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MICROBIOLOGICAL AND EPIZOOTOLOGICAL CHARACTERIZATION OF MATERIALS FROM WASTEWATER TREATMENT PLANT

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Abstract: Studies were carried out for the presence of microorganisms of major groups, containing pathogenic agents with epizootological importance, in order characteristic of their dynamics at different levels in the treatment plant and assessing the environmental safety of the final materials. For this purpose were traced the changes in the quantities of microorganisms contained in the materials from successive levels of processing. It was found that the purified water inlet and outlet does not contain *Salmonella enterica*. Presence of *Escherichia coli* and *Clostridium perfringens* beyond the requirements of the regulation *(Decree N339, 2004)* was not established, as well as *Enterococcus* spp. The tested sludge contained microorganisms from these groups and could not be deposited in the soil without prior aerobic or anaerobic processing. The draining in the nature of treated water at the outlet of plant does not hide environmental risk. In addition to monitoring of the sanitary indicative bacteria, the following of the main groups of Gram-negative and Gram-positive microorganisms in the water and sewage sludge allows more reliable assessment of their decontamination. Furthermore, the new approach for reporting the results in a unit of dry matter of the studied materials, proposed here, allows their more exact comparison.

Key words: waste water treatment plant, microflora, epizootological assessment

Introduction

The wastewater treatment is a contemporary method for their manufacture. Its application is important for protection of the environment (soil and water) from contamination by toxic substances, heavy metals and causes of infections that may be contained in such water. This is essential for the support healthy and stable ecosystems. The main function of the plant is to accelerate the natural process of self-purification of water. Due to the growth of population, industry and livestock, such treatment is required for accelerating the purification of the large amounts of wastewater *(EPA, 2004)*.In order to prevent the spread of pathogens is important their reduction or inactivation in the final products, postponed in nature. The most common possibilities for recast include anaerobic degradation, aerobic digestion and composting. Incineration also is used, but to a much lesser extent *(Langenkamp and Part, 2001; EPA, 2004)*.

The aim of this work was to perform a microbiological assessment of the wastewater and sludge of individual purification steps in urban wastewater treatment plant in terms of their epizootiological safety.

Materials and Methods

Samples from different stages of processing in urban wastewater treatment plant near to Sofia, obtained during the first half of April, were examined. The materials were indicated as follow: incoming water after precipitating (IW); primary sludge (PS); secondary sludge (SS); mixed sludge (MS), dewatered by belt filter presses; stayed mixed sludge (SMS); input into the digester (methane tank) (ID); purified water (PW) in the output of the plant.

Microbiological studies were conducted in accordance with the Ordinance on the terms and conditions for use of sludge from wastewater treatment through its use in agriculture *(Decree N339, 2004)*. The titers of *Escherichia coli* and *Clostridium perfringens* also were established. Additionally were tracked the quantities of bacteria from the genera *Staphylococcus, Enterococcus, Pseudomonas*, Gram-negative aerobic bacteria, fungi, and the total number of microorganisms.

Selective media (Scharlau - Antisel, Bulgaria) were used for isolation and quantitative determination of the microorganisms from the studied groups and types. The following media have been chosen: Mueller Hinton agar for counting the total number of microorganisms in the examined material, Eosin Methylene Blue agar for *E. coli* and Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus

Staphylococcus, Sabouraud agar for fungi, selective medium for enterococci, Salmonella-Shigella agar for *Salmonella enterica* and selective agar for *Clostridium perfringens* (Merck -Bio Lab, Bulgaria).

Quantitative determination of the microorganisms was carried out using the conventional method in serial tenfold growing dilutions of the investigated material in a sterile saline solution. Cultures of them were made on the selected media, three for each medium and dilution. After incubation at 37° C for 24-72 h under aerobic and anaerobic conditions (with Anaerocult ® A mini - Merck-Bio Lab, Bulgaria) the mean arithmetical number of developed colonies was determined and the quantities of colonies forming units (CFU-colony forming units) in 1 ml or 1 g of starting material were calculated. The corresponding quantities of microorganisms in 1 g dry matter in each of the studied materials also were calculated. For this purpose, the number of detected CFU was multiplied by the quotient obtained according to the percentage of dry matter in the material.

Results and Discussion

The results of the studies of the quantitative changes in the total number of microorganisms, as well as of Gram-negative bacteria in the materials from the various stages of treatment plant, are presented in Figure 1.

