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Treatment of Industrial Textile Wastewater in Biological Aerated Filters – Microbial Diversity Analysis

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Abstract
Investigated herein was the biodegradation of highly contaminated textile wastewater on a laboratory scale, with biological aerobic filters as a single treatment and in combination with the coagulation/flocculation process. Among the three support materials tested (Intalox saddles, ceramsite and beach shavings), the highest organic carbon compound removals (above 60% measured as COD and TOC) and steady operation were obtained for ceramsite. Effective and stable biological treatment was possible thanks to the development of biofilm of high bacterial and fungal diversity. The biodiversity of microflora was estimated on the basis of metagenomic analysis. The coagulation process with PAX 18 was effective in total phosphorus depletion (94%), while the coagulant Epoly CRD enabled up to 99% colour removal. The best results were obtained after the combined treatment, in which biodegradation was followed by coagulation (PAX 18). Such a combination enabled the removal of 98% of BOD_5, 87% of COD, 88% of TOC, 48% of the total nitrogen, 98% of the total phosphorus, 98% of toxicity (towards Vibrio fisheri) and above 81% of colour.

Key words: *Biological Aerated Filters, metagenomic analysis, textile wastewater, toxicity, coagulation.*

List of abbreviations

ABG – Aerobic Bacterial Granule
ABTS – 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
BAF – Biological Aerated Filter
BOD_5 – five day biological oxygen demand
CF – coagulation/flocculation process
COD – chemical oxygen demand
EC_{50} – half the maximal effective concentration
Epoly CRD – coagulant of unknown composition
Flopam EM 532 – anionic flocculant
H – Shannon-Wiener index
HRT – hydraulic retention time
ITS – internal transcribed spacer
NGS – next generation sequencing
PACl – polyaluminium chloride coagulant
PAX 18 – one of the commercially used PACl
PCR – polymerase chain reaction
PCR-DGGE – polymerase chain reaction coupled with denaturing gradient gel electrophoresis
rRNA – ribosomal RNA
SAC – spectral absorption coefficient
TN – total nitrogen
TOC – total organic carbon
TP – total phosphorus
TU – toxic units

Introduction

The textile industry is known for releasing big amounts of significantly contaminated wastewater. Apparently, the wet processes used in dyehouses are responsible for enormous water demand as well as wastewater production in this industry branch. The most sustainable approach to solving the problem of textile effluents is the closing of the water cycle within the factory.

Water recovery in a dyehouse is a very complicated task due to the following:

- There are plenty of different chemicals released into water during the wet processes – natural fibre impurities, sizing agents, preparation agents, surfactants, carboxylic acids, thickeners, complexing agents, salts and dyes [1];
- The wastewater composition varies due to the type of fibre used – different fibres are dyed with various dyes which need specific auxiliaries (in particular, the application of reactive dyes requires high salt concentration; moreover, detergents and auxiliary agents are also used);
- The wastewater composition changes according to the season of the year and fashion trends;
- Users demand textiles whose colour is resistant to sweat, light, water and oxidising agents.

Despite the fact that researches concerning textile wastewater purification (with the main emphasis on dye removal) have been conducted for dozens of years, there is still a need to invite and implement new, more economical and ecologically friendly systems. The best results are obtained when effluents coming from different units (e.g. dyers, printers), and even baths from dyers (e.g. washing, rising, dyeing, etc.), are segregated and treated separately [1]. Most often such a wide selection of wastewater streams is not possible. However, there is a need to find a solution which is not only environmentally friendly but also economically feasible. One of the possible systems is to divide effluents into two streams – one containing mainly baths from rinsing in dyers and the other consisting of wastewater from printers and finishing apparatus together with baths after washing and dyeing. The first stream may be cleaned and recycled, while the second – treated and discharged [2].

There are a lot of processes that enable wastewater treatment: physical, chemical, physico-chemical, electrochemical and biological. Experiments conducted on aqueous solutions of dyes or even on synthetic wastewater are not that representative of industrial conditions and do not enable implementation of their processes in factories [3]. Therefore, it is crucial to perform investigations on industrial textile wastewater. Closing of the water cycle demands the integration of different methods, among which the most common membrane processes are the main part of the systems used [4]. However, some researchers claim that

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advanced oxidation processes are more suitable for water reuse as they do not produce waste streams [3, 5, 6].

