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Comparison of the Results from Microscopic Tests Concerning the Quality of Activated Sludge and Effluent

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Abstract: Physicochemical studies on wastewater quality and microscopic analyses of activated sludge are necessary to properly evaluate the condition of activated sludge. The aim of this study was to evaluate the application of the sludge biotic index to determine when a change in treatment quality is short-lived, caused by, e.g., a change in influent quality or quantity, and when it indicates adverse changes in the biocenosis of activated sludge, which would very likely result in the lower efficiency of wastewater treatment in the near future. The objects of the study were two identical parallel running small wastewater treatment plants. The following indicators of contamination were analyzed: the chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), and total suspended solids (TSS). The authors additionally carried out a microscopic analysis. The study confirmed a correlation between the sludge biotic index and the removal efficiency of COD and BOD_5; however, no correlation was found between the sludge biotic index and the removal efficiency of total suspended solids. The presence of metazoan microorganisms coincided in time with a good effluent treatment efficiency. When their quantity declined, a decrease in the efficiency of wastewater treatment was also observed.

Keywords: activated sludge; microscopic analysis; small wastewater treatment plant; sludge biotic index; treatment efficiency

1. Introduction

1.1. Activated Sludge in Biological Wastewater Treatment

The first research on activated sludge was carried out in England and the United States at the end of the 19th century. In the 1880s, the primary scientific objective was to remove odours from wastewater. Significant experiments were conducted between 1912 and 1913 at Lawrence Experimental Station of Massachusetts State Board of Health (the USA); however, they did not produce satisfactory results. Edward Arden and William T. Lockett, members of the River Committee of the Manchester Corporation, UK, modified the research. Subsequently, they coined the term "activated sludge" and introduced it to the literature [1].

The interest in biological wastewater treatment, particularly using the technology of activated sludge, has increased in recent years [2–4]. The activated sludge process is efficient and economical, and as a result, it prevails among currently used biological systems. The method has been used in both flow reactors and sequential batch reactors (SBRs) for about twenty years [5].

Activated sludge is a suspension of flocs in which bacterial cells are crucial active ingredients [1]. Sludge flocs consist of living and dead microorganisms such as bacteria (including filamentous bacteria), protozoa (ciliates, flagellates, amoebae), metazoa (rotifers, nematodes), fungi, and Actinobacteria. Flocs also include non-decomposed organic particles and inorganic particles such as sand [6,7].

Physicochemical studies on wastewater quality and microscopic analyses of activated sludge are necessary to properly evaluate the condition of activated sludge. In order to determine its condition, on-site options are limited to the activated sludge volume after 30 min sedimentation. However, field methods are primarily designed for assessing the sedimentation properties of activated sludge [8]. The operation of wastewater treatment plants that use activated sludge should be based on comprehensive control of the processes involved. A fundamental aspect of this control ought to be microscopic tests of activated sludge. A microscopic analysis enables finding the causes of poor sedimentation by identifying filamentous microorganisms. Incorrect morphology indicates the inappropriate composition of the influent and/or altered environmental conditions in the reaction chamber [9]. Depending on the frequency and type of occurring microorganisms, it is possible to specify [10]:

The good condition of activated sludge that is characterised by:

- high content of ciliates—settled and freely floating,
- high content of *Bacillus* species, *Flavobacteria*, *Pseudomonas* bacteria, and zoogleal bacteria,
- occurrence of rotifers and nematodes,
- low population number of flagellates and amoebae,
- lack of fungi and filamentous bacteria;

The average condition of activated sludge that is characterised by:

- low content of ciliates,
- occurrence of transparent flagellates and amoebae,
- occurrence of fungi and filamentous bacteria;

The poor condition of activated sludge that is characterised by:

- low content of ciliates,
- frequent occurrence of filamentous bacteria, flagellates, amoebae and fungi.

Studies on the operation of small wastewater treatment plants (SWTP) often prove to be highly problematic. A frequent cause is the lack of appropriate measuring equipment, as well as the high variation in influent quality and quantity [11–15]. The pilot facility replicated the operation of a standard SWTP and was subjected to typical physicochemical measurements and less commonly performed microbiological tests, i.e., microscopic analysis. The experiment was aimed at demonstrating whether the use of a sludge biotic index can determine when the change in treatment quality is short-lived—caused by, e.g., a change in influent quality or quantity—and when it is informative about adverse changes in the biocenosis of activated sludge, which would very likely result in a lower efficiency of wastewater treatment in the near future.

1.2. Microorganisms of Activated Sludge

Bacteria are found in the form of single cells or micro-colonies and are the largest group of microorganisms. Their concentration ranges between 1.6×10^9/mL and 2.4×10^{10}/mL. The most common types of bacteria are members of the following genera: *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Achromobacter*, and *Zooglea*. Zoogleal bacteria play an important role in activated sludge due to the contribution to slime growth.

Filamentous microorganisms are mainly bacteria and fungi. They take part in forming immobilized biomass (biofilm) and structuring activated sludge flocs. When found in activated sludge, they may cause serious operational problems. As single-celled organisms, filamentous bacteria reproduce into strands (chains) and contribute to the swelling of sludge. They can be observed under conditions of a low oxygen concentration or in rotten wastewater, in activated sludge, and in biofilm [16]. Examples of these bacteria are members of the following genera: *Nocardia*, *Sphaerotilus*, and *Thiothrix*.

