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## ANALYSIS OF THE PERIPHYTON COMMUNITIES AT THE MUNICIPAL WASTEWATER TREATMENT PLANT – CASE STUDY

### ANALIZA ZBIOROWISK PERYFITONU MIEJSKIEJ OCZYSZCZALNI ŚCIEKÓW

**Abstract:** There was studied the composition of periphyton and its quantitative development during different stages of wastewater treatment in the flow type purification plant with the biological part working as a modified bardenpho system (Hajdow, Lublin). The periphyton samples were taken from the walls of the object at all main stages of water purification. In the composition of periphyton there were identified the following groups of organisms: algae, fungi, flagellates, testate amoeba, ciliates, rotifers and nematodes. Towards the end of purification process in periphyton of successive chambers, the part of metazoa and protozoa has increased while the abundance of flagellates has decreased. In all of the studied sampling points, the basis of periphyton community was formed by protozoa and metazoa: their part ranged from 75 to 95 %. The periphyton structure regardless of forming conditions shows similar tendencies, determined by the general conditions of a system, in which from initial stages of the purification till its end the amount of organic matters in environment decreases.

**Keywords:** periphyton, communities, municipal wastewater treatment plant (WWTP), protozoa, metazoa

## Introduction

The periphyton is observed on all surfaces dipped into the water. It creates disturbances for technical facilities (*eg* water pipes, industrial water coolers etc.), pollutes water with biological components of fouling, but also could provide biological

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treatment of wastewater and indicates the quality of purification plant work. The biofilm was studied mostly in purification systems, where it was the main instrument and process factor of sewage treatment.

Many publications are devoted to studies of physical characteristics and physiological activity of biofilms and bacterial component of them [1–7]. At the same time relatively small amount of works provides information concerning other organisms that together with bacteria are regular components of biofilm and have certain influence on bacteria life activity and on purification process performed by the biofilm community. For example the work of Madoni [8] contains data about biofilms protozoa from rotating biological contactors (RBC) and percolating filters. The results of studies of protozoa in percolating filters have been given by some authors [9–13].

The lists of species found in RBC systems were mentioned by such authors as Madoni, Madoni and Ghetti [14, 15]. The results of research of ciliate populations and their distribution in RBC systems can be found in the following works: [15–22]. It was elicited that heterotrophic flagellates and free-swimming ciliates such as *Cyclidium glaucoma* and *Colpidium colpoda* are dominant at the early stages of wastewater purification when load of organic is higher, while shell amoebae and attached ciliates such as *Epistylis plicatilis* and *Zoothamnium procerius* dominate at the finale stage that is characterized with low BOD<sub>5</sub> level. There are given data concerning biomass of ciliates in biofilms of RBC [12]. Moreover, there are also evidence that besides of bacteria, the most numerous and important organisms in biofilm of RBC are ciliates [23].

Metazoa are also an important part of biofilm community, taking in consideration that they often reach big quantities and are an influential links of trophic chains [24–28].

Today, there is not enough information to specify which role does periphyton plays in treatment facilities as bioreactors with activated sludge working in flow-mode or as an SBR. About periphyton – biofilm growing unconsciously at the wall of purification facilities submerged in wastewater, it is known much less than about activated sludge that is functioning in bioreactors. Within the periphyton structure in bioreactors there are such groups of organisms to find as bacteria, fungi, algae, rhizopods, flagellates, ciliates, rotifera, nematodes [29].

At the same time there are many questions that are still awaiting answers as for example – how far is the process of biofilm and activated sludge organisms exchange (components of biocoenosis) influences the forming of activated sludge structure. How does biofilm react to negative (destructive) factors that periodically influence the water treatment system (eg adverse temperature, adverse pH, disturbances in aeration process, chemical toxicants etc.) and if organisms of periphyton play any important role in restoration of activated sludge biocoenosis.

The work deals with the study of main periphyton groups structure and their quantitative development at different stages of wastewater purification. The analysis of structure dynamics and diversity of main periphyton groups according to purification stage can provide additional information concerning stability of mentioned processes and likely destabilization of activated sludge structure and properties.

## Materials and methods

The research material comprised periphyton sampled from the main line of technological system of the “Hajdow” mechanical-biological wastewater treatment plant in Lublin (south-eastern Poland). The studies of periphyton during main stages of sewage purification were conducted in spring when activated sludge was not in optimal conditions. In the aeration chamber the initial stages of activated sludge bulking and foaming were observed.

