DE GRUYTER OPEN
DOI: 10.1515/eces-2014-0043 ECOL CHEM ENG S. 2014;21(4):605-616

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BIOINDICATION ASSESSMENT OF ACTIVATED SLUDGE ADAPTATION IN A LAB-SCALE EXPERIMENT

BIOINDYKACYJNA OCENA ADAPTACJI OSADU CZYNNEGO W WARUNKACH EKSPERYMENTU LABORATORYJNEGO

Abstract: The adaptation of activated sludge from the Hajdow sewage treatment plant in a laboratory SBR was studied. The structure of the ciliate assembly was considered as a criterion. 32 ciliate species were found during the experiment. The composition and changes in the ciliate community structure during the process of activated sludge adaptation was examined. In the process of adaptation, reduction was observed in the number of ciliate species together with an increase in assembly total abundance. The decrease in the Shannon diversity index and equitability index in the adaptation process was observed. In the process of adaptation, two states of ciliate assembly were marked out - unstable transient period and stable period. During the transient period, reduction of ammonium utilization efficiency down to 50% and its subsequent increase up to 80% in the stable period were observed. In the transient period, the Simpson dominance index remained low but increased in the stable period. At a temperature of 10°C, the transient period lasted from six to nine days. After the stabilization process, the diversity of the ciliate assemblage remained at a lower level. Rarefaction methods showed that the number of potential ecological niches of ciliate amounted to 30 in the adaptation period, whereas there were only 15-20 ecological niches in adapted sludge.

Keywords: bioindication, activated sludge, ciliates community, biocenotic indices, adaptation, SBR-type bioreactor, lab-scale experiment

Introduction

Wastewater treatment is a constantly modified and improved process that requires scientific research. Most of such investigations are performed in a laboratory environment using appropriate equipment [1-4]. When activated sludge is transferred to a different environment, its community undergoes a period of intense transition needed for adaptation [5]. Activated sludge for laboratory is often taken from large-scale wastewater treatment plants (WWTP). A majority of papers published on this subject deal with activated sludge adaptation to the influence of various toxic substances. In a number of papers, the aspects of

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adaptation of activated sludge microorganisms to the effect of phenol [6, 7], high concentrations of ammonium [8], organic solids accumulated in the system [9], toxic organic micro-pollutants [10], bisphenol-A [11] and other [12-16] are examined. In the work of Bajenov and Kanunnikova [17], adaptation of activated sludge to low oxygen concentrations was also studied. A detailed account of the process of activated sludge adaptation is provided in the work of Anielak and Piaskowski [18]. Setting of adaptation regimes and periods makes it possible to conduct the research without considering the reaction of activated sludge to stress caused by repeated volume reductions and stabilization of the main factors. To avoid mistakes in the experimental results, it is important to assess quantitatively in advance whether conditions in the activated sludge have stabilized [19, 20]. The common practice assumes that the optimal period for adaptation lasts 2-2.5 sludge ages [21-23]. However, a body of evidence proves an assumption that the period of adaptation should last for 9 weeks [24]. However, in each case, the time and quality of adaptation may depend on the primary state of activated sludge, season, and the other factors [25, 26]. This necessitates searching for criteria that facilitate controlling the adaptation process [27, 28]. In this respect, it is interesting to use the fully automated image analysis procedure for characterization of the activated sludge composition [29, 30]. The aim of this work is to study the transformations of the most representative and diverse group of prokaryotes (ciliates) in the process of activated sludge adaptation to the conditions of the laboratory SBR.

Materials and methods

The experiment was carried out in a SBR-type laboratory reactor with autonomous control of oxygen supply and recording of its concentration, as well as automatic recording of pH and redox potential. The laboratory SBR consists of three chambers with a volume of 2 dm^3 each; in addition, the temperature was stabilized at 10°C using a water jacket. The mixing and aeration systems are provided centrally, individually for each chamber.

The SBR reactor had the following modes of operation. The cycle of the reactor lasted 12 hours, the operating cycle consisted of six successive phases: I - fill (10 minutes), II - mix (180 minutes), III - react (420 minutes), IV - settle (90 minutes), V - decant (10 minutes), and VI - idle (10 minutes). The mixture in the chamber comprised activated sludge - 0.6 dm^3, raw sewage - 0.5 dm^3, and purified wastewater - 0.7 dm^3. Each time of decantation (end of cycle) pumped out 0.5 dm^3 of purified wastewaters; at the same time, raw sewage was pumped into the chamber during the filling phase (at the beginning of the next cycle).

