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CHANGES IN THE STRUCTURE OF ACTIVATED SLUDGE PROTOZOA COMMUNITY AT THE DIFFERENT OXYGEN CONDITION

ZMIANY W STRUKTURZE ZBIOROWISKA PIERWOTNIAKÓW OSADU CZYNNEGO W ZRÓŻNICOWANYCH WARUNKACH TLENOWYCH

Abstract: Several experiments were performed in the laboratory condition using an SBR bioreactor modelling the expected conditions, created by malfunction of certain bioreactor elements, thus the different oxygen condition. In the course of the experiments, the concentrations of ammonia nitrogen, nitrates(III), nitrates(V), TOC, and TC were systematically measured. Besides physico-chemical parameters, the structure of activated sludge community was analyzed. In the samples, the number and species composition of protozoa (ciliates) were determined. Each of the three measuring series conducted for various types of process conditions was repeated three times. The activated sludge used for inoculation of the bioreactor was sampled at Hajdow WWTP in Lublin. The results obtained are the average of three repetitions of every experimental series. On this ground, we may conclude that the number of ciliates shows a high correlation with the O_2 concentration, pH and TOC.

Keywords: SBR bioreactor, activated sludge, community, protozoa, wastewater purification, oxygen concentration

Bioreactors for wastewater purification by the activated sludge method used in laboratory conditions allow, beside experiments in the standard conditions, conducting tests that reflect extreme situations commonly affecting the process of wastewater treatment. However, these conditions result in an interesting influence on active sludge or biofilm organisms. Bioreactors with integrated removal of carbon, nitrogen, and phosphorus working in laboratory provide alternating anaerobic, anoxic, and aerobic conditions [1]. They also allow carrying out research under conditions that exploiters try to prevent at the wastewater treatment plants (WWTPs), as they may cause disturbances in biological

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treatment processes. These include emergencies, *eg* damage or shut down of stirring and aeration systems. Such situations rarely occur in devices on a technical scale. However, they may be characterized by a significant excess of the standard values and the most important process parameters. Therefore, they are interesting from a scientific point of view and allow obtaining information concerning the impact of selected factors upon activated sludge organisms. A series of experiments were conducted in the laboratory SBR bioreactor simulating various situations possible during malfunction of several elements of the bioreactor (mixing or aeration system). The temperature during the experiment was maintained in the range of 20-21°C. The concentrations of nitrites, nitrates, TOC, TC and pH were systematically measured.

In parallel with the physical and chemical indicators, the population of activated sludge microorganisms was studied. The species composition and the abundance of ciliates were determined [2, 3].

It is known that the protozoa communities in activated sludge can be treated as bioindicators of wastewater purification process and their community composition varies depending on many environmental factors [4-15]. Oxygen is one of the most important factors for activated sludge organisms. Their community consists of species that do not need high oxygen concentrations, but at the same time, they are usually not anaerobic. Therefore, emergencies in the aeration system accompanied by a decrease in the oxygen concentration affect the functioning of activated sludge biocoenosis.

For a majority of organisms, quantitative changes in populations are caused by the processes of reproduction and extinction. In the case of protozoa, the number of species in populations can also grow or fall due to encystment-excystment processes [16]. In response to different stimuli, many ciliates are able to develop different kinds of cysts, for example, resting, reproductive, *etc*. [16-20]. The cyst-phase in the protozoan life cycle can significantly expand their possibilities and positively influences the rate of their response to changes in habitat conditions as well as dynamics of quantitative changes in their populations [21, 22].

Each of the three series of experiments on the various process conditions was repeated tree times - starting from variable qualitative and quantitative characteristics of the activated sludge sampled in Lublin WWTP Hajdow. The results obtained are the average of three single series of measurements. The experiment was conducted using municipal sewage after the mechanical stage of the WWTP. The sampling point was located at the outlet of the primary settling tank.

Materials and methods

The samples of activated sludge used for inoculation of the bioreactor chambers were taken at the external recirculation channel in Hajdow WWTP, which purifies sewage from Lublin and Świdnik - cities of eastern Poland (daily discharge *Qd* mean year value ca. 60,000 m^3/day). The activated sludge was loaded into three thermostatically controlled SBR-type bioreactors with active capacity of each chamber 8 dm^3, where it was adapting for one month.

After adaptation in each of the tanks the following conditions were implemented:

Chamber 1. (Aeration chamber) Throughout the experiment, in the first tank the activated sludge was systematically stirred and enriched with oxygen. The concentration of oxygen was kept within the limits of 90% of saturation.

