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COMMUNITY COMPOSITION AND ABUNDANCE OF PROTOZOA UNDER DIFFERENT CONCENTRATION OF NITROGEN COMPOUNDS AT “HAJDOW” WASTEWATER TREATMENT PLANT

ZBIOROWISKA PIERWOTNIAKÓW I ICH LICZEBNOŚĆ W OCZYSZCZALNI ŚCIEKÓW „HAJDÓW” PRZY RÓŻNYCH STĘŻENIACH ZWIĄZKÓW AZOTU

Abstract: The protists notably ciliates and testate amoebas in the conditions of modernized wastewater treatment plants “Hajdow” (Lublin, Poland) and the nitrogen removal efficiency were studied. Sampling took place every week during the period of August-September 2010. The total of 31 taxa of ciliates and 3 taxa of testate amoebas were identified. Most of the species were registered during the whole research period. Significant abundance reached the following species: *Acinertia uncinata*, *Aspidisca cicada*, *Aspidisca lynceus*, *Vorticella aquadulcis*, *Vorticella infusionum*, *Arcella vulgaris*, *Euglypha acanthophora* and *Pyxidicula operculata*. In September increase in efficiency of nitrogen removal process was registered. The research shows that the increase of nitrogen reduction efficiency was accompanied by increase of ciliates quantity, mainly due to peritrichid ciliates.

Keywords: activated sludge, wastewater treatment plants, sewage purification, ciliates, testate amoeba, nitrogen compounds

Introduction

Realized through bacteria activity, the utilization processes of organic matters, that domestic sewage contains, run with the help of large number of prokaryotic organisms, most abundant among them are Ciliata, Amoeba, Flagelata, Rotifera, Nematoda and Tardigrada [1-4]. Among organisms listed, the highest level of abundances is achieved by protozoa, at the same time; they remain the required component of the activated sludge biocoenosis [5-8]. Protozoa is of a primary importance through the all stages of sewage treatment,

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starting from process of activated sludge flocks forming [8-12]. As bacteria consumer, protozoa participates in processes of organic matters destruction and at the same time stimulates the growth of bacterial populations, supporting their biochemical activity [13-16]. Obligatory presence and quite high ecological plasticity of this group in conjunction with species variety makes them good indicators of processes in bioreactor chambers [17-20]. However, regularly, the control of treatment quality is realized by means of controlling the effectiveness of several chemical matters degradation, nitrogen compounds in the first place [21]. According to control of the activated sludge flock properties - size, shape or sedimentation properties, the classic methods containing the microscopic analysis as well as automated methods e.g. laser diffraction may be applied [22-25].

In spite of well-studied mechanism of bacterial breakdown of nitrogen compounds, the effectiveness of this process depends also on many factors, including the eukaryotic organisms activity and first of all protozoan activity [14, 26-31]. On account of this, the process of nitrogen compounds removal (nitrification and denitrification process) as a component of general metabolic activity in bioreactors is under careful examination [8, 32-38]. In turn, various factors, interacting with each other in bioreactors, are able to considerably affect the structure of microflora, protozoa, and other hydrobionts [39, 40]. The changes in activated sludge organisms structure can significantly impact the *N*-removal efficiency [41, 42].

Existing interconnections between the efficiency of sewage treatment and organisms structure in activated sludge on the one hand and undeveloped mechanisms of effective control over these structures on the other hand, make researches of activated sludge biological component dynamics very urgent and topical. In this work was studied the connection between nitrogen compounds concentration in advanced bioreactor and the composition of activated sludge organisms - testate amoebas and ciliates, which are commonly used as bioindicators [19].

Materials and methods

The study was conducted at WWTP "Hajdow" in Lublin (Poland). This plant apply the cycle, for *N*-removal, alternating aerobic and anoxic zones to develop nitrifying/denitrifying bacterial populations.

Samples were collected once a week during two months period (August and September) in 2010. Activated sludge samples for microscopic examination were collected from all tracks of bioreactors from the beginning to the final processes: station 1 - inlet into bioreactor; station 2 - outlet from denitrification chamber; station 3 - outlet from nitrification chamber; station 4 - outlet from denitrification chamber II; station 5 - outlet from nitrification chamber II (outlet from bioreactor). The sludge samples were collected using a Ruttner bathometer 1 m below the surface of sewage. Containers with samples of activated sludge were at least half filled to avoid oxygen limitation, and then were transferred to the laboratory in bag-refrigerator. Samples of influent and effluent were also collected, before and after the biological treatment for analysis of physical-chemical parameters.

Samples were processed immediately after the arrival to the laboratory. Removal efficiency data [%] were calculated from the measured influent and effluent physical-

chemical parameters. The content of nitrogen forms in sewage was determined by the spectrophotometer HACH DR 2800 in the research laboratory of Lublin University of Technology, Poland, with the use of individual techniques of light absorption emission for each kind of nitrogen.

The frequency and abundance were calculated for ciliates and testate amoeba species. Number of protozoa has been assessed in subsamples with 50 microliter capacity. It was calculated depending on the category of abundance. Protozoa, which quantity reached 20 representatives per 50 microliter (*ie* one specimen per one passage of objective lens over 18x18 mm coverglass) were counted in 3 subsamples. Protozoa with quantities of 10-20 specimens per 50 microliter, were counted in 5 subsamples. Calculation of small population protozoa - less than 10 specimens per 50 microliter was made in 7 subsamples. In 7 sub-samples were counted also colonial protozoa. The colonies number as well as number of zooids in colony were registered. All ciliated protozoa were identified on living material with a microscope at appropriate magnification, although when necessary silver staining method was used [43]. In calculations of testate amoebas only living cells were applied. Subsamples data were averaged out.