The samples of periphyton from the walls of main chambers were analyzed to calculate the quantities of main hydrobiont groups, such as flagellates, testate amoebae, rotifers, nematodes, ciliates, algae and fungi. Ciliates were analyzed according to their ecological groups – attached ciliates, crawling ciliates, swimming ciliates.

The samples were taken with a scraper ca. 15 cm below the sewage surface in each device. The periphyton was sampled from an area of approximately 100 cm<sup>2</sup>.

The sampling points were: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber.

The samples were placed in containers, which were filled up to 1/3 of the volume with clarified wastewater sampled from the periphyton collection site. The samples were transferred to the laboratory for microscopic analyses and stored in a refrigerator at a temperature of 7 °C before use.

The samples were analysed under a transmitted-light with bright-field using optical microscope Opta-Tech with a magnification of 100, 250, and 400x. The method of data preparation for counting the organisms involved taking digital images of 21 non-overlapping crosswise fields of view along the major axes of symmetry of the cover slip for each of the magnifications applied. Digital archiving of each field of view allowed possible re-verification of the counting results if necessary.

Organisms analysed in the fields of view were identified and classified into the following morphological previously mentioned groups: fungi, flagellates, algae, testate amoebae, ciliates, rotifers, and nematodes. The number of the organisms within all fields of view from material sampled in one point was regarded as one replicate. Presented in article data are averaged from two months of experiment – sampling period one week.

## Results and discussion

Using data concerning the number of different groups of organisms in periphyton there was analyzed the similarity of conditions at the studied sampling points applying PCA method. The sampling points are grouped as shown on Fig. 1.

Consecutive location of sampling points № 1, 2, 4, 5, 6 on the picture reflects the gradual changes in environment from the start of purification process (screen chamber) till its ending (aeration chamber). These two points are united with conditions of flowage. Either raw sewage or sewage with activated sludge flows through these chambers uninterruptedly.

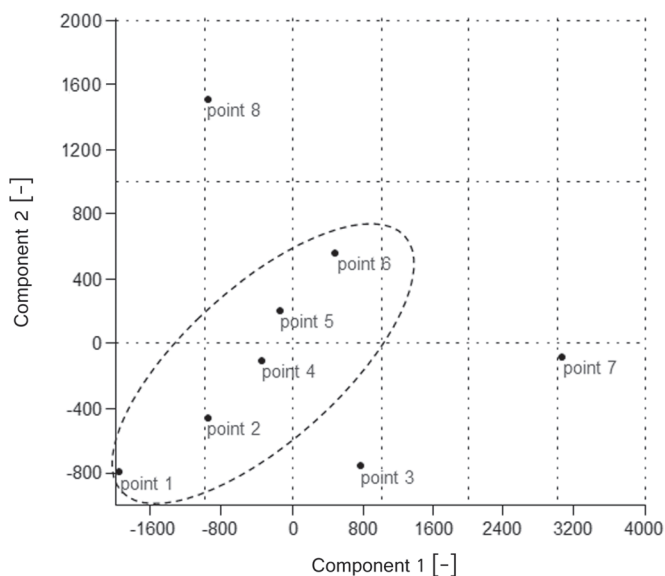


Fig. 1. PCA ordination diagram of the sampling points of the treatment plants "Hajdow". The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

The specific of condition differences in primary clarifier chamber (point 3), secondary clarifier (point 7) and recirculation chamber (point 8) caused the forming of other periphyton group inside them. Points' 3 and 7 obvious moving main group is probably caused by the fact that conditions in primary clarifier develop during the sedimentation of raw sewage that came through preliminary mechanical treatment. Meanwhile, in the secondary clarifier periphyton undergoes the influence of sewage after treatment. The conditions in recirculation chamber form, under the influence of turbulent, stream of concentrated activated sludge. In this way, the diagram confirms significant influence of chambers conditions on periphyton.

The analysis of periphyton communities in studied points enabled to reveal the whole number of differences in their structural organization. The total abundance of organisms in periphyton showed that points 3, 7 and 8 drop out of the general line (Fig. 2). The total abundance of organisms in periphyton showed that points 3, 7 and 8 drop out of the general line (Fig. 2). In fact attention can be focused on the increase in the total abundance in primary and secondary clarifiers (point 3 and point 7) and on noticeable reduction of abundance in recirculation chamber. Probably these differences are connected to conditions in the settling tanks (clarifiers) where the water flow is minimal.

