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Abundance and Structure of Ciliated Protozoa Community at the Particular Devices of "Hajdów" WWTP

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1. Introduction

Ciliates are generally dominant protozoa in activated sludge; the most common are attached forms and forms that crawl over the surface of the sludge flocks. The experimental work demonstrated that ciliated protozoa are essential for the production of a good quality effluent [3].

Communities of organisms in the system of activated sludge are an object of intensive study for more than 50 years. Multiple researches showed that protozoa are good indicators of treatment process [14, 16, 24] and determine activated sludge quality [3, 5, 17, 20]. At the same time the process of domestic and industrial effluents treatment is improving by changes in technological schema, aimed at increase of sludge organisms' activity. For that reason, protozoa communities' studies continue. However, the reactions of activated sludge communities often remain uncertain and unpredictable.

The process conditions in water treatment plant depend on many factors, which contribute to the changes in structure and quantity of organisms. To understand the processes and their dynamics it is important to have information about the structure and dynamic in the stable (optimal) conditions. There are many works directed towards studying the activated sludge species abundance and structures [14, 18, 23] and influence of factors upon them [1, 4, 12]. But there are very few researches

that would embrace different stages of waste water treatment. This affirms the current importance of the undertaken investigations.

2. Materials and methods

The aim of this work was to monitor the ciliated protozoa under the conditions of modernized wastewater treatment plant Hajdów.

Twelve locations in technological facilities of WWTP "Hajdów" in Lublin were investigated. Samples were taken from: chamber inlet in front of rake screens (Inlet), chamber outlet behind rake screens (M1), chamber inlet in front of sand separator (M2), chamber outlet behind sand separator (M3), outlet behind primary sedimentation tank (M4), outlet from anaerobic chamber (AC), mix chamber (MC), inlet into bioreactor B1 (denitrification chamber), outlet from nitrification chamber B2 (6th chamber of bioreactor), outlet from nitrification chamber B3 (9th chamber of bioreactor), recirculation canal (RC), outlet behind secondary sedimentation tank (Outlet).

Sludge samples were collected using Ruttner bathometer, from depth of 0.5 m below the surface of sewage. Sampling was conducted with one week interval during three months – August, September, and October, 2010.

The content of nitrogen compounds in sewage was determinated by the spectrophotometer HACH DR 2800 in the research laboratory of Lublin University of Technology (Poland), with the use of individual procedures of light absorption for each kind of nitrogen compounds. Content of nitrogen compounds in samples was determined immediately after delivery to the laboratory, in next 0.5 hour after sampling. The structure and quantity of protozoa were identified in first 5 hours after sampling. Before the beginning of researches samples had been preserved in refrigerator.

Microorganisms' abundance was determined «in vivo» with a sub-sampling technique: a 25-µl of mixed solution was taken with an automatic micropipette. Subsamples were examined three times for numerous protists and five times for not numerous protists which density was less than 5 individuals in 25 µl. The identification of species was mainly carried out "in vivo" under an optical microscope with passing-through light, at appropriate magnification. The nuclear apparatus was

stained with acidulous solution of methyl green (0.1% methyl green in 1% acetic acid), although when necessary silver staining method was applied [25, 26]. Ciliophora were identified according to Kahl [13] and Foissner et al. [6-9].

Based on these calculations of similarity, the hierarchical cluster-analysis was conducted.

Species occurrence in samples was calculated according to the following formula:

$$C = N_a/N$$

where:
N_a – number of samples with ”a” species,
N – total number of samples.

For euconstant were taken the species whose occurrence exceeded 75,1 – 100,0% [10].

The rarefaction curves were computed using Diversity and Richness 3.03 software. For statistical analysis the programs STATISTICA 8.0 and PAST 1.81 [11] were used.

3. Results and Discussion

The researches have covered the key stages of purification conducted at WWTP “Hajdów”. The samples have been taken starting from the point where wastewater enters until the point where purified effluent comes into the discharge canal to the river. On the grounds of averaged data describing the composition and abundance of protozoa, the sampling stations were divided into two clusters (Fig.1).

First cluster on quite low level of similarities combined the stations of prestage mechanical treatment (M1-M4) and stations at purification plant inlet and outlet. The combination of sampling points, which is a place where untreated sewage enters, and station, where treated sewage discharged into the river Bystrica, was determined by extremely low number of present organisms before the start of treatment as well as after it. Low number of ciliate species and their abundance was observed at the stations of mechanical purification and in the primary sedimentation tank (M4). The second cluster grouped the stations of activated sludge circulation.

