MICROSCOPIC ANALYSIS OF ACTIVATED SLUDGE IN INDUSTRIAL TEXTILE WASTEWATER TREATMENT PLANT

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Abstract:

The relationship between a quality of activated sludge microbiota and wastewater treatment plant (WWTP) operational stability has been defined in the past few decades. However, this dependence is not so clear in the case of industrial wastewater treatment. In this article, a very specific example of industrial textile wastewater treatment plant (ITWTP) is analyzed. Textile effluents are well known as highly contaminated wastewater containing many biodegradable compounds. Microscopic analysis included flocs morphology examination, attempts to evaluate the Sludge Biotic Index (SBI), and identification of dominant filamentous microorganisms. Routine operational control of ITWTP covered pH, temperature, redox potential, dissolved oxygen and COD measurements. The average ecosystem existing in the described ITWTP differed significantly compared to municipal WWTPs. The flocs were smaller and irregular. Filamentous bacteria did not cause foaming although filaments index reached 4. Nostocoida limicola I dominated with significant amounts of type 0041 and type 021N. The evaluation of SBI was impossible as the most of protozoan was in the form of cysts. The overall microbiota diversity correlated with COD removal in activated sludge unit of ITWTP.

Keywords:

Textile wastewater treatment, activated sludge, microscopic analysis

1. Introduction

The textile production is known as a highly water-consuming industry branch [1]. On the one hand, it demands massive quantities of high-quality water (80–100 m³/Mg of finished textile [2]). On the other hand, it generates wastewater contaminated with a wide range of chemicals, such as dyes, sizing agents, preparation agents, surfactants, or salts [3]. Thus, the textile effluents contain both biodegradable and non-biodegradable compounds [4]. As a result, their treatment demands a combination of different methods—biological, chemical, and physicochemical. Although the textile wastewater treatment is a very complicated task that demands sophisticated systems [5], the partial closing of the water cycle within the factory is possible [6].

Among the variety of techniques available, an activated sludge process (ASP) is the most commonly used method in industrial practice [5] as an element of the system. The main principle of ASP is contacting activated sludge (AS) with the contaminated wastewater in aerated or mixed tanks followed by the AS separation. The treated wastewater is separated from AS in secondary settlers (the most popular option among municipal wastewater treatment plants) or by membrane filtration (micro- or ultrafiltration). AS is a complex biocenosis consisting mainly of bacteria and protozoa [7]. The bacteria have the metabolic abilities to oxidize or reduce organic and inorganic contaminants [1], combined with microbial growth (energy consumption and mass assimilation) [8]. Moreover, extracellular polymeric substances (EPS) produced by bacteria or absorbed from wastewater form AS flocs [8], which bind living or dead bacteria, precipitated salts, inorganic particles (e.g., sand) and organic fibers [9]. Filamentous microorganisms constitute the backbone to which floc-forming bacteria adhere [10]. Apart from very weak forces on the outer part of the flocs, the interior is stabilized by chemical binding forces in which divalent cations (e.g. Ca²⁺) play a significant role [9]. Since bacteria are introduced into the flocs, the mass transfer phenomena also play an important role in biodegradation [11]. The additional mechanism of AS wastewater purification is based on the adsorption of contaminants on the surface of AS flocs or even flocculation [12]. The composition of EPS affects the surface properties of AS flocs—surface charge, hydrophobicity, or flocculating activity [8]. The final efficiency of pollutant removal depends on all processes: biodegradation, adsorption, and separation.

The morphological characteristics of flocs play a key role in the treatment and determine the efficiency of all the above-mentioned processes, in particular solid–liquid separation in the secondary settler [13]. The smaller flocs, the better mass transfer efficiency—the better availability of pollutants for microorganisms. However, very small flocs (so called pin point flocs) are not able to sediment in the secondary settling tanks.
The range of floc sizes varies between 0.5 and 1000 μm, but the typical range (for well-mixed AS systems) was found to be 10–70 μm [11]. The high-size flocs quite often are very irregular in shape and have lower density. They may float instead of settling [9]. The irregular shape of flocs is most often connected to imbalance between EPS producing and filamentous bacteria [7]. In the case of significant overproliferation of the filamentous microorganisms, the flocs may be bound together into agglomerates. In the biodegradation and adsorption, this phenomenon can be helpful, but it also causes very poor settling properties [10]. The excessive abundance of filamentous bacteria may lead to foaming and bulking of AS [9].