Concerning decrease in the organisms' abundance in the recirculation chamber one can assume that intensive splashing of concentrated activated sludge on the walls of the chamber may mechanically and adversely affect the periphyton. An increase in the total abundance at certain points as well as its decrease are obviously determined not by an

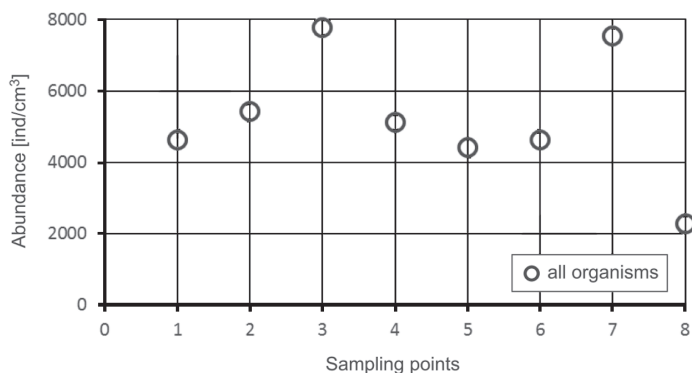


Fig. 2. Total abundance of the organisms in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

even increase of each system component but by the reaction of certain groups for which the conditions become more optimal. For example nematodes do not show distinct dependence of abundance on the conditions, although there was some increase of them observed in the secondary clarifier.

At the same time rotifers have clearly increased their populations' density exactly in periphyton conditions in clarifiers (Fig. 3.).

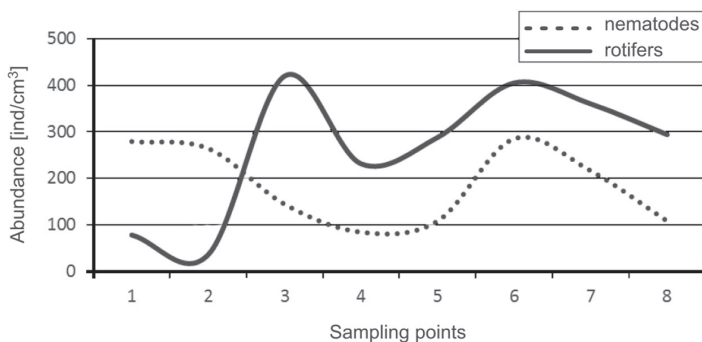


Fig. 3. Abundance of nematodes and rotifers in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

Quite an independent tendency showed testate amoebae and flagellates. The average values of testate amoebae abundance were decreasing insignificantly from the beginning till the end of purification process. Meanwhile by flagellates this tendency was quite explicit (Fig. 4). Since the beginning of the process their abundance has decreased by 30 times. It is obvious that small flagellates propagate fast in the water rich in organic matters and with minimum oxygen saturation. All this matches with their high abundance during those stages of purification, where activated sludge was not involved.

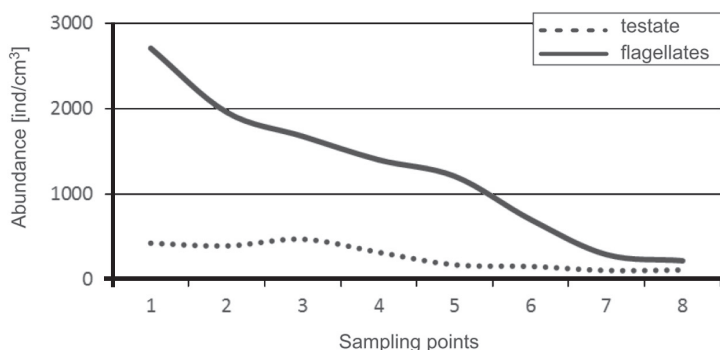


Fig. 4. Abundance of testate amoeba and flagellates in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

Ciliates reached the maximum abundance in the clarifiers Fig. 5.