Fungi are not typical organisms of activated sludge, but they may occur in wastewater with a low pH and nitrogen deficiency. Incoming industrial or toxic wastewater can create suitable conditions for the development of fungi. Actinobacteria, as the form of filamentous microorganisms, may also adversely affect the properties of activated sludge.

Protozoa are unicellular eukaryotic organisms ranging in size from 2 to 2000 μm. They are characterised by various morphological features, including specialised organelles. There are three basic protozoan groups in activated sludge: ciliates, flagellates, and amoebae. Protozoa are heterotrophs and feed mainly on bacteria and other small microorganisms [17]. It is commonly believed that the primary function of these organisms is to improve the quality of outflow by eliminating bacteria that are dispersed in a liquid phase or on the edges of flocs [18]. An important consequence is also that they help to eliminate pathogenic bacteria [19].

The most common protozoans in activated sludge are ciliates, which are completely or partially covered with cilia. Cilia are used to move and nourish. There are three types of ciliates. Stalked ciliates can occur in colonies or as single organisms. They stick to the surface of flocks using a petiole (foot/leg segment). The structure includes a mouth (peristome) surrounded by cilia. The most common types of stalked ciliates belong to the *Vorticella*, *Carchesium*, and *Opercularia* groups. Crawling ciliates occurring in activated sludge belong to the *Aspidisca* genus. Ciliates with reduced mobility are characteristic of sludge with a small number of scattered bacteria. The energy demand of these forms is smaller. Free swimming ciliates, usually in motion in the liquid phase of sludge (between flocks), are observed when there is a large number of scattered bacteria. The most common types of ciliates include *Lionotus*, *Colpidium*, *Paramecium*, *Euplotes*, and *Chilodonella* [6,7,20]. Other literature reports indicate that *Euplotes* and *Chilodonella* are classified as crawling and bacterivorous ciliates [21,22].

Moving protozoa mix the liquid in the vicinity of colonies. As a result, they reduce their limitations due to the rapid consumption of essential substances in their environment. According to the literature [23], the movement of membranellae of these filtering ciliates is important for transporting substances in the sludge environment. Fried and Lemmer [24] carried out research on the effects of this phenomenon in biological reactors. The test results demonstrate that water currents, induced by *Epistylis coronata* ciliary structures, were noticeable, even at the distance of >500 μm from ciliates. The maximum speed was 180 $\mu m \cdot s^{-1}$.

Among multicellular organisms, there is a notable presence of nematodes and rotifers. Nematodes are fast moving and are easily seen due to their size: 500 to 3000 μm. Typically, with low contamination loading, there is a more frequent occurrence of nematodes. Rotifers are active organisms that can be up to 1000 μm long. Their head, which has circularly arranged cilia, serves to move and collect food. The rear part, commonly known as a leg, can also be a motor organ. A higher population number of rotifers can be observed with low sludge loading with contaminants and good aeration conditions [7,25].

2. Materials and Methods

2.1. Research Facility

The objects of the study were two identical parallel running small wastewater treatment plants, WTP1 and WTP2, working in the SBR system. These wastewater treatment plants are research facilities located in the municipal sewage treatment plant. Raw sewage (wastewater from municipal sewage treatment plants after treatment through the grit chamber) was mixed with tap water to approximate the value of basic contamination indicators typically found in small wastewater treatment plants.

Afterwards, wastewater was separated into two technological lines. Within 24 h, approximately 0.6 m^3 of sewage in 12 different doses flows to each treatment plant.

The wastewater treatment plants have a primary settling tank with a volume of 1.2 m^3 and a biological reactor with a volume of 2.2 m^3 (Figure 1). The wastewater treatment plants work in a 24-h cycle. Wastewater pre-treated in the primary settling tank flows by gravity into the reactor chamber. Aeration is cyclical, from 7.00 to 2.30. The sedimentation phase lasts 2 h, from 2.30 to 4.30. Decantation of effluent takes place once a day.

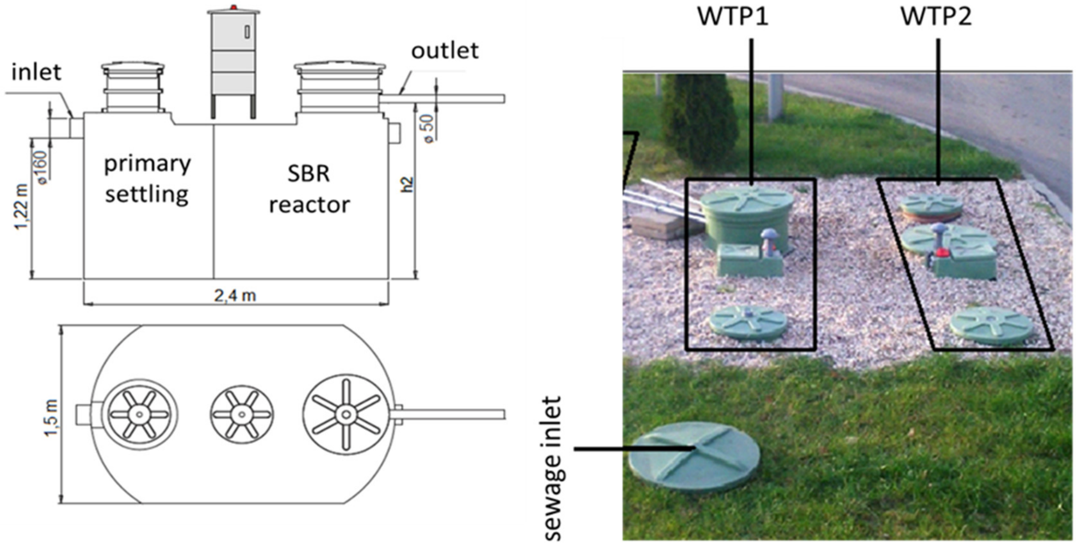


Figure 1. Scheme of wastewater treatment plants.