$N\text{-}NH_3$ and TSS (Total Suspended Solids) were measured using a HACH DR 3600 spectrophotometer from HACH-Lange. The analysis was based on the methodology developed by HACH-Lange. The turbidity was measured using nephelometry CyberScan TN-100 EUTECH Instruments. The measurement of contamination was performed immediately after the idle phase.

The activated sludge and sewage from the Hajdow wastewater treatment plant (Lublin, Poland), sampled within winter period at temperature 10°C, was used as material for this study.

The studies were carried out with an Olympus CX41 microscope. The ciliates were identified in vivo. For diagnosis, the method of Galliano was used. The nuclear apparatus of the ciliates was stained with methylene green.

The ciliates were counted in a volume of 25 mm^3 (microsamples). The calculation was performed in five replicates. The data were averaged and recalculated to 1 cm^3. Data for colonial protozoa are presented as the number of zooids in 1 cm^3.

Ciliate species richness in the different cycles was compared using rarefaction curves. Hierarchical cluster analyses based on the Euclidean similarity index (dendrogram) were used to compare the species assemblages at the different cycles [31]. These analyses (rarefaction curves, cluster analysis) were performed using the software program Past 1.57 [32].

Species richness (*S*), Shannon-Weaver index of diversity (*H*), equitability (*j* = evenness) ($j = H/\ln S$), and Simpson dominance index were used to compare ciliate assemblages between the cycles [33-37].

Canonical correspondence analyses (CCA) was carried out based on ciliate abundance. Multivariate analysis was performed using the software program Past 1.57 [32].

Results and discussion

The sludge was sampled at the final stage of purification, when factorial fluctuations in the environment are minimal. The stability conditions at the final stage of purification presuppose the existence of an unstressed community of activated sludge. The community of activated sludge ciliates was analyzed. Their structure was considered as the initial structure. The composition of the assemblage of ciliates and the dynamics of their abundance during the experiment are shown in Table 1.

The important measure of the quality of sewage treatment is a effectiveness of reduction ammonium [38-41]. From the beginning of the experiment, the treatment efficiency of wastewater added to the SBR was monitored by the reduction of N-NH_3. The dynamics of changes in ammonium utilization efficiency can be well described with a polynomial equation with a high value of the approximation coefficient - R^2 (Fig. 1). The efficiency of nitrogen utilization was calculated based on its content at the beginning of the cycle after adding and mixing of wastewater with an activated sludge and content of nitrogen at the end of the cycle in supernatant after sedimentation.

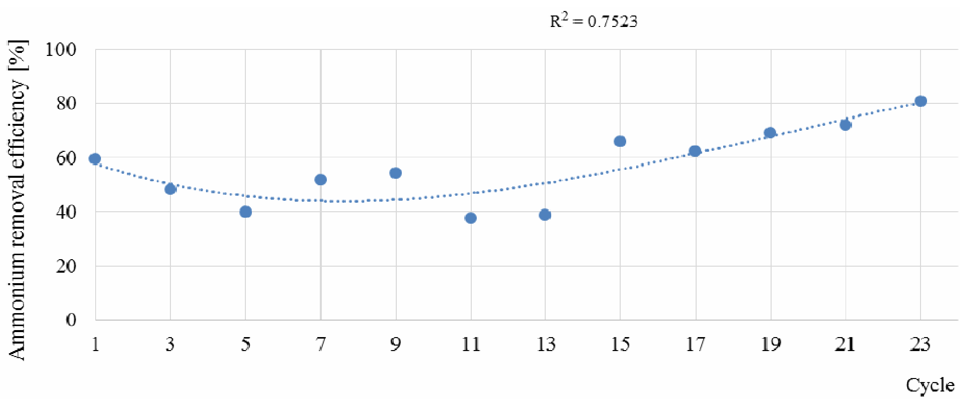

Fig. 1. Ammonium removal efficiency in the process of adaptation of the activated sludge in the SBR