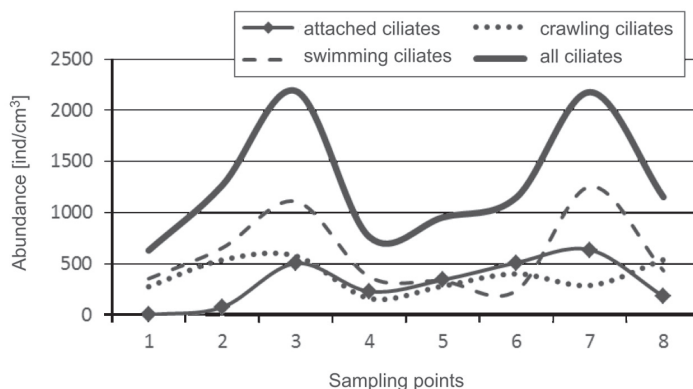


Fig. 5. Abundance of ciliates in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

At the same time the general tendency was caused mainly by specimen of free-swimming ciliates. The specimen of attached ciliates and crawling ciliates did not show pronounced increase in abundance in conditions of clarifiers. At the same time attached ciliates specimen were only minimally present at the initial stages of purification and significantly reduced their abundance in the conditions of recirculation chamber. Such a relation between various forms of ciliated protozoa can be partially explained by the fact that attached ciliates present in the periphyton must propagate within it too, while free-swimming ciliates as well as crawling ciliates can penetrate the periphyton from the outside and rapidly increase their abundance. This can be considered from the perspective of ability of ciliates from different ecologic groups to act as bioindicators. Treatment facilities such as aerotanks usually operate on the basis

of heterotrophic communities and their autotrophic component is minimized. Therefore, the question of establishing a potential part of autotrophes in periphyton of treatment plants is of a big interest.

The nature of the quantitative representation of algae and fungi recorded at various points during this study is presented in Fig. 6.

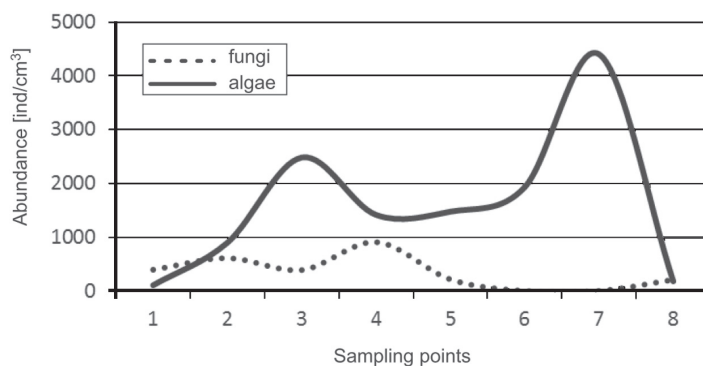


Fig. 6. Abundance of algae and fungi in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

The figure shows that ecological optimum in the conditions of clarifiers had algae; their abundance grew a lot in primary clarifier where water transparency was the highest among all studied points. As to fungi, the decrease of their number during purification indicates that optimum for these organisms are eutrophic and anoxic conditions. The role of heterotrophic and autotrophic organisms in the periphyton structure in the conditions of treatment plant is shown in Fig. 7.

According to proportion in all the chambers the basis of the periphyton community was formed with protozoa and metazoa. Their part at various purification stages was from 75 % to 95 %. Together with fungi the part of heterotrophic organisms in periphyton can reach 99 %. The part of fungi in various stages of purification was not higher than 8 %, while autotrophic organisms formed from 1 % to 25 % of all organisms' abundance. Since PCA analysis was performed based on the relative number of organism groups in the gradient of conditions that are determined by a continuous process of purification, it can be concluded that consolidation of most organisms groups (lower right quarter of the diagram) is caused by their connection with dynamics of the processes (Fig. 8).

At the same time considerable remoteness of flagellates and algae from the main group indicates resultant influence of external factors on them. It is clear that algae directly depend on illumination intensity and flagellates – on the level of organic matters. Thus, the flagellate dynamics will be rather determined by disturbances of the purification process or excessive inflow of wastewater. According to Curds [30] flagellates predominate in the system in the early stages only because of their lower energy requirements. Heterotrophic flagellates are therefore recognized as indicators of