The study on the two parallel wastewater treatment systems was conducted in three stages:

I. In the period from July to December, all conditions for performing the processes at WTP1 and WTP2 were the same: alternately an aerobic phase (15 min) and an anaerobic phase (15 min), with the periodic removal of small volumes of excess sludge.
II. In the period from December to April, all conditions for performing the processes at WTP1 and WTP2 were the same: alternately an aerobic phase (30 min) and an anaerobic phase (30 min). Excess sludge was removed periodically to maintain the sludge concentration of approx. 2 kg/m^3.
III. In the period from April to June, the conditions for performing the processes were the same except for the aeration time—the aerobic phase in WTP2 was shortened by 17%. WTP2 had alternately an aerobic phase (20 min) and an anaerobic phase (40 min), while WTP1 kept its previous settings. Excess sludge was removed periodically to maintain the approx. sludge concentration of 2 kg/m^3.

2.2. Laboratory Methods of Sludge Testing

Laboratory tests were carried out from July 2014 to June 2015. In the analytical laboratory of the Department of Hydraulic and Sanitary Engineering at the University of Life Sciences in Poznań, an analysis of the following indicators of contaminants was carried out: the chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), and total suspended solids (TSS). COD and BOD_5 were determined through spectrophotometry, and TSS by a direct weight measurement. The authors additionally carried out a microscopic analysis. Observations of activated sludge flocks were performed with a Zeiss AXIOSTAR PLUS (Carl Zeiss, Germany) light microscope, at a magnification of 100× and 400×, in unstained preparations.

For testing, a standard microscope slide was replaced with a Fuchs-Rosenthal counting chamber, which enabled the rapid determination of the number of microorganisms in sludge samples. The depth of the chamber is 0.2 mm, and the total area of a computational grid is 16 mm^2. The number of microorganisms in 1 dm^3 of sludge was calculated using the following Equation (1):

$$\frac{Number\ of\ cells\ in\ 16\ fields\ of\ the\ chamber\ 0.0032l}{0.0032l} = number\ of\ cells\ in\ 1\ \mathrm{dm}^3 \quad (1)$$

2.3. Sludge Biotic Index

The sludge biotic index (SBI) is a scale for showing the condition of activated sludge, taking into account the sensitivity of basic protozoan groups and the presence of other indicative microorganisms. Indirectly, SBI draws attention to the physicochemical and technological parameters of the treatment process, which translates into the diversity of species in activated sludge. Based on the table available in the literature, the biological quality of sludge is defined on a scale from 0 to 10. The material is evaluated on the basis of key groups, the density and number of taxa occurring in activated sludge. The assigned value allows for the classification of sludge as one of four classes. The first-class sludge is colonised and stable with very good biological activity, while the fourth-grade sludge found in aeration chambers is characterised by poor biological treatment [26].

In the first step, all ciliates, live amoebae, rotifers, other metazoa, and big flagellates were counted. The next step was to determine the density of individual species and groups per millilitre of sludge and select the dominant group, followed by selecting the appropriate range of the population number. The limit value is one million individuals per dm^3 of sludge. Another important element is the number of taxa taken into account when analysing the material: the greater the taxonomic diversity found in wastewater, the higher the SBI results from the calculation [21].

3. Results

3.1. Physicochemical Analysis

For the purpose of the study, the following indicators were tested: the chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), and total suspended solids (TSS). The charts show the concentration values of parameters in wastewater entering the SBR (after the primary settling tank—sample collection during the flow of sewage) and in effluent. In addition, they include the maximum permissible concentration values of contaminants in effluent according to Polish requirements for treatment plants in areas up to 2000 PE (population equivalents) [27].

Figures 2–4 illustrate the values of BOD_5, COD, and TSS in wastewater samples at successive collection times. According to Polish law, the acceptable BOD_5 value is 40 mg O_2/dm^3 and COD is 150 mg O_2/dm^3. The permissible TSS value is 50 mg/dm^3. Although the tested treatment plants met the requirements for the removal of organic compounds (BOD_5 and COD), WTP2 was an exception during the last period of research (shortened aeration time). The average treatment performance for the biological part expressed in BOD_5 was 94% for WTP1 and 86% for WTP2. The chemical oxygen demand for WTP1 and WTP2 was, respectively, 87% and 80%. However, the studied facilities encountered a problem with the value of total suspended solids in effluent. There were two reasons for this phenomenon. In the first period of research, it was caused by the long retention time of biomass in the system, whereas in the last period of research, it originated from the deterioration of the activated sludge volume after 30 min sedimentation. The biological part of the treatment plant achieved the average removal efficiency of total suspension at 58% and 36% for WTP1 and WTP2, respectively.