Several guides were used for identification [44-52]. Relationship between considered groups of protista and chemical compounds were explored through correlation analysis. For statistical analysis STATISTICA 8.0 and CANOCO 4.5 software was used.

Results and discussion

As researches showed, the N-removal efficiency at “Hajdow” WWTP is quite high. Mean values of N - NH₄⁺ efficiency of ammonium compounds utilization are presented in Table 1. Nitrogen N-NH₄⁺ utilization reached the level of approximately 70% in August and 80% in September. Such an increase of utilization efficiency occurred on the background of temperature fall of activated sludge by 3°C (±1.5°C) at an average. Herein should be noted that described N-removal efficiency is pertained to the concentration of N-NH₄⁺ at the inflow and the outflow of the bioreactor, not a whole WWTP. Also this efficiency is counted for dynamic value at the moment of sampling, not a proportional daily-average.

Table 1
Means and standard deviation (SD) of ammonium compounds removal efficiency at WWTP “Hajdow”

August	Efficiency [%]	September	Efficiency [%]
06.08.10	75.26	02.09.10	79.01
13.08.10	66.35	08.09.10	72.59
20.08.10	66.84	15.09.10	84.52
27.08.10	78.19	29.09.10	89.78
Mean	71.02	Mean	81.73
SD	5.97	SD	7.38

The number of ciliates and testate amoebas taxa, detected in bioreactor in August and September did not differ notably ($t = -1.55$; $p = 0.19$), but their abundance in September increased comparing with August ($t = -4.39$; $p = 0.01$). Mean number of taxa on different treatment stages in bioreactor in compared periods is showed in Figure 1 and the mean abundance in Figure 2.

The total 31 taxa of ciliates and 3 taxa of testate amoebas were registered during the research period (Table 2).

Table 2

Mean and standard deviation (SD) of abundance of ciliates and testate amoebas (indiv./cm³) in activated sludge from WWTP "Hajdow". Trophic groups: Ba - bacterivorous, P - predator, Fl - consumer of heterotrophic flagellates. Ecological groups: A - attached, Cr - crawling, Sw - free-swimming

Taxa	Trophic group	Ecological group	August		September		August - September	
			Mean	SD	Mean	SD	Max	Min
Ciliates								
<i>Acinera uncinata</i> Tucolesco, 1962	Ba	Cr	1029	711	1343	550	2617	150
<i>Acineta fluviatilis</i> Stokes 1885	P	A	-	-	47	42	53	4
<i>Acineta ornata</i> Sand, 1899	P	A	62	44	-	-	117	5
<i>Acineta tuberosa</i> (Pallas, 1766)	P	A	101	152	41	30	600	7
<i>Amphileptus pleurosigma</i> (Stokes, 1884)	P	Cr	94	9	-	-	100	87
<i>Aspidisca cicada</i> (Mueller, 1786)	Ba	Cr	766	490	691	328	2400	150
<i>Aspidisca lynceus</i> (Mueller, 1773)	Ba	Cr	211	169	556	371	1467	17
<i>Carchesium polypinum</i> (Linnaeus, 1758)	Ba	A	755	888	86	52	2250	33
<i>Chilodonella uncinata</i> (Ehrenberg, 1838)	Ba	Cr	349	249	291	265	967	43
<i>Cinetochilum margaritaceum</i> (Ehrenberg, 1831)	Ba	Sw	-	-	51	4	53	48
<i>Dendrosoma radians</i> Ehrenberg, 1837	P	A	15	3	-	-	17	13
<i>Epistylis chrysemidis</i> Bishop & Jahn, 1941	Ba	A	-	-	141	111	267	33
<i>Epistylis coronata</i> Nusch, 1970	Ba	A	139	92	164	223	783	17
<i>Epistylis entzii</i> Stiller, 1935	Ba	A	-	-	206	92	267	100
<i>Epistylis plicatilis</i> Ehrenberg, 1831	Ba	A	161	161	245	232	883	28
<i>Euplotes affinis</i> (Dujardin, 1841)	Ba, Fl	Cr	99	73	52	36	223	5
<i>Holophrya discolor</i> Ehrenberg, 1833	P	Sw	56	30	44	13	100	17
<i>Litonotus fusidens</i> (Kahl, 1926)	P	Cr	182	318	19	11	900	5
<i>Litonotus lamella</i> (Mueller, 1773)	P	Cr	49	40	36	24	150	3
<i>Opercularia articulata</i> Goldfuss, 1820	Ba	A	429	387	205	135	1167	7
<i>Opercularia coarctata</i> (Claparède & Lachmann, 1858)	Ba	A	-	-	150	24	167	133
<i>Opercularia minima</i> Kahl, 1935	Ba	A	216	235	79	33	627	17
<i>Plagiocampa rouxi</i> Kahl, 1926	Ba	Sw	46	30	40	19	113	8
<i>Tokophrya lemnaeum</i> (Stein, 1859)	P	A	-	-	56	4	61	53
<i>Tokophrya quadripartita</i> (Claparède & Lachmann, 1859)	P	A	78	10	-	-	86	71
<i>Uronema nigricans</i> (Mueller, 1786)	Ba	Sw	-	-	50	5	53	46
<i>Vorticella aquadulcis</i> -Complex	Ba	A	357	299	2812	3168	11733	33
<i>Vorticella convallaria</i> -Complex	Ba	A	244	182	19	13	600	8
<i>Vorticella elongata</i> Fromentel, 1874	Ba	A	79	53	167	82	233	42
<i>Vorticella infusionum</i> -Complex	Ba	A	208	171	1134	1929	8333	17
<i>Vorticella microstoma</i> -Complex	Ba	A	168	98	137	121	467	17
Testate amoebas								
<i>Arcella vulgaris</i> Ehrenberg, 1832	Ba	Cr	4751	3301	4118	3082	10490	667
<i>Euglypha acanthophora</i> (Ehrenberg, 1838)	Ba	Cr	481	348	1025	516	2500	33
<i>Pyxidicula operculata</i> Ehrenberg, 1838	Ba	Cr	440	403	1475	1328	5167	63

