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THE NUMBER AND SIZE OF SAMPLES REQUIRED TO MEASURE THE SAPROBE POPULATION AT VARIOUS POLLUTANT CONCENTRATIONS IN SEWAGE

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Abstract: The present work focuses on problems connected with the location and sampling method for pecton (biofilm) in sewage treatment plants. We also discuss the amount and quantity of pecton necessary to compose a representative sample. Comparisons of other selected contamination indicators in place of pecton sampling, are also presented. Research carried out at the WWTP "Hajdow" demonstrated that everything (starting from grid chambers), coming into contact with sewage surfaces is covered with biofilm This biological formation does not cause any significant changes in sewage quality due to its relatively small surface compared to the sewage flux. As presented in the following analysis, pecton can be used for bioindication of sewage quality. This is possible because the organisms forming these communities use substances contained in flowing sewage as nutritional substrates. In such cases the wastewater purification level in biological sewage treatment plants can, in a way similar to rivers, be determined based on bioindication methods using existing similarities between the prevailing processes and organisms.

Keywords: wastewater quality, bioindication, activated sewage sludge, biofilm

INTRODUCTION

The use of water for both industrial processes and domestic purposes, and the need to dispose of rain water from urbanized areas are dominant sources of wastewater. Sanitary and industrial sewage, incidental waters, storm and infiltration waters form urban wastewater, flowing to a treatment plant through a sewer system and then to the recipient after treatment in the wastewater plant (Hvitved-Jacobsen, 2002, Łagód et al., 2004).

An urban sewer system generally consists of two subsystems: a sewer transport subsystem and treatment subsystems. From a biotechnological point of view, they should be treated as one (Hvitved-Jacobsen, 2002). Saprobiont communities populate both subsystems and are mainly formed by bacteria, fungi, protozoa, rotifers. Sometimes they may also contain representatives of Metazoa such as: nematodes, oligochaetes, tardigrades, gastrotriches, arachnids, copepods, cladocers and turbellarians. Different saprobionts occur in sewer system incidentally or live there permanently, forming a biofilm on the bottom and walls of sewer pipes. Incidental and biofilm-originated organisms form live suspensions in raw wastewater (Łagód et al., 2004, 2006, 2007).

The colonisation of sewer systems by saprobe species, as any other living environment, is widely accepted and described. Therefore, partial treatment of wastewater during its flow through sewer system is important as shown by a decrease in pollutant load due to microbial activity (Hvitved-Jacobsen, 2002, Łagód et al., 2004, 2006, 2007).

In the wastewater treatment subsystem saprobionts form a pecton – a biofilm on walls and beds and also present in the activated sludge of aeration tanks (Curds and Cockburn, 1970a, 1970b, Klimowicz, 1972, 1983, Montusiewicz et al., 2007). Pecton formation does not cause any significant changes in the sewage quality due its relatively small surface comparing to the flux, but can, as initial analysis has indicated, function as a useful parameter in bioindication research. This arises from the fact that the organisms of this formation (communities) use the substances contained in flowing sewage as nutritional substrates in the individual steps of the plant.

Species richness and dominance structure in saprobiont communities can be used for the bioindication of the wastewater quality in the sewer system and for the bioindication of wastewater treatment efficiency (Curds and Cockburn, 1970a, 1970b, Łagód et al., 2004, 2006, 2007, Montusiewicz et al., 2007). Similarly, saprobe communities are useful for the measurement of pollution levels of water bodies (Gorzel and Kornijów, 2004). Structural indices calculated on species abundance are useful tools for biomonitoring of wastewater quality and wastewater treatment efficiency. The abundance of morphological-functional groups representing taxonomic levels higher than species level can also be used for this purpose (Łagód et al., 2004, 2006, 2007, Montusiewicz et al., 2007). It is possible to build and calibrate a model of sewage biodegradation in sewer systems on the basis of the proliferation of microbial population and development description. Microbial activity in sewers can be treated as a pre-treatment process before the wastewater treatment plant facilities. Saprobionts can be useful as indicators of the state of activated sludge and wastewater treatment efficiency.

Hence, this work attempts to find a correlation between the population of saprobes and level of pollution in sewage, based on a small number of samples and their microscopic evaluation.

MATERIALS AND METHODS

Measurement data used for the purpose of this work is taken from the author's previous research on microfauna in activated sludge system carried out at the municipal wastewater treatment plant "Hajdów". The number of microbe individuals in communities of activated sludge and pecton micro-fauna were counted based on the following ten morphological-functional groups, representing easily identified taxonomic levels higher than the species level:

1. attached ciliates (Peritricha)
2. crawling ciliates (Hypotricha)
3. swimming ciliates (Holotricha)
4. flagellates
5. amoebas
6. nematodes
7. rotifers
8. algae
9. fungi
10. diatoms

Safety precautions were required during the collection of representative samples. The surface area of pecton (biofilm) samples can be determined using the Greig-Smith grid where the following areas in cm^2 are the n-th powers of 2 (2^{-1}, 2^0, 2^1, 2^2 ...2^n). The preferred area is the one where no new taxa appears. During the present study this exact method was used. We concluded that in Lublin City, under conditions enabling examination of the pecton formation, the sampling surface should be at least 9 cm^2 (because $2^3 = 8cm^2$).

Typically, during our experiments pecton was sampled using an iron blade from the same location just below the sewage surface at the same time (9 a.m.). The pecton sample was placed in a sterile plastic container filled with 10 cm^3 of sewage to 1/3 volume. 2/3 of the volume was left for air necessary to provide oxygen for the organisms. Prepared containers were immediately taken to the laboratory where samples were analyzed and recorded as photos and videos.