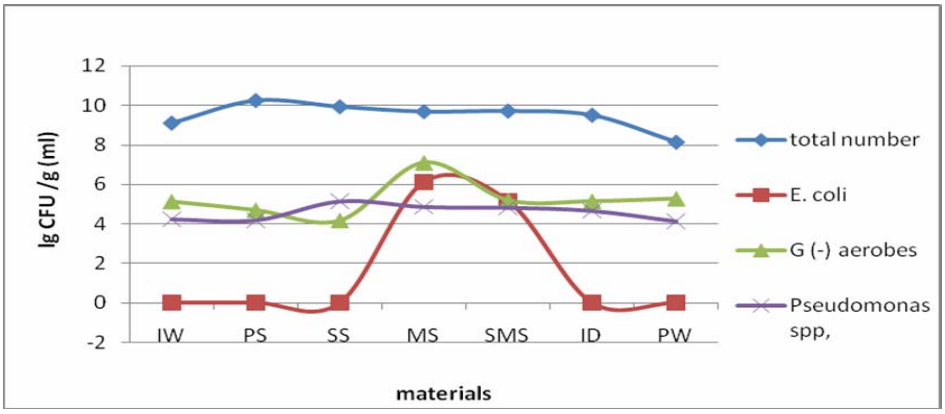

Figure 1. Quantities of microorganisms (total and Gram-negative bacteria) in the examined materials of the various steps of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

The graphs of Figure 2 show the quantitative change of the microorganisms from the same groups, represented in a unit of dry matter in the materials.

The content of dry matter in the tested samples in % was as follows: IW - 0,00049; PS – 4,9; SS – 0,9; MS – 41,81; SMS – 56,46; ID – 2,96; PW – 0,00048.

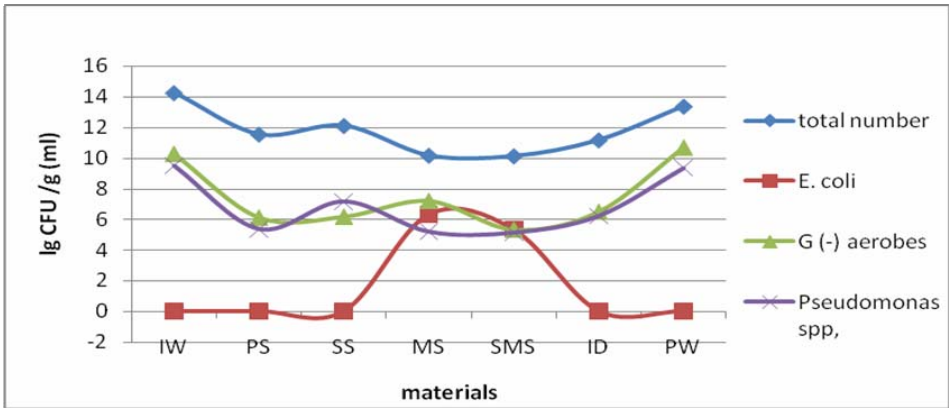

Figure 2. Quantities of microorganisms (total and Gram-negative bacteria), presented in a unit of dry matter in the materials of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

Figures 3and 4 show the data from the quantitative studies of Gram-positive organisms in the samples from the steps of the treatment plant, presented in a unit fresh and dry material respectively.

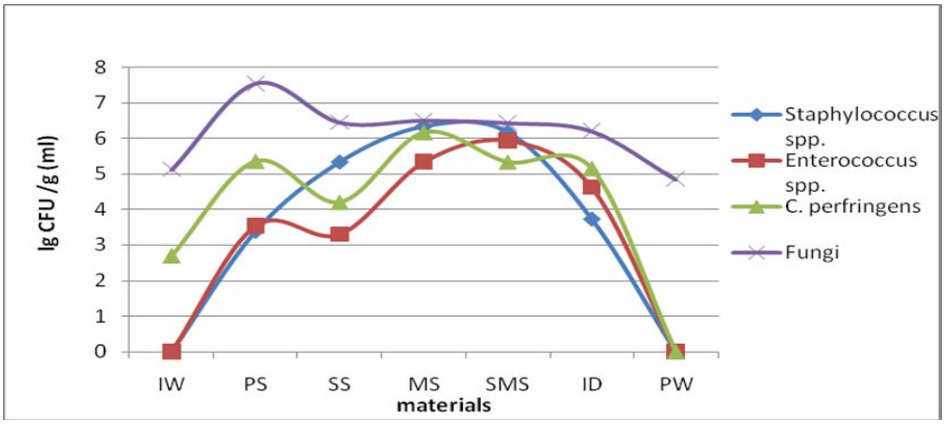

Figure 3. Quantities of Gram-positive microorganisms in the examined materials from various stages of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

From the graphs of the figures is seen that the number of saprophytic microorganisms was higher in the materials with high water content. Sanitary indicative bacteria showed relatively high levels in the examined sludge.

Salmonella enterica was not isolated from the studied materials at all levels of recast. In the examined water samples (input and output) *E. coli* and *Clostridium perfringens* were not established in quantities exceeding the set out in the requirements of Bulgarian current Ordinance on the manner of utilization of sludge from wastewater treatment through its use in agriculture *(Decree N339, 2004)*. From these samples was not isolated and *Enterococcus* spp. Draining in the nature of the treated water at the output of plant does not hide environmental risk. As shown in the data of other authors *(Langenkamp and Part, 2001; Talahassee, 2010)*, subject to the requirements of modern techniques for purifying of wastewater, the risk of waterborne diseases greatly reduced.