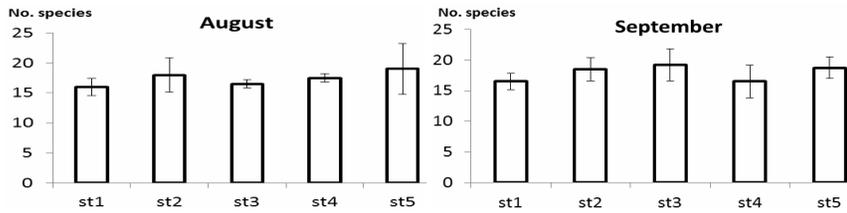


Fig. 1. Mean number and standard deviations of species at different stations in bioreactor

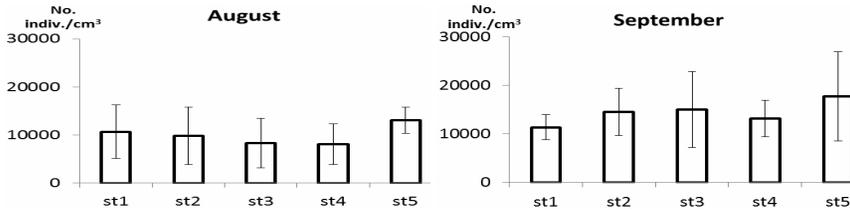


Fig. 2. Mean abundance and standard deviations of protists at different stations in bioreactor

During the whole research period the following populations were numerous: *Acinertia uncinata*, *Aspidisca cicada*, *A. lynceus*, *Chilodonella uncinata*, *Opercularia articulata*, *Vorticella aquadulcis*, *V. infusionum*, *Arcella vulgaris*, *Euglypha acanthophora* and *Pyxidicula operculata*. Also not numerous populations of *Epistylis coronata*, *E. plicatilis*, *Euplotes affinis*, *Holophrya discolor*, *Litonotus lamella*, *L. fusidens* were constantly registered.

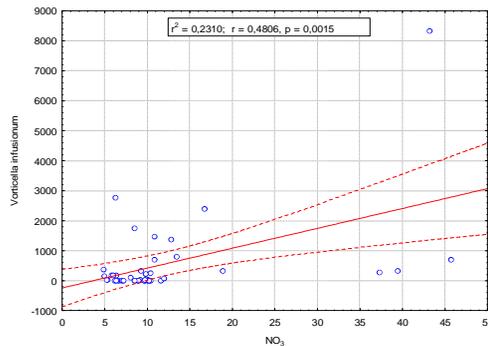


Fig. 3. Correlation of *Vorticella infusionum* abundance with nitrate concentration in activated sludge during the study

Some studies showed the dependence between flagellates (specifically bodonids), small amoebas and high level of nitrification. The same dependence with ciliates is still not proved [42]. Our researches also showed that there is no correlation between changes in concentration of nitrogen compounds and changes in quantity of most registered protozoa species. Just few species demonstrated weak correlation with nitrogen compounds

concentration. Positive correlation relationship with nitrate concentration had *Vorticella infusioformis* only (Fig. 3).

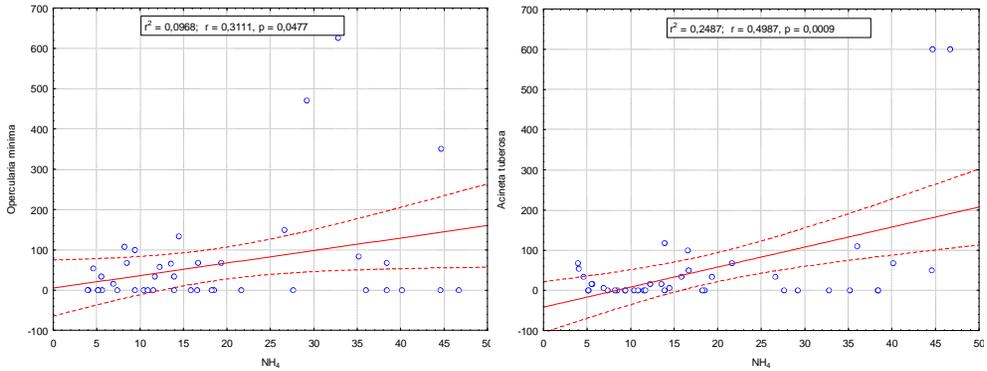


Fig. 4. Correlation of *Opercularia minima* and *Acineta tuberosa* abundance with ammonium concentration in activated sludge during the study

Positive correlation with ammonium showed *Opercularia minima* and *Acineta tuberosa* (Fig. 4). At the same time *Epistylis plicatilis* and *Euglypha acanthophora* showed negative correlation with ammonium (Fig. 5).