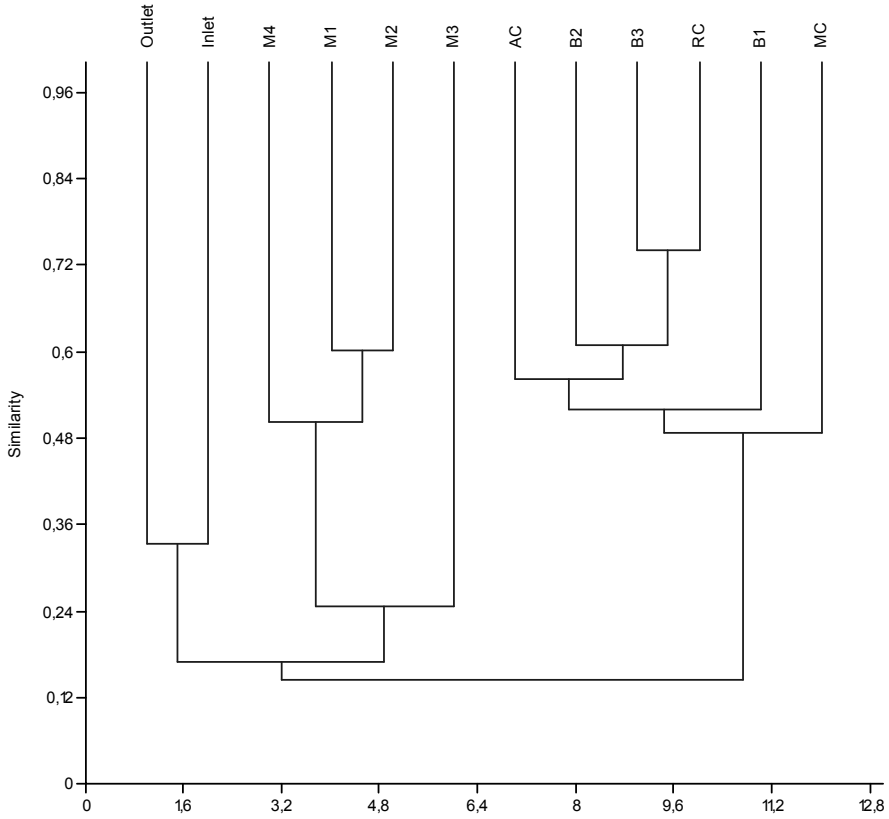


Fig. 1. Dendrogram of similarity between sampling points at the particular devices of Hajdów WWTP (Jaccard similarity index, algorithm – paired group)

Rys. 1. Dendrogram podobieństwa pomiędzy punktami poboru próbek w poszczególnych urządzeniach oczyszczalni ścieków Hajdów (miara podobieństwa Jaccarda, algorytm grup parowanych)

One of the important criterions of purification efficiency control is the process of nitrogen compounds removal. In the modern upgraded purification plants the efficiency of nitrogen and ammonium hydrate removal exceeds 90% [21]. During the research period the nitrogen compounds concentration at Hajdów WWTP was reducing by 8-10 times at the average from the beginning of the treatment process to its end (Fig. 2), and efficiency of the process achieved 89-93%.

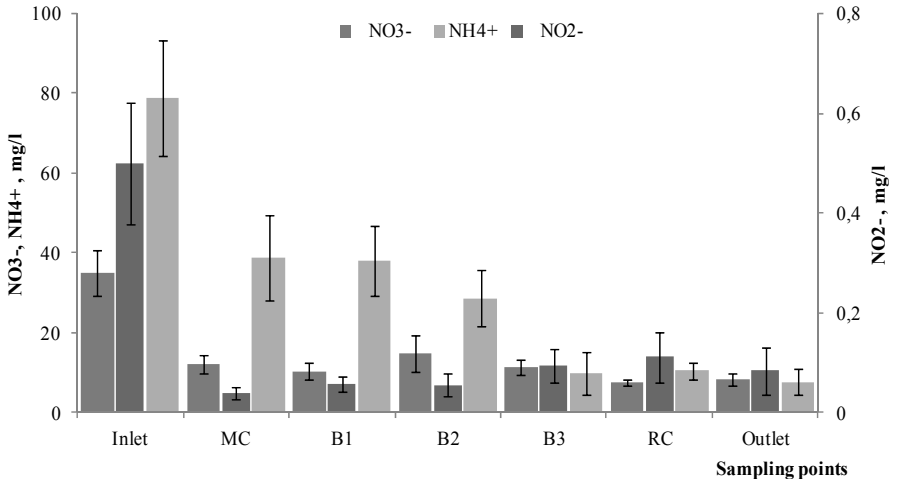


Fig. 2. Changes of nitrogen compounds concentration over the course of facilities of Hajdów WWTP