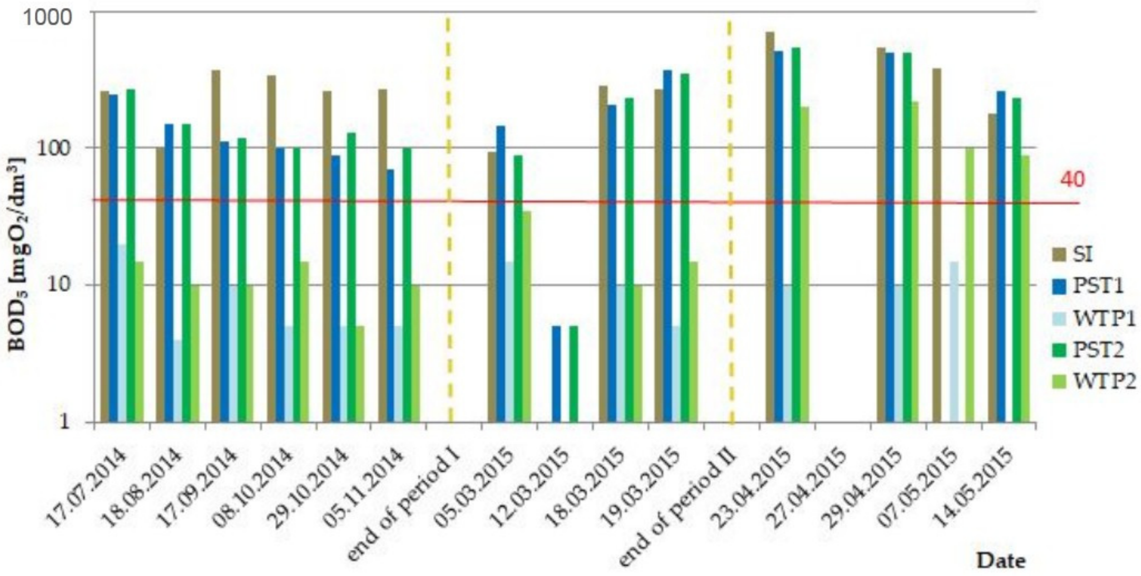


Figure 2. Biochemical oxygen demand (BOD_5) in sewage inlet (SI), after primary settling tank (PST1 and PST2), and after WTP1 and WTP2.

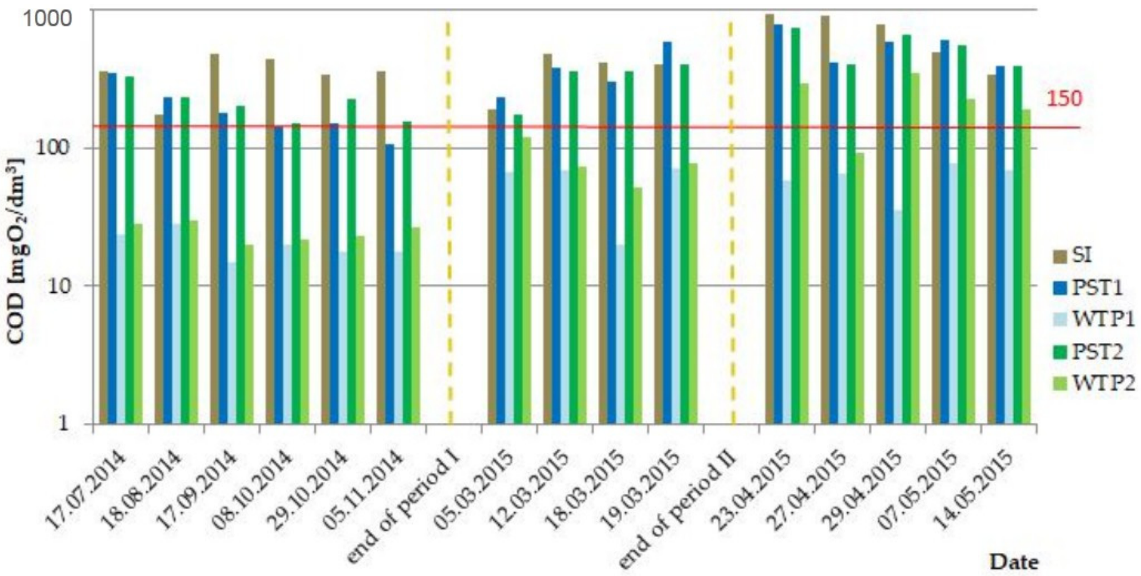


Figure 3. Chemical oxygen demand (COD) in sewage inlet (SI), after primary settling tank (PST1 and PST2), and after WTP1 and WTP2.