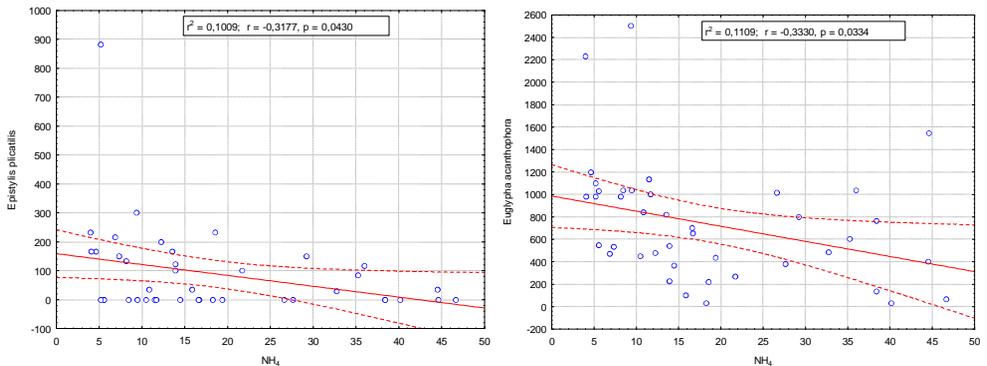


Fig. 5. Correlation of *Epistylis plicatilis* and *Euglypha acanthophora* abundance with ammonium concentration in activated sludge during the study

The graph made with CANOCO 4.0 software using CCA method based on the matrix of averaged quantities, demonstrates dependence of 4 ciliate species allocation on the rates of N- NH_4^+ and NO_3^- (Fig. 6). Variables were selected using a manual forward stepwise procedure. Only significant variables were included (Table 3).

According to the quantitative development and the ratio between crawling and attached forms of bacterivorous, activated sludge from WWT plants “Hajdow” relates to the 1st quality class in Sludge Biotic Index (SBI) [19], and is characterized as good colonized, stable and with high biological activity. During research period the proportion of taxa

number of crawling, free-swimming, and attached protozoa in activated sludge was quite stable (Fig. 7).

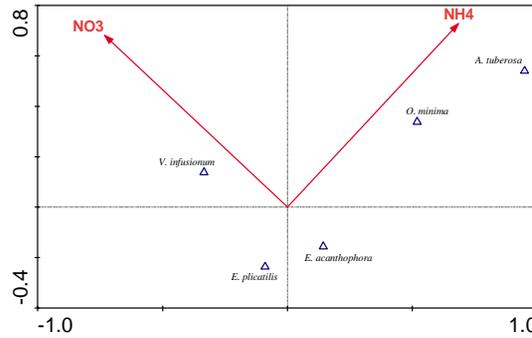


Fig. 6. CCA ordination plot for the 4 ciliate species and 1 testate amoeba - *Euglypha acanthophora* in the treatment process, using the environmental variables (parameters of $N-NH_4^+$ and NO_3^-) selected by forward stepwise procedure

Table 3

The variance ratios (F) and the significance (P) listed in order of manual forward selection by CANOCO

Variables	F	P
NH_4^+	5.987	0.002
NO_3^-	5.987	0.002
NO_2^-	0.377	0.806

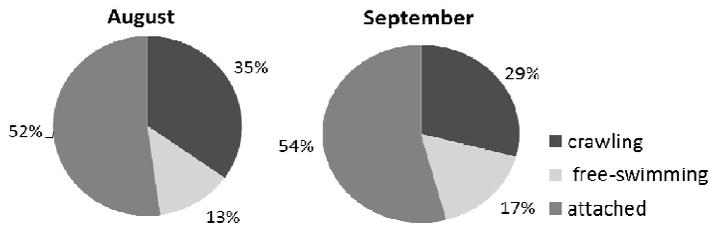


Fig. 7. Mean percentage of ciliates from different ecological groups (based on taxa number) in activated sludge

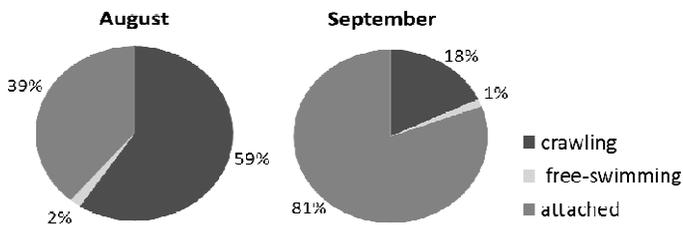


Fig. 8. Mean percentage of ciliates from different ecological groups (based on abundance) in sludge

At the same time the proportion of protozoa quantities from different ecological groups from August to September changed substantially. So, in August crawling forms prevailed (59%), and in September their share decreased by 18%. The changes in quantity of attached forms had the opposite tendency. In August their part came to 39%, and in September to 81% (Fig. 8).

The correlation analysis between ecological groups and nitrogen compounds concentration reveal no dependence. At the same time on the low level of correlation between the concentration of nitrogen compounds and trophic groups of protozoa was detected. Thus predators number showed weak positive connection with ammonium, bacterivorous - with nitrates (Fig. 9).