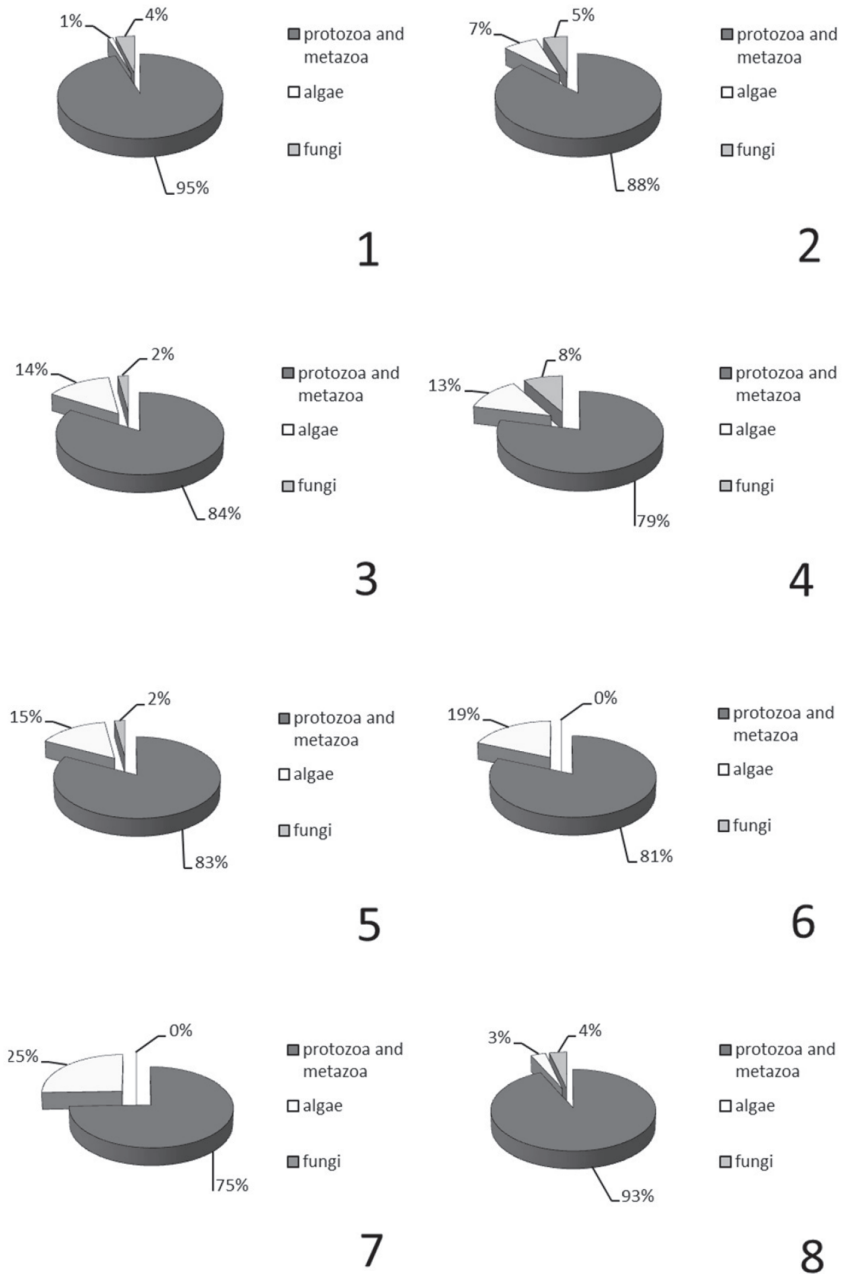


Fig. 7. The ratio of the quantitative representation of different groups of organisms in the structure of periphyton community along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber



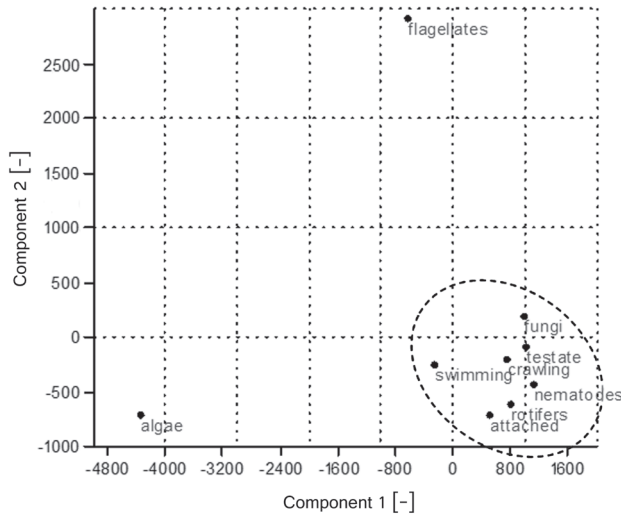


Fig. 8. PCA ordination diagram of the different groups of organisms in the structure periphyton community in the conditions of treatment plant

malfunction of treatment facilities or their overload [8, 12]. There is direct relation between dynamics of algae, fluctuations in illumination intensity and efficiency of water clarification process. During the study period there was a tendency observed – towards the end of purification process the part of metazoa and protozoa in periphyton of successive chambers was increasing (Fig 9). Similar results were obtained at RBC type water treatment plant [23].