Rys. 2. Zmiany stężeń związków azotu w kolejnych urządzeniach ciągu technologicznego oczyszczalni ścieków Hajdów

It is known, that dynamic of ciliate population structure in activated sludge is determined by stability of purification process, and ciliate development influences the purification efficiency. Because of that the changes in structure and quantity of activated sludge protozoa are most often used for the control over purification process. Although difficulties in interpretation of protozoa community respond to changes in bioreactors prompts to look for alternative objects for the bioassays, e.g. biofilm at the surfaces of WWTP devices [15, 19, 22], the protozoa of activated sludge remain the main object of purification process bioindication [16, 17, 23, 24].

There were 30 taxa of ciliate detected in wastewater of mechanical part and in the activated sludge during the research period. The type of change in abundance of protozoa, starting from the chamber where the activated sludge mixes with an untreated wastewater and ending by recirculation canal, is represented on the graph (Fig. 3).

Meanwhile the changes in protozoa quantity occurred not at all sampling points due to realization of reproductive potential of populations. Thus the high numbers of protozoa in anaerobic chamber (AC) are caused by arriving of concentrated activated sludge from recirculation

canal. Decrease of abundance in the mix chamber (MC) and at the inlet into 1st chamber of bioreactor (B1) is the result of activated sludge dilution by mechanically treated wastewater. The increase observed in the quantity of protozoa at the recirculation canal (RC) results from their high concentration due to the removal of purified effluent from final sedimentation tank.

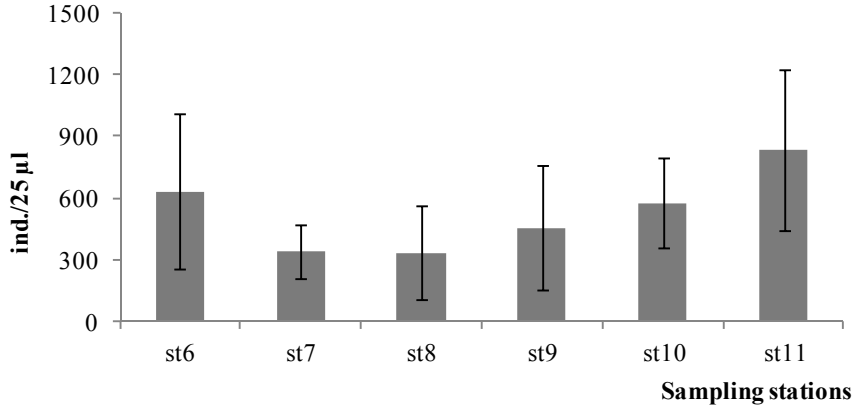


Fig. 3. Ciliate abundance in the particular devices of Hajdów WWTP
Rys. 3. Liczebność orzęsków w kolejnych urządzeniach oczyszczalni Hajdów

Considering the specificity of activated sludge circulation, the realization of protozoa reproductive potential is the most clearly observed within the system of bioreactor chambers. Our research showed that at the end of purification process the ciliate abundance varied from 18 000 to 48 000 individuals per milliliter. This is close to the quantities observed at the final stage of purification process under conditions of normally functioning treatment plant in Cracow, Poland, where abundance of ciliate in aerated bioreactor chamber reached 11 000 – 33 000 individuals per milliliter [21]. The control over ciliate abundance at the initial stage of activated sludge development, after its mixing with mechanically treated wastewater, allowed to calculate the efficiency of quantity growth during the sludge movement through the chambers of bioreactor. The numbers of protozoa at the initial stage of purification fluctuated from 8 000 to 20 000 individuals per milliliter. The total quantity of protozoa increased by 80-85 % towards the end of purification. The high numbers of protozoa a priori imply the high level of bacteria grazing. However

according to the research results, high level of grazing doesn't negatively affect $N\text{-}NH_4^+$ removal in a wastewater treatment plant [21]. The number of ciliate species detected from the beginning of the process until its end was increasing (Fig.4). The tendencies described above persisted during the whole studied period.