Chamber 2. (Mixing chamber) In the second tank of the bioreactor, the aeration was not performed, however, the activated sludge was constantly stirred. Apparently, due to the mixing, the oxygen saturation in the sludge was kept within limits of 0.5-1 mg/dm^3.

Chamber 3. (Control chamber) In the third tank, neither stirring nor aeration was performed. The activated sludge stayed as a sediment at the bottom of the tank. The activated sludge was only mixed at the time of biological material sampling. In the tank, three anoxic conditions were preserved.

The samples for the experiment were taken once a day. The samples for chemical analysis (in all three tanks) were taken simultaneously at the time when the aeration and stirring in the first tank were shut down and the activated sludge was accumulating at the bottom as a sediment. The biological samples for microscopic analysis were taken when the activated sludge in first and second tanks was being stirred. In the third tank, the activated sludge sediment was carefully mixed before sampling for proper evaluation of the number of organisms present in it.

The organisms were counted immediately after sampling. Calculations were carried out for 3-5 subsamples. When the number of organisms decreased, the number of calculations was extended to 7 subsamples. One subsample had a volume of 25 microliters. The results were averaged and brought to 1 milliliter (1 cm^3).

Besides the oxygen concentration in the SBR bioreactors, the concentrations of ammonium, nitrites, and nitrates were controlled. Measurements of the nitrogen compounds mentioned were performed using a HACH DR2800 spectrophotometer HACH-Lange. The analysis was based on the methodology developed by HACH-Lange cuvette tests. At the beginning of the experiment, the wastewater supply in the SBR tank was stopped as well as the supply of organic matter. Afterwards, the response of ciliates to cessation of organic supply in the various SBR operation conditions was studied.

Results and discussion

Since species react differently to the same environmental conditions, the study of the dynamics of active and encysted ciliate populations in nature could be very important in ecological researches. Thus, the quantitative data cumulated now cannot be generalized and must be evaluated one by one.

Before the beginning of the experiment, the composition of ciliates species was analyzed as well as the quantitative characteristics of their populations. The species present in the activated sludge and their average numbers are presented in Table 1.

Because of different physical-chemical parameters, changes in structures of organisms communities occur differently depending on analyzed bioreactor chamber. This confirms the possibilities of using analyzed groups of protozoa as bioindicating organisms [23-31].

Table 1
List of ciliates observed in the three chambers and their mean abundance (A) and relative frequencies [%] (F) (A = number of individuals per cm^3, F = percentage of samples containing the species)

Taxa	Chamber					
	1		2		3	
	A	F	A	F	A	F
Acineria uncinata Tucolesco, 1962	516.81	100	62.10	50	43.82	66.7
Acineta fluviatilis Stokes, 1885	0.48	8.3	0.48	8	0.48	8
Amphileptus fusidens Kahl, 1926	1.14	25	-	-	-	-
Aspidisca cicada (Müller, 1786)	165.19	100	74.29	50	75.25	83.3
Aspidisca lynceus (Müller, 1773)	0.95	16.7	0.48	8.3	0.48	8.3
Carchesium polypinum (Linnaeus, 1758)	12.10	50	0.48	8.3	0.48	8.3
Chilodonella uncinata (Ehrenberg, 1838)	94.57	100	8.57	25	10.00	25
Crossacineta ornata Sand 1899	0.95	8.7	0.95	8.3	0.95	8.3
Epistylis coronata Nusch, 1970	47.33	91.7	31.43	41.7	1.62	16.7
Epistylis entzii Stiller, 1935	31.57	41.7	-	-	-	-
Epistylis plicatilis Ehrenberg, 1831	49.71	75	-	-	-	-
Holophrya discolor Ehrenberg, 1834	17.72	66.7	8.57	33.3	12.95	75
Litonotus lamella (Müller, 1773)	5.6	66.7	-	-	1.42	16.7
Opercularia articulate Goldfuss, 1820	2.84	41.7	1.43	16.7	0.95	8
Opercularia coarctata (Claparède & Lachmann, 1858)	8.67	25	-	-	3.33	58.3
Opercularia minima Kahl, 1935	5.15	50	5.67	75	5.71	75
Plagiocampa rouxi Kahl, 1926	-	-	-	-	1.28	25
Podophrya sp.	3.14	33.3	-	-	2.36	41.7
Pseudovorticella elongata (Fromentel, 1876)	14.67	91.7	2.86	16.7	3.81	41.7
Tokophrya quadripartita (Claparède & Lachmann, 1859)	20.74	100	11.76	100	13.35	100
Uronema nigricans (Müller, 1786)	-	-	1.4	16.7	9.13	75
Vorticella aquadulcis-complex	51.45	91.7	18.10	16.7	26.72	50
Vorticella convallaria (Linnaeus, 1758)	2.76	25	-	-	-	-
Vorticella infusionum Dujardin, 1841	1.43	8.3	2.86	16.7	3.31	41.7
Vorticella octava-complex	1.42	16.7	0.48	8.3	2.86	41.7
Total	1056.4		231.8		222.1	

