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STUDY ON ACTIVATED SLUDGE FLOCS MORPHOLOGY AND COMPOSITION IN A FULL-SCALE WASTEWATER TREATMENT PLANT IN POLAND

This work is a comprehensive study of a full-scale municipal wastewater treatment plant (WWTP) in the city of Zgierz (Poland) aiming at the estimation of the impact of seasonal changes of temperature on flocs morphology, biomass concentration, quantity of filamentous bacteria, content of polyhydroxybutyrate (PHB) and composition of microinvertebrates in the activated sludge system. Two short-term measurement campaigns, in winter and in summer, were carried out under dry weather conditions. It occurred that lower temperatures (11.1–14.6 °C) had hardly any effect on flocs size or concentration of activated sludge biomass in the full-scale activated sludge system treating municipal wastewater. They did not aggravate the biosynthesis of PHB either. However, decreasing temperature favored the growth of filamentous bacteria and led to the elevation of the sludge volume index (SVI). In spite of this, it did not induce any bulking events. Irrespective of the season, the groups of crawling ciliates, attached ciliates and testate amoebae, indicating good performance of the full-scale plant tested, were predominant.

1. INTRODUCTION

The conventional activated sludge systems are still most frequently used for the secondary treatment of municipal wastewater. Bacteria able to form aggregates (flocs) play a key role in these systems. The size and density of such aggregates are regarded the critical parameters influencing the settling properties of activated sludge and the efficiency of wastewater treatment processes. Thus, the morphology of activated sludge flocs and its measurement are of particular importance. Many works concerning the

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application of automated image analysis procedures for the determination of morphological parameters of activated sludge flocs have been published so far [1–6]. It was proved that image analysis was the appropriate tool to characterize both aggregates and filamentous bacteria [7]. The elaborated procedures of automated image processing and analysis were applied for the monitoring of lab-scale as well as full-scale wastewater treatment plants [3, 5]. It occurred that they allowed the detection of bulking events in full-scale wastewater treatment plants [3]. Oliveira et al. [8] found that filamentous bulking events were characterized by a floc population shift towards larger sizes and lower solidity and circularity values, while washout phenomena were characterized by smaller flocs and the increase in their solidity. At the same time, Koivuranta et al. [9] owing to the application of image analysis procedures showed that activated sludge flocs were bigger and rounder, and had fewer filaments in summer than in winter. The aforementioned findings proved the usefulness of image analysis techniques for the continuous examination of activated sludge biomass.

One of the most important and still not fully understood issues concerned the plausible correlation between flocs morphology and operational parameters of activated sludge systems. Barbusinski and Koscielniak [10] found that after substrate overload event, activated sludge flocs were more prone to breakup (pin-point effect). Wilén et al. [11] observed that higher organic loading rate improved settling and compaction properties of activated sludge flocs. Regarding other important environmental and operational parameters, it was found that at lower temperatures (in winter) biochemical activity of activated sludge biomass decreased and, as a consequence, the structure of flocs was weakened [11, 12]. Lower dissolved oxygen (DO) concentrations (0.5–2.0 mg/l) aggravated the settling properties in comparison with higher DO concentrations (2–5 mg/l) [13]. The main reason for this phenomenon was the excessive growth of filamentous bacteria that generally have lower values of saturation constant for dissolved oxygen than floc-forming bacteria.

Apart from bacteria, protozoans and metazoans play an important role in the maintenance of the activity of the microbial community of activated sludge. They are absolutely necessary to ensure the appropriate efficiency of biological wastewater treatment processes. In 1994, Madoni [14] elaborated the sludge biotic index (SBI), which expressed the correlation between the presence and abundance of microinvertebrates with operational parameters and performance of biological wastewater treatment plants. SBI has been increasingly used by both scientists and WWTP workers to control and monitor activated sludge systems [15, 16]. Its application makes the description of microbial communities of activated sludge more complete. However, the studies presented heretofore focused either on the composition and morphology of bacterial aggregates or the appearance and frequency of microinvertebrates.