Highly contaminated streams that are not reused and concentrates from the membrane processes should be treated by low-cost techniques. Biodegradation and coagulation/flocculation processes are assumed as the most cost effective ones [7, 8].

The biodegradation may be performed with activated sludge, pure cultures of fungus or bacteria, consortia of plants and bacteria (constructed wetlands), or with biofilms (consortia of bacteria, fungus and even algae). The bioprocesses are conducted in aerobic, anaerobic or sequentially anaerobic and aerobic conditions. The mechanisms of degradation depend on the types of dyes and biomass used. Disperse, vat, direct and basic dyes can be adsorbed onto activated sludge or flocculated [9]. The bacterial biodegradation of azo dyes requires a combination of two stages: first an anaerobic one, leading to a reduction in azo bonds and formation of aromatic amines, and second aerobic, enabling oxidation of the aromatic amines evolved [10]. Fungi are capable of removing dyes by biosorption and biodegradation [11]. They solubilises the insoluble substrates by producing extra-cellular, highly oxidative enzymes such as laccases, manganase peroxidase or lignin peroxidase [12]. Biofilms are formed on a natural or synthetic support. Carrier materials can be stationary (fixed-bed bioreactors) or in motion (moving bed or fluidising bed bioreactor). In the biofilms very complex consortia of microorganisms can cooperate. As a result the biofilm biodegradation efficiency is more stable than in the case of activated sludge treatment. Dealing with complex wastewater demands mixed microbial communities as they are more effective in pollutant degradation and less sensitive to toxicants, in comparison to pure cultures [13]. Fixed-bed bioreactors, e.g. biofilters may be a more economically reasonable solution than moving-bed bioreactors as they demand less energy. Additionally, a thicker layer of biofilm may rise on the stationary support, which enables the occurrence of gradients of oxygen, as well as other substrates and products within the biofilm. As a result several zones (aerobic, anoxic or anaerobic) may be formed, and consequently the decolourisation of dyes may occur [14]. Biofilters were successfully used for the treatment of industrial textile effluents [15-17]. However, there was no microbial diversity analysis of the biofilm developed in the BAFs treating this type of wastewater.

Nowadays, the biodiversity of microflora is estimated on the basis of metagenomic analysis. Firstly, the polymerase chain reaction coupled with denaturing gradient gel electrophoresis (PCR-DGGE) were used to study the diversity and complexity of a microbial community treating textile wastewater [13, 18]. Recently, instead of DGGE, next generation sequencing (NGS) is used [19]. Both methods were also used to observe the microbial community structure in BAFs [20-22]. However, there are no literature data concerning the bacterial and fungal diversity in BAFs treating industrial textile effluents.

Due to their economical and instrumental feasibility, coagulation and flocculation processes are widely used for the removal of suspended or colloidal forms of pollutants [8]. They are sometimes used as the main wastewater treatment but most often as the pretreatment step [8, 23, 24]. They involve the addition of chemicals to alter the physical state of dissolved and suspended solids and facilitate their removal by sedimentation [25]. As the main drawback of those processes is sludge production, main research stress is put on the sludge amount and optimization of properties [8, 25]. They are capable of wastewater decolourisation [26], however, the effectiveness of the colour removal varies due to the dye type [25] as coagulation mainly enables the removal of suspended solids [27]. Generally, inorganic coagulants and synthetic or natural polymers are used [8]. It was established that pre-hydrolysed metallic salts are often more effective than hydrolyzing metallic salts, such as aluminium sulphate (alum), ferric chloride and ferric sulphate [25]. As an example, PACl products are similar to alum, but they are partially pre-neutralised, contain Cl^- instead of SO_4^{2-} and have a rapid aggregation velocity with bigger and heavier flocs. As a result they show better colour removal efficiency in the wide pH range of 7-10 [25].

In this study a wastewater stream containing effluents from printers and finishing machines together with baths after washing and dyeing (from dyers) was treated by two low-cost methods: biodegradation in BAFs and the coagulation/flocculation process. The efficiencies of the single processes as well as their combinations were investigated. Moreover, due to the fact that there is a lack of literature data concerning the biodiversity of the biofilm developed in BAF treatment of industrial textile wastewater, metagenomic analysis of the biomass found in the raw wastewater as well as in the biofilm growing on a ceramsite filling was performed.

The experiments presented are a part of investigations on an integrated system that enables a water cycle closing up to 40% of the water used in the factory dyeing of cotton. The results obtained for the recycled stream (less contaminated) were described previously by Sójka-Ledakowicz and co-workers [2].

