Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2022.28102

248 (2022) 39–48
February

Composition and variability of the activated sludge biocenosis in membrane biological reactors

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Received 8 September 2021; Accepted 3 January 2022

ABSTRACT

The operating parameters of membrane biological reactors (MBRs) differ significantly from those of conventional activated sludge (CAS) reactors, especially with regard to solids retention time (SRT) and food-to-mass ratio. This has an impact on the structure of the activated sludge biocenosis and its susceptibility to seasonal variations. The aim of this research was to find out the structure and variability of the activated sludge biocenosis in a small wastewater treatment plant using MBR reactors situated in southern Poland. The results were compared with the results obtained from a CAS wastewater treatment plant with a similar capacity and showed that the sludge biocenosis in MBRs differs significantly from that in CAS reactors. There was a noticeable difference in the ratio of attached to crawling ciliates in the sludge from MBRs and CAS, and it was also shown that the MBR biocenosis, despite the long SRT, undergoes stronger seasonal changes during the year, with a clear deterioration of the biocenosis structure in the autumn and winter period and a visible improvement in the spring. Despite this, no noticeable impact of changes in the MBR biocenosis structure was found with regards to the technological efficiency of the wastewater treatment process.

Keywords: Biological wastewater treatment; Activated sludge; Membrane biological reactors; Biocenosis; Taxonomic structure; Microscopic analysis

1. Introduction

Membrane filtration technology at the level of micro- and ultrafiltration is now increasingly used for the treatment of municipal wastewater. This is due, on the one hand, to the high stability and efficiency of membrane filtration in phase separation and, on the other hand, to the constantly decreasing costs of the membrane modules and the membranes themselves. The capacities of the largest wastewater treatment plants (WWTPs) using this technology reach as much as 1 million m^3/d (e.g., Beichu WWTP in China). The most common form of membrane application is the membrane biological reactor (MBR) with suspended activated sludge, in which membrane filtration replaces secondary sedimentation. These MBRs come in many options, and new modifications are constantly proposed for them, including biofilm MBRs, hybrid MBRs with suspended and fixed biomass, anaerobic MBRs (AnMBRs) or electrochemical MBRs (eMBRs), for example [1–4]. It seems that the future prospects for the development of this technology are very good, which results both from the progress in the development of new membrane materials (e.g., nanomaterials) and the increasing acceptance of this technology among operators of WWTPs [5]. The noticeable decrease in the operating costs in recent years, resulting mainly from the reduction of the required transmembrane pressure (TMP), also has a large impact.

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Activated sludge is a very complex biocenosis including autotrophic and heterotrophic bacteria, fungi and protozoa. While the bacterial communities have been characterized in various activated sludge systems, little is known about archaeal communities in activated sludge. However, there are many arguments that they can play an important role in the degradation of some pollutants and the removal of the nitrogen load [6]. For the proper operation of activated sludge systems, it is important to maintain appropriate proportions between different microorganism groups in the sludge biocenosis. Systems with conventional activated sludge (CAS) have been studied for many years, but the MBR systems differ significantly in the structure of the biocenosis. The specificity of the activated sludge in MBR starts with the size of the sludge flocs, which is 240 μm for the MBR system and only 160 μm for the CAS [7]. Information on sludge ecology in MBRs is still very scarce [8]. Most of the research in this field focuses on bacteria, rarely analysing other microorganisms such as fungi and protozoa. For this, molecular biology techniques based on 16S ribosomal RNA gene sequencing, followed by bioinformatics analysis of the isolated sequences or fluorescent *in situ* hybridization (FISH) tests, are commonly used. Even among the above, they often focus on the biofilm formed on the surface of the membrane, to reduce fouling of the membrane (e.g., [9,10]).

Mesquita et al. performed an extensive review of quantitative image analysis (QIA) and chemometric techniques used for activated sludge characterization. The review showed that QIA can be a powerful tool, with great potential for use in wastewater treatment, especially in activated sludge systems providing near real time information. This is especially true for enhanced biological phosphorus removal (EBPR) systems and the systems with granular sludge, where image analysis procedures can be a way to overcome the lack of information on the nature of the microbial population. The combination of microscopic techniques with chemometric techniques and multivariate statistical analysis is particularly promising, but they also noted that more research was needed with regard to linking microscopic observations with standard analytical parameters. An example of such an approach was proposed by Asgharnejad and Sarrafzadeh [12] with a novel method for activated sludge quantification based on RGB colour values in macroscopic imaging.