In this work, the investigations on the composition and morphology of flocs, and the composition of microinvertebrates were integrated in order to provide a comprehensive description of activated sludge from the full-scale plant treating municipal wastewater. Additionally, this study aimed at the estimation of the impact of seasonal variations of temperature and operational parameters on flocs morphology and composition of activated sludge. The latter comprised the number of filamentous bacteria, the concentration of polyhydroxybutyrate and the values of sludge biotic index. It is one of the first works presenting the comprehensive analysis of activated sludge system in the full-scale WWTP.

2. MATERIALS AND METHODS

WWTP studied and measurement campaigns. The object of the study was the Wastewater Treatment Plant in Zgierz (WWTP Zgierz) treating municipal wastewater. The biological step consisted of one five-zone bioreactor and secondary clarifier run in the Phoredox process configuration [17]. The total volumes of each zone were as follows: 857.5 m^3 – anaerobic zone, 3536 m^3 – first anoxic zone, 15 850 m^3 – first aerobic zone, 3118 m^3 – second anoxic zone, and 795 m^3 – second aerobic zone. The average pollutant load corresponded to approximately 94 000 PE in this plant. In the period of the studies, the average inflow of wastewater was 8540±1453 m^3/day. The hydraulic retention time of wastewater was at the level of 13±1 h.

Table 1

Operational parameters of WWTP Zgierz in the measurement campaigns with the statistical differences between them expressed using *p*-value (ANOVA test)

Characteristic feature	January 2015	June–July 2015	*p*-value ($\alpha = 0.05$)
Range of average daily flow rate, m^3/day	7120–11230	8000–11970	0.162
Internal recirculation, %	570–860	520–830	0.506
External recirculation, %	92–98	93–96	0.501
COD of influent, mg O_2/dm^3	630–3930	980–3760	0.922
BOD_5 of influent, mg O_2/dm^3	440–1200	540–800	0.689
N_{tot} of influent, mg N/dm^3	68–74	66–87	0.289
P_{tot} of influent, mg P/dm^3	8.9–11.4	7.2–14.4	0.666
Average sludge loading rate, g BOD/(g TS·day)	0.10–0.16	0.09–0.19	0.483
Dissolved oxygen in the second aeration chamber, mg O_2/dm^3	2.28–5.95	2.85–6.56	0.0507
Temperature in activated sludge chamber, °C	11.1–14.6	20.2–23.4	$6.03\cdot10^{-9}$
SRT, day	9.7–11	15.7–20.8	$1.66\cdot10^{-7}$
TS, g/kg	3.94–4.90	3.60–4.81	0.0693
SVI, aeration chamber, cm^3/g TSS	75–98	47–71	$7.15\cdot10^{-6}$

Two short-term measurement campaigns, in the winter (from 17 to 31 January 2015) and in the summer (from 27 June to 11 July 2015), were carried out. Both measurement campaigns were conducted under dry weather conditions. However, the ambient temperatures were in the strong contrast to each other. In the winter campaign the maximum ambient temperatures were in the range from –11.5 to 4.1 °C, while the minimum ambient temperatures varied from –14.6 to 2.7 °C. In the summer campaign, they were from 19.5 to 32 °C and from 9.9 to 20 °C, respectively.

During each measurement campaign, the activated sludge was sampled three or four times a week, and in this way seven independent sets of samples were obtained. Every time activated sludge biomass was taken from the anaerobic (first part of the Phoredox process) and the second aerobic (last part of the Phoredox process) zones of the activated sludge chamber. In parallel, the operation of the WWTP Zgierz was carefully observed during both measurement campaigns. It concerned particularly the composition of influent (COD, BOD_5, total nitrogen and phosphorus concentration) and such operational parameters as sludge loading rate, temperature, dissolved oxygen and SRT. These data together with the results of their statistical elaboration are presented in Table 1.