Materials and methods

Wastewater

The experiments were performed on wastewater taken from an industrial dye house (Z.W. Biliński Sp. J., Poland). Raw wastewater was collected in a 1 m^3 tank for 24 hours (an automatically switched on/off pump dosed the wastewater once per hour) before a coagulation/flocculation unit. Pretreated wastewater was collected after processing by an industrial coagulation/flocculation installation, which was described previously [28]. ***Table 1*** shows brief details of the characteristics of the wastewater used.

Experimental set-up and procedures

The biodegradation processes were conducted at an ambient temperature in upflow BAFs with a working volume of 15 dm^3 (height – 86 cm and diameter – 15 cm). The equipment was described previously by Wrębiak et al [29]. Volumetric flow rates of wastewater were set between 5 and 7.5 $dm^3 \cdot d^{-1}$. Compressed air was introduced concurrently with a volumetric flow rate equal to 20 $dm^3 \cdot min^{-1}$.

Biodegradation of raw wastewater

In the first run of experiments, three BAFs treating raw wastewater were run in parallel with 72 h HRT. One traditional filling used in the chemical industry (Intalox ceramic saddles, specific surface area – 254 $m^2 \cdot m^{-3}$) was compared with two cheap, natural support materials: ceramsite (diameter of 9 ÷ 13 mm) and beech shavings (size – 15 × 50 mm). No

external inoculum was introduced into the reactors. At start-up the low-loaded stream was recirculated with an addition of glucose (1 g·dm^{-3}). As a result, after one month of the operation, the autochthonous microorganisms created a biofilm. Afterwards, investigations on the efficiency of the textile wastewater biodegradation were conducted. After three months of stable operation (in terms of organic removal efficacy), the biofilm samples were taken from the BAF filled with the ceramsite support in order to perform metagenomic analysis. Biofilm samples were collected at two points – from the middle of the BAF and from its top layer.

Biodegradation of raw and pretreated wastewater

On the basis of the result obtained in the first part of the experiments, the ceramsite filling was chosen for the next investigations. In the second set of the experiments, two BAFs were used: one treated raw wastewater, while the second – wastewater after coagulation in the industrial installation located in dye house Z. W. Biliński Sp. J. using PAX 18 as a coagulant. Taking into account the lower organic loads in the pretreated wastewater, the HRT was shortened to 48 h.

Coagulation/flocculation processes on a laboratory scale

In the third part of the investigations, the coagulation/flocculation processes were implemented for both raw and biologically pretreated wastewater (in BAF filled with cermasite).

The chemical coagulation/flocculation processes were investigated on a laboratory jar test apparatus – JLT6, produced by Fisher Scientific (USA). CF experiments were performed in three steps: fast mixing (1 min, 120 rpm), slow mixing (15 min, 15 rpm) and sedimentation (30 min).

The effectiveness of two types of coagulants was compared: one already applied in industry – PAX 18 (partially preneutralised polyaluminium chloride used with anionic flocculant Flopam EM 532) and a new one – Epoly CRD (unknown composition, described as a decolourising and clarifying agent – both a flocculant and coagulant [30]). Coagulation with PAX 18 was conducted with different coagulant doses (from 0.8 to 2.0 cm^3·dm^{-3}) at a constant flocculant dose (20 cm^3·dm^{-3} of 0.3% Flopam EM 532). Epoly CRD was investigated in the range from 0.4 to 1.2 g·dm^{-3} without a flocculant.

Analytical methods

The following analytical means were applied to the raw as well as treated wastewater:

- pH: SenTix® 20 electrode connected to WTW meter Multi 720 (Germany),
- conductivity: TetraCon® 325 electrode connected to WTW meter Multi 720 (Germany),
- Sludge Volume Index: standard method [31],
- BOD_5: dilution method: standard method [32],
- COD: standard dichromate method, LCK314, spectrophotometer DR 5000, Hach (USA),
- TOC: four-channel NDIR detector with built-in analyser IL550TOC-TN, Hach (USA),
- TN: chemiluminescent detector with built-in analyser IL550TOC-TN, Hach (USA),
- TP: colorimetric method, LCK 348, spectrophotometer DR 5000, Hach,
- spectrophotometric colour measurements: according to PN-EN ISO 7887:2002, UV-VIS spectrophotometer Helios Thermo (Thermo Fisher Scientific, USA).