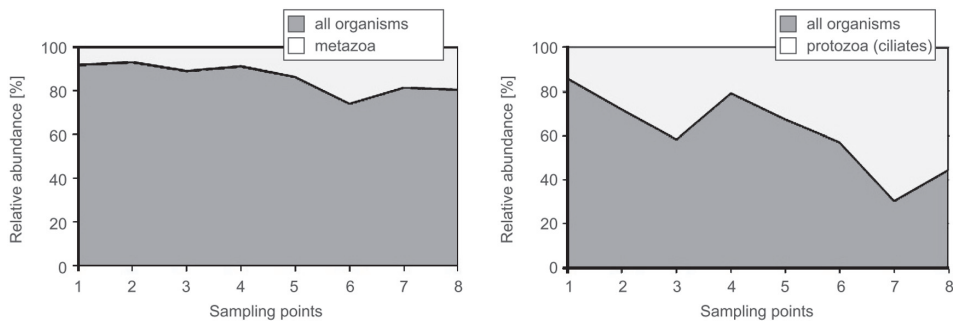


Fig. 9. Part of metazoan (A) and ciliates (B) in the total abundance of organisms in periphyton along the treatment plant. The sampling points: 1 – screen chamber, 2 – grit chamber, 3 – primary clarifier, 4 – anaerobic chamber, 5 – anoxic chamber, 6 – aeration chamber, 7 – secondary clarifier, 8 – recirculation chamber

In periphyton (RBC) the most numerous group of organisms was ciliates (55–95 % of the total quantity). In the periphyton conditions of flow type purification plant in the

modified system bardenpho, equal to plant Hajdow protozoa make up from 14 % to 69 %. Similarly behaved the small flagellate – in treatment plant Hajdow as well as in (RBC) their number was reducing by the end of purification process (Fig. 4). Thus, the structure of periphyton irrespective of its forming conditions shows similar tendencies, determined by the general conditions of system in which from the initial stages and till the end, the amount of organic matters in environment decreases.

## Summary and conclusions

The periphyton community in successive chambers of flow type treatment plant with the biological part functioning in the modified system bardenpho, reached maximum abundance in primary and secondary clarifiers. Concerning clarifiers, the maximum abundance was reached by algae, rotifers and ciliates. The abundance of flagellates from the start to the end of the purification process decreased of more than 30 times. Towards the end of purification process of periphyton in successive chambers, the amount of protozoa and metazoa has increased. In all of the studied sampling points, the basis of periphyton community was formed by protozoa and metazoan, their part made up 75–95 %. The part of heterotrophs reached 99 % and part of autotrophes varied from 1 % to 25 %

## Acknowledgments

Research project partly financed from the budget for science in the years 2012–2013 within the “Diamond Grant” program. Grant no. 0013/DIA/2012/41.

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## ANALIZA ZBIOROWISK PERYFITONU MIEJSKIEJ OCZYSZCZALNI ŚCIEKÓW

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**Abstrakt:** W pracy zaprezentowano badania składu peryfitonu i jego rozwój ilościowy podczas kolejnych etapów oczyszczania ścieków w miejskiej oczyszczalni ścieków Hajdów w Lublinie, której część biologiczna pracuje w technologii zmodyfikowanego systemu bardenpho. Próby peryfitonu pobierano z powierzchni ścian obiektów na wszystkich głównych etapach oczyszczania ścieków. W składzie peryfitonu zostały zidentyfikowane następujące grupy organizmów: glony, grzyby, wiciowce, ameby skorupkowe, orzęski, wrotki i nicienie. W kolejnych analizowanych urządzeniach zlokalizowanych w ciągu technologicznym oczyszczalni peryfiton wykazywał wzrost ilości organizmów w obrębie grup metazoa i pierwotniaków, podczas gdy liczebność wiciowców uległa zmniejszeniu. We wszystkich badanych punktach pomiarowych podstawa zbiorowisk peryfitonu utworzona była przez pierwotniaki i metazoa, ich udział wahał się od 75 do 95 %. Struktura peryfitonu badanych urządzeń wykazuje podobne tendencje, uzależnione od warunków panujących w poszczególnych urządzeniach, w którym od pierwszych etapów oczyszczania aż do jego końca ilość dostępnych substancji organicznych maleje.

**Słowa kluczowe:** peryfiton, zbiorowiska, komunalna oczyszczalnia ścieków, pierwotniaki, metazoa