Image analysis procedures. Three automated image analysis procedures were applied in order to describe the morphology and composition of activated sludge flocs. They aimed at the measurement of size (i.e., area and diameter) and shape (circularity calculated from the area and perimeter as follows $4\cdot\pi\cdot\text{area}/(\text{perimeter})^2$) of flocs in procedure No. 1, quantification of filamentous bacteria (procedure No. 2) and determination of the value of field area (F_A, procedure No. 3). In all procedures, the observations were made under a light microscope Nikon Eclipse Ni. The RGB images were snapped, processed and analyzed with the help of NIS ELEMENTS AR software (Nikon). Procedure No. 1 including the definitions of morphological parameters measured has been presented elsewhere [17].

Procedure No. 2 consisted of three stages: Neisser staining (a), the capture of microscopic images (b), digital image processing and analysis (c) [18]. As a result, the following indicators of filamentous bacteria were calculated: total filamentous length per image (TL/image), total filamentous bacteria length per volume (TL/vol) and the ratio of filaments to the aggregate area (Fil/Agg). Field area (F_A) was defined as the ratio of the total area of flocs to the total area of the field of view [1]. The automated procedure for the calculation of F_A (procedure No. 3) was similar to the procedure No. 1 and consisted of the same steps, with only one difference concerning the enumeration of objects. In procedure No. 3, all visible objects, including those which crossed the border of the image, were enumerated and taken into account for the calculation of F_A.

Sludge biotic index (SBI) was evaluated with the use of a light microscope Nikon Eclipse Ni with the objective 20×. After homogenization, 25 μL of sludge was transferred into a microscopic slide and all the organisms found were counted. The taxonomic groups were morphologically identified and the value of SBI was calculated according to the guidelines presented by Madoni [14].

Other measurements and analyses. The values of operational parameters as well as the results of most of the physicochemical analyses of wastewater and sludge were obtained from the WWTP studied. They were made within the measurement campaigns. Flow rates of influent and recirculation streams, pH and temperature were measured online. Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total nitrogen (N_{tot}), total phosphorus (P_{tot}), total solids (TS), total suspended solids (TSS), volatile solids (VS) and volatile suspended solids (VSS), sludge volume index (SVI) were determined with the use of the standard methods [19].

Polyhydroxybutyrate (PHB) concentration was determined by the liquid chromatography according to Karr et al. [20] with the use of the modified methodology proposed by Rodgers and Wu [21]. It comprised the following stages: 1) removal of water from activated sludge sample by centrifugation and addition of ethanol, 2) acid hydrolysis with concentrated sulfuric acid, 3) digestion at 100 °C, 4) dilution of the sample and determination with UPLC® acquity system (Waters, USA) on Aquity BEH C_{18} column at 40 °C. Elution (flow rate equal to 0.2 cm^3/min) was made with water acidified with formic acid (at 0.1%). Photodiode array detection (PDA) at $\lambda = 210$ nm was applied. Crotonic acid, which is the product of hydrolysis of PHB and dehydration of its monomer, was used as the standard. PHB concentration was expressed as mg of crotonic acid/dm^3. The accuracy of the determination was 0.01 mg/dm^3.

Statistical elaboration of data. The data comprising the characteristics of the influent and operational parameters of the studied WWTP were subjected to the statistical elaboration. Its main goal was to find, whether there were any statistically relevant differences in the operation of the WWTP Zgierz between the winter and summer campaign. It was checked with the use of one-way analysis of variance (ANOVA) implemented in MS Excel (Analysis ToolPak) software [22]. The confidence level of 95% was assumed, i.e., $\alpha = 0.05$. The results of this analysis are given in Table 1. ANOVA ($\alpha = 0.05$) was also applied in order to compare the results of both measurement campaigns. It comprised all measured indicators in the paper. They were analyzed and compared statistically in order to estimate the effect of season, particularly temperature, and oxidoreductive conditions on their values. Also the mean values and standard deviations were calculated. Standard deviations (σ) presented in Figs. 1–3 express the variability of the values of the morphological parameters shown in these figures.