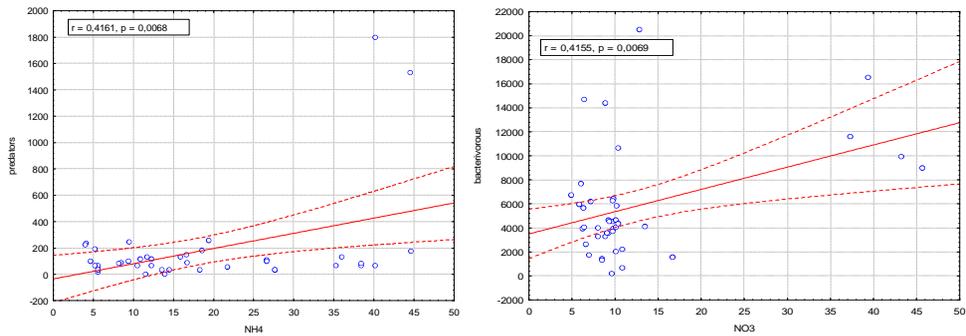


Fig. 9. Correlation between the ecological groups of protists and nitrogen compounds in activated sludge

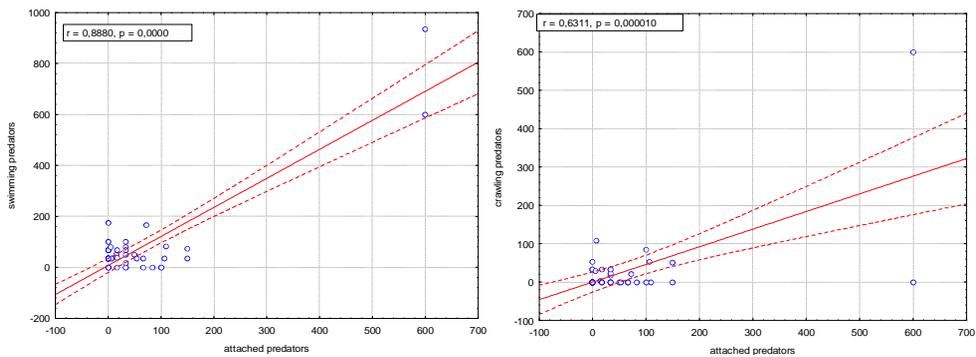


Fig. 10. Correlation between different ecological groups of predators in activated sludge

In spite of the different way of food acquisition, that different ecological groups of predators use, it was revealed the high positive correlation between their developments (Fig. 10).

Also high positive correlation was demonstrated by attached and crawling bacterivorous ciliates (Fig. 11).

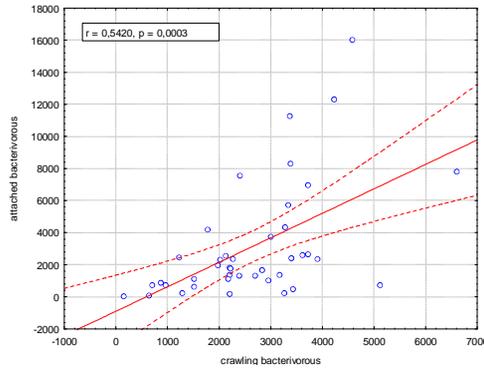


Fig. 11. Correlation between different ecological groups of bacterivorous ciliates in activated sludge

At the same time the correlation between predators and their prey - bacterivorous was lower. Predators from all ecological groups exhibited a positive correlation with the crawling bacterivorous only. With other possible preys no connection was found. At the Figure 12 the connection between free-swimming, crawling and sessile predators and the quantity of crawling bacterivorous, which were constantly present in the significant concentrations is shown.