The taxonomic structure of the activated sludge community depends on many factors, including plant capacity, chemical composition of the incoming wastewater, process temperature and other operating conditions [13–17]. The studies performed by Jo et al. [8] on data from ten full-scale MBRs showed that bacterial communities significantly differed among the studied plants. Liu et al. [18] concluded that the richness of the biocenosis increases with the growing complexity of the activated sludge system. Moreover, the communities found in the sludge and membrane biofilm were very different, with biofilm biocenosis being more heterogeneous. Many sources indicate a close relationship between the composition of the activated sludge biocenosis and the susceptibility of membranes to fouling [2,7,19]. Witzig et al. [19] monitored the microbiological structure of an aerobic MBR system treating municipal wastewater for over a year and reported high variability in the floc structure and the ratio between free suspended and aggregated cells. They also noticed high variability in the presence of filamentous bacteria in the sludge and that protozoa and metazoa rarely occurred in the sludge community. Similar studies were repeated by Baek and Pagilla [20], confirming the observations by Witzig et al. [19] on the bacterial community structure and that the bacteria present in the highly concentrated MBR biomass use most of the energy supplied to maintain metabolism, while limiting growth. The results of the tests carried out by Arévalo et al. [21] in a pilot-scale MBR with hollow fibre membranes showed high fragmentation of flocs with abundantly dispersed bacteria. Two different study phases (I and II) corresponded to different solids retention times (SRTs), of 25 and 35 d, respectively (Table 1). At the SRT of 35 d, they observed the constant predominance of small flagellates, carnivorous ciliated protozoa and rotifers.

In the routine operation of many WWTPs, no taxonomic analyses of activated sludge or microscopic observations are performed at all. The evaluation of the activated sludge condition is based solely on physicochemical parameters. As the activated sludge biocenosis is formed spontaneously and its composition reflects the parameters of the WWTP, regular microscopic inspection should be carried out in parallel with physicochemical tests. Bacteria are the main component of the activated sludge biocenosis, responsible for the degradation of pollutant loads. However, only a few can be observed under a microscope. They are mainly filamentous and spiral bacteria. Most of the bacteria responsible for decomposing organic matter and reducing the content of nutrients in sewage are micro-fouling forms occurring in the structure of activated sludge flocs. Since they are small, colourless and have little contrast, they cannot be identified by light microscopy. Zoogleal and monocolony-forming bacteria are also difficult to quantify. Selected groups of bacteria visible under the microscope can be quantified. These are free-floating (planktonic) bacteria that indicate a very high food-to-mass ratio (F/M) of the sludge or its unstable operation, spiral bacteria that indicate a low oxygen concentration, and spiral bacteria whose overgrowth results in poor performance of CAS systems. The excess of bacteria is effectively removed by large protozoa, especially ciliates, and small protozoa, especially rotifers. In conclusion, in a properly functioning sludge, the number of observable bacteria should be small, and the number of cilia and tissues should be large enough. Therefore, protozoa and metazoa, which are large, diverse and characteristic groups of microorganisms play a valuable diagnostic role. The observation of protozoa especially provides important information about the condition of the activated sludge. Their species composition, proportions of individual communities and morphological features allow assessment of the sludge SRT, organic loading, presence of toxic substances (including SO_2) and level of oxygenation. Unfortunately, most protozoa tolerate variability in environmental factors well, and only a few have well-documented preferences for their use as indicators. Out of about 250 described species of protozoa identified in the activated sludge, only a quarter occur regularly. Usually, 70% of them are ciliates and 10%

Table 1
Frequency (Freq.) and predominance (Pred.) of protozoa and small metazoa in MBR activated sludge (cited from [21])

Classification	Taxa	Phase I		Phase II	
		Freq. (%)	Pred. (%)	Freq. (%)	Pred. (%)
Small flagellates		100		100	63
Large flagellates	*Paranema* spp.	14		37	
Free-swimming ciliates	*Colpoda* spp.			12	
	Pseudochilodonopsis fluviatilis			12	12
	Uronema spp.			25	
	Paramecium spp.	14			
	Not identified			87	
Crawling ciliates	*Aspidisca turrita*			12	
	Aspidisca cicada	86	71	62	
	Acineria uncinata			37	
	Chilodonella uncinata			50	
	Trochilia minuta			25	12
Attached ciliates	*Vorticella convallaria*	43		50	12
	Vorticella microstoma			100	
	Vorticella infusionum	86	14	75	
	Opercularia spp.	71		37	
	Epistylis spp.	14		12	
	Carchesium spp.			12	
Carnivorous ciliates	*Coleps hirtus*	43	14	37	
	Tokophrya spp.	14		37	
	Litonotus spp.	100		100	
Small metazoa	Rotifers	71		100	