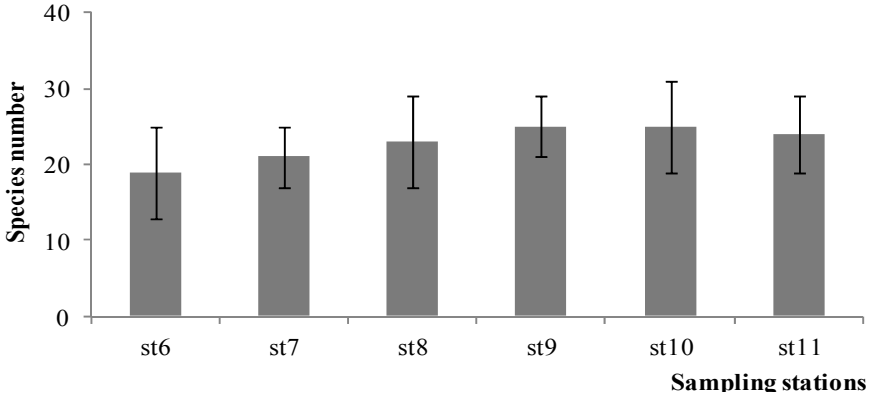
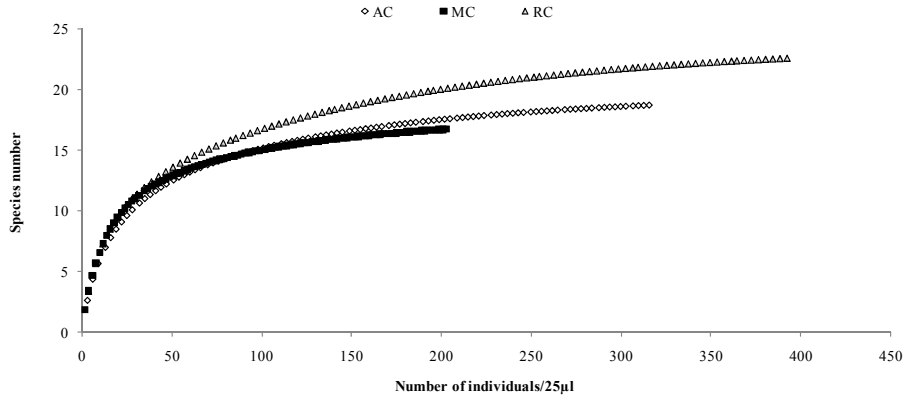


Fig. 4. Number of ciliate species in particular devices of Hajdów WWTP
Fig. 4. Ilość gatunków orzęsków w kolejnych urządzeniach oczyszczalni Hajdów

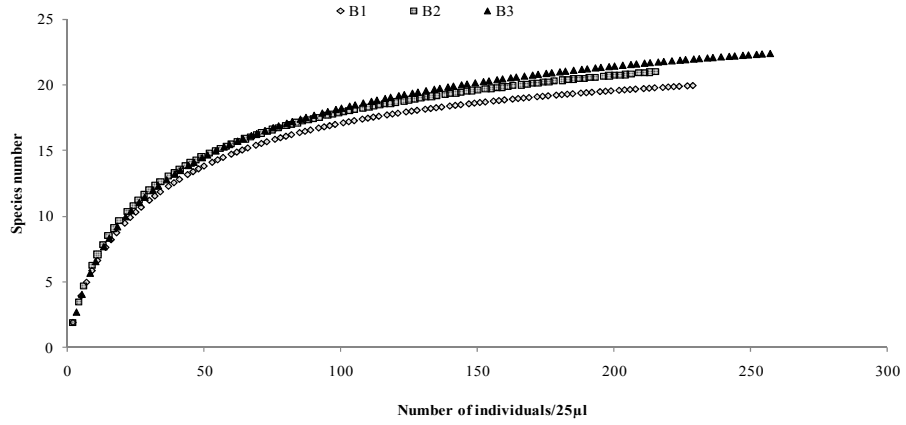
The diversity of ciliated protozoa in the activated sludge was evaluated using the rarefaction method. The type of species accumulation curves in the anaerobic chamber, mix chamber and recirculation canal is shown at the Fig. 5A.

