and/or limed. Hygienisation the sludge with lime decreased the number of bacteria in municipal sediments and caused an increase in sediment samples from food industry compared to the non-limed ones (Fig. 1).

Pre-treatment of sewage also affects the quantitative and qualitative diversity of microorganisms. Dewatering of sludge with the flocculant had an impact on the numbers of meso- and psychrophilic bacteria. The presence of a flocculant in sewage sludge from coke industry caused an increase in the number of meso- and psychrophilic bacteria, yeasts and filamentous fungi compared to the nondewatered sediment (Figs. 2 and 3). On the other hand, in municipal sludge a decrease and in sediments from the food industry- an increase could be seen in the number of these groups of bacteria after liming (Fig. 2). However, the number of yeast and filamentous fungi in this sewage sludge was decreased (Fig. 3). In municipal sediments, there was no yeast, in sediments from the food industry - one type of yeast, while in the coke sludge in the presence of flocculant - three types of yeast. In the

municipal and from food industry sediments dominated fungi: *Aspergillus niger* and *Cladosporium sp.*, whereas in coke wastewater - *Aspergillus amstelodami, Paecilomyces javanicus* and *Acremonium sp.*

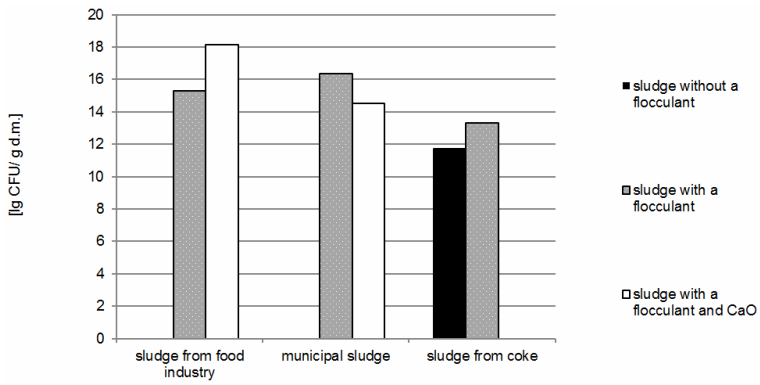

Fig. 1. The total number of bacteria [lg CFU/g d.m.] in the studied sewage sludge

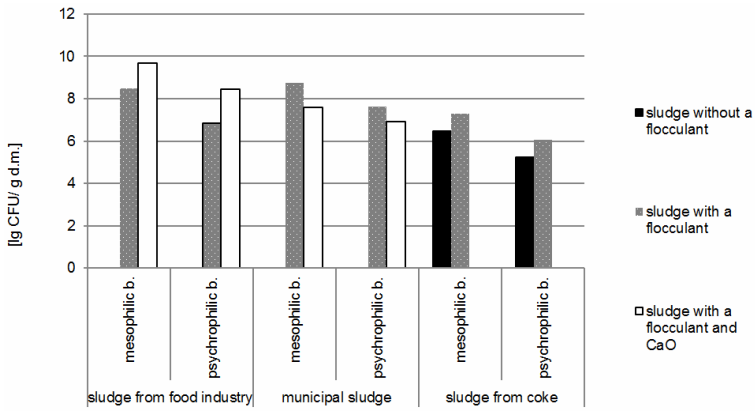

Fig. 2. The total number of mesophilic and psychrophilic bacteria [lg CFU/g d.m.] in the studied sewage sludge

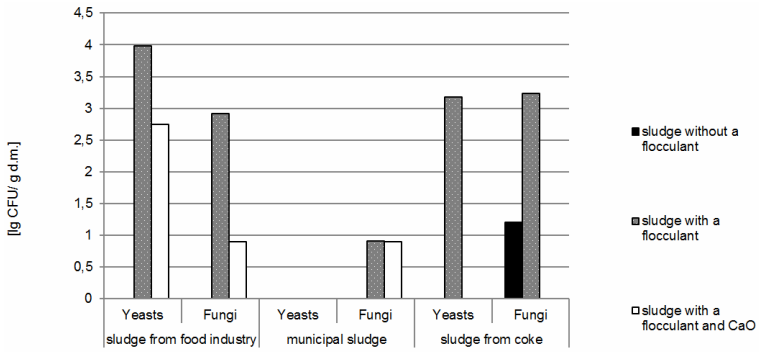

Fig. 3. The total number of yeast and filamentous fungi [lg CFU/g d.m.] in the studied sewage sludge

Among the isolated microorganisms, depending on the origin of the sludge was found the dominance of the various morphological groups of bacteria (Fig. 4). The highest number in the studied sediments were gramnegative rods, with the exception of coke sludge without flocculant (dominated grampositive rod-shaped cocci) (Fig. 4).