are amoebas. The number of protozoa is noticeably lower than the number of bacterial cells, but they are many times larger, so they can be identified and quantified. Ciliates use organic food particles, bacterial cells or other protozoa as food, especially undesirable small flagellates. As a result, they play an essential role in the renewal of the activated sludge biocenosis. However, ciliates are a diverse group of protozoa, which can be divided into stalked, crawling and free-living species. The latter have the highest nutritional requirements and should not dominate in a sludge of good F/M ratio. Amoebas prefer a higher sludge F/M ratio and tolerate low oxygen concentrations well. On the other hand, testate amoebas surrounded by a type of shell are typical organisms abundant in sludge of low F/M and are an indicator of proper nitrification. Summing up, a well-working activated sludge should be dominated by crawling and stalked ciliates remaining in balance with each other. In addition, a moderate number of metazoa and shell amoebas is also desirable [22–27].

2. Materials and methods

2.1. Description of the MBR plant

The WWTP investigated in this study is a medium-size plant treating typical municipal wastewater, with a capacity equal to 6,200 person equivalents (PEs) and two MBRs installed. The treatment technology is aimed at the removal of organic pollutants, biological nitrification and denitrification, and chemical precipitation of phosphorus. Incoming wastewater is mechanically treated with a 6 mm vertical screen and then with a 3 mm sieved grit chamber. Mechanically treated wastewater is retained in a 220 m^3 equalization tank and pumped into two MBRs (A and B) operating in parallel. Each MBR has a total volume of 606 m^3 and includes anoxic and aerobic zones. Each anoxic zone is equipped with the mixers to keep activated sludge in suspension. The aerobic zones have disc air diffusers installed to aerate the activated sludge. Microfiltration flat-sheet membranes of 0.2 μm pore size and a total area of 3,696 m^2 are installed at the end of the aerobic zone. The membranes operate in a cycle of 50 min/h at an average TMP of 0.01–0.04 bar. The membrane modules are continuously cleaned with a dedicated coarse-bubble aeration system. The return sludge is recirculated from the end of the aerobic zone to the beginning of the anoxic zone. The excess sludge is periodically removed from the MBR and pumped to a separate aerobic chamber for its biological stabilization and then dewatered with a centrifuge to reduce its volume. Major operational parameters of the analysed WWTP are presented in Table 2. The 3D diagram of the MBR configuration is shown in Fig. 1.

2.2. Sample collection and analysis

The activated sludge samples were collected over a period of eight months, from November 2018 until June

Table 2
The design and actual parameters of the analysed facility

Parameter	Design value	Actual value
Wastewater flow, m^3/d	862	680
Person equivalent (PE)	6,200	3,740
Biological reactor volume, m^3	1,212	1,212
Anoxic volume, m^3	279	279
Aerobic volume, m^3	933	933
Solids retention time (SRT)	25	54
Food to mass ratio (F/M), g BOD_5/g MLSS d	0.04	0.025
Mixed liquor suspended solids (MLSS), kg/m^3	8	9–15
Dissolved oxygen conc. in the aerobic zone, g O_2/m^3	2	0.4–1.4
Temperature in aerobic zone, °C	12	11.2–20