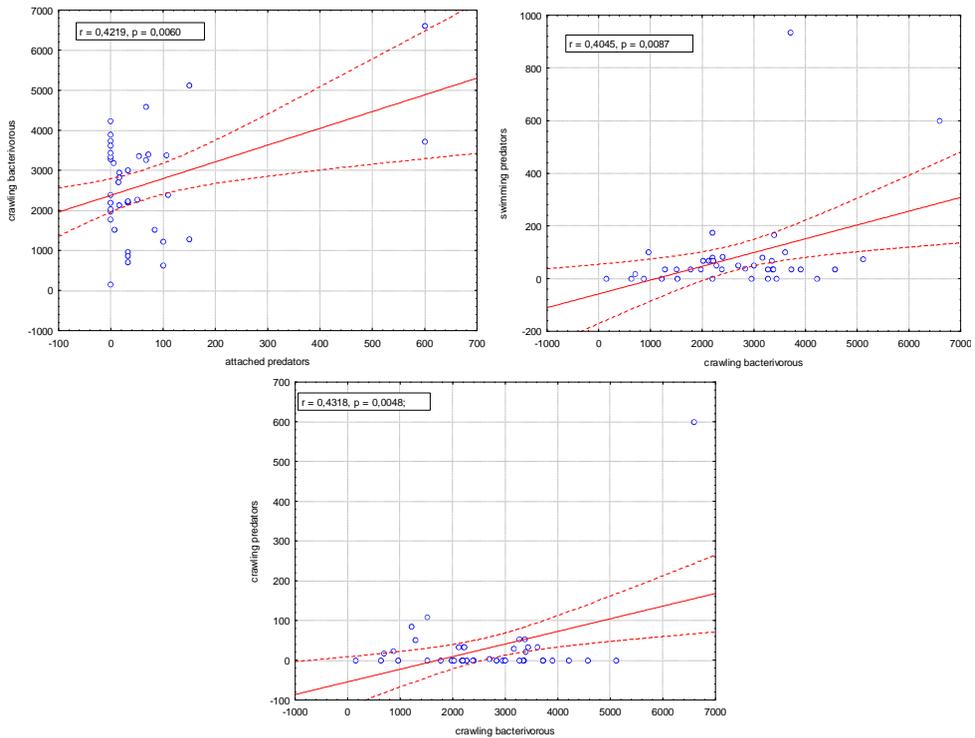


Fig. 12. Correlation between the predators and bacterivorous in activated sludge

Although in August and September the pollution level was the same, the effectiveness of purification process in September increased by 10% (Table 1). The increase of effectiveness was accompanied with considerable quantity increase of sessile bacterivorous, namely peritrichid ciliates (Ciliophora: Peritricha). This can be considered as proof of positive influence of nitrifying bacteria grazing on effectiveness of ammonium nitrogen utilization process [38]. Whereas it is quite complicated to interpret the reasons of Peritricha quantity surge inside the community that exist in stable environment and with no food deficiency. It is interesting, that phenomenon of periodical spring and autumn quantity surges of sessile Peritricha and other ciliates are characteristic for natural water bodies [53-55]. Perhaps, the increase of sessile Peritricha quantity in activated sludge is caused by its their seasonal biological rhythm.

Conclusions

Research reveals that the efficiency of ammonium removal process was definitely higher when the domination of sessile bacterivorous forms was observed. A relationship between changes in quantity of some protozoa populations, their ecological groups and changes in concentrations of certain nitrogen compounds in activated sludge was also noted.

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References

- [1] Kinner NE, Curds CR. Development of protozoan and metazoan communities in rotating biological contactor biofilms. *Water Res.* 1987;21(4):481-490. DOI: 10.1016/0043-1354(87)90197-7.
- [2] Lee NM, Welander T. Use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment. *Biotechnol Lett.* 1996;18(4):429-434. DOI: 10.1007/BF00143465.
- [3] Selivanovskaya SY, Petrov AM, Egorova KV, Naumova RP. Protozoan and metazoan communities treating a simulated petrochemical industry wastewater in a rotating disc biological reactor. *World J Microbiol and Biotechnol.* 1997;13(5):511-517. DOI: 10.1023/A:1018505107030.
- [4] Martín-Cereceda M, Pérez-Uz B, Serrano S, Guinea A. Dynamics of protozoan and metazoan communities in a full scale wastewater treatment plant by rotating biological contactors. *Microbiol Res.* 2001;156(3):225-238. DOI: 10.1078/0944-5013-00105.
- [5] Madoni P. Estimates of ciliated protozoa biomass in activated sludge and biofilm. *Bioresour Technol.* 1994;48(3):245-249. DOI: 10.1016/0960-8524(94)90153-8.
- [6] Łagód G, Chomczyńska M, Montusiewicz A, Malicki J, Bieganski A. Proposal of measurement and visualization methods for dominance structures in the saprobe communities. *Ecol Chem Eng S.* 2009;16(3):369-377.
- [7] Chomczyńska M, Montusiewicz A, Malicki J, Łagód G. Application of saprobes for bioindication of wastewater quality. *Environ Eng Sci.* 2009;26(2):289-295. DOI:10.1089/ees.2007.0311.
- [8] Jaromin K, Babko R, Łagód G. Abundances of protozoa on particular devices of "Hajdow" WWTP on the background of the nitrogen compounds concentration. *Proc ECOpole.* 2010;4(2):403-408.
- [9] Curds CR. The role of protozoa in the activated-sludge process. *Amer Zool.* 1973;13(1):161-169.
- [10] Arregui L, Serrano S, Linares M, Pérez-Uz B, Guinea A. Ciliate contributions to bioaggregation: laboratory assays with axenic cultures of Tetrahymena thermophila. *Internat Microbiol.* 2007;10:91-96.
- [11] Arregui L, Linares M, Pérez-Uz B, Guinea A, Serrano S. Involvement of crawling and attached ciliates in the aggregation of particles in wastewater treatment plants. *Air Soil Water Res.* 2009;1:13-19.