As indicated by the results of experiment, in the conditions of deficit in organics, the populations of ciliates respond to the aeration rate with an increase in abundance (Fig. 1). During the first 48 hours, growth of ciliate population's quantity was observed in the first tank of the SBR. Already on the fifth day, the number of ciliates fell and reached the initial level. By the end of the experiment, their general number had become four times lower than the initial quantity.

Concerning the organic matter in chamber 1, the content of NH_4 decreased tenfold on the first day and later the process of degradation thereof was held up. The decrease in the NH_4 concentration was naturally followed by an increase in NO_3. The slow reduction of the quantity of protozoa after two days obviously reproduced the rate of nutrition resource (bacteria) reduction. Within the ciliated protozoa community, bacteriophages dominated.

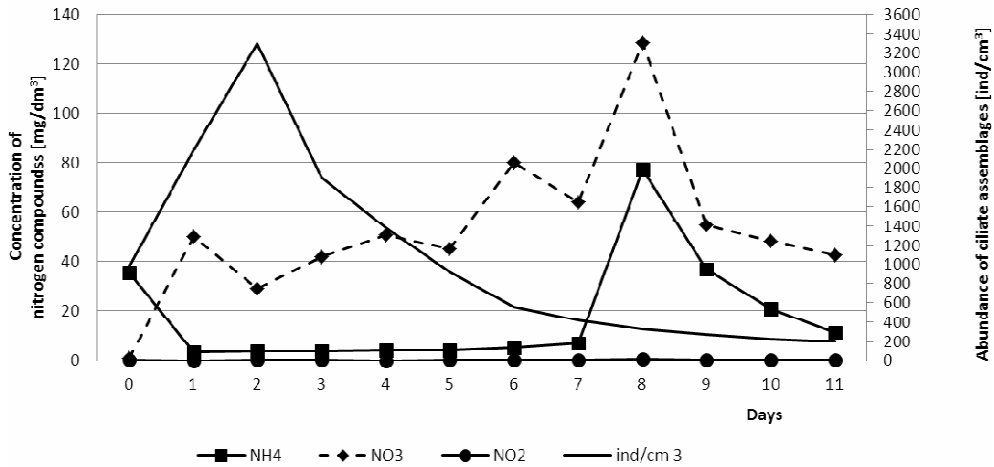

Fig. 1. Changes in the abundance of ciliates during the experiment compared with changes in the concentration of nitrogen compounds (chamber with mixing and air supply)

The ciliated protozoa in the conditions of chamber 2 were obviously not short of oxygen during the first 24 hours, which apparently provoked the initial phase of their quantitative growth (Fig. 2).