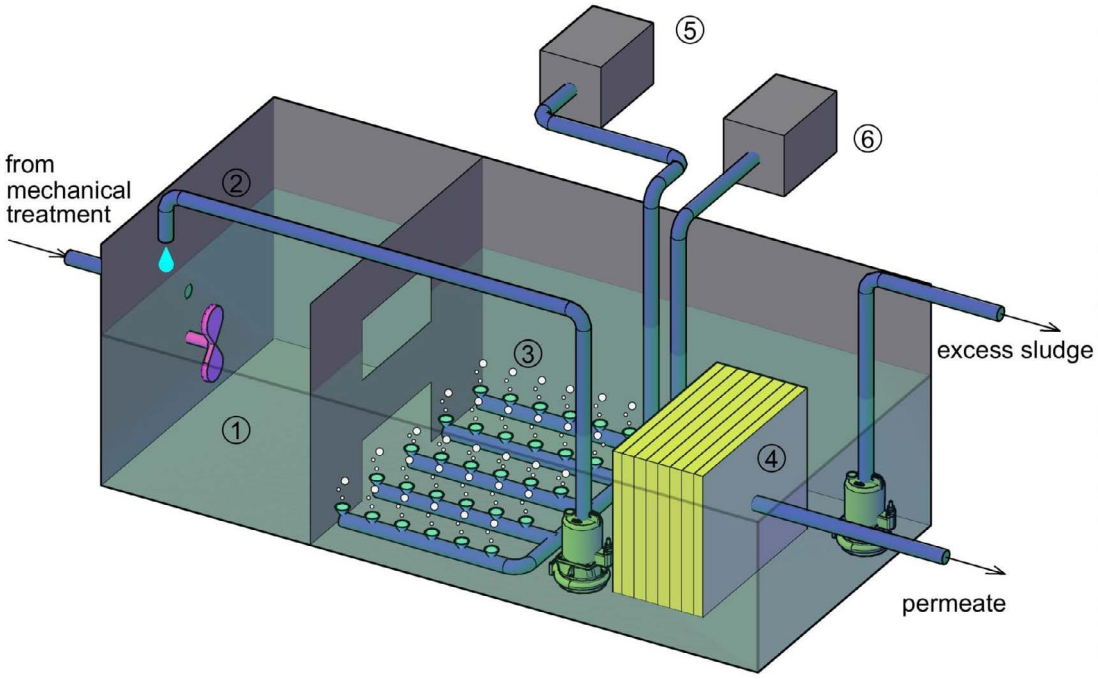

Fig. 1. Diagram of the MBR in the analysed plant (1 – anoxic zone; 2 – return sludge; 3 – aerobic zone; 4 – membrane modules; 5 – blower; 6 – membrane modules blower).

2019. The samples were taken from both MBRs (A and B) at the same time, as the operational parameters in the reactors were not identical. Both reactors have the same characteristics, but during the tests, reactor A received about 25% more wastewater than reactor B. Despite different loading both reactors operated with similar technological efficiency, obtaining the required quality of treated wastewater. This is due to the adoption of design parameters that ensure high operational stability. Nevertheless, it could be expected that there might be differences in the taxonomic picture of the activated sludge in both reactors.

The sludge samples were transported to the laboratory in containers providing air access. The microscopic examination was performed up to 3 h after a sludge sample was collected. A wet mount was used for observing living activated sludge. All observations were made in triplicate in the bright field using magnifications 64x, 160x and 640x, trying not to extend individual observation beyond 20 min. If necessary, immersion oil was applied to the slide and the observation was made under a magnification of 1,600x. Staining of fixed activated sludge smears was not performed. The floc size was measured using a Moticam BTU8 Camera with Motic Images Plus 3.0 ML software. The quantitative analysis was performed by placing a defined volume of activated sludge (50–100 μL) under the microscope and counting the microorganisms. Determination of quantitative indexes for individual groups of microorganisms was performed on the basis of the generally accepted recommendations of Madoni [26], Eikelboom [27] & Fiałkowska et al. [28] and other commonly

accepted solutions. The index of filamentous organisms was established by comparing the microscopic image with reference images, in accordance with commonly accepted methods [26].

3. Results and discussion

3.1. Characteristics of the biocenoses in the MBRs

A common feature of the activated sludge from both reactors is the similar morphological picture of the flocs, which are small to medium in size, rather open and irregular. The flocs contain a relatively small number of filamentous organisms. Only in the spring samples did the number of filaments increase significantly. Similarly, in both sludges, the number of spiral bacteria (*Spirillae* and *Spirochaetae*) is very moderate, which may indicate proper oxygen conditions. Looking at the sludge taxonomic structure, a certain evolution of both biocenoses can be noticed over the period of observation from November to June. However, it has a different character in each analysed MBR.

In the activated sludge samples taken from reactor A, considerable variability of the micro fauna is noticeable (Table 3). In the autumn samples, the number of attached (sedentary) ciliates (Fig. 2e and h), which usually constitutes the dominant group of ciliates, decreases dramatically. On the other hand, throughout the period from the end of winter to early summer, the dominant position of crawling cilia remains, which has a positive effect on the structure of the flocs. The small number of free-swimming ciliates may indicate a minor amount of the free-floating bacteria that they feed on. However, the microscopic image does not show any free-floating bacteria. The number of flagellates also decreases in winter and increases in spring, but without exceeding the values considered as acceptable. However, small dinoflagellates and large bacteriophageous flagellates are absent. Flagellates are decreasing in the late winter samples, but there is a corresponding rapid multiplication of amoeba genera *Amoeba* and *Saccamoeba* (Fig. 2a). Usually, both of these groups appear in large numbers at a high sludge F/M ratio and low dissolved oxygen concentration.