3. RESULTS AND DISCUSSION

A full-scale WWTP is a stochastic system affected by many factors, particularly, environmental conditions and operational parameters. Therefore, in this study it was first checked, whether there were any statistically significant differences in the operation of the WWTP Zgierz between the winter and summer measurement campaigns. It occurred that apart from the temperature in the bioreactor and sludge retention time (p-value below 0.05 in both cases), the differences in the values of the other operational parameters in two measurement campaigns were not statistically significant (Table 1). Thus, these two factors, temperature and sludge retention time, might have affected the results of the physicochemical and microscopic analyses performed in this work to the highest extent. It should be noted that also other operational parameters and/or indicators characterizing influent (e.g., COD, the toxicity of wastewater) might have an effect on activated sludge properties and wastewater quality. However, upon the results of ANOVA, they were not analyzed.

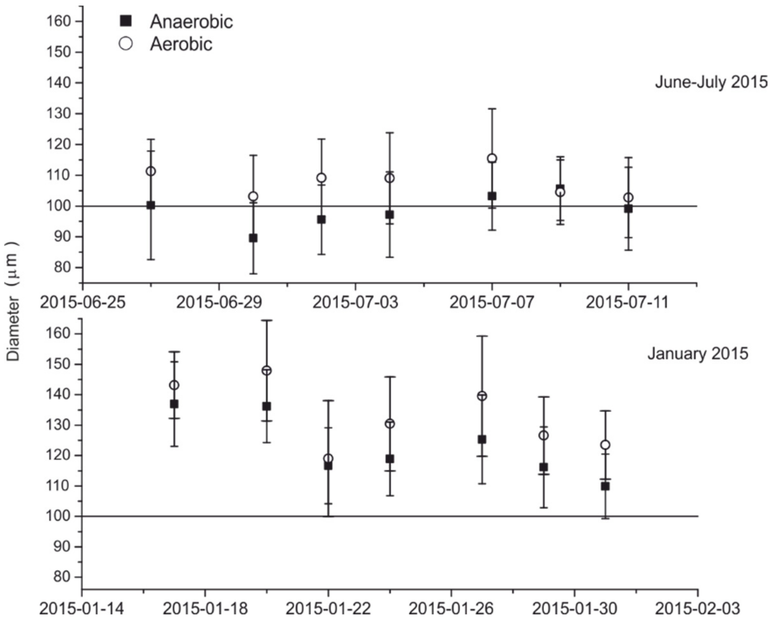

Fig. 1. Diameters of activated sludge flocs in the WWTP Zgierz

In the summer campaign, the mean values of diameters of activated sludge flocs varied from 89.5 to 115.4 µm, whereas in the winter they were higher, from 109.9 to 197.9 µm. Figure 1 shows that during the winter season the mean diameters of flocs were above 100 µm, while in the summer campaign most of the mean values of diameters of flocs were less than or equal 100 µm. The difference in the diameter of activated sludge flocs between two campaigns occurred to be statistically relevant (p-value < 0.05). It occurred that lower temperatures from 11.1 to 14.6 °C in the bioreactor contributed to the decrease in flocs dimensions. Furthermore, it was observed that lower temperatures and lower sludge age from 9.7 to 11 day did not interfere with bioflocculation processes when other operational parameters of the activated sludge system were at a similar level. Liao et al. [23] observed that there was no unequivocal effect of SRT on floc size in the sequencing batch reactor (SBR), however at lower SRTs (4–9 day) the variability in size was broader than that at higher SRTs (16–20 day). It was most probably connected with a relatively unstable microbial community at lower SRTs, which was accompanied by higher concentration of extracellular polymeric substances in activated sludge [23]. Also lower temperatures favored the synthesis of extracellular polymers [11] and, as a result, facilitated bioflocculation processes.

Comparing the diameters of flocs in two studied activated sludge chamber zones, which had different oxidoreductive conditions, it was found that flocs were bigger in the aerobic conditions irrespective of the season and other environmental and operational parameters (Fig. 1). In the winter campaign, the differences in flocs diameters between anaerobic and aerobic zones were not statistically relevant (p-value equal to 0.104), in contrast to the summer campaign (p-value equal to 0.00472). These findings are in agreement with the previous observations in the long-term study [17]. The values of standard deviation of the mean diameter shown in Fig. 1 were at similar level irrespective of the season and the oxidoreductive conditions.