Other potentially important indicators of ASP performance and efficiency are protozoa. They are not directly active in biodegradation of contaminants. However, they consume free-swimming bacteria clarifying treated wastewater and rejuvenate the population of floe-forming bacteria [14]. The dependence between the protozoa population and the operational stability of municipal wastewater treatment plants (WWTPs) can be found in a number of literature sources [9, 14-16]. It was established that the higher content of ciliates (stalked and freely swimming), the better AS condition and stable WWTP operation [14]. Additionally, some sessile ciliates, namely Opecificula spp and Vorticella microstoma, are able to survive stress conditions (e.g., high loadings, toxic substances, and metals) and their domination indicates operational problems in WWTP [15]. Massive abundance of small heterotrophic flagellates is also associated with the poor performance of biological processes [9].

The methods, such as Sludge Biotic Index (SBI), developed for municipal WWTPs cannot be directly applied in industrial WWTPs [17-19]. In pulp and paper industry WWTP, Bernat et al. [17] observed good quality of effluent independently of dominant protozoa. dos Santos and coworkers [19] performed the investigation on WWTP treating 70% of textile wastewater and 30% of domestic wastewater. They stated that only the dominance of small flagellates correlated with discrepancy of the effluent quality. However, they observed a correlation between feed-to-microorganisms ratio and abundance of Vorticella microstoma and Aspidiscica cicada.

Moreover, the standard approach to AS microfauna analysis does not work in the case of membrane biological reactors (MBRs). Arevalo et al. [20] stated that the operation conditions of MBR caused the fragmentation of flocs and abundance of freely swimming bacteria. As a result, small flagellates, carnivorous ciliated protozoa, and rotifers dominated, which had no correlation to the effluent quality. Additionally, the settling properties of flocs are not so important as membranes stop in the MBR all flocs, including dispersed bacteria [21]. The foaming caused by the filamentous bacteria also does not influence the separation efficiency of MBR [22].

This study aimed to the applicability testing of standard AS microscopic analysis in the ASP as a part of the industrial textile wastewater recycle system in Biliński Textile Company, Poland. To the best of authors’ knowledge, this is the first such a deep and long-term AS analysis of industrial APS of high volumetric flow. Particularly, the flocs morphology analysis, Sludge Biotic Index estimation and the filamentous bacteria identification tests were applied.

2. Experimental Section

2.1. Description of installation

The AS was taken from an installation called “Water Renewal Line” (WRL) located in Biliński Textile Company, Poland. WRL is used for recirculation of about 40% of wastewater generated under textile processing in this company. Apparently, the selected, low-loaded with chemicals baths from textile bleaching, washing, and dyeing are recirculated (such as rinsing after washing and dyeing of fibers). Figure 1 shows the schematic diagram of WRL. The separated low-loaded wastewater stream is directed through rotary drum filtration unit (1), equalization tank (2), and heat exchanger (3) into biological treatment module (4), which consists of three chambers: two aerated (I and II) and one anoxic (III). The biologically treated effluent is pumped through the set of ultrafiltration membranes (5) to separate the AS. The retentate (containing AS) is recirculated into the biological module (Chamber I). The excess sludge is separated in a vertical settler (7). The permeate (purified wastewater) is being pumped through two transitional tanks (6 and 8) and subjected to ozonation (9). Finally, the purified wastewater is directed to buffer tank (10) from where it can be disposed as a process water for textile-processing operations.