The spectral absorption coefficient is referred to as SAC (unit: m^{-1}). Determination of the SAC was performed according to DIN 38 4043 and PN-EN ISO 7887:2002.

The toxicity towards *Vibrio fisheri* was measured as the differences in the bioluminescence of bacteria incubated (5 min at 15 °C) in the control and diluted wastewater samples, using a Microtox Model 500 analyser (Modern Water Inc., USA). The "Basic test 81.9%" procedure was employed, which enables the calculation of EC_{50} (by means of Microtox Omni software, Modern Water Inc., USA). The results obtained were expressed as toxic units (TU) calculated with the following formula:

$$TU = \frac{1}{EC_{50}} \cdot 100$$

Amplicon sequencing and bioinformatics analysis

For this study, DNA was extracted from samples by bead beating in a FastPrep24 instrument with a DNA Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The amount of DNA was measured using s NanoDrop spectrophotometer (Thermo Scientific nowadays Thermo Fisher Scientific, USA). The DNA concentration of amplicons was checked by agarose gel electrophoresis.

The microbiota composition was sequenced using a Illumina MiSeq platform. Primers targeting the V3-V4 hipervariable region of 16S rRNA and the ITS region of 18S rRNA contained additional adapters compatible with the Nextera Index Kit (Illumina, USA). The following primer sequences were used: 16S_F: 5' – CCTACGGGNGGCWGCAG – 3' and 16S_R: 5' – GACTACHVGGGTATCTAATCC–3';ITS_F:GTGARTCATCGARTCTTTG and ITS_R: TCCTCCGCTTATTGATATGC. These primers also contained overhang adapter sequences attached to the 5' end of the primers, compatible with the MiSeq flow cell adapters (Illumina).

PCR reactions were carried out in 25 µL consisting of 12,5 µl of 2x KAPA HiFi HotStart ReadyMix kit (KAPA BIOSYSTEMS, USA), 2,5 µl of genomic DNA (~5 ng/µL), 5bµl of each primer (1 µM) and nuclease-free water. A C1000-Touch^{TM} Thermal Cycler (BioRad, USA) was used. PCR amplification was initiated with a denaturation step at 95 °C for 3 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; and a final elongation at 72 °C for 5 min. To incorporate primers with adapters and indexes, PCR reactions contained 25 µl of 2 x KAPA HiFi HotStart ReadyMix kit (KAPA BIOSYSTEMS, USA), 5 µl of primers P5 and P7 (Nextera Index Kit), 5 µl of the PCR product and nuclease-free water, for a total volume of 50 µl. Cycle conditions applied were as follows: 95 °C for 3 min; 8 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, and elongation at 72 °C for 5 min. Purification of the amplified PCR products was performed using AMPure XP beads (Beckman Coulter Genomic nowadays Genewuz Inc., USA). Prior to library pooling, clean constructs were quantified using a Qubit fluorometer (Invitrogen). 8 pM libraries were sequenced with the usage of MiSeq Reagent Kit v2 (500 cycles).

The raw dataset containing pair-ended reads with corresponding quality scores was merged using PEAR software [33]. Next, it was trimmed with a quality lower than 30 and converted to a fasta format using a FASTX – Toolkit. Data were analysed and interprated with the use of

specialised software packages: Quantitative Insights into Microbial Ecology (QIIME) [34] (1.8.0) with 90% of identity and UPARSE [35]. The Greengenes (13.8) 16S rRNA and 18S rRNA gene collection was used as a reference database [36]. Custom python and QIIME scripts were used for the subsequent analysis steps.

Results and discussion

Biodegradation of raw wastewater

The wastewater used in this study was characterised by a relatively high BOD_5/COD ratio (0.29 ± 0.06, ***Table 1***) in comparison to the mixed textile wastewater collected from the equalisation tanks. For example, Gil Pavas and co-workers treated wastewater with a BOD_5/COD ratio equal to 0.14 by means of physicochemical methods [27], while Mokhtar and co-workers investigated wastewater of BOD_5/COD as low as 0.03 [37]. In our case the wastewater may be considered as biodegradable by selected microorganisms [38]. The experiments performed in BAFs with different fillings confirmed the above-mentioned assumption. Despite the fact that the raw wastewater had a significant toxicity towards *Vibrio fisheri* (TU equal to 27 ± 1.6, Class IV – high acute toxicity according to Persoone and co-workers, [39]), the microorganisms forming the biofilms in BAFs were capable of degrading up to 62% of organic carbon compounds measured as COD and 64% as TOC (***Table 2***). Kornaros and Lyberatos [17] obtained similar results in a biological trickling filter treating textile wastewater – they achieved 60% removal of COD. Moreover, the toxicity of the wastewater dropped by about 92% in a reactor filled with ceramsite and by about 94% in a reactor filled with beech shavings. As a result the biologically treated wastewater passed to Class III – acute toxicity (according to Persoone et al. [39]). Although the BAF with beech shavings showed better TN and colour removal efficiencies (***Table 2***), the beech shavings after three months of stable operations were partially decomposed and had to be replaced by new material. At the same time the BAF filled with the ceramsite worked stably without clogging. The BAF with the ceramsite support was chosen for further experiments.

