Teka Kom. Ochr. Kszt. Środ. Przyr. – OL PAN, 2016, 13, 27–32

SEASONAL CHANGES OF CILIATES IN ACTIVATED SLUDGE (WASTEWATER TREATMENT PLANT „BIEŁAWIN", CHEŁM)

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Abstract. The primary objective of the present study was to determine the seasonal dynamics of ciliates in activated sludge. Studies were carried out in order to verify the hypothesis that fertility of a habitat may significantly influence the seasonal dynamics of the abundance of ciliates, as well as the number and intensity of correlations between physic-chemical parameters and ciliates. It seems that the values of numbers of ciliates were seasonally changeable. The highest numbers of ciliates were found in spring and summer, however the lowest numbers of ciliate communities were noted in winter. The studies showed that protozoa community is determined by ammonia mainly in summer. In spring and winter additional factors may be important. Probably suspended solid, total organic carbon and concentration of appropriate food (bacteria and flagellates) are the major regulator of abundance of ciliates.

Key words: wastewater treatment plant, activated sludge, ciliates

INTRODUCTION

Biological wastewater treatment processes rely on the natural self-purification ability of microbial communities. Nevertheless, they differ from aquatic ecosystems due to certain characteristics, such as a strong flow of organic matter into the system, accelerated decomposition processes, and the prevalence of heterotrophic organisms [Madoni 2011]. Protozoa, especially ciliates, are generally predominant taxa when activated sludge performs adequately [Kexin *et al.* 2007]. Most of ciliates found in activated sludge system are ubiquitous appearing all over the word [Madoni *et al.* 1993]. Due to the clear predominance of protozoa in activated sludge, they are recognised as fulfilling the main function in matter and energy flow [Kexin *et al.* 2007]. Those microorganisms are significant consumers of bacteria, flagellates, and algae, and participate in mineralisation of organic matter and circulation of biogenic compounds [Mieczan *et al.* 2012]. The ma-

jority of ciliates present in biological water treatment feed upon dispersed populations of bacteria. The ciliates in activated sludge can be subdivided into four groups: free-swimmers, crawlers, attached and carnivorous [Madoni *et al.* 1993, Madoni 2011]. Moreover, ciliated protozoa are considered important bioindicators of the activated sludge process, due to their association with physical and chemical characteristics of the habitats. The presence of ciliated protozoa reflects the improvement in the effluent quality with respect to the significant reduction in BOD, suspended solid and pathogens [Tyagi *et al.* 2008]. Any information on the seasonal relations between ciliates and environmental parameters in activated sludge is particularly scarce [Madoni *et al.* 1993]. The primary objective of the present study was, therefore, to determine the seasonal dynamics of ciliates in activated sludge. Studies were carried out in order to verify the hypothesis that fertility of a habitat may significantly influence the seasonal dynamics of the abundance of ciliates, as well as the number and intensity of correlations between physical and chemical parameters and ciliates.

MATERIAL AND METHODS

Fieldwork was done on a monthly in winter, spring and summer 2015 in wastewater treatment plant „Bieławin" located in Chełm (eastern Poland). The production line of the sewage treatment plant contains: primary clarifier, anaerobic reactor, aerobic reactor, cascade biological reactors, secondary clarifier, station of cleaning sewers thoroughly on filters, digesters, station of dehydrating deposit, container of biogas, torch of biogas. In each season three samples were collected from the cascade biological reactors. Al the samples were analyzed for physical-chemical and microbiological (ciliates) as per standard methods. Temperature and oxygen were determined *in situ* using the multifunction device equipped with an integrated head CX-461 (Elmetron, Poland). The remaining factors (ammonium nitrogen, nitrates) were analysed in the laboratory using a spectrophotometer VEGA 400 equiped with thermoreactor Spectroquant TR320 (Merck, Germany) [Golterman 1969]. Samples of mixed liquor for microscopic analysis were taken from the outlet end of the aeration tanks. Technique of Madoni *et al.* [1993] was used which involved: 25 µL sub-samples of activated sludge were taken with 100 µL automatic micropipette and a minimum a four replicates of this volume were counted under phase-contrast illumination (100 × magnifications). The sludge was kept constantly homogenized and aerated for the entire duration of analysis, to keep all the solids in suspension. Ciliates counts were performed every time within 6 h sample collection. The differences of physical and chemical water parameters among seasons were analysed by means of one-way ANOVA. Tukey's multiple range test (at $P < 0.05$) was used to compare means when significant differences were found. The analysis was performed

using PAST software. Pearson`s correlation coefficients were calculated in order to specify the interactions between ciliates, physical and chemical parameters.