2.2. Analytical methods

2.2.1. Technological analysis

The standard operational control of biological module included wastewater level (level controller, Apar), temperature and redox (redox platinum electrode with temperature sensor ERPt-13, Hydromet) and dissolved oxygen (Oxymax COS61D, Endress+Hauser) measurements in each chamber and Chemical Oxygen Demand (COD) analysis (cuvette tests Hach-Lange, spectrophotometer DR3900 Hach-Lange) for inlet and third chamber. Additionally, the biochemical oxygen demand (BOD₅, OxiTop, WTW), total organic carbon (TOC, four-channel NDIR detector built in analyzer IL550TOC-TN, Hach), total nitrogen (TN, chemiluminescent detector built in analyzer IL550TOC-TN, Hach), and toxicity (toward luminescence bacteria Vibrio fisheri, Microtox Model 500 analyzer) analysis were performed periodically, also for inlet and third chamber.

2.2.2. Microscopic analysis

Microscopic analysis of samples was performed on the same day of sampling. Immediately after taking samples, the microfauna community was analyzed in triplicates using microscope CX22Led Olympus (equipped with digital camera and Motic Image Plus 3.0 software). For the count of small flagellates, a Fuchs-Rosenthal chamber was used, following the guidelines by Madoni [15]. The abundance of other protozoa and metazoans was estimated from the analysis of subsamples
3. Results and discussion

3.1. Overall performance of biological treatment

Although WRL influent consists of "cleaner" baths from dyers, it is highly variable in time. Table 1 presents hourly parameter variations. The great diversity can be observed, inter alia, in pH values. It can be seen that during one single day, pH could vary with 0.025 cm³ volume of mixed liquor, calculated per 1 cm³ of AS. The attempts for estimation of SBI were undertaken in accordance with guidelines by Madoni [15]. The morphological analysis of flocs and identification of filamentous bacteria were performed using a microscope Olympus BX40 equipped with digital camera and phase contrast. The flocs size and the filament thickness were measured using Micro Image 4.0 software (Media Cybernetics for Olympus). The identification of dominant filamentous organisms was performed according to the procedure proposed by Eikelboom [9]. Gram and Neisser staining was employed. Both fresh samples and samples after staining were observed in clear field under the magnification of 1000x.

| Sample ID | H1  | H2  | H3  | H4  | H5  | H6  |
|-----------|-----|-----|-----|-----|-----|-----|
| Time      | 7:30| 8:30| 9:30| 10:30| 11:30| 12:30|
| pH (-)    | 6.49| 7.96| 10.57| 7.38| 7.35| 8.63|
| Conductivity (mS/cm) | 1.76| 1.86| 2.46| 2.10| 1.74| 4.37|
| Redox potential (mV) | 165.1| 112.7| 88.7| 232.1| 246.8| 217.0|
| Chlorides (mgCl/dm³) | 0.97| 1.02| 1.30| 1.09| 0.89| 2.31|
| COD (mgO₂/dm³) | 468| 787| 1166| 945| 1276| 1760|
| BOD₅ (mgO₂/dm³) | 177| 110| 231| 160| 459|
| TOC (mgC/dm³) | 158| 199| 316| 248| 160| 37.1|
| TN (mgN/dm³) | 12.6| 14.7| 27.3| 9.8| 8.2| 37.1|
| TP (mgP/dm³) | 1.29| 0.53| 2.68| 1.21| 1.51| 3.46|
| BOD₅/COD (-) | 0.38| 0.09| 0.15|
between 6.49 and 10.57 (Table 1). To avoid this adverse effect, biological unit has 24 h retention time, which gives it significant buffering capacity. Figure 2 shows pH differences in the inlet wastewater and both aerated chambers measured daily in October 2019. It can be seen that significant fluctuations of pH in raw wastewater up to 5 units (between 7.00 and 11.90) were smoothed to 0.80 unit in amplitude in the last biological chamber (between 7.75 and 8.55). During 1 year of WRL operation, the mean pH value in the last biological chamber was 7.98 with standard deviation equal to 0.26, while minimal and maximal observed values were 7.40 and 8.55, respectively. As a rule, the conditions were on the border of the optimal pH range for AS microorganisms, which is estimated as 6.5–8 [23]. However, AS is a complex consortium of microorganisms, so it has better adaptation ability than single microbial culture. Moreover, the AS composition changes depending on the conditions and available substrates. For example, in the biofilm treating textile wastewater halo- and alkaliphilic bacteria *Nitrincola* ssp. and *Marinilactibacillus* ssp. accounted for 45% of all bacteria species [24]. Results of the earlier investigations [25] conducted in sequence batch reactors (SBRs) revealed that AS microorganisms are able to degrade textile wastewater with pH as high as 8.96 ± 0.18.