Table 1

Comparison of efficiency values of the analyzed system

	Hajdow WWTP		**Transient period**		**Adapted period**	
Species	**X***	**S****	**X***	**S****	**X***	**S****
Acineria incurvata (Dujardin, 1841)	190.21	63.25	26.8	13.5	20.4	7.9
Acineria uncinata (Tucolesco, 1962)	371.69	88.2	294.0	78.0	1061.0	236.1
Acineta fluviatilis (Stokes, 1885)	34.12	14.2	6.1	6.1	–	–
Apoamphileptus claparedii (Stein, 1867)	40.25	12.3	–	–	–	–
Apsiktrata gracilis (Penard, 1922)	40.25	24.24	224.9	43.2	0.7	1.1
Aspidisca cicada (Müller, 1786)	410.78	375.069	919.5	84.3	1410.0	35.9
Aspidisca lynceus (Müller, 1773)	100.23	23.094	102.2	33.3	37.3	40.4
Carchesium polypinum (Linnaeus, 1758)	142.29	51.36	134.3	29.1	136.0	34.8
Cinetochilum margaritaceum (Ehrenberg, 1831)	94.25	23.094	29.7	20.8	46.4	30.0
Crossacineta ornata (Sand, 1899)	20.13	15.3	26.6	11.1	13.3	8.5
Epistylis coronata (Nusch, 1970)	350.98	69.24	114.7	66.3	28.9	38.6
Epistylis plicatilis (Ehrenberg, 1831)	100.23	39.21	5.8	7.8	–	–
Euplotopsis affinis (Dujardin, 1841)	–	–	3.0	3.0	6.7	1.6
Holophrya discolor (Ehrenberg, 1834)	53.23	29.36	1.1	1.8	217.2	37.2
Litonotus fusidens (Kahl, 1926)	–	–	5.9	4.0	–	–
Litonotus lamella (Műller, 1773)	65.64	25.36	62.9	19.7	78.1	44.0
Metacystis sp.	13.11	11	39.8	8.2	–	–
Opercularia articulata (Goldfuss, 1820)	–	–	–	–	2.2	3.6
Opercularia coarctata (Claparède & Lachmann, 1858)	–	–	5.3	8.9	–	–
Opercularia microdiscus (Faure-Fremiet, 1904)	40.25	31.1	8.3	13.9	–	–
Plagiocampa rouxi (Kahl, 1926)	53.23	27.69	5.5	5.5	34.1	13.3
Pseudovorticella elongata (Fromentel, 1876)	205.5	147.5	151.5	50.4	23.3	17.2
Thigmogaster potamophilus (Foissner, 1988)	360.87	278.3	131.3	49.9	5.3	5.3
Thuricola kellicottiana (Stokes, 1887)	40.25	12.8	16.4	6.5	7.3	10.9
Tokophrya lemnarum (Stein, 1859)	20.13	12.3	17.8	4.7	28.9	15.7
Tokophrya quadripartita (Claparède & Lachmann, 1859)	20.13	19.1	8.7	2.9	8.7	2.9
Urotricha farcta (Claparède, Lachmann, 1859)	20.13	18.3	23.3	4.3	–	–
Vorticella aquadulcis-complex	100.23	29.33	89.4	58.3	34.5	30.2
Vorticella convallaria-complex	–	–	13.1	17.4	6.9	9.2
Vorticella microscopica (Fromentel, 1874)	90.78	24.3	4.8	8.1	–	–
Vorticella microstoma-complex	20.13	13.2	28.9	12.9	11.7	12.8
Vorticella octava-complex	63.33	36.14	43.3	23.9	1.1	1.8

* - average, ** - standard deviation

During the first eleven cycles, the efficiency of N-NH_3 utilization remained around the level of 50% (Fig. 1). Starting from the thirteenth cycle, the efficiency grew to 70-80%, which corresponds to good, stable work of SBR.

Concerning suspended matters, the efficiency of reduction thereof after the first cycle reached 97-99% and stabilized at this level (Fig. 2).

The fact that the efficiency of purification in the process of adaptation remained stably high can be explained with as follows: suspended matters mainly consist of bacteria; removal thereof is the function of ciliates and rotifers, and their quantity in the SBR remained high or was increasing until the end of experiment.