RESULTS AND DISCUSSION

In the cascade biological reactors the concentration of oxygen was highest in spring and summer, whereas the oxygen content was lowest during winter (ANOVA, F = 12.36, P = 0.021). Temperature fluctuated between 11 and 13.7°C. In turn, suspended solid was significantly differentiated, attaining from 4.5 mg L^{-1} to 6.3 mg L^{-1} (ANOVA, F = 22.74, P = 0.003). The highest suspended solid occurred in summer, but was decidedly lower in spring or winter. Nutrients reached the highest values during the winter and spring periods, and considerably lower in summer (ANOVA, F = 28.11, P = 0.0021–0.033). The concentration of ammonia fluctuated between 0.09 mg L^{-1} in summer and 0.12 mg L^{-1} in winter. The highest contents of nitrates occurred in summer, but was decidedly lower in spring (Table 1). A total of 10 ciliate species were identified in the sample.

Table 1. Physical and chemical characteristic of sewages from cascade biological reactors (average values for period winter – summer 2015)

Parameters		Period		
		winter	spring	summer
Oxygen	mg L^{-1}	1.97	2.4	2.29
Temperature	°C	11	13	13.7
TSS	mg L^{-1}	4.5	5.6	6.3
BOD	mg L^{-1}	6.2	9	14.2
Ammonia	mg L^{-1}	0.12	0.10	0.09
Nitrate	mg L^{-1}	9.29	18	8.05

The highest species richness (7 taxa) occurred in summer. Decidedly lower numbers of taxa (4) were observed in winter (Table 2). The diversity analysis revealed a mean Shannon-Wiener diversity index (H) of 2.45. The highest diversity was measured in summer (H = 2.3), and the lowest diversity was observed in *winter* (H = 0.71). The most frequent were crawling species: *Chilodonella uncinata, Stylonychia mytilus-complex* and stalked ciliates – *Vorticella microstoma* (Fig. 1). The highest abundance of ciliates were noted in spring and summer with the dominance of *Stylonychia mytilus-complex.* The density of ciliates during winter was the lowest. All these species together can be used as bioindicators for high effluent quality as they displayed strong association with low effluent concentrations of BOD and TSS [Tyagi *et al.* 2008]. Generally, the abundance of ciliates were correlated with the nutrients concentrations (from

Table 2. Species composition and abundance* of ciliates in activated sludge in investigated wastewater treatment plant

Taxa	Periods		
	winter*	spring*	summer*
Amphileptus sp.			1
Chilodonella uncinata		13	2
Cinetochilum sp.		1	
Euplotes octocarinatus	10		3
Euplotes sp.		6	
Litonotus sp.			3
Stylonychia mytilus-complex	10		27
Vorticella campanula		6	6
Vorticella convallaria	7		21
Vorticella microstoma	3	8	
No. of taxa: 10	4	5	7
Total abundance: ind. mL^{-1}	30	34	97

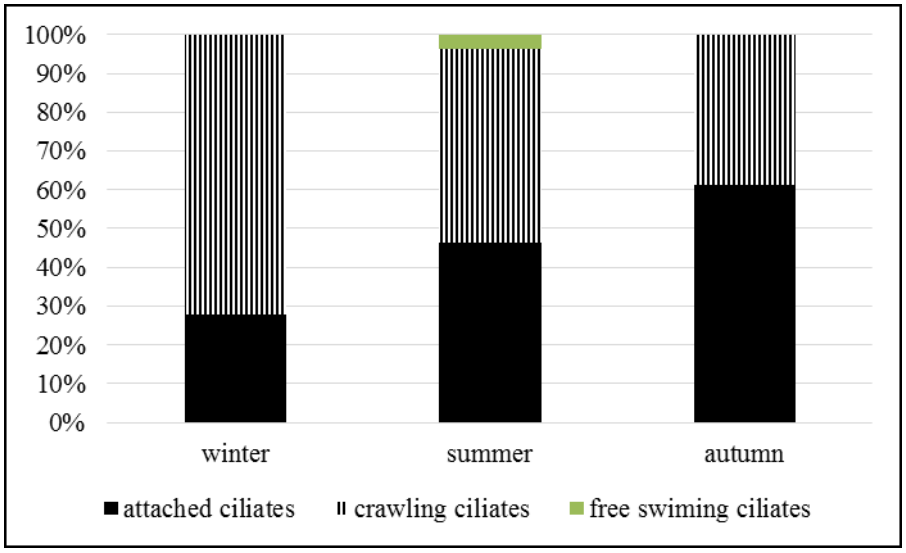

Fig 1. Domination structure of functional groups of ciliates in activated sludge in investigated sewage treatment