- [12] Wilén BM, Lumley D, Mattsson A, Mino T. Relationship between floe composition and flocculation and settling properties studied at a full scale activated sludge plant. *Water Res.* 2008;42:4404-4418.
- [13] Curds CR. A theoretical study of factors influencing the microbial population dynamics of the activated-sludge process - I The effects of diurnal variations of sewage and carnivorous ciliated protozoa. *Water Res.* 1973;7(9):1269-1284.
- [14] Güde H. Grazing by protozoa as selection factor for activated sludge bacteria. *Microbial Ecol.* 1979;5:225-237. DOI: 10.1007/BF02013529.
- [15] Jürgens K, Pernthaler J, Schalla S, Amann R. Morphological and compositional changes in a planktonic bacterial community in response to enhanced protozoan grazing. *Appl and Environ Microbiol.* 1999;65(3):1241-1250.
- [16] Rønn R, McCaig AE, Griffiths BS, Prosser JI. Impact of protozoan grazing on bacterial community structure in soil microcosms. *Appl and Environ Microbiol.* 2002;68(12):6094-6105. DOI: 10.1128/AEM.68.12.6094-6105.2002.
- [17] Curds CR, Cockburn A. Protozoa in biological sewage-treatment processes - II. protozoa as indicators in the activated-sludge process. *Water Res.* 1970;4(3):237-249.
- [18] Al-Shahwani SM, Horan NJ. The use of protozoa to indicate changes in the performance of activated sludge plants. *Water Res.* 1991;25(6):633-638. DOI: 10.1016/0043-1354(91)90038-R.
- [19] Madoni P. A sludge biotic index (SBI) for evaluation of activated sludge plants based on the microfauna analysis. *Water Res.* 1994;28:67-75. DOI: 10.1016/0043-1354(94)90120-1.
- [20] Martín-Cereceda M, Serrano S, Guinea A. A comparative study of ciliated protozoa communities in activated-sludge plants. *FEMS Microbiol Ecol.* 1996;21(4):267-276. DOI: 10.1111/j.1574-6941.1996.tb00123.x.
- [21] Salvado H, Gracia MP, Amigó JM. Capability of ciliated protozoa as indicators of effluent quality in activated sludge plants. *Water Res.* 1995;29(4):1041-1050. DOI: 10.1016/0043-1354(94)00258-9.
- [22] Eikelboom DH, van Buijsen HJJ. *Microscopic Sludge Investigation Manual*. 1st edition (in Polish). Szczecin: Sejdel-Przywecki; 1999.
- [23] Liwarska-Bizukojc E. Application of image analysis techniques in activated sludge wastewater treatment process. *Biotechnol Lett.* 2005;27:1427-1433. DOI: 10.1007/s10529-005-1303-2.
- [24] Hopkins BM. A quantitative image analysis system. *Opt Eng.* 1976;15:236-240.
- [25] Bieganowski A, Łągód G, Ryzak M, Montusiewicz A, Chomczyńska M, Sochan A. Measurement of activated sludge particle diameters using laser diffraction method. *Ecol. Chem. Eng. S* 2012;19(4):597-608. DOI: 10.2478/v10216-011-0042-7.
- [26] Kinner NE, Curds CR, Meeker LD. Protozoa and metazoan as indicators of treatment efficiency in rotating biological contactors. *Water Sci Technol.* 1989;20:199-2004.
- [27] McClure NC, Fry JC, Weightman AJ. Survival and catabolic activity of natural and genetically engineered bacteria in a laboratory-scale activated-sludge unit. *Appl Environ Microbiol.* 1991;57(2):366-373.
- [28] Salvadó H, Gracia MP. Determination of organic loading rate of activated sludge plants based on protozoan analysis. *Water Res.* 1993;27(5):891-895. DOI: 10.1016/0043-1354(93)90154-A.
- [29] Forney LJ, Liu WT, Guckert JB, Kumagai Y, Namkung E, Nishihara T, Larson RJ. Structure of microbial communities in activated sludge: potential implications for assessing the biodegradability of chemicals. *Ecotoxicol and Environ Safety.* 2001;49(1):40-53. DOI: 10.1006/eesa.2001.2034.
- [30] Lee S, Basu S, Tyler CW, Wei IW. Ciliate populations as bio-indicators at Deer Island treatment plant. *Advances in Environ. Res.* 2004;8:371-378. DOI: 10.1016/S1093-0191(02)00118-1.
- [31] Moussa MS, Hooijmans CM, Lubberding HJ, Gijzen HJ, van Loosdrecht MCM. Modelling nitrification, heterotrophic growth and predation in activated sludge. *Water Res.* 2005;39(20):5080-5098. DOI: 10.1016/j.watres.2005.09.038.
- [32] Ratsak CH, Maarsen KA, Kooijman SALM. Effects of protozoa on carbon mineralization in activated sludge. *Water Res.* 1996;30:1-12. DOI: 10.1016/0043-1354(95)00096-4.
- [33] Lee NM, Oleszkiewicz JA. Effects of predation and ORP conditions on the performance of nitrifiers in activated sludge systems. *Water Res.* 2003;37:4202-4210. DOI: 10.1016/S0043-1354(03)00341-5.
- [34] Petropoulos P, Gilbride KA. Nitrification in activated sludge batch reactors is linked to protozoan grazing of the bacterial population. *Can J Microbiol.* 2005;51(9):791-799. DOI: 10.1139/W05-069.
- [35] Gerardi MH. *Wastewater Bacteria*. New Jersey: John Wiley and Sons, Inc., Hoboken; 2006:77-101.
- [36] Pogue AJ, Gilbride KA. Impact of protozoan grazing on nitrification and the ammonia- and nitrite-oxidizing bacterial communities in activated sludge. *Can J Microbiol.* 2007;53(5):559-571. DOI: 10.1139/W07-027.
- [37] Pérez-Uz B, Arregui L, Calvo P, Salvadó H, Fernández N, Rodriguez E, Zornaza A, Serrano S. Efficiency of nitrogen removal and protist communities: the potential for Introduction of novel biological index. *Proc of*