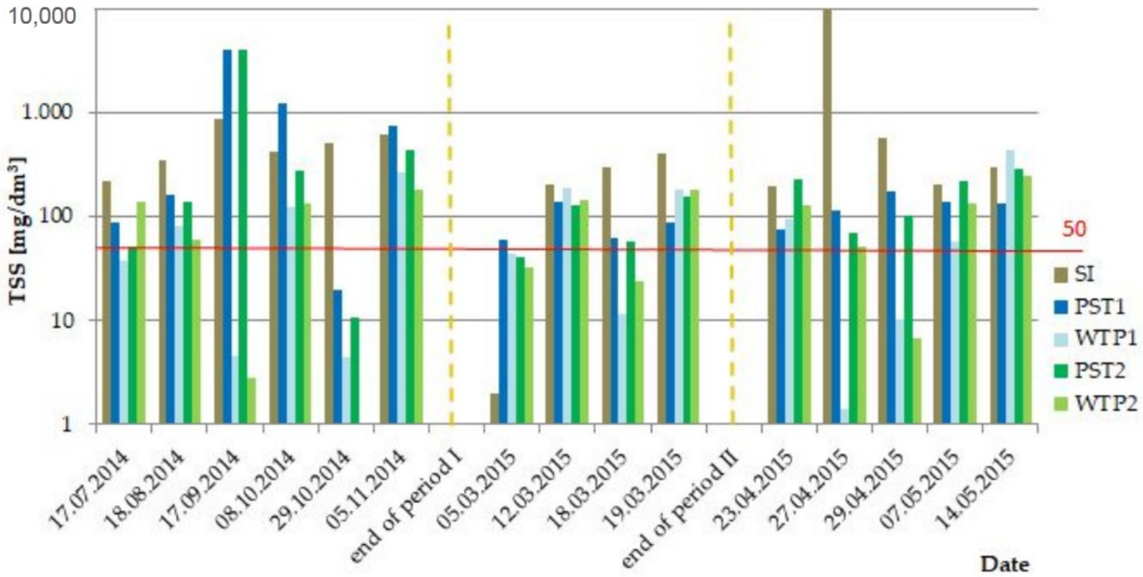


Figure 4. Total suspended solids (TSS) in sewage inlet (SI), after primary settling tank (PST1 and PST2), and after WTP1 and WTP2.

3.2. Microscopic Analysis

The microscopic analysis included activated sludge working in the biological part of the sequential biological reactor (SBR). The estimated number of microorganisms and wastewater treatment efficiency for WTP1 are presented in Figure 5.

WTP1 is characterised by a high BOD_5 and COD removal efficiency. During the analysis carried out from August 2014 to June 2015, different groups of microorganisms were identified. The first two research periods were characterised by a significant number of stalked and free swimming ciliates. Despite good conditions and a satisfactory removal efficiency (stabilisation of the system), rotifers were not observed. Metazoa organisms were most abundant in the system in October and November 2014 (long retention time of biomass in the systems). A decrease in their number was observed after removing a considerable portion of sludge from the chamber (February/March) and maintaining a constant concentration of sludge in both systems at approx. 2 kg/m^3. In March, there were no nematodes in the activated sludge, but an increase in the number of stalked ciliates was observed (Figure 6a). Furthermore, instability of the system was reflected in a sudden and short-lived increase in the number of stalked ciliates. The highest number of stalked ciliates was recorded at the time when the treatment efficiency for COD exhibited the lowest value (approx. 70%). The presence of nematodes and rotifers in the system was no longer observed. The largest number of taxonomic groups was reported in October and November. This was related to correct operation of the treatment plant. A high efficiency of organic removal was observed as early as in August. Rotifers (Figure 6b) as indicative microorganisms appeared in stable sludge with good biological activity properties and a long retention time of biomass in the bioreactor.

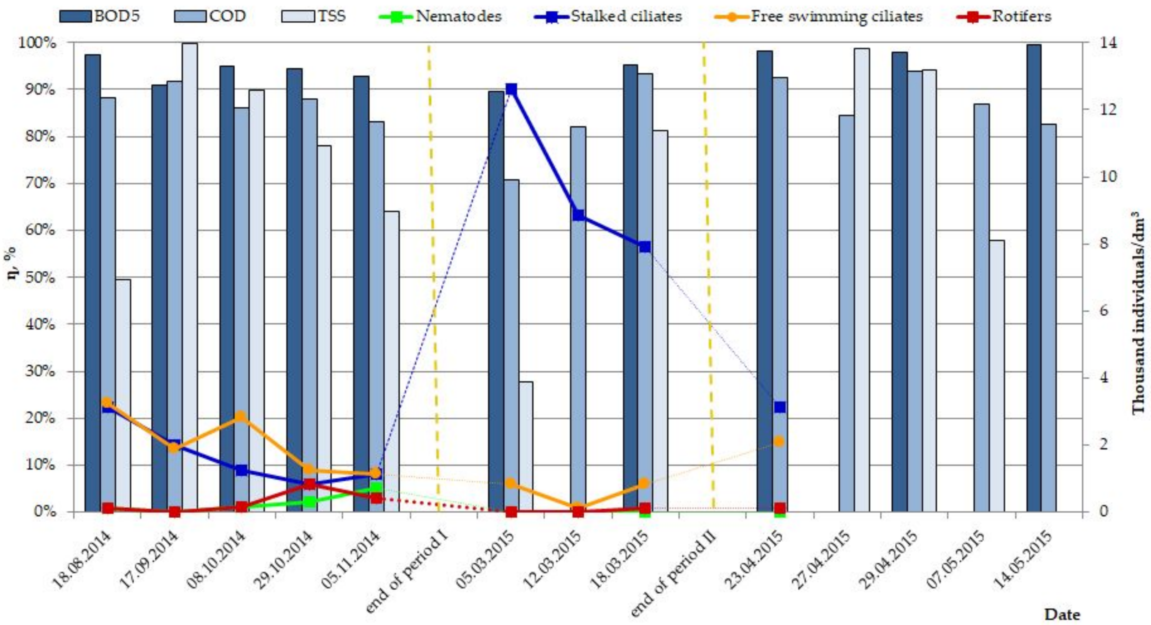
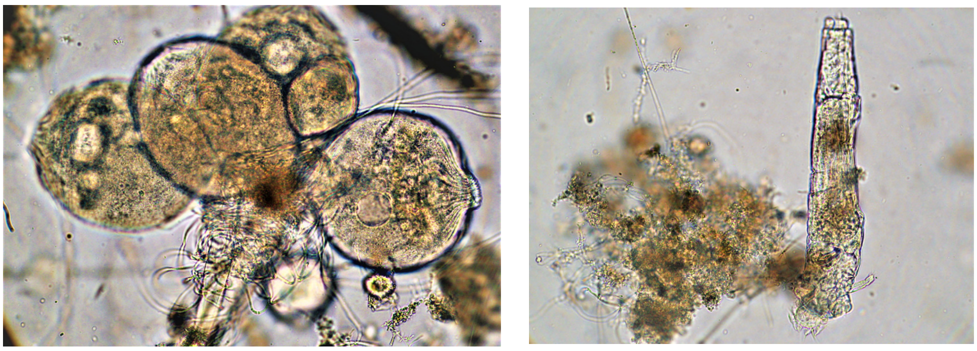


Figure 5. Abundance of organisms in the activated sludge, efficiency of organic compounds removal, and efficiency of total suspended solids removal in WTP1.