$r = 0.31$, $P \leq 0.05$ to $r = 0.53$, $P \leq 0.01$). However, the number of significant correlations between the ciliates and abiotic parameters was different amongst seasons. In summer the relations between protozoa and environmental parameters were stronger. Ciliate density correlated positively with the concentrations of nitrate and ammonia ($r = 0.58$, $r = 0.63$, $P \leq 0.01$). In spring, there was a significant and positive correlation between ciliates density and concentrations of total suspended solid ($r = 0.35$, $P \leq 0.05$). So far, comparative data concerning seasonal changes ciliates in active deposit in the savage treatment are very scarce. This study suggests significant relationships between the species richness of ciliates and season. This results consistent with the results of other seasonal studies [Madoni *et al.* 1994]. We observed differences in ciliate communities among seasons. In spring the most abundant species were *Chilodonella uncinata* and *Vorticella microstoma*. *Stylonychia mytilus-complex* and *Vorticella convallaria* were primarily observed in winter and summer. The spring and summer peaks may be linked to the increase in nutrient concentration. These patterns also partly match previous observations. For example, Madoni [2011] found a peak in the number of ciliates in spring and autumn and a lower activity during the mid-winter. Among the observed environmental variables, the most significant were nutrients and suspended solid. The importance of nutrients has also been reported by other studies [Mieczan *et al.* 2012]. Nutrients concentration may affect ciliates and their potential food resources – bacteria, microalgae, other protozoa, micrometazoa and fungi [Mitchell *et al.* 2000]. This study shows that ciliates respond to the same major environmental gradients in Poland as in other parts of the world. The strongest relationship was found between ciliate communities and both nitrate and ammonium. Also ascertained was a significant influence of TSS on the occurrence of ciliates. Similar to these results, a correlation between ciliates and some nutrients was found in activated sludge plant in China [Zhou *et al.* 2006]. In wastewater treatment, ciliate communities were related to NO_3^-, and to a combination of physical variables, e.g. temperature, and chemical variables such as pH, N and TOC [Madoni 2011].

CONCLUSIONS

It seems that the values of numbers of ciliates were seasonally changeable. The highest numbers of ciliates were found in spring and summer, however the lowest numbers of ciliate communities were noted in winter. The studies showed that protozoa community is determined by ammonia mainly in summer. In spring and winter additional factors may be important. Probably suspended solid, total organic carbon and concentration of appropriate food (bacteria and flagellates) are the major regulator of abundance of ciliates. This study suggested that microfauna chiefly composed of attached and crawling ciliates with small free-swimming ciliates, represents the efficient working of activated sludge.

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SEZONOWA ZMIENNOŚĆ ORZĘSKÓW W OSADZIE CZYNNYM (OCZYSZCZALNIA ŚCIEKÓW „BIEŁAWIN", CHEŁM)

Streszczenie. Zasadniczym celem badań było poznanie sezonowej dynamiki zmian orzęsków zasiedlających osad czynny w oczyszczalni ścieków. W oparciu o prowadzone badania weryfikowano hipotezę, iż żyzność ścieków w istotny sposób wpływa na sezonową zmienność tych mikroorganizmów oraz na siłę korelacji pomiędzy pierwotniakami a wybranymi właściwościami fizycznymi i chemicznymi. Zarówno największą różnorodność gatunkową, jak i największą liczebność orzęsków stwierdzono wiosną i latem, z kolei w okresie zimy były one najmniejsze. Analiza korelacji wykazała, że obfitość orzęsków w okresie letnim najsilniej korelowała z zawartością w ściekach amoniaku. Wiosną i zimą prawdopodobnie inne czynniki determinowały występowanie tych pierwotniaków. Były to głównie stężenia zawiesiny, materii organicznej oraz obfitość potencjalnego pokarmu (m.in. bakterii i wiciowców).

Słowa kluczowe: oczyszczalnia ścieków, osad czynny, orzęski