Generally, it can be seen that reactor A entering the winter period causes serious disturbances in the activated sludge structure (no metazoa at all). This may result from a high F/M ratio or unstable operation of the activated sludge. In the March samples, a noticeable change in the biocenosis structure is observed in reactor A. The number of flagellates is increasing, and the number of amoebas is declining. At the same time, the numbers of ciliates, testate amoebas (Fig. 2f) and metazoa (Nematodes and Rotifera, Fig. 2c) start to increase. Thus, the processes of sludge biocenosis regeneration begin in spring. *Aspidisca* sp. dominates among the ciliates (Fig. 2g), as it has high oxygen requirements and prefers low F/M values. Further signs of improvement are observed in the June samples: no amoebas and a large number of ciliates and testate amoebas, the presence of which is beneficial. Small invertebrates are numerous in the sample and, as they are long-living organisms, their presence proves the stable operation of the activated sludge in reactor A.

The activated sludge samples taken from reactor B in the period from November to July showed a similar variability as in the case of samples from reactor A (Table 4). In November, when the winter season starts, there are relatively few ciliates and flagellates in the sludge. Among the protozoa, testate amoebas are found in significant numbers, which disappear with time. Sessile ciliates do not exist, which is quite an unusual situation. There are few metazoans, and they are represented almost entirely

Table 3
Changes in the reactor A biocenosis

Reactor A	Number of organisms in 1 mL of sludge and indexes			
	November 2018	January 2019	March 2019	June 2019
Ciliates				
Attached	5,100 [44%]	167 [5%]	100 [4%]	500 [5%]
Crawling	5,450 [47%]	3,137 [94%]	2,350 [85%]	9,400 [93%]
Free-swimming	1,050 [9%]	33 [1%]	300 [11%]	200 [2%]
Ciliates total	11,600	3,337	2,750	10,100
Flagellata	10,350	133	21,800	23,700
Amoebas	100	4,133	50	0
Testate amoebas	350	333	700	400
Nematodes	0	33	0	2,000
Rotifera	50	567	0	1,000
Tardigrada	0	0	0	0
Gastrotricha	0	0	0	0
Filamentous organisms (index)	1.0	1.0	3.0	1.0
Spiral bacteria (index)	0.0	n.d.[a]	1.0	0.0
Average floc size in μm (floc index)	n.d.[a]	116.0 (1.0)	125.4 (1.0)	97.5 (1.0)