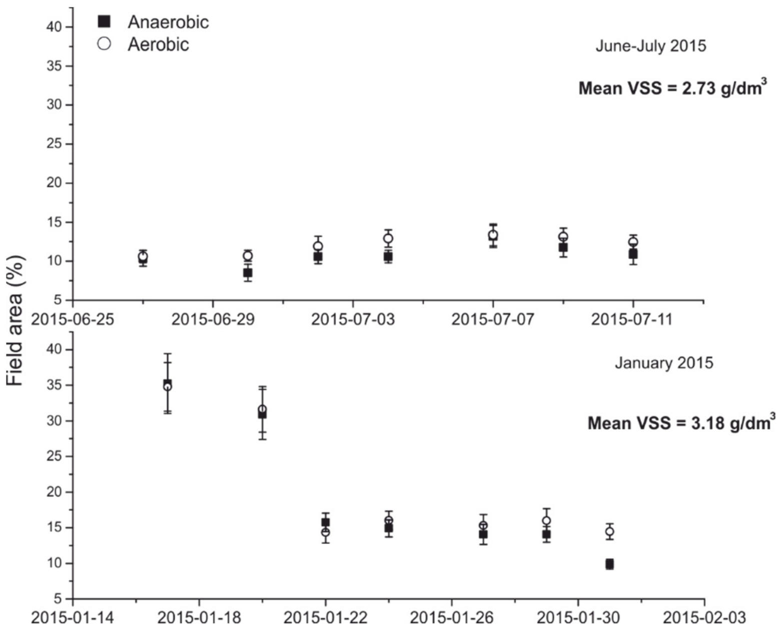

Fig. 2. Estimation of field area in the activated sludge system of the WWTP Zgierz

The variations of field area (F_A) obtained in both campaigns coincided with the changes of flocs size. Higher field areas were found in the winter campaign and their mean values varied broadly from 9.8 to 35.2% (Fig. 2). In the summer campaign, the values of field area were more stable and remained between 8.5 and 13.4% (Fig. 2). ANOVA revealed that the differences between the field area in the winter and summer campaigns were statistically relevant ($p < 0.05$). Field area is regarded an indicator of biomass concentration in activated sludge systems. In some works, the linear correlations between field area and the concentration of total or volatile suspended solids were found [1]. Although in this work such relation was not statistically relevant (R^2 usually below 0.850), it was found that the increase of the field area was accompanied by the increase of VSS concentration. On average, higher concentration of biomass expressed as VSS and higher values of field area were observed in the winter campaign. As the effect of temperature on the processes performed by ordinary heterotrophic bacteria was estimated as moderate [24], the most plausible reason of the increase of biomass quantity at lower temperatures was the sludge age. The elongation of SRT in the summer season causes the decrease of the yield and active fraction of biomass because of the increased importance of death and decay at longer SRTs [25].

The performed quantification of filamentous bacteria revealed that lower temperatures and lower SRT favored the expansion of filamentous bacteria (Fig. 3). It was confirmed by all indicators of filaments quantity used in this study and the values of sludge volume index (Table 1). The application of ANOVA showed that the differences between the values of each indicator in the winter and summer periods were statistically significant. For example, the p-values were 0.00874 and 0.00334 for TL/image in the anaerobic and aerobic zones, respectively. It indicates that the main reason for the expansion of filamentous bacteria in the winter campaign was temperature. This observation was also confirmed by other research groups. Filamentous bacteria constituted on average 24% of the entire activated sludge bacteria in full-scale WWTPs in Denmark [26]. Seasonal variations in the abundance of filamentous bacteria in the WWTPs tested in Denmark were relatively small, however, more filaments were observed in winter than in summer [26]. Koivuranta et al. [9] observed a similar phenomenon as well. Also, Jones and Schuler [27] noticed that in cold weather the ability to settle of activated sludge was worse than in warm weather in most of the full-scale WWTPs tested in central New Mexico (USA). In some cases, it was accompanied by the higher content of filamentous bacteria [27].