The protozoa diversity is higher in recycled sludge: the number of species grows by maximal quantities, starting from 350 ind./25 µl. The minimal diversity of species achieved by low quantities of protozoa (150 ind./ 25 µl) was observed in the chamber where sludge mixes with sewage (MC). During the activated sludge movement through the chambers of bioreactor, by the end of purification process (from B1 to B3) the tendency towards the increase of ciliate diversity in the activated sludge structure was observed (Fig. 5B). The maximal species diversity was reached at the end of wastewater purification process at the station B3 with the quantity of protozoa 250 ind/25µl. In general at all chambers the quite stable structure of ciliate assemblage was observed. This structure

becomes insignificantly more complex towards the end of purification process.



A



B

Fig. 5. Rarefaction diversity estimates of ciliates collected at the sampling points

Rys. 5. Krzywe rarefakcji dla zbiorowisk orzęsków wybranych urządzeń

The analysis of ciliate occurrence enabled to mark out the group of euconstant ciliates, with an occurrence in the samples higher than 75% [10]. The part of euconstant ciliates populations was numerous and part had small numbers. The group of not numerous euconstant species was composed of *Euplotes affinis*, *Litonotus lamella*, *Holophrya discolor*, *Plagiocampa rouxi*. The numerous group included 6 species – *Aspidisca*

cicada, *Aspidisca lynceus*, *Chilodonella uncinata*, *Acineria uncinata*, *Vorticella aquadulcis*, *Opercularia articulata*.

Quantities of numerous euconstant species at the purification stage from station B1 to station B3 were steadily increasing. The pattern of abundance growth of several euconstant species populations is shown at the Fig. 6. The density of not numerous populations of predaceous species, such as *Holophrya discolor* and *Litonotus lamella* remained relatively stable at all stages of purification. The share of euconstant ciliates in the protozoa community of activated sludge was fluctuating from 26% to 38% (Fig. 7).

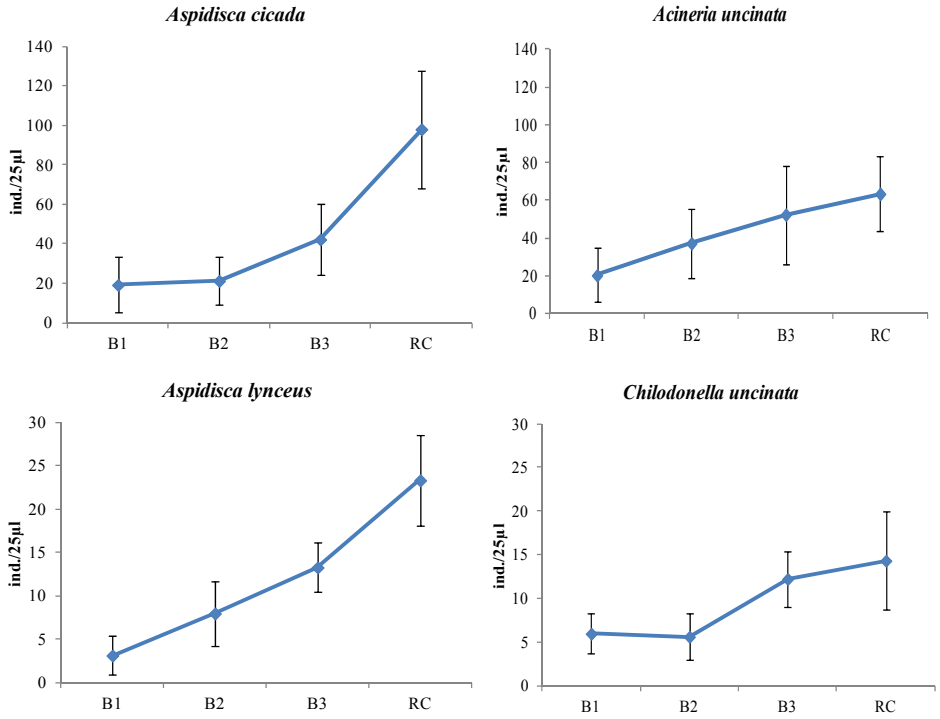


Fig. 6. Abundances of euconstant ciliate species in particular devices of Hajdów WWTP

Rys. 6. Liczebność dominujących gatunków orzęsków w kolejnych urządzeniach oczyszczalni Hajdów