In the coke sludge with the presence of a flocculant an increase in the number of potentially pathogenic bacteria was observed compared to the raw one. In samples of sewage sludge with a flocculant although the liming an increase in the number of potentially pathogenic bacteria was also observed (Fig. 5).

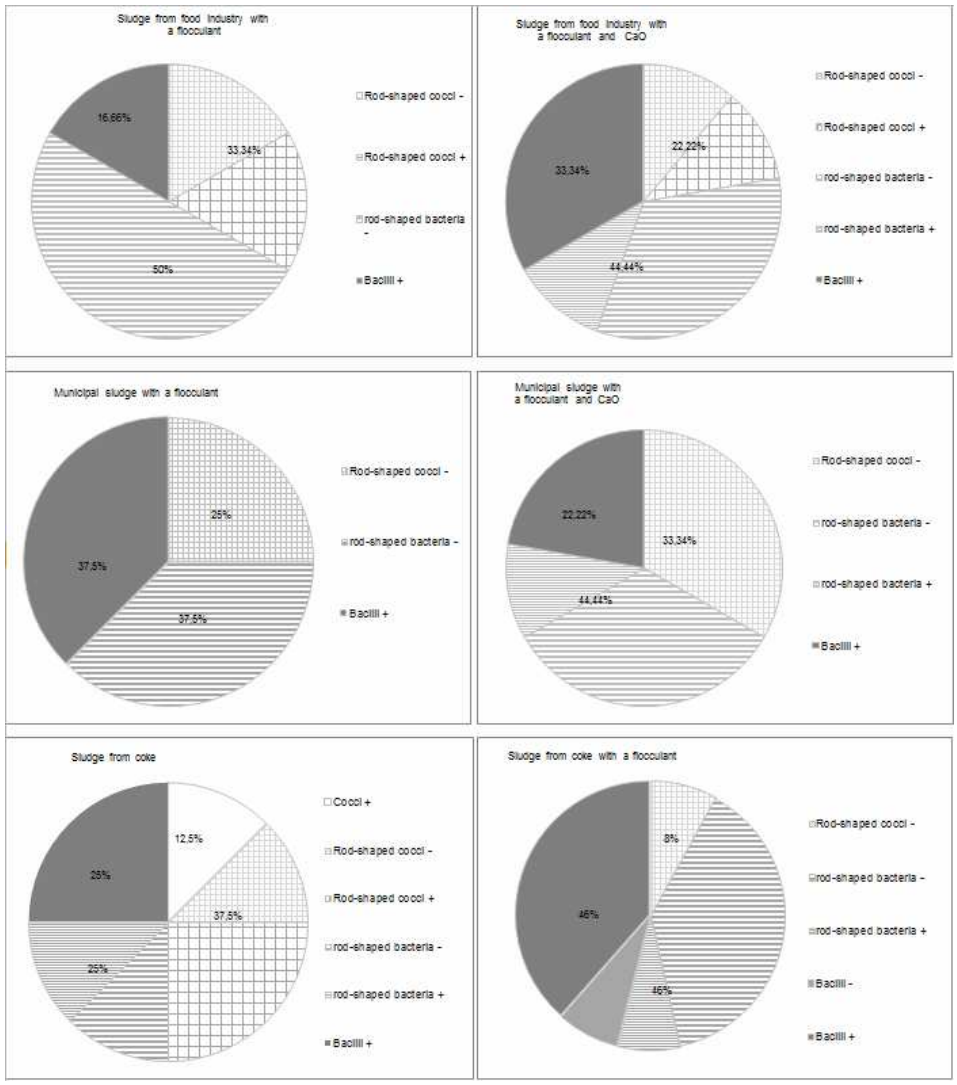

Fig. 4. The composition of the bacterial population [%] in the studied sewage sludge