Metagenomics analysis

NGS was implemented as a novel and precise tool for metagenomic analysis.

Table 1. *Brief details of characteristics of textile wastewater.*

Parameter		Unit	Raw wastewater		Pre-treated wastewater	
			Mean value	SD	Mean value	SD
pH		–	8.48	0.63	7.1	0.45
Conductivity		mS cm^{-1}	15.32	5.22	10.91	2.02
BOD_5		mgO_2 dm^{-3}	283	108	176	67
COD		mgO_2 dm^{-3}	990	250	462	129
BOD_5/COD		–	0.29	0.06	0.32	0.03
TOC		mgC dm^{-3}	358	90	159	106
TN		mgN dm^{-3}	97	57	54	54
TP		mgP dm^{-3}	3.5	2.0	0.64	1.26
SAC	436 nm	m^{-1}	63	14	38	39
	525 nm	m^{-1}	75	23	46	49
	620 nm	m^{-1}	90	43	56	67
Toxicity units		–	27	1.6	9.7	2.5

Table 2. *Efficacy of biodegradation in BAFs for different support materials.*

Parameter		Intalox saddles		Ceramsite		Beech shavings	
		Effluent	Removal, %	Effluent	Removal, %	Effluent	Removal, %
pH, –		8.44±0.20	–	8.39±0.12	–	8.22±0.16	–
Conductivity, mS cm^{-1}		16.81±1.62	–	16.8±1.8	–	16.48±1.53	–
BOD_5, mgO_2 dm^{-3}		10.01±4.55	94.97±3.26	5.6±2.0	97.3±1.1	4.1±1.6	97.8±1.6
COD, mgO_2 dm^{-3}		330±45	48±12	248±38	62±8	278±63	58±11
TOC, mgC dm^{-3}		99.7±11.4	52.3±16.9	76.1±13.4	64.1±10.8	84.2±20.9	61.5±13.5
TN, mgN dm^{-3}		35.9±27.3	26.3±30.6	36.0±27.5	28.2±21.1	17.9±13.4	64.6±27.8
SAC, m^{-1}	436 nm	53.4±17.7	19.7±12.8	47.8±20.0	20.4±8.1	42.4±13.8	28.7±17.5
	525 nm	50.1±27.8	20.3±21.4	42.3±31.6	19.8±15.7	31.0±17.5	35.9±23.5
	620 nm	37.0±20.9	18.3±24.8	33.9±24.6	19.9±26.2	20.8±11.0	37.6±21.5

The Shannon-Wiener index (H), which has been used since the mid-1950s as a measure of diversity [40], was calculated on the basis of an assigned number of reads. In the case of bacteria, the H index revealed high values – 3.91 ± 0.12 for the biofilm and 2.31 for the wastewater. Chaudhari and co-workers [19] achieved an even higher H index for microflora forming aerobic bacterial granules (ABG) – between 3.18 to 6.10. Whereas Punzi and co-workers observed H values between 1.55 and 2.09 for the biomass attached [13]. It is possible that the NGS technique used in this study was more precise than the DGGE used by Punzi and co-workers.