(**a**) (**b**)

Figure 6. Observed microorganisms of activated sludge: (**a**) Stalked ciliates; (**b**) Rotifer.

The estimated number of microorganisms and wastewater treatment efficiency for WTP2 are presented in Figure 7. The removal efficiency of organic compounds on the first five research dates was satisfactory and exceeded 80% for both COD and BOD_5. This was reflected in the number of identified taxonomic groups. Apart from stalked and free swimming ciliates, rotifers were also observed. From August, the number of free swimming ciliates began to stabilise. A decisive decrease in wastewater treatment efficiency was observed in March (5 March 2015) in both treatment plants. The microscopic analysis, which recorded the occurrence of only free swimming ciliates, provided a further confirmation of operational problems. Another deflection of the treatment system related to WTP2 occurred in the third period of research—in April. The efficiency of organic removal expressed in BOD_5 and COD was slightly over 60% (day of microscopic analysis). During the last period of research, only the occurrence of free swimming ciliates was recorded in activated sludge. The efficiency of total suspension solids removal was not satisfactory, which cannot be attributed to the number of occurring microbial groups.

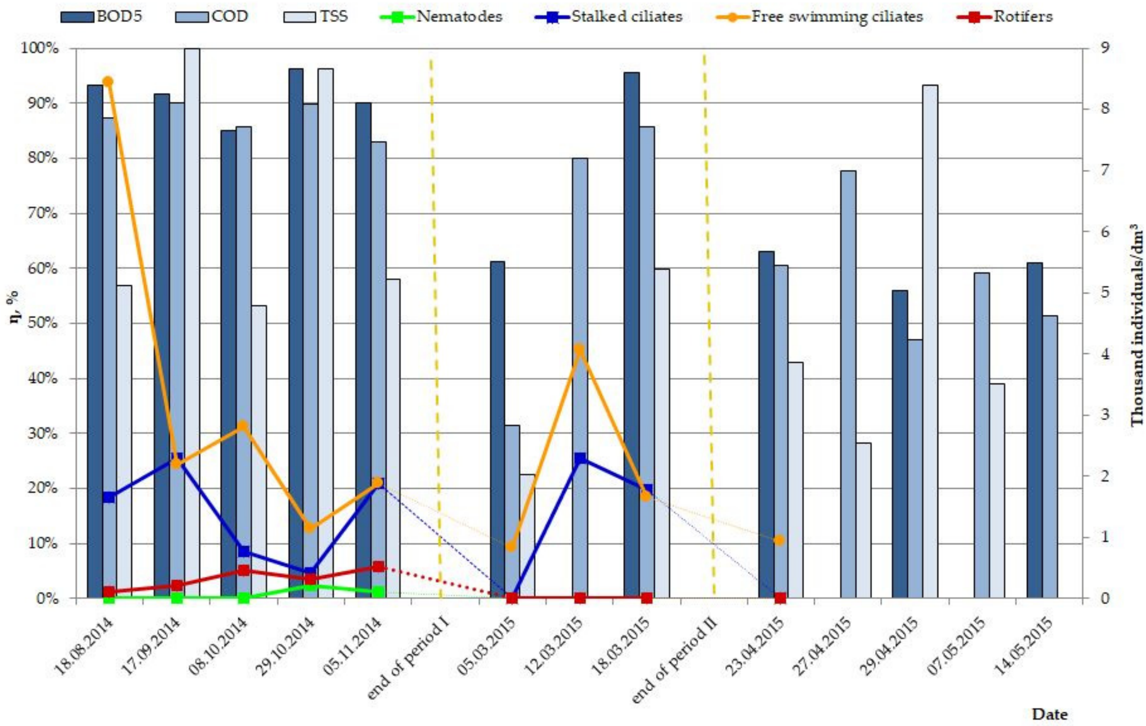


Figure 7. Abundance of organisms in activated sludge, efficiency of organic compounds removal, and efficiency of total suspended solids removal in WTP2.

3.3. Sludge Biotic Index

The sludge biotic index (SBI) was calculated on the basis of an enhanced microscopic analysis. The value of SBI assigned activated sludge in WTP1 (Figure 8) to class II, which indicates the deteriorating biological activity of the material. Nevertheless, the class is characterised by well-colonised and stable sludge with good removal efficiency properties. During the period of research, the SBI value was decreasing, although it did not exceed the bottom limit of class II for sludge quality. On the basis of research (Figures 8 and 9), it has been assumed that there is a correlation between the value of the biotic sludge index and the removal efficiency of organic compounds, whereas a correlation between SBI and the suspension removal efficiency has not been found. A plausible explanation for the high concentration of suspension in effluent in the first period of research was the long retention time of biomass in the system, and in the last period, the excessive growth of filamentous microorganisms. Filamentous microorganisms primarily induce poor sedimentation of sludge [28–31].