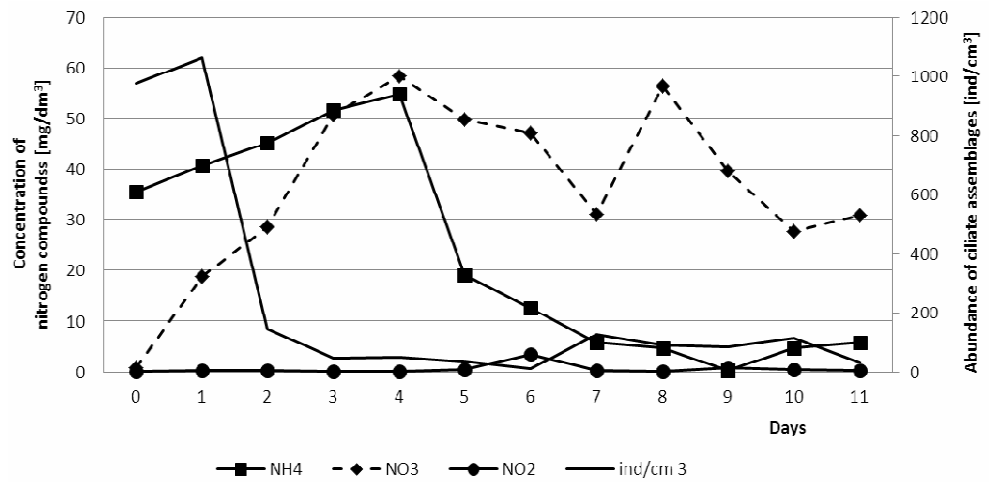

Fig. 2. Changes in the abundance of ciliates during the experiment compared with changes in the concentration of nitrogen compounds (chamber with mixing but without of air supply)

However, already during the next day, their number decreased to a level that the community usually reached on the tenth day in aerated chamber 1. Until the end of the experiment in chamber 2, the number of protozoa remained at the minimal level.

Obviously, the abrupt decrease in oxygen in the system not only affects protozoa but also suspends the anaerobic processes of organic decomposition, which is followed by slight retention of the high NH_4 concentration in the system.

As shown by the growth of the NO_3 concentration in the system, the biochemical activity remained stable during 4 days and then went down dramatically.

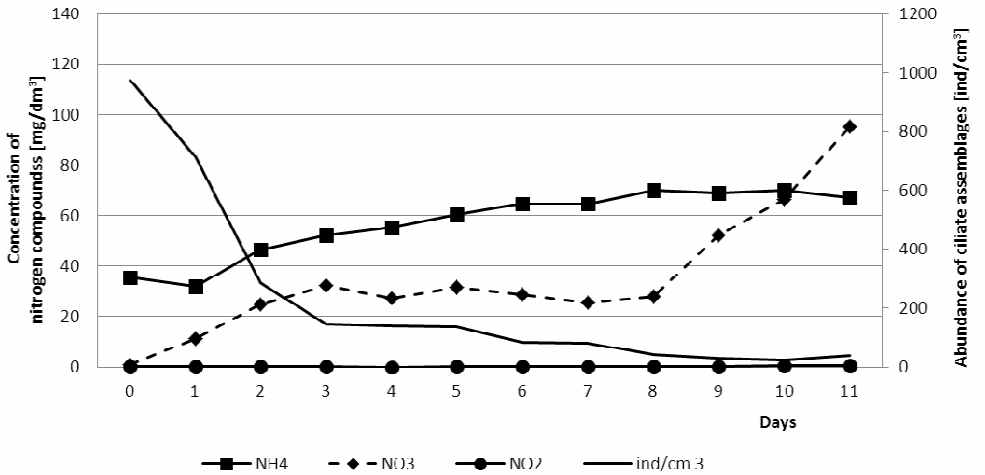

Fig. 3. Changes in the abundance of ciliates during the experiment compared with changes in the concentration of nitrogen compounds (control chamber without mixing and without air supply)

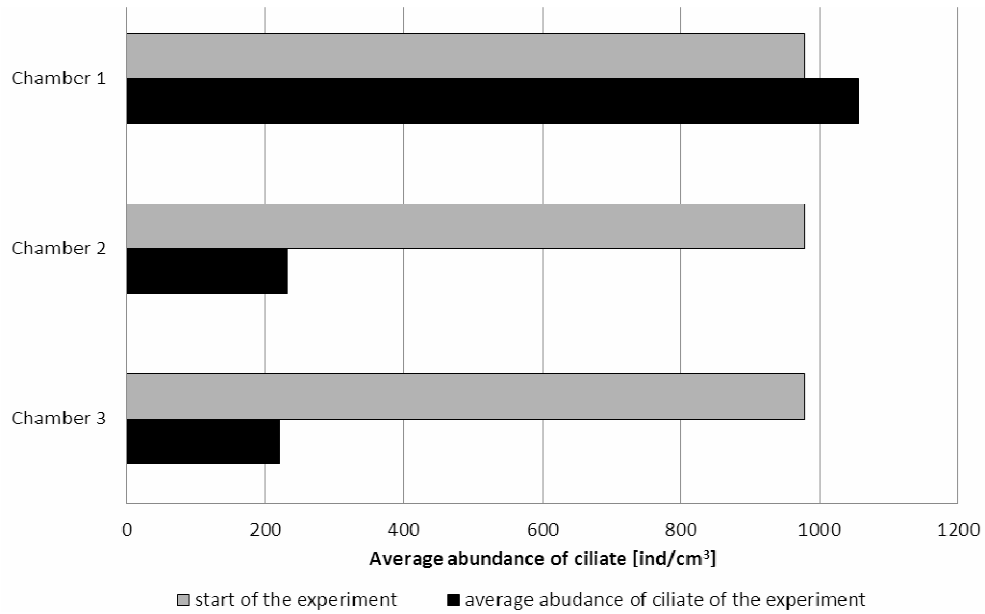

Fig. 4. The total average abundance of ciliates and the abundance of ciliates at the beginning of the experiment