[a]n.d. – not detected

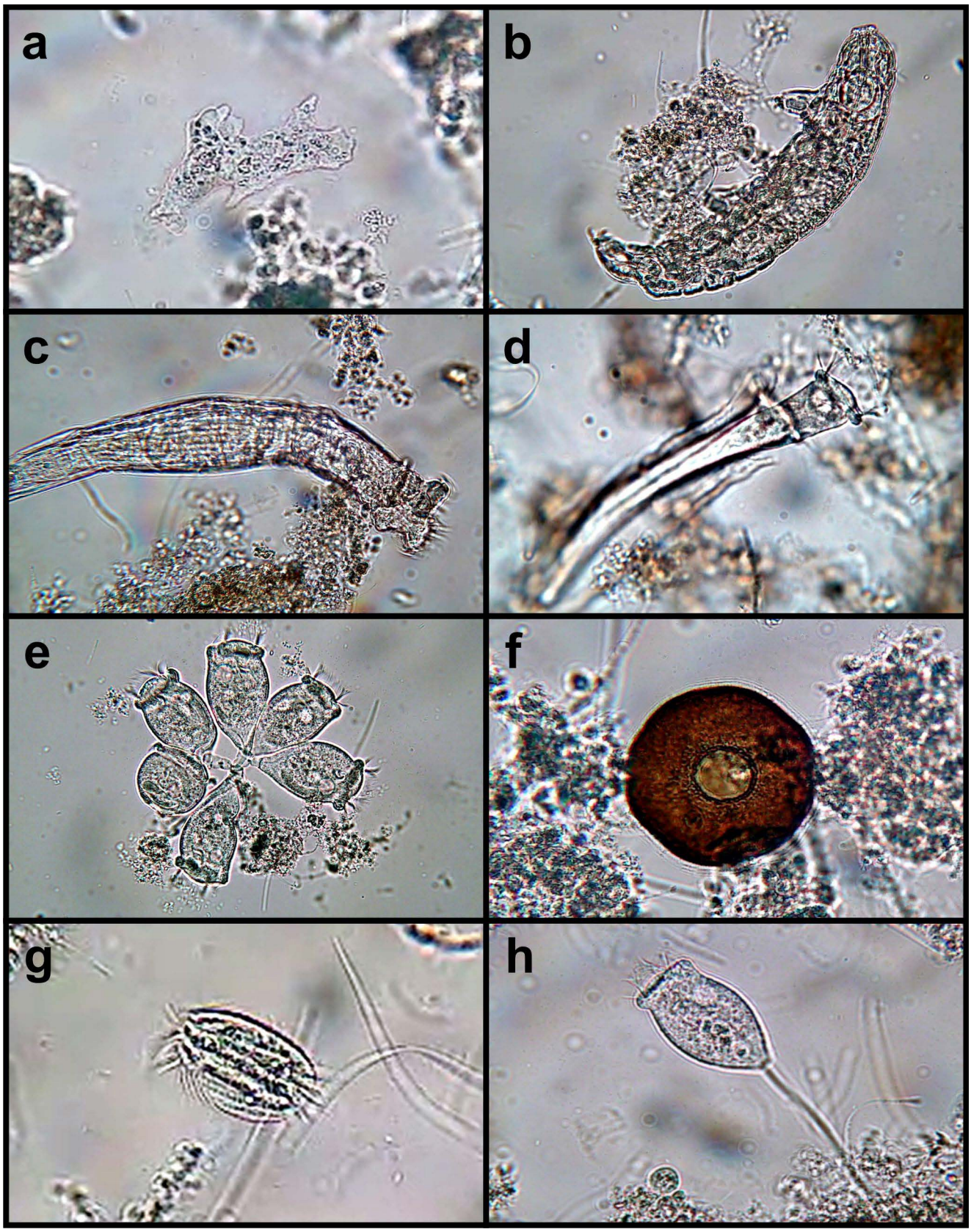

Fig. 2. Typical representatives of protozoa and metazoa present in the investigated activated sludge samples. (a) *Saccamoeba* sp., (b) representative of Tardigrada, (c) *Rotaria* sp., (d) *Thuricola folliculata*, (e) *Epistylis* sp., (f) *Arcella* sp., (g) *Aspidisca* sp., and (h) *Vorticella* sp.

Table 4
Changes in the reactor B biocenosis

Reactor B	Number of organisms in 1 mL of sludge and indexes			
	November 2018	January 2019	March 2019	June 2019
Ciliates				
Attached	0 [0%]	2,800 [88%]	750 [37.5%]	2,200 [49%]
Crawling	1,350 [93%]	200 [6%]	1,150 [57.5%]	1,500 [33%]
Free-swimming	100 [7%]	200 [6%]	100 [5%]	800 [18%]
Ciliates total	1,450	3,200	2,000	4,500
Flagellata	2,200	4,767	59,900	9,100
Amoebas	0	133	0	0
Testate amoebas	3,550	1,700	100	100
Nematodes	0	33	0	4,000
Rotifera	50	233	50	1,000
Tardigrada	450	33	0	0
Gastrotricha	0	0	0	0
Filamentous organisms (index)	1.0	1.0	3.0	1.0
Spiral bacteria (index)	0.0	n.d.[a]	1.0	0.0
Average floc size in μm (floc index)	n.d.[a]	84.0 (1.0)	121.1 (1.0)	86.5 (1.0)

[a]n.d. – not detected

by tardigrades. The presence of numerous testate amoebas (Fig. 2f) and tardigrades (Fig. 2b) proves that the activated sludge works well at a low F/M ratio. The lack of free-swimming ciliates probably results from the activity of tardigrades and crawling ciliates, which eliminate free-floating bacteria and some filamentous bacteria, as well as the lower F/M ratio in the reactor. In the winter samples, there are noticeable changes in the sludge biocenosis. Initially, the tardigrades disappear and do not come back. The number of testate amoebas is also decreasing. Virtually all ciliates are represented by *Thuricola* sp. (Fig. 2d) and testate amoebas – *Euglypha* sp. The presence of these genera proves that the activated sludge is still working well. The sludge still seems to be stable in terms of living conditions, and is well oxygenated and low loaded.