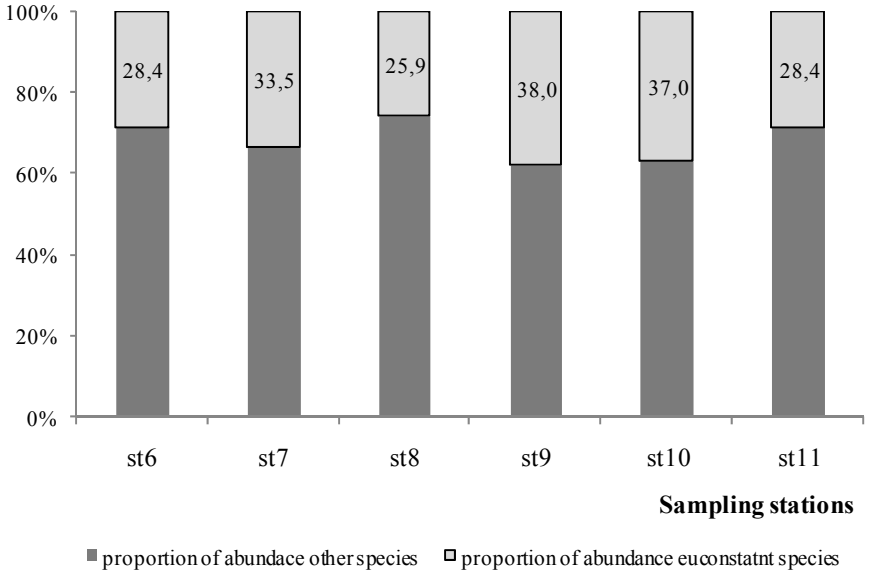


Fig. 7. Proportion of euconstant ciliate species abundance in total abundance of ciliates

Rys. 7. Proporcje pomiędzy liczebnością dominujących gatunków orzęsków a liczebnością pozostałych gatunków

4. Summary

Thereby, in the conditions of steady functioning treatment plant the protozoa community trends towards preservation of quite stable structure. The overall number of ciliated protozoa at the beginning of purification process varied from 8 000 to 20 000 individuals per milliliter, while at the end of the process ciliate quantity maximally reached 48 000 individuals per milliliter. From the start to finish of purification process the number of protozoa in WWTP increased by 80% at an average.

About ¼ to $^1/_3$ of ciliate assemblage was represented by euconstant species. During the movement of activated sludge through the chambers of bioreactor, the trend towards increase of detected species number was observed. The maximal expected number of species - 25 was realized in the last chamber of bioreactor by the protozoa quantity of 250 individuals per 25 microliters and in recycled sludge by the quantity of 350-400 individuals per 25 microliters.

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Liczebność i struktura zbiorowisk orzęsków w urządzeniach oczyszczalni ścieków Hajdów

Abstrakt

Kompozycja, liczebność oraz bioróżnorodność zgrupowań orzęsków występujących w kolejnych urządzeniach oczyszczalni ścieków Hajdów były analizowane w ramach niniejszej pracy. Podczas całego okresu badań praca oczyszczalni była stabilna, przebiegając bez zakłóceń oraz sytuacji awaryjnych. Próbki materiału biologicznego do badań pobierane były począwszy od punktu w którym ścieki surowe napływają do oczyszczalni, aż do punktu w którym oczyszczone ścieki odprowadzane są do odbiornika – kanału zrzutu do rzeki. W trakcie badań zidentyfikowano w analizowanym materiale 30 gatunków orzęsków. Podczas przemieszczania się osadu czynnego wraz z oczyszczanymi ściekami przez kolejne komory bioreakcji, występuje stały trend wzrostu liczby gatunków oraz bioróżnorodności zbiorowisk orzęsków. Od ¼ do 1/3 liczby orzęsków w zbiorowiskach stanowią gatunki najczęściej występujące w oczyszczalniach ścieków. Całkowita liczba orzęsków występujących na początku procesu oczyszczania wahała się pomiędzy 8 000 a 20 000 osobników na mililitr, zaś ich maksymalna ilość na końcu procesu oczyszczania osiągała wartość 48 000 osobników na mililitr. Średnio pomiędzy początkiem a końcem procesu oczyszczania ścieków liczba orzęsków w analizowanym materiale biologicznym zwiększała się o około 80%. Krzywe rarefakcji wskazują, iż maksymalna spodziewana liczba ganków na poziomie 25 osiągana jest w punkcie pomiarowym zlokalizowanym na odpływie z bioreaktora, przy liczebności organizmów równej 250 osobników w 25 mikrolitrach, zaś w osadzie recyrkulowanym przy liczebności 350-400 osobników w 25 mikrolitrach.