- the Int Wastewater Treatment, Monitoring and Reclamation: Key Role Played by the Waste Water Treatment Plant. ISIRIM/ LIFE, 2009:1-9.
- [38] Pajdak-Stós A, Fiałkowska E, Fyda J, Babko R. Resistance of nitrifiers inhabiting activated sludge to ciliate grazing. *Water Sci Technol.* 2010;61(3):573-580. DOI: 10.2166/wst.2010.868.
- [39] Madoni P, Ghetti PF. The structure of Ciliated Protozoa communities in biological sewage-treatment plants. *Hydrobiology* 1981;83(2):207-215. DOI: 10.1007/BF00008268.
- [40] Stasinakis AS, Thomaidis N, Mamais D, Papanikolaou EC, Tsakon A, Lekkas TD. Effects of chromium (VI) addition on the activated sludge process. *Water Res.* 2003;37(9):2140-2148. DOI: 10.1016/S0043-1354(02)00623-1.
- [41] Madoni P, Davoli D, Chierici E. Comparative analysis of the activated sludge microfauna in several sewage treatment works. *Water Res.* 1993;27(9):1485-1491. DOI: 10.1016/0043-1354(93)90029-H.
- [42] Pérez-Uz B, Arregui L, Calvo P, Salvadó H, Fernández N, Rodríguez E, Zornoza A, Serrano S. Assessment of plausible bioindicators for plant performance in advanced wastewater treatment systems. *Water Res.* 2010;44:5059-5069. DOI: 10.1016/j.watres.2010.07.024.
- [43] Foissner W. Basic light and scanning electron microscopic methods for taxonomic studies of Ciliated Protozoa. *Europ J Protistol.* 1991;27:313-330.
- [44] Kahl A. *Urtiere Oder Protozoa: Wimpertiere oder Ciliata (Infusoria).* Die Tierwelt Deutschlands, Jena, G.Fischer, 18, 21, 25, 30, 1930-1935.
- [45] Stiller J. Szájkoszorús Csillóskó-Peritricha. *Fauna Hung.* 1971;105:1-245.
- [46] Foissner W, Blatterer H, Berger H, Kohmann F. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band I: Cyrtophorida, Oligotrichida, Hypotrichida, Colpodea.- Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 1991;1(91):478-478.
- [47] Foissner W, Berger H, Kohmann F. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band II: Peritrichida, Heterotrichida, Odontostomatida.- Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 1992;5(92):502-502.
- [48] Foissner W., Berger H. and Kohmann F.: Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band III: Hymenostomata, Prostomatida, Nassulida.- Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 1994;1(94):548-548.
- [49] Foissner W, Berger H, Blatterer H, Kohmann F. Taxonomische und ökologische Revision der Ciliaten des Saprobiensystems - Band IV: Gymnostomatea, Loxodes, Suctoria.- Informationsberichte des Bayer. Landesamtes für Wasserwirtschaft. 1995;1(95):540-540.
- [50] Foissner W, Berger H. A user-friendly guide to the ciliates (Protozoa, Ciliophora) commonly used by hydrobiologists as bioindicators in rivers, lakes, and waste waters, with notes on their ecology. *Freshwater Biol.* 1996;35:375-482.
- [51] Lee JJ, Leedale GF, Bradbury P. *The illustrated guide to the protozoa.* Society of Protozoologists, 2nd ed. Allen Press. Inc., 2000.
- [52] Adl SM, Simpson AGB, Farmer VA, Andersen RA, Anderson OR, Barta JR, et al. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryotic Microbiol.* 2005;52(5):399-451. DOI: 10.1111/j.1550-7408.2005.00053.x.
- [53] Esteban G, Téllez C, Bautista LM. Dynamics of ciliated protozoa communities in activated-sludge process. *Water Res.* 1991;25:967-972. DOI: 10.1016/0043-1354(91)90145-G.
- [54] Babko R, Łagód G, Jaromin-Gleń KM. Abundance and structure of ciliated protozoa community at the particular devices of "Hajdów" WWTP. *Ann. Set Environ. Protect.* 2012;14:56-68.
- [55] Babko RV, Kuzmina TM. Ciliata (Protista, Ciliophora) of Epiphyton of Higher Aquatic Plants in a Small River. *Hydrobiol J.* 2004;40(4):22-38.

ZBIOROWISKA PIERWOTNIAKÓW I ICH LICZEBNOŚĆ W OCZYSZCZALNI ŚCIEKÓW „HAJDÓW” PRZY RÓŻNYCH STĘŻENIACH ZWIĄZKÓW AZOTU

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Abstrakt: Przedstawiono analizę zgrupowań pierwotniaków obejmującą orzęski oraz ameby skorupkowe, zasiedlające kolejne urządzenia głównego ciągu technologicznego oczyszczalni ścieków „Hajdów” na tle zmian w stężeniach związków azotu. Badania prowadzono po modernizacji części biologicznej realizowanej w celu podniesienia skuteczności usuwania ze ścieków związków biogenych, a w szczególności związków azotu. Pobór próbek prowadzony był raz w tygodniu w okresie sierpień-wrzesień 2010 roku. W badanym materiale biologicznym wyodrębniono 31 gatunków orzęsków oraz 3 gatunki ameb skorupkowych. Większość ze wspomnianych gatunków pierwotniaków odnotowywana była przez cały okres badań. Najbardziej licznie w analizowanym materiale biologicznym reprezentowane były następujące gatunki: *Acinertia uncinata*, *Aspidisca cicada*, *Aspidisca lynceus*, *Vorticella aquadulcis*, *Vorticella infusionum*, *Arcella vulgaris*, *Euglypha acanthophora* i *Pyxidicula operculata*. We wrześniu odnotowany został wzrost skuteczności procesu usuwania związków azotu. Prezentowane badania wskazują, iż wzrostowi stopnia usuwania ze ścieków związków azotu towarzyszył wzrost ilości orzęsków odnoszący się głównie do form peritricha.

Słowa kluczowe: osad czynny, oczyszczalnie ścieków, orzęski, ameby skorupkowe, związki azotu