In the March samples, similar changes in the biocenosis structure are observed in reactor B as in reactor A. All microorganisms typical for a properly functioning activated sludge disappear. The number of ciliates is decreasing, the metazoans are practically absent, and the number of testate amoebas is also falling. Meanwhile, the number of flagellates is growing to about 60,000 individuals per 1 mL. It can be assumed that the maximum number of flagellates in a well-functioning activated sludge does not exceed 50,000 individuals per 1 mL. All this may indicate a low dissolved oxygen concentration, high sludge F/M ratio and a generally unstable operation of reactor B. However, in the June samples, everything returns to normal. The number of dinoflagellates is decreasing, and the number of attached and crawling ciliates increases. Nematodes and rotifers also return to the typical level for a well-functioning activated sludge (2,000–3,000 individuals per 1 mL).

The observed average size of the activated sludge flocs in both MBRs was similar and ranged from 84 to 125 μm. This value is clearly lower than that reported in the literature, where the values are given as 240 μm for MBR and 160 μm for CAS [7].

3.2. MBR vs. CAS systems

The taxonomy of the activated sludge samples from the MBR were compared with samples taken from a CAS reactor from a similar WWTP of 1,500 m^3/d capacity and PE equal to 6,950. The results of the test were obtained from the plant operator. The CAS plant uses 3 mm sieves and a grit chamber for mechanical treatment, two multiphase activated sludge reactors with anaerobic, anoxic and aerobic zones, and vertical-flow secondary settling tanks for biomass separation. However, the activated sludge process is run with values typical of CAS systems for the major technological parameters, namely: a higher F/M ratio (0.15 g BOD_5/g MLSS d), shorter SRT (8–10 d) and smaller mixed liquor suspended solids (MLSS) (4.5 kg MLSS/m^3). The sludge samples were taken during a comparable period of time, including winter, spring and summer conditions from February through to July (Table 5).

In most of the collected sludge samples, no significant variation in the taxonomic composition is observed over most of the sampling time. The flocs are small to medium-sized and contain a significant amount of filamentous organisms. Small invertebrates are practically absent. Attached ciliates are definitely dominant in the ciliate structure, while crawling ciliates are practically absent until mid-summer. This was quite different from the sludge from both MBRs, where both types of ciliates are present in significant numbers for most of the time (Fig. 3). The presence of attached ciliates is usually beneficial, and they are the most characteristic component of the activated sludge biocenosis. However, if they appear *en masse* and gain dominance over other organisms of activated sludge, this

Table 5
Changes in the biocenosis of activated sludge in the reference CAS reactor

Classification and indexes	*Ciliates* distribution and indexes					
	Feb 2016	Mar 2016	Apr 2016	May 2016	June 2016	July 2016
Ciliates (index)	1.5	3.0	1.5	1.0	1.0	2.0
Attached	95%	95%	100%	80%	100%	15%
Crawling	0%	0%	0%	20%	0%	85%
Free-swimming	5%	5%	0%	0%	0%	0%
Flagellata (index)	0.5	0.0	0.5	1.0	1.0	0.5
Amoebas (index)	0.0	0.0	0.0	0.0	0.0	0.0
Testate amoebas (index)	0.0	0.0	0.0	0.0	0.5	2.5
Nematodes (index)	0.0	0.0	0.0	0.0	0.5	1.0
Rotifera (index)	0.0	0.0	0.0	0.5	0.0	0.5
Tardigrada (index)	0.0	0.0	0.0	0.0	0.0	0.0
Gastrotricha	0.0	0.0	0.0	0.0	0.0	0.0
Filamentous organisms (index)	0.5	1.0	1.5	2.0	1.0	1.5
Spiral bacteria (index)	0.0	0.5	1.0	0.0	0.0	0.0
Floc size (index)	1.5	1.5	1.5	1.0	1.5	2.0