The number of individuals belonging to the morphological-functional groups in particular points of sampling were determined using an optical light microscope with bright field of view, and a magnification of 400. Particular groups of individuals were counted along the test line. Only those individuals which crossed the line or touched it from the right side were counted. The microorganisms from each sample were counted in ten view fields.

The data presented in table 2 is derived from standard measurements conducted by the WWTP "Hajdów" technical staff in their normal quality control procedures.

RESULTS

The results show that representatives of all morphological-functional groups, are present in WWTP facilities, especially in activated sludge reactors if wastewater is treated to a significant degree and effluent BOD_5 values do not exceed 20 gO_2 m^{-3}. All the acquired data is presented in tables 1 and 2 as well as in the graphical illustration – figure 1.

Table 1. Number of organisms belonging to the identified morphological-functional groups in samples taken from the facilities of WWTP "Hajdów", on 09/08/2006

Facilities	**Morphological-functional groups**									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Before screen			3	36	1		2		4	
After screen			2	28	1			1		
Before grit chamber	1		3	15	1					
After grit chamber			7	8	4	1	1			
Primary settler	1		77	11	1			1		
Inflow to anaerobic chamber	8	1	21	1	8		1	3		21
Outflow from anaerobic chamber	6	1	20		4		6	1		9
Inflow to aerobic chamber	5	2	25	2		1	2	30		68
Outflow from aerobic chamber	7	2	21	1	2	1	4	14		38
Final settler			150	2	5	1	2	16		355
Outflow from WWTP	1		8	3	2			3		70

Variations in the pollution level in the different facility of the wastewater treatment plant change the number of morphological-functional groups as well as the number of microbes belonging to these groups. The examination of biofilm settled on the walls of equipment located in the different facilities shows that not all of the studied groups were present in all samples. The largest numbers of m-f groups were found in aerobic chambers, a lesser number of m-f groups after the screen, before the grid chamber and after the primary settler. Purified wastewater in the final settler and outflow from the WWTP had a number of m-f groups in pecton at the medium level compared to other facilities. In general, the number of morphological-functional groups was similar in all WWTP facilities but a particular community has a different taxonomic composition. The quality of sewage in particular facilities influenced the number of microorganisms as well as their morphological-functional group. The smallest number of saprobes was found in samples taken from grid chambers; the largest came from the final clarifier.

Table 2. Pollution index at the facilities of WWTP "Hajdów", sampled on 09/08/2006

Facilities	Pollution index	Value (mg/dm^3)
Before screen	BOD_5	520
	COD	1123
	N	83,43
	P	14,3
Primary settler	BOD_5	382
	COD	680
	N	70,63
	P	14,5
Outflow from WWTP	BOD_5	4,9
	COD	64
	N	22,42
	P	0,26

While the experiment was being conducted, the treatment at WWTP removed 99% of BOD_5, 94.3% of COD, 73.1% of N and 98.2% of P from sewage. Because of this, it can be said that differences in pollution level at the sampling sites were significant and had a strong influence on changes in the number of saprobiont microorganisms and morphological-functional groups. Surprisingly, aerobic photoautotrophic microorganisms were present in facilities which were believed to be anaerobic or anoxic. This could be explained by unintentional aeration during turbulent flow of wastewater or presence of O_2 in recirculated activated sewage sludge.

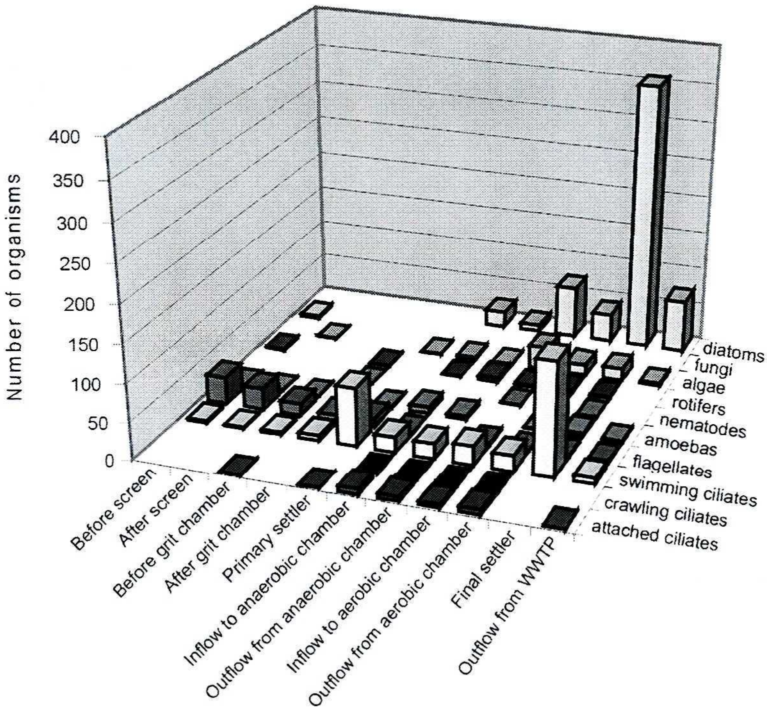

Figure 1. Number of organisms present in different facilities and their pollution level

CONCLUSIONS

Based on the results presented above, we conclude the following:

- the number and type of saprobic microorganisms strongly depends on the actual parameters of the wastewater being treated,
- the structure of morphological – functional groups varies in different facilities of the wastewater treatment plant; the resulting communities have structures which depend on the actual and local conditions,
- our results can be used for biomonitoring and bioindication of processes operating in wastewater treatment plants,
- for biomonitoring and bioindication purposes in WWTP, the saprobe microfauna count in 10 view areas of a representative sample of pecton (ca. 9 cm^2) is sufficient.

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