In the winter campaign, the variability of the indicators of filamentous bacteria was higher than it was in the summer campaign. For example, the mean values of TL/vol in the winter campaign varied widely from 0.372 to 1.814 mm/μL, while in the summer campaign they were from 0.186 to 0.329 mm/μL. Higher quantity of filamentous bacteria in the winter campaign might have contributed to the higher values of field area and their higher variability in this period of time (Figs. 2 and 3).

This work has shown that anaerobic conditions favored the expansion of filaments to a higher extent than the aerobic ones (Fig. 2). However, the differences between them were not statistically relevant (p-value > 0.05). The higher quantity of filamentous bacteria in the anaerobic zone was connected with the higher ability of filamentous bacteria to grow in the oxygen limitation conditions than it was in the case of floc-forming bacteria. It was confirmed by very low values of half-saturation constants, i.e, at the level of 0.01 mg O_2/dm^3 for filamentous bacteria [28].

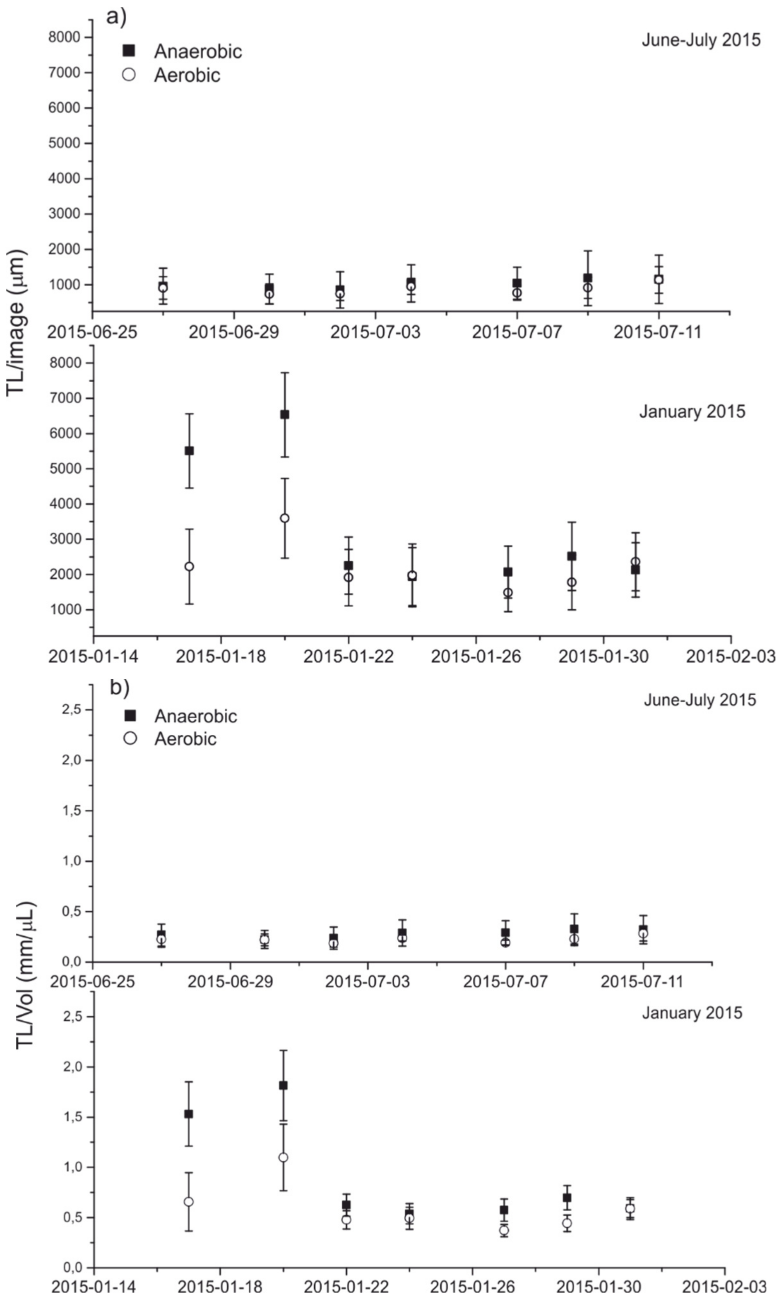

Fig. 3. Quantification of filaments in the WWTP Zgierz expressed as (a) TL/image and (b) TL/Vol