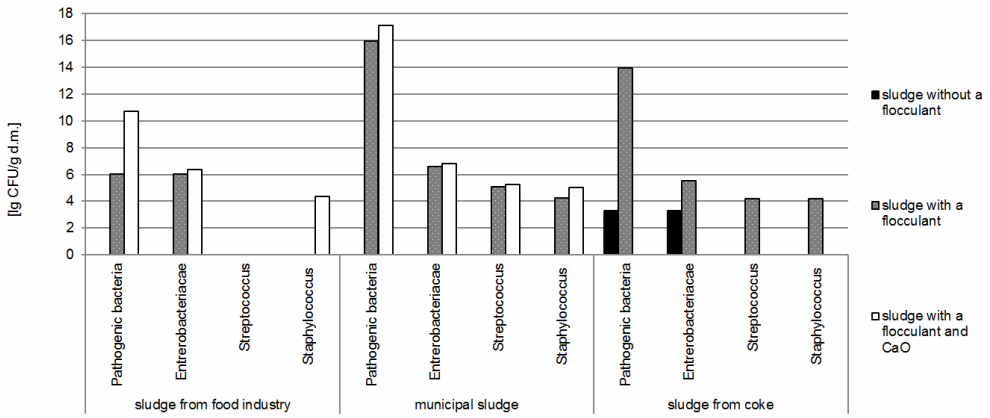

Fig. 5. The total number of potentially pathogenic bacteria [lg CFU/g d.m.] in the studied sewage sludge

Conclusions

Techniques for sewage sludge treatment towards their hygienisation and reduce the amount of sediment supporting by flocculants and/or liming affect the quantitative and qualitative composition of microorganisms. In the dewatered sludge with a flocculant, with the exception of sediments from the food industry, there was the highest total number of microorganisms, both meso- and psychrophilic bacteria, yeast, filamentous fungi and potentially pathogenic bacteria. However, in the sediments from food industry an increase in the number of bacteria was observed after simultaneous application of liming. Among the isolated microorganisms, depending on the origin of the sludge was found the dominance of gramnegative rods, with the exception of coke sludge without a flocculant (dominated grampositive rod-shaped cocci).

In the municipal and from food industry sewage dominated fungi: *Aspergillus niger* and *Cladosporium sp*. and in coke sediments - *Aspergillus amstelodami, Paecilomyces javanicus* and *Acremonium sp.* In these latter the greatest variety of yeasts was also observed.

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RÓŻNORODNOŚĆ MIKROBIOLOGICZNA OSADÓW ŚCIEKOWYCH

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Abstrakt: Osady ściekowe mogą być skupiskiem różnych grup mikroorganizmów, w tym potencjalnie chorobotwórczych, dlatego ich przeróbka powinna być prowadzona w sposób chroniący pracowników i środowisko przed zagrożeniem sanitarnym. Celem badań była mikrobiologiczna ocena ilościowa i jakościowa osadów ściekowych komunalnych i pochodzących z przemysłu spożywczego oraz koksowni po przeróbce z dodatkiem flokulantu i/lub wapna palonego. Materiał badawczy stanowiły próbki pobrane bezpośrednio z oczyszczalni ścieków. Ocenę ilościową i jakościową bakterii mezofilnych i psychrofilnych, drożdży, grzybów strzępkowych oraz mikroorganizmów potencjalnie chorobotwórczych przeprowadzono metodą hodowlaną. Techniki przeróbki osadów ściekowych w kierunku ich higienizacji oraz zmniejszenia ilości osadów poprzez wspomaganie flokulantami i/lub wapnowanie miały wpływ na skład ilościowy i jakościowy mikroorganizmów. W badanych osadach ściekowych odwadnianych flokulantem, z wyjątkiem osadów z przemysłu spożywczego, występowała najwyższa ogólna liczba mikroorganizmów, zarówno bakterii mezo-, jak i psychrofilnych, drożdży i grzybów strzępkowych oraz bakterii potencjalnie chorobotwórczych. Natomiast w osadach przemysłu spożywczego wzrost liczebności bakterii obserwowano dopiero po równoczesnym zastosowaniu wapnowania. Wśród wyizolowanych mikroorganizmów, w zależności od pochodzenia osadu ściekowego, stwierdzono dominację pałeczek gram-ujemnych z wyjątkiem osadów koksowniczych bez flokulantu. W osadach komunalnych i pochodzenia spożywczego dominowały grzyby: *Aspergillus niger* oraz *Cladosporium sp*., a w osadach koksowniczych *Aspergillus amstelodami*, *Paecilomyces javanicus* i *Acremonium* sp. W tych ostatnich obserwowano również największą różnorodność drożdży.

Słowa kluczowe: osad ściekowy, ocena mikrobiologiczna, różnorodność mikrobiologiczna