In the conditions of chamber 3 (with no aeration and no stirring), where activated sludge stayed as sediment on the bottom, there was no protozoan growth phase and during two days abundance of ciliate became 4 times smaller (Fig. 3).

In chamber 3, the decrease in the quantity of infusorians to 200 individuals per cm^3 was found on the sixth day only, while in chamber 3 with non-stop stirring, the quantity reached 200 individuals per cm^3 already after two days. Starting from the sixth day of the experiment in chamber 3, slow reduction of individuals and the general number of species took place. The critical decrease in the active cells of protozoa was observed on the ninth day.

Thus, mixing of the activated sludge without aeration does not provide preservation of the active forms of protozoa. Moreover, the degradation of the ciliate community in chamber 3, where the activated sludge was not mixed, was slower. However, the average abundance of ciliates in chambers 2 and 3 was similar (Fig. 4). The average abundance of ciliates in chamber 1 was significantly higher than in chambers 2 and 3.

During the investigations, a correlation was shown between ciliates and the parameters of the measured process. Based on the calculations conducted with the STATISTICA software, it was established that the coefficient r for dependences between the number of ciliates and dissolved oxygen correlation was about 0.853 and r^2 was equal to 0.728, at p of ca. 0.0146. For the same group of organisms, the dependence between the number of individuals and pH was characterized by a value of $r = 0.877$, $r^2 = 0.769$, and $p = 0.0096$, together with the correlation between the abundance of the group and TOC at the level of $r = 0.758$, $r^2 = 0.574$, and $p = 0.0484$. Considering the concentration of nitrogen compounds, noticeable dependence is evident between the abundance of ciliates and NO_2 characterized by $r = 0.699$, $r^2 = 0.489$, and $p = 0.08$.

Conclusions

Based on the results obtained from the three series of the experiments performed in triplicate in different process conditions, it can be concluded that nutrient deficit and malfunction of the aeration system leads to encystment or death of a majority of ciliates present in activated sludge on the second day. Simultaneously, at proper aeration, the number of protozoa increases significantly during the first two days. The increase induced by oxygen availability is followed by a decline in the ciliate abundance to the initial level within the next two days. As indicated by the analyses of the activated sludge organisms sampled in the chamber without mixing and aeration, the deficiency in nutrient supply results in a significant reduction in the number of protozoa already after two days. During the experiment, the protozoan species structure was stable for four days.

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ZMIANY W STRUKTURZE ZBIOROWISKA PIERWOTNIAKÓW OSADU CZYNNEGO W ZRÓŻNICOWANYCH WARUNKACH TLENOWYCH

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Abstrakt: W pracy przedstawiono wyniki badań prowadzonych w laboratoryjnym bioreaktorze SBR, symulującym warunki występujące w przypadku awarii urządzeń stanowiących wyposażenie bioreaktora (systemu mieszania i systemu napowietrzania). Analizowano skład chemiczny ścieków, w tym stężenia związków azotu (azot amonowy, azotany(III) i azotany(V)), a także stężenia związków organicznych wyrażanych jako ogólny węgiel organiczny (OWO) i węgiel całkowity. Oprócz wskaźników chemicznych analizowany był również zespół organizmów osadu czynnego. W pobieranych próbkach określano ilość pierwotniaków (orzęski) w wymienionej grupie. Każdą z trzech serii pomiarowych prowadzonych dla różnych warunków procesowych powtarzano trzykrotnie. W eksperymencie wykorzystano osad czynny pobierany z oczyszczalni ścieków Hajdów w Lublinie. Na podstawie uzyskanych wyników badań można stwierdzić, że liczebności analizowanych zbiorowisk orzęsków wykazują związek ze stężeniem tlenu, pH oraz wartością OWO.

Słowa kluczowe: bioreaktor typu SBR, osad czynny, zespół organizmów, pierwotniaki, oczyszczanie ścieków, stężenie tlenu