The H index shows a significantly higher bacterial biodiversity of the biofilm in comparison to the raw wastewater, which is also visible in the number of species – 182 ± 8 in the biofilm and 53 in the wastewater. In the case of bacteria almost 100% of the reads were classified into taxonomic categories – at the order level (***Figure 1***). 81% of the reads were assigned to the families lev-

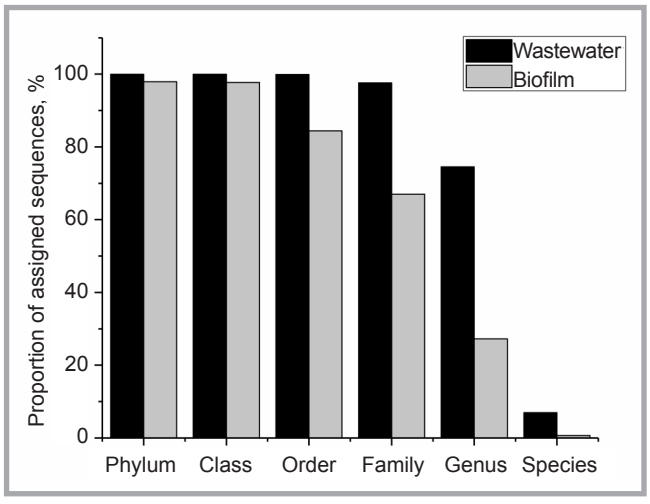

Figure 1. *Proportion of sequences successfully classified and assigned at six taxonomic levels – bacterial community.*

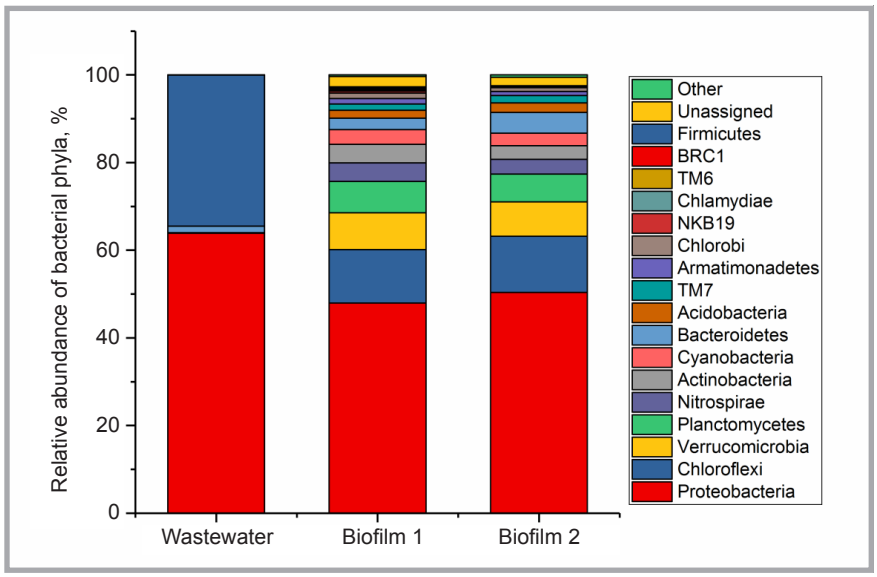

Figure 2. *Bacterial phyla found in wastewater and biofilm samples: biofilm 1 – middle of BAF, biofilm 2 – top of BAF.*

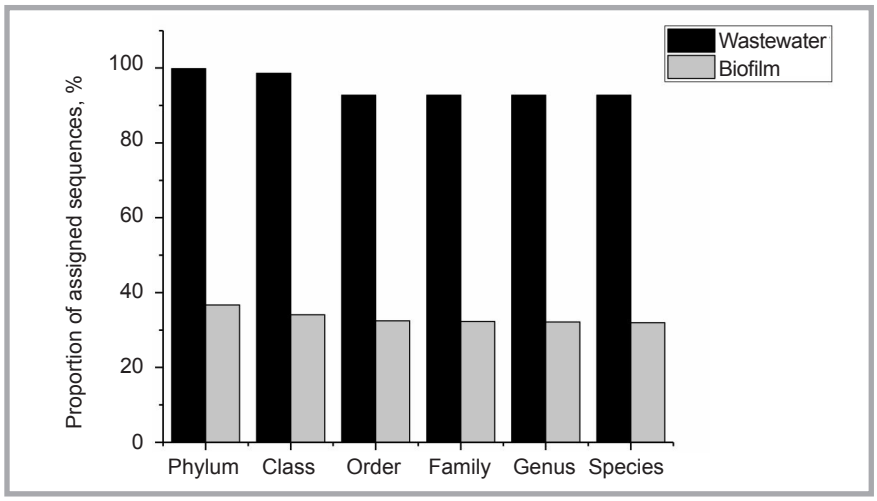

Figure 3. *Proportion of sequences successfully classified and assigned at six taxonomic levels – fungal community.*