Activated sludge found in WTP2 during seven analyses was identified as second-class material. The analysis of SBI indicates its deteriorating biological activity. The sludge biotic index value decreased with the decline in the removal efficiency of organic compounds (Figure 9). Among other factors, it resulted from the removal of a significant portion of activated sludge from the bioreactors at the turn of February and March 2015 (the end of the first period of research), after which the sludge was maintained at a fairly low level of approx. 2 kg/m^3 (2nd and 3rd periods of research). This was equivalent to an increase in organic sludge loading. As a result of the aforementioned operational changes and a significant reduction of aeration time for WTP2 during the last period of research, the sludge was assigned to quality class IV. The results of analytical measurements of effluent confirmed the deterioration of biological activity of the sludge.

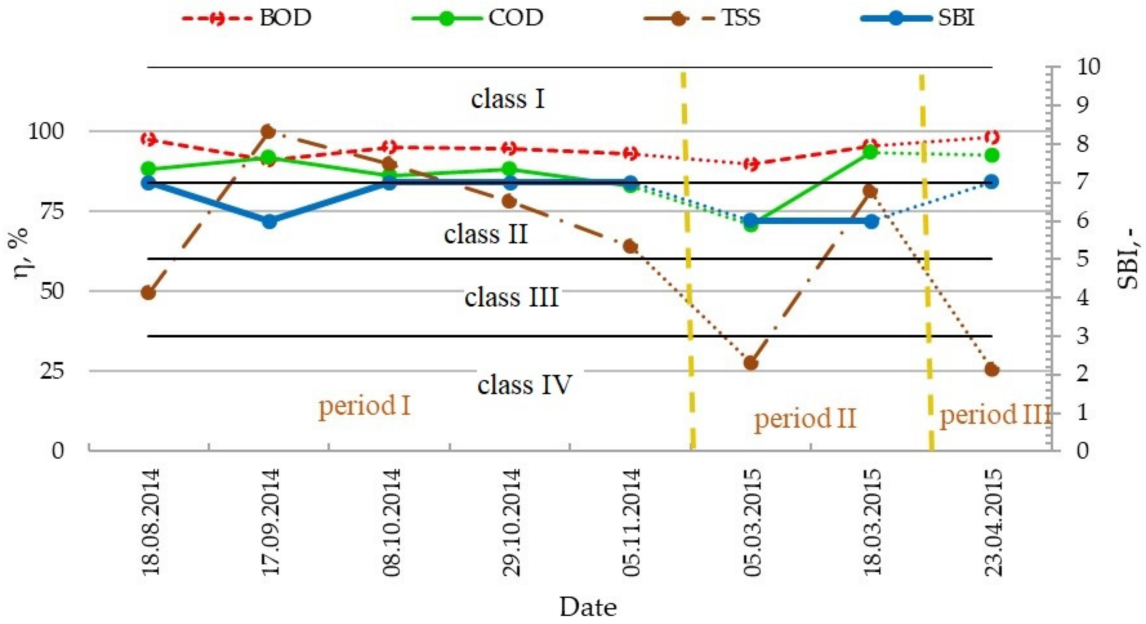


Figure 8. Sludge biotic index (SBI), efficiency of organic compounds (BOD_5, COD) removal, and efficiency of total suspended solids (TSS) removal in the WTP1.

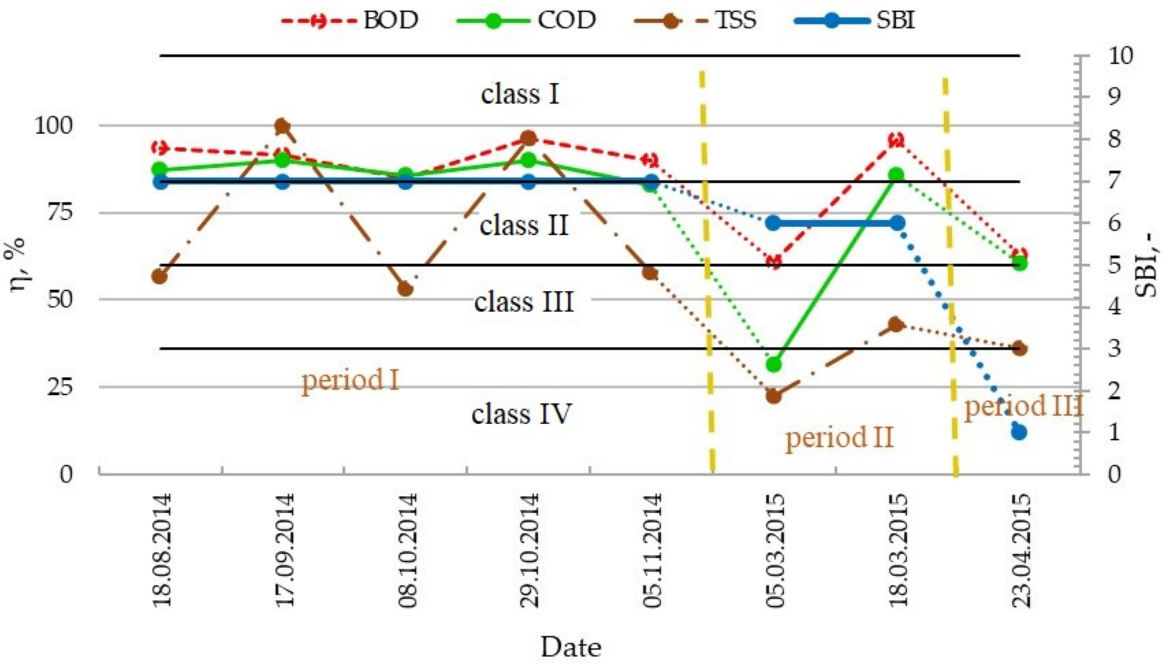


Figure 9. Sludge biotic index (SBI), efficiency of organic compounds (BOD_5, COD) removal, and efficiency of total suspended solids (TSS) removal in WTP2.