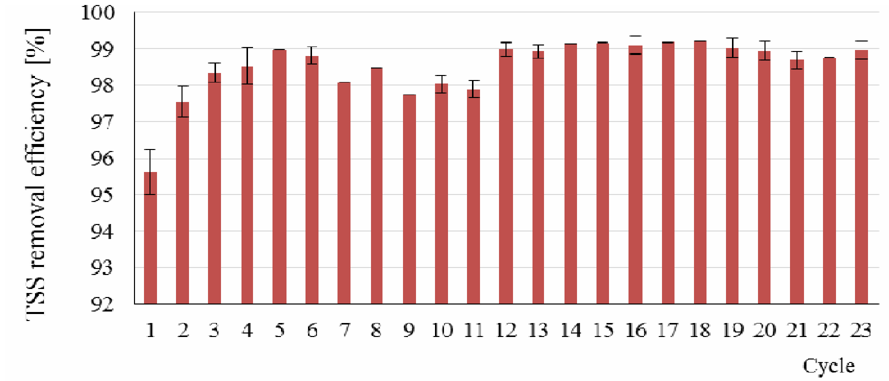

Fig. 2. TSS removal efficiency in the process of adaptation of the activated sludge in a SBR with standard deviation bars

The abundance of ciliates was controlled every two cycles. As can be seen from Figure 3, the number of species declined substantially in the course of the experiment.

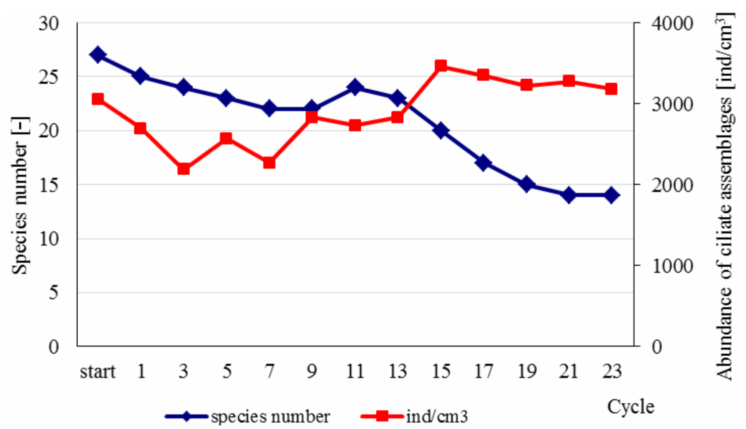

Fig. 3. The abundance of ciliate assemblages [ind/cm^3] and their species number in the period of the experiment

The reduction of the species number was followed by an increase in their abundance starting from cycles 11-13.

Indexes calculated on the basis of abundance and species representation showed that the dominance increased together with the equalization decline (Fig. 4). Declining equalization and growing dominance were accompanied by a decrease in the overall diversity (Fig. 5), which is natural at significant reduction in the number of present species.

SBR technology is used all over the world successfully for big installations of Wastewater Treatment Plants (WWTP) or as a small laboratory installation. Undoubtedly, in order to solve the number of problems occurring during activated sludge studies, it is reasonable to conduct investigations in a laboratory scale. The transfer from the industrial to laboratory scale requires a period of adaptation of the activated sludge community to the conditions of the SBR [19, 20]. The adaptation period should be neither too short, or else sludge will not be stabilized, nor too long, or else experiment will be time consuming. It is important to establish an average time required for the adaptation of activated sludge in the conditions of a laboratory SBR. The cluster analysis was used to study the structural rearrangements of the ciliate assembly during 23 cycles (Fig. 6).

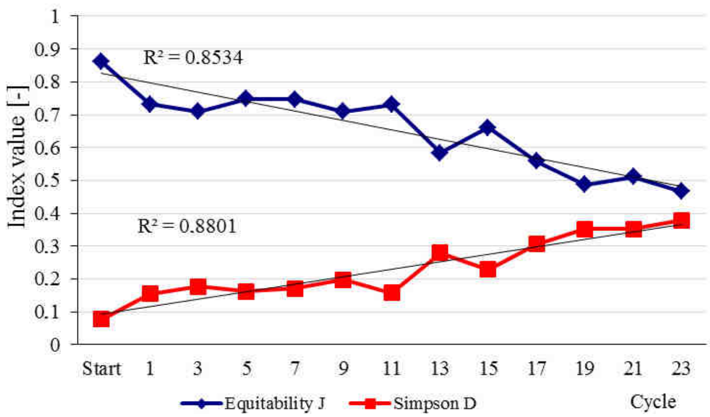

Fig. 4. The Simpson dominance index and equitability (*J*) index calculated on the basis of the species composition of the ciliate assemblage