As most dyeing processes demand the addition of salt (such as NaCl or Na₂SO₄), the RWL influent has a higher conductivity than municipal wastewater. The mean conductivity was 3.61 ± 1.28 mS/cm (within 1 year, minimum 1.64 mS/cm, maximum 9.16), while the conductivity values found in the literature varied between 0.58 and 1.37 mS/cm [26, 27]. Apart from pH and conductivity, the temperature values were also in the range unprecedented in municipal WWTPs. The mean temperature value was 30.61°C (standard deviation 3.76°C; minimum 13.6°C, maximum 39.7°C). Correspondingly, it can be expected that mesophilic microorganisms are dominating in the biological unit.

The COD values in the inflowing wastewater varied between 468 and 1,760 mgO₂/dm³ for one single day (Table 1) and between 658 and 1,425 mgO₂/dm³ within 1 year (measured once per day, Figure 3). It resulted in a quite significant fluctuation in the removal efficiency between 32 and 84% (Figure 3). However, the average removal was satisfactory (67 ± 11%) and remained stable for most of the year (Figure 3). There was one breakdown of AS unit operation—after the production break in winter (days between 197 and 225, Figure 3). The mean COD value in the last biological chamber was 321 ± 110 mgO₂/dm³. As in RWL, the biological unit is followed by ultrafiltration and ozonation processes, the overall COD removal efficiency exceeded 93% and varied in a narrow range between 91 and 97%.

The biodegradability of wastewater may be estimated by means of BOD₅ to COD ratio. The inflow to biological unit was characterized by BOD₅ to COD ratio 0.21 with standard deviation 0.11. It is stated in the literature that wastewater with BOD₅:COD in the range 0.2–0.5 is biodegradable with selected microorganisms. For the effluents with BOD₅:COD below 0.2, chemical pretreatment is recommended [28], which means that organic loads of wastewater treated in RWL may be only partly biodegraded, what was confirmed by COD removal effectiveness.

### 3.2. Microscopic analysis

The microscopic analysis was performed once per month over the half of the year—between December 17, 2019 and May 27, 2020. The surplus sample was taken after the breakdown in AS unit operation.
3.2.1 Flocs morphology

The flocs formed in the both chambers were distinctly irregular, open, and weak (Figure 4). Their structure is caused mainly by the abundance of filamentous microorganisms. All of the abovementioned features are characteristic of AS with poor settling properties [9]. However, it is not a significant problem for the WRL operation, as the biomass is separated in the ultrafiltration unit. Moreover, the separation process may be an additional reason for such flocs morphology [20].

In the case of membrane separation, the massive abundance of freely swimming bacteria and very small flocs are much more problematic [20]. According to Eikelboom and coworkers [9], although the size of flocs varied significantly over time (Figure 5), the mean value was in the range of medium-sized flocs (25–250 μm). However, the size distribution of flocs (Figure 6) also shows the occurrence of very small flocs (below 25 μm). The freely swimming bacteria were not visible in significant quantities.