Despite higher content of filamentous bacteria in the winter season, particularly in the anaerobic zone, their presence did not induce any undesired phenomena like bulking events. It was confirmed by the values of SVI, which were in the range from 47 to 98 cm^3/g TSS. Although higher quantity of filamentous bacteria was accompanied by higher values of SVI (cf. Table 1 and Fig. 3), a linear correlation between these two parameters was not found. As it was shown by da Motta et al. [2] and Mesquita et al. [6], it was difficult to find a global quantitative correlation between SVI and amount of filamentous bacteria, although such relations existed in the limited ranges of the values of these parameters. Nevertheless, SVI in the winter campaign was higher than the one found in the summer campaign and the differences between the values of SVI in both campaigns were statistically relevant (p-value equal to $7.15\cdot10^{-6}$, Table 1).

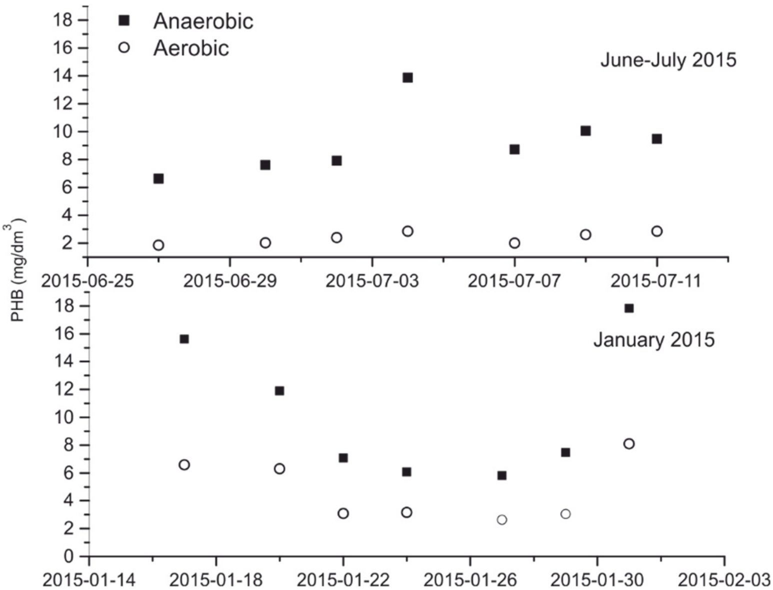

Fig. 4. PHB concentration in the WWTP Zgierz.
Standard deviations (σ) from 0.07 to 0.29 mg/dm^3 in the winter season and from 0.04 to 0.21 mg/dm^3 in the summer season are not visible in the figure

The content of PHB in activated sludge was at a similar level irrespective of the season (Fig. 4). In the winter campaign, PHB concentrations were on average 10.26±4.79 mg/dm^3 under anaerobic conditions and 4.69±2.22 mg/dm^3 under aerobic ones, while in the summer campaign they were 9.18±2.36 mg/dm^{3}and 4.30±1.93 mg/dm^3, respectively. There were no statistically significant differences between the values of PHB concentration in the winter and summer campaigns ($p > 0.05$). It indicated that environmental conditions and operational parameters in the range studied did not have any influence on the biosynthesis of PHB by activated sludge biomass. The only difference between both campaigns was that in the winter campaign concentration of PHB varied in the broader range than in the summer one, which was reflected by the higher values of standard deviations (σ) as mentioned above. The concentration of PHB was, on average, above twice higher in the anaerobic conditions in comparison to the aerobic ones. This difference was relevant statistically ($p = 0.0181$ in the winter or $p = 0.00121$ in the summer campaign). It was in agreement with the other study showing that the concentration of PHB in the anaerobic zone was usually from two to four-fold higher than in the aerobic zone of activated sludge systems [21]. This phenomenon was somewhat expected because the WWTP studied belongs to the systems of enhanced biological phosphorus removal (EBPR).