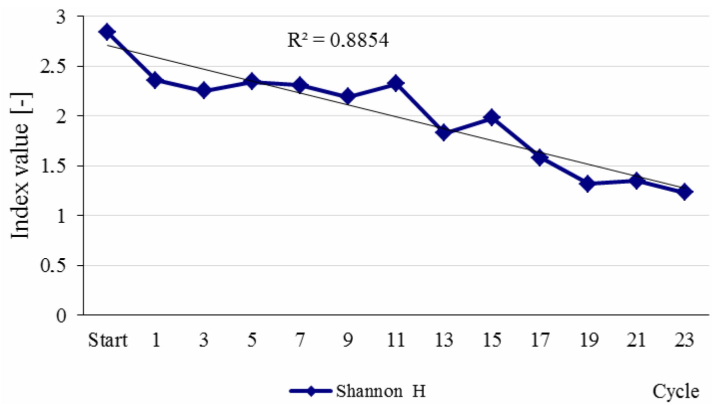

Fig. 5. The Shannon diversity index demonstrated the ciliate species diversity in the different subsequent cycles

The sample from the treatment plant demonstrates a specific independent structure. In the process of adaptation, the ciliate assembly split into two clusters. The first combined six cycles from 1 to 11 and the second - six cycles from 13 to 23.

In the process of adaptation, there are two states of ciliate assembly to define - a transient period of an unstable structure and a stable period. The presence of the two periods during the experiment was also confirmed by other methods of analysis. Canonical correspondent analysis showed that the structure of ciliate assembly split into two states (Fig. 7).

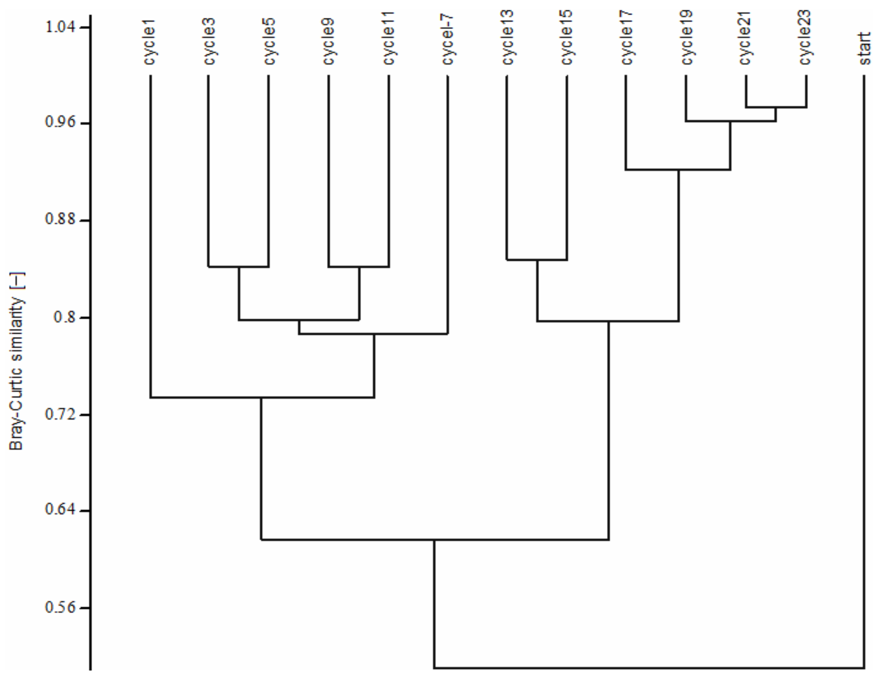

Fig. 6. The dendrogram of the 0(start)-23 cycles calculated on the basis of the ciliate assemblage abundance, using the paired group linkage and Bray-Curtic similarity