4. Discussion

Despite the fact that bacteria are the most important active ingredients of sludge, they do not provide much information. Acquisition of data on activated sludge is possible due to the differentiation of protozoa in terms of their shape, movement, and size. Any change in their biocenosis may indicate possible problems within the system [21]. There are many benefits of the microscopic observation of activated sludge. Changes in the biocenosis of activated sludge might be a sign of the inadequate composition of influent. Observation of microorganisms enables independent evaluation of sludge loading with contaminants. An increase in the number of microorganisms with a decrease in their diversity may indicate a gain in loading. A decline in the number of contaminants may be manifested by a decrease in the quantity of micro-fauna with an increase of its diversity.

The effectiveness of use of the sludge biotic index in assessing the condition of activated sludge and thus the effectiveness of treating wastewater from various sources has been confirmed and reported in numerous scientific publications. This indicator has even been applied for researching slaughterhouse wastewater treatment [32]. The authors have identified SBI as a very useful indicator for determining both the condition of activated sludge and the analysis of biological working conditions of treatment plants. Identification of micro-fauna in mixed wastewater (domestic and industrial) has been recognized as an important element by many researchers [22,33–36].

Based on the results of this study, it can be stated that the sludge biotic index reflected the operating conditions of the treatment plants, particularly in the case of aeration and the sludge concentration in the reactor. With a relatively short retention time of biomass in the bioreactor (WTP1 and WTP2) and too short aeration (WTP2), the SBI was systematically reduced to the lowest class of sludge quality. The mentioned correlation has been noted, among others, by dos Santos et al., [37] while conducting research on textile wastewater treatment (70% industrial sewage and 30% domestic sewage). He demonstrated that the temporal variability of the sludge biotic index was well correlated not only with sludge concentration and dissolved oxygen concentration, but also with the inflow of total suspended solids.

Hu et al. [34] conducted—as in the present study—experiments on two parallel wastewater treatment systems. On the basis of lengthy research (14 months) and a very thorough analysis, where nearly a hundred species of microorganisms were identified, they observed a significant correlation between COD, BOD_5, and suspended solids, as well as the presence and abundance of protozoan and metazoan organisms.

The results of the present study do not confirm the theory that the presence of protozoa is strongly correlated with BOD_5 in the outflow [38–40]. Similarly, as in the studies of Dubber and Gray [41] and Yiannakopoulou [42], we could not unambiguously prove the above correlation, although it periodically occurred during the research. To a certain degree, the doubts about use of the SBI were confirmed by research conducted in SBR wastewater treatment plants by Papadimitriou et al. [43,44]. The study covered wastewater largely from industry and heavily loaded with organic substrates.

The results of some Polish studies [9] indicate that the usefulness of the indicator to monitor the status of activated sludge and the quality of sewage in municipal wastewater, including sewage treatment after the settling tank, is quite limited. These researchers found that the change in sediment forms is a more sensitive indicator of the wastewater quality. For the purpose of a slightly later publication (the comparison of various wastewater treatment systems), the same team of researchers along with Bernat [45] used the SBI to determine the condition of activated sludge while admitting that it does not always accurately reflect the reality. These authors acknowledge that neither their experience nor the reference literature is unequivocal and further research is necessary. Similar conclusions can be drawn from the studies by Leal et al., [46] conducted within one year on various treatment systems on a weekly basis, including the microscopic analysis of sludge, physicochemical properties of wastewater, and treatment efficiency. On the basis of 54 analyses, these researchers confirmed the significant usefulness of microbiological tests for assessing the work of treatment plants and the usefulness of the biotic index to determine the condition of activated sludge, despite certain limitations, e.g., the SBI class was decreasing more slowly than the efficiency of wastewater treatment.

5. Conclusions

The presence of metazoan microorganisms coincided in time with a good effluent treatment efficiency. When their quantity declined, a decrease in the efficiency of wastewater treatment was also observed.

(1) A large number of taxonomic groups reflects the high efficiency of wastewater treatment for COD and BOD_5.
(2) A decrease of the diversity of microorganisms into one clearly dominant group (e.g., free swimming ciliates or flagellates) indicates operational problems.
(3) Operational changes significantly affect the diversity of microorganisms, and hence the sludge biotic index.
(4) Microscopic studies allow the prediction of effluent quality.
(5) This study confirms the correlation between the sludge biotic index and removal efficiency of COD and BOD_5; however, a correlation between the sludge biotic index and removal efficiency of total suspended solids was not found.

Author Contributions: Maciej Pawlak and Aleksandra Sowinska conceived and designed the research theme. Aleksandra Sowinska, Maciej Pawlak, Marta Pacholska, and Jakub Mazurkiewicz collected the data and designed methods. Aleksandra Sowinska, Jakub Mazurkiewicz, and Maciej Pawlak analyzed the data and interpreted the results. Aleksandra Sowinska, Maciej Pawlak, and Jakub Mazurkiewicz wrote and edited the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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