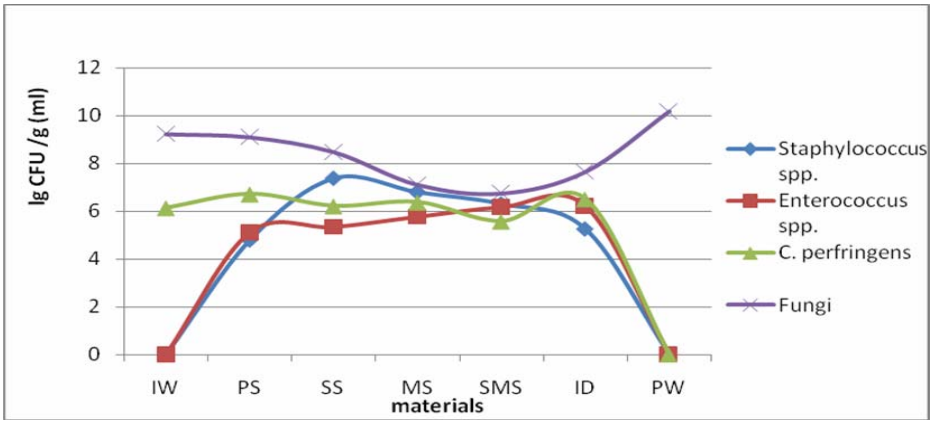

Figure 4. Quantities of Gram-positive organisms, presented in a unit of dry matter of the examined materials. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

The studied sludge, however, contained relatively high amounts of microorganisms from these groups (*E. coli, Clostridium perfringens, Enterococcus* spp.) and should not be deposited in the soil without prior aerobic or anaerobic processing. As can be seen from the data presented, the quantities of microorganisms from all monitored groups increased in primary and secondary sludge, as their content was highest in the mixed sludge. The established high microbial content in the activated sludge is natural, since it is related with the role of these microorganisms in the aerobic decomposition of the organic compounds, which is carried out at this stage of purification. The incoming into the digester material contained microorganisms from the groups with pathogenic representatives in amounts less with about 1 lg compared to these in previous level of processing. During anaerobic digestion their quantity is subjected to further reduction on account of the development of specific anaerobic microbial types. The dynamics of the fungi was closely related to that of the total number of microorganisms. Similar were the graphs of motion of Gram-negative aerobes, staphylococci, enterococci and *Clostridium perfringens*.

In our view, the representation of the results in a unit of dry matter of the studied materials, proposed here by us, allows their more exact comparison.

These results demonstrate that the practice of landfilling the final dewatered sludge is necessary and proper from an environmental perspective as its microbial content is high, including sanitary illustrative families and kinds of bacteria. The incoming in the bioreactor material was with a lower content of microorganisms from all traced groups, compared to the previous levels. Our former studies *(Popova et al., 2009)*, associated with the decontamination of

organic material from livestock under continuous mesophilic anaerobic degradation process, showed that the resulting compost was safe from the epidemiological point of view and could be used to fertilize the soil. Fastest and most reliable results in this direction gives the thermophilic anaerobic degradation mode, as well as mesophilic regime, combined with pasteurization of input and output material *(Popova et al., 2012)*.

Conclusion

Tracking of the main groups of Gram-negative and Gram-positive microorganisms in the wastewater and sewage sludge enables more reliable assessment of their decontamination.

The proposed new approach for reporting of the results in a unit of dry matter of the tested materials allows a more accurate comparison of the content of microorganisms therein.

At abidance of technology of purification, the treated water at the exit of the plant does not contain *E. coli*, enterococci and *Clostridium perfringens* and its discharge into natural waters is safe.

The studied sludge contained microorganisms from these species and can't be deposited in the soil without prior aerobic or anaerobic processing.

Acknowledgment

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Mikrobiološka i epiziootološka karakterizacija materijala iz postrojenja za prečišćavanje otpadnih voda

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Rezime

Istraživanja su sprovedena na prisustvo mikroorganizama velikih grupa, koje sadrži patogene agente epizootološkog značaja, u cilju utvrđivanja

karakteristika njihove dinamike na različitim nivoima u postrojenju i procene ekološke bezbednosti završnih materijala. U tu svrhu su se pratile promene u količini mikroorganizama koje se nalaze u materijalima sa sukcesivnim nivoima obrade.

Utvrđeno je da prečišćeni ulaz i odvod vode ne sadrži *Salmonela enterica*. Prisustvo *Escherichia coli* i *Clostridium perfringens* izvan zahteva propisa (Uredba N339, 2004) nije osnovana, kao i *Enterococcus* spp. Testiran mulj sadrži mikroorganizme iz ovih grupa i ne može da se deponuje u zemljištu bez prethodnog aerobnog ili anaerobnog tretmana. Odvođenje u prirodu tretirane vode na izlazu iz postrojenja ne krije rizik po životnu sredinu.

Pored praćenja sanitarno indikativnih bakterija, praćenje glavne grupe gram-negativnih i gram-pozitivnih mikroorganizama u vodi i kanalizacionom mulju omogućava pouzdaniju procenu njihove dekontaminacije. Osim toga, novi pristup za izveštavanje rezultata po jedinici suve materije ispitivanih materijala, predloženo u radu, omogućava njihovo preciznije poređenje.

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