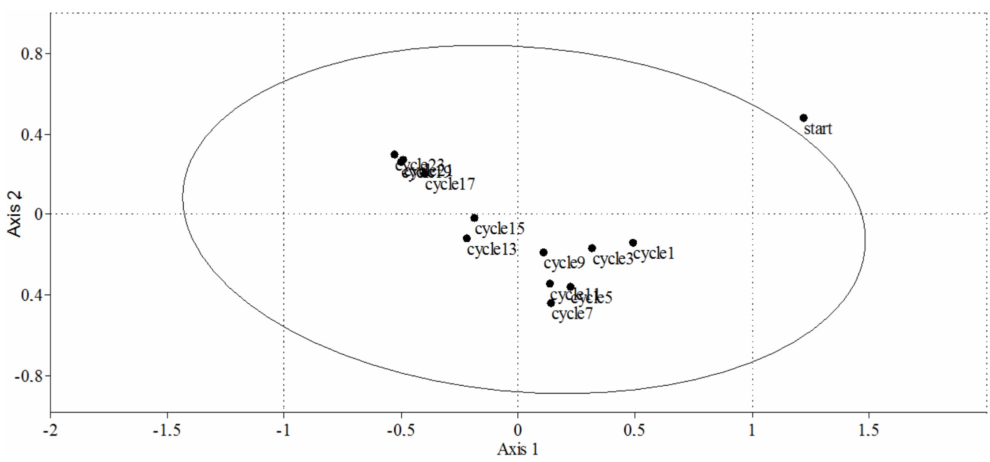

Fig. 7. CCA ordination diagram with the first two axes for the 0(start)-23 cycles data (environmental variables are not shown)

Moreover, between the transient period and adapted sludge, there is a state of transition, which lasts during cycles 13-15, *ie* two days. Hereby, the adapted sludge can be observed starting from cycle 17, *ie* on the ninth day after the beginning of the adaptation process.

Comparison of species diversity at the different stages of adaptation gave the following results.

Analysis of the protozoan structure with the method of rarefaction showed that the maximum potential number of species in a treatment plant at the moment of sampling was about 28 with total ciliate abundance 1000 ind./cm^3 (Fig. 8A). An increase in the number of expected species up to 30 at the same 1000 ind./cm^3 was observed on the basis of the average values during the period of adaptation from cycle 1 to cycle 11 (Fig. 8B).

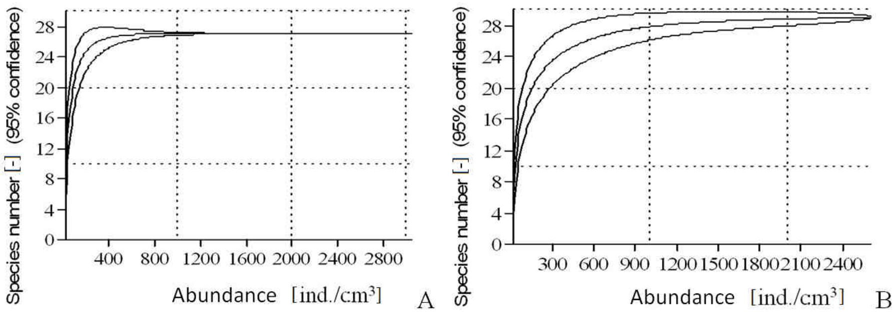

Fig. 8. The rarefaction curves show the potential number of species with 1000 ind./cm^3 ciliate abundance in the treatment plant at the moment of sampling (A); average values of ciliate abundance during the period of adaptation from cycle 1 to cycle 11 (B)

A possible reason is that the populations of the present species did not react to the emerging conditions for a new structure of the assembly and retained the same quantities that they had in the conditions of Hajdow.

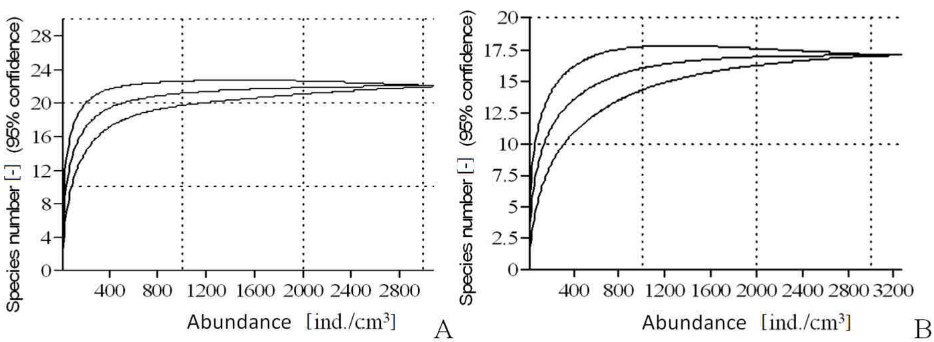

Fig. 9. The rarefaction curves show ciliate diversity during the period from cycle 13 to 15 at the abundance of 1000 ind./cm^3 (A); the average values of ciliate abundance for the period of cycles 17-23 (B)

As shown by the rarefaction curves, the ciliate diversity during the period from cycle 13 to 15 remained at the level of 20 at the abundance of 1000 ind./cm^3 (Fig. 9A).