The results of the estimation of SBI are presented in Table 2. The total abundance of the individuals varied from 1.19 to 3.40 organisms/cm^3 (Table 2).

Table 2

Estimation of SBI for the WWTP Zgierz

Season	Total [1/cm^3]	No. of identified taxonomic units	Key groups and taxonomic units	SBI
Winter	1.19–1.95	5 or 6	crawling ciliates (*Aspidisca* spp., *Euplote*s spp.); attached ciliates (*Epistylis* spp., *Opercularia* spp.); testate amoebae (*Arcella* spp.); small metazoans, mainly nematodes	6 (five times) 8 (twice)
Summer	2.69–3.40	7 or 8	crawling ciliates (*Aspidisca* spp., *Euplote*s spp.); attached ciliates (*Epistylis* spp., *Opercularia* spp., *Vorticella* spp.); free-swimming ciliates (*Paramecium* spp.); testate amoebae (*Arcella* spp.); small metazoans, mainly rotifers and tardigrades	8 (every time)

The number of the individuals was higher in the summer season (the mean abundance was 2.96±0.26) than in the winter one (the mean abundance 1.54±0.28). The mean richness of taxonomic units was in the range from 5 to 8 and was also higher in the summer season. However, the groups of crawling ciliates, attached ciliates and testate amoebae were predominant in each sample tested irrespective of the season. Their presence indicated good performance of the WWTP studied. Both ciliate groups usually co-dominated the microfauna in activated sludge systems, while testate amoebae were dominant in sludge characterized by low loading rate, high sludge retention time and high DO in the aeration tank [14]. At the same time, the higher abundance of free-swimming ciliates in the summer period in comparison with the winter one resulted most probably from higher sludge loading rate and lower dissolved oxygen concentration in the summer than in the winter campaign (Table 1). It must be emphasized that free-swimming ciliates did not dominate in activated sludge but were only the associated key group of organisms (Table 2). Referring the values of SBI to the quality classes of activated sludge distinguished by Madoni [14], it occurred that in the winter campaign sludge quality corresponded usually with the second class (one exception was found), whereas in the summer campaign it corresponded with the first class.

In both campaigns, the effluent had a high-quality meeting the requirements of Polish and European Union legislation [29]. For example, COD of the effluent was in the range from 28 to 76 mg O_2/dm^3, the concentration of total nitrogen varied from 5.15 to 7.42 mg N/dm^3 and concentration of phosphorus was from 0.22 to 0.59 mg N/dm^3. All indicators of the treated wastewater were below the acceptable limit for such type of wastewater treatment plant in Poland (in accordance with the water permit granted to the WWTP in Zgierz).

4. CONCLUSIONS

Despite the seasonal variations of temperature and changes of the operational parameters, activated sludge biomass occurred to be resistant to them and able to hold the appropriate morphology and composition that did not deteriorate wastewater treatment processes. Activated sludge flocs from the WWTP studied were on average of medium size (the mean diameter varied from 89.5 to 197.9 μm) and high compactness. A relatively small quantity of filamentous bacteria and high richness of taxonomic units of protozoans (SBI equal to 6 or 8) were found. These properties of sludge were observed irrespective of the seasonal variations of temperature.

Lower temperatures (11.1–14.6 °C) and the variations in the operational parameters neither decrease the flocs size nor the concentration of activated sludge biomass. They also did not aggravate the biosynthesis of polyhydroxybutyrate.

Cold weather and low SRT favored the growth of filamentous bacteria in the full scale activated sludge system treating municipal wastewater. As a result, it contributed to the increase of sludge volume index, however, it did not induce any bulking events.

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