3.2.2. Sludge Biotic Index

The attempt to SBI estimation was unsuccessful as the most of protozoa were visible in the form of cysts. The decrease in temperature together with changes in oxygen concentration most likely contribute to a rapid cyst formation in the analyzed samples. Additionally, the treated wastewater contains a lot of inorganic salts, which can also cause osmotic stress. Although the probes were analyzed directly after sampling, it was impossible to determine the number of all individuals belonging to protozoan groups (big flagellates, freely swimming, crawling and stalked ciliates). As a result, instead of typical SBI estimation, the quantification of cysts and living forms of protozoan groups were performed. At the beginning of the observation, only cysts and rotifers were observed (Figure 7). However, the COD removal was high—above 70%. After 2 weeks, the company had a demurrage in production, which consequently resulted in the breakdown in the AS unit operation. Due to the lack of inflow, the temperature dropped down more than 10°C. It resulted in the decrease in numbers of cysts, complete rotifers disappearance, and significant COD removal efficiency deterioration (Figure 7). Afterward, the biodiversity of AS was consequently rising, together with COD removal values (Figure 7). After 2 weeks, the lack of flagellates of large size was noticed, but crawling ciliates and rotifers appeared. After 1 month, large flagellates and stalked ciliates were visible again. During the following months, the AS biodiversity maintained at almost constant level (Figure 7). The number of small flagellates remained stable—below 10 counted along the diagonal of Fuchs-Rosenthal chamber. In summary, it was impossible for the textile WWTP to use the SBI estimation in the analysis of municipal WWTP operation. However, the overall AS biodiversity showed a kind of correlation to COD removal efficiency.

3.2.3. Filamentous organisms

The filamentous organisms were abundant in significant amounts—Filament Index (FI) changed between 2 (Figure 8a) and 4 (Figure 8b). FI was estimated according to the methodology proposed by Eikelboom et al. [9], a predominant filamentous organism was identified as *Nostocoida limicola I*. It was outnumbering other filaments bacteria in all analyzed samples. As its dominance was more visible by FI 4 (between February and May) and less distinct by FI 2, it can be concluded that *Nostocoida limicola I* determined...
Mechanical processing of the AS in the membrane modules.

Specific floc morphology could be observed due to enhanced stress caused by a high content of inorganic salts and the temporary temperature drop (days 197–225) resulted in serious mesophilic conditions for microorganisms. Consequently, the Biotic Index estimation was impossible for this type of AS. Moreover, the microscopic observation of overall microflora biodiversity showed the correlation to the removal of organic carbon compounds in the AS unit of the described ITWTP.

4. Conclusions

This study presented an extensive, long-term analysis of AS originating in an industrial textile wastewater plant. To the best of the authors' knowledge, it was the first wide-ranging study on AS of industrial origin. The attempts of AS characterization in terms of morphology and microbial consortium constitution led us to insightful observations.

Summarizing the results, it can be concluded that both AS morphology and biocenosis structure were strongly dependent not only on the wastewater specifics but also on the treatment plant technical construction. The wastewater treated in described textile WWTP may be characterized as slightly biodegradable, significantly loaded with electrolytes and organic carbon compounds. Based on the presented BOD/COD ratio and the results of COD reductions during the operational time of 350 days, it can be concluded that wastewater is only partly biodegradable.

The average temperature, 30.61°C, was significantly higher in ITWTP compared to municipal WWTP, which creates the mesophilic conditions for microorganisms. Consequently, the temporary temperature drop (days 197–225) resulted in serious AS consortium breakdown. Probably due to increased osmotic stress caused by a high content of inorganic salts and the temperature changes after sampling made the microorganisms more prone to cysts incubation.

Moreover, the biomass is separated from the wastewater by means of ultrafiltration process in this treatment plant, which is not so common in the municipal WWTPs. Consequently, the specific floc morphology could be observed due to enhanced mechanical processing of the AS in the membrane modules.

In the context of overall ITWTP performance, the standard analysis of floc morphology and the filamentous organism abundance and types identification did not provide complete information. However, it would be valuable if the biomass had been separated gravitationally. Unfortunately, the Sludge Biotic Index estimation was impossible for this type of AS. Nevertheless, the microscopic observation of overall microflora biodiversity showed the correlation to the removal of organic carbon compounds in the AS unit of the described ITWTP.

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