Based on the average values of ciliate abundance for the period of cycles 17-23, the rarefaction curves showed diversity close to 15 species (Fig. 9B).

Obviously, the process of activated sludge stabilization is accompanied by harmonization of the conditions, and the structure of ciliate assembly remains at the level of species diversity lower than the starting one.

Thus, it was observed that the protozoan assembly of activated sludge from the sewage treatment plant in the conditions of a small-volume SBR undergoes structural changes because of simplification, universalization, and unification of the living space and factorial determination that reduces the number of potential eco niches. This makes the structural reorganization of ciliate assemblies a significant criterion of activated sludge adaptation.

It is obvious that later, together with sludge ageing, its structure will undergo changes. This might be a subject of a further investigation of great interest.

Conclusions

- In the process of adaptation, reduction is observed in the number of ciliate species together with an increase in the total assembly abundance.
- A decrease in the Shannon index from 3 to 1 was observed.
- During the adaptation time, the equitability in the ciliate assembly falls and the dominance increases.
- On the basis of the structural changes in the assembly, it was demonstrated that the process was composed of two stages - a transient period and a stable period.
- The transient period lasted from 11 to 15 cycles.
- During the transient period, reduction of ammonium utilization efficiency down to 50% and its subsequent increase in the stable period up to 80% was observed.
- In the Hajdow wastewater treatment plant, a decrease in the ciliate species number from 28 to 15 in the stable period was observed during the adaptation process.

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.

The work was presented at International Scientific and Technical Conference Water Treatment Technologies - Technical, Biological and Ecological Aspects, Kyiv 3-5 December 2013.

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BIOINDYKACYJNA OCENA ADAPTACJI OSADU CZYNNEGO W WARUNKACH EKSPERYMENTU LABORATORYJNEGO

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Abstrakt: Zaprezentowano badania dotyczące procesu adaptacji osadu czynnego pobranego w miejskiej oczyszczalni ścieków Hajdów (Lublin, Polska) do warunków pracy w laboratoryjnym bioreaktorze typu SBR. Jako kryterium wspomnianej adaptacji (wpracowania) przyjęto parametry struktury zbiorowiska orzęsków osadu czynnego. Podczas eksperymentu w analizowanym osadzie czynnym zidentyfikowano 32 gatunki orzęsków, zaś bazując na ich liczebności, analizowano zmiany następujące w strukturze zbiorowiska w kolejnych etapach badań. Podczas etapu adaptacji obserwowano redukcję liczby gatunków następującą równocześnie ze wzrostem liczebności całego zbiorowiska orzęsków, stąd też w kolejnych dniach widoczny był spadek wartości indeksu różnorodności Shannona i indeksu równomierności. Podczas adaptacji osadu zaobserwowano wyraźne rozgraniczenie struktury zbiorowiska orzęsków - niestabilnej etapu przejściowego oraz struktury stabilnej. W trakcie okresu przejściowego adaptacji zaobserwowano także znaczny spadek efektywności usuwania azotu amonowego - do poziomu 50%, a potem jej sukcesywny wzrost do stabilnego poziomu 80%. W pierwszym ze wspomnianych etapów indeks dominacji Simpsona przyjmował niskie wartości, natomiast w drugim wyraźnie wzrastał. Dla temperatury 10°C okres przejściowy trwał od sześciu do dziewięciu dni, po tym okresie zbiorowisko orzęsków osadu czynnego cechowało się obniżonym poziomem różnorodności. Opracowanie danych za pomocą metody rarefakcji wykazało, że w trakcie okresu adaptacji osadu czynnego do warunków pracy w bioreaktorze laboratoryjnym liczba potencjalnych niszy ekologicznych dla orzęsków osiągała poziom 30, natomiast dla osadu o stabilnej strukturze po okresie adaptacji poziom z zakresu 15-20.

Słowa kluczowe: bioindykacja, osad czynny, zbiorowiska orzęsków, indeksy biocenotyczne, adaptacja, bioreaktor typu SBR, eksperyment laboratoryjny