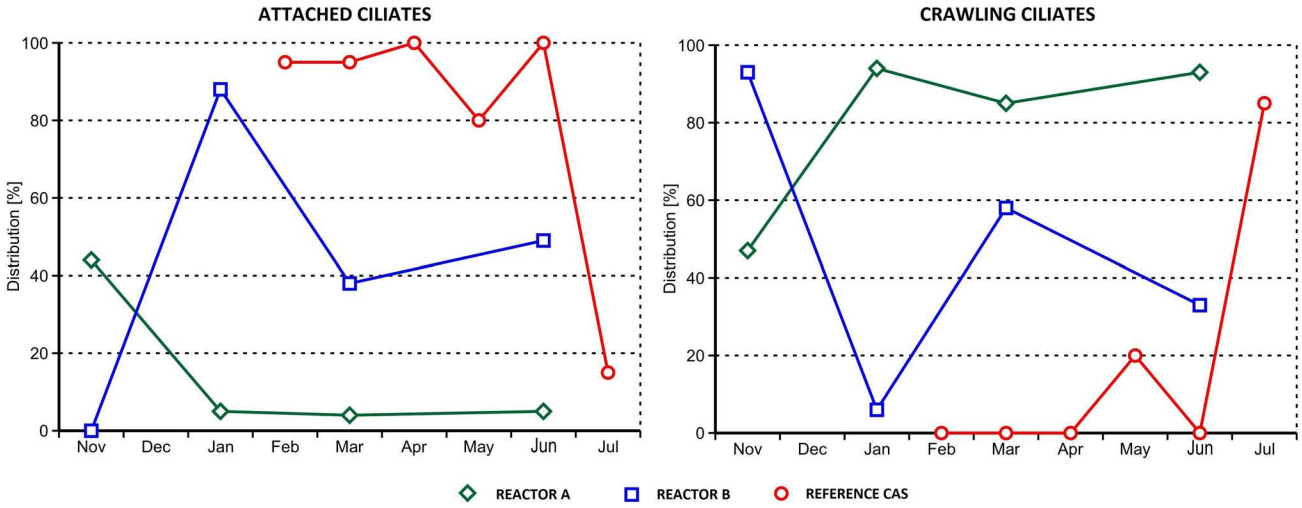

Fig. 3. Change in the biocenosis composition in the studied MBR reactors and the reference CAS reactor during the research.

may indicate a sudden increase in F/M ratio or its instability. Nematodes and rotifers appear in the CAS reactor only in mid-summer. The number of testate amoebas is also increasing and the species structure of ciliates changing at this time, which is evidence of positive changes taking place during this season of the year. It should be noted that despite the observed differences in the biocenosis of the activated sludge in MBR and CAS reactors, they did not have a noticeable impact on the technological performance of both reactors.

4. Conclusions

The average size of the sludge flocs observed in both MBR reactors was similar and was clearly smaller than the values reported in the literature. The microscopic analysis of the activated sludge biocenosis structure in the MBRs showed that it depends on the operating conditions. The taxonomic structure of the MBR sludge also clearly changes with the seasons of the year: it is clearly disturbed in winter and early spring (January-March), while a clear improvement occurs only at the beginning of summer (June). An unexpectedly high number of tardigrades were only in one of the analysed MBRs (B) in the autumn–winter period, and these were gradually replaced by nematodes and rotifers with the onset of spring. It is difficult to explain unequivocally, but this was most likely related to the smaller F/M sludge loading in reactor B compared to reactor A. There was also a very marked seasonal increase in the number of flagellates in the spring in reactor B, and also to a slightly

lesser extent in reactor A; however, this subsided with the arrival of summer.

Such significant changes in the structure of the activated sludge biocenosis in both reactors can be explained by various reasons. Firstly, in winter the average wastewater temperature is lower than in summer. The temperature of wastewater flowing into the MBR WWTP in January–February was 11.2°C, while in June it increased to 19°C–20°C. This means a significant change in the conditions for the growth of microorganisms. The winter period is usually the time when filamentous organisms dominate and samples collected in March confirm this observation. Another factor may be changes in oxygen concentration. In the winter period, the average oxygen concentration in the tested MBRs remained at the level of ~0.4 g O_2/m^3, while in June it was ~1.2 g O_2/m^3. This could be the reason of the winter decline of metazoa and other oxygen-requiring microorganisms. In June, their share in the sludge biocenosis increased significantly. The winter oxygen deficit caused a disturbance of nitrogen transformations, which indirectly also proves the existence of unfavorable conditions for the development of a stable activated sludge biocenosis.

The microscopic analysis of the activated sludge samples from the CAS reactor over a similar period of time showed that there were no such clear changes in the sludge taxonomic structure with the seasons, as in the MBR sludge. A noticeable, beneficial change in the taxonomic structure of the sludge took place only in July, that is, significantly later than in the MBRs. This indicates faster sludge succession in the MBRs and, at the same time, a greater sensitivity of this sludge to changes of season, despite a much longer SRT than in the CAS systems. It should be noted that the observed seasonal changes in the structure of the activated sludge biocenosis in the MBRs did not have a noticeable impact on the technological efficiency of the biological treatment processes. This proves the high technological stability of the MBR process and the resistance to changes in the composition of the activated sludge.

Funding

This work was partially funded by Schwander Polska Sp. z o.o. under the contract no. Ś-4/707/2018/P with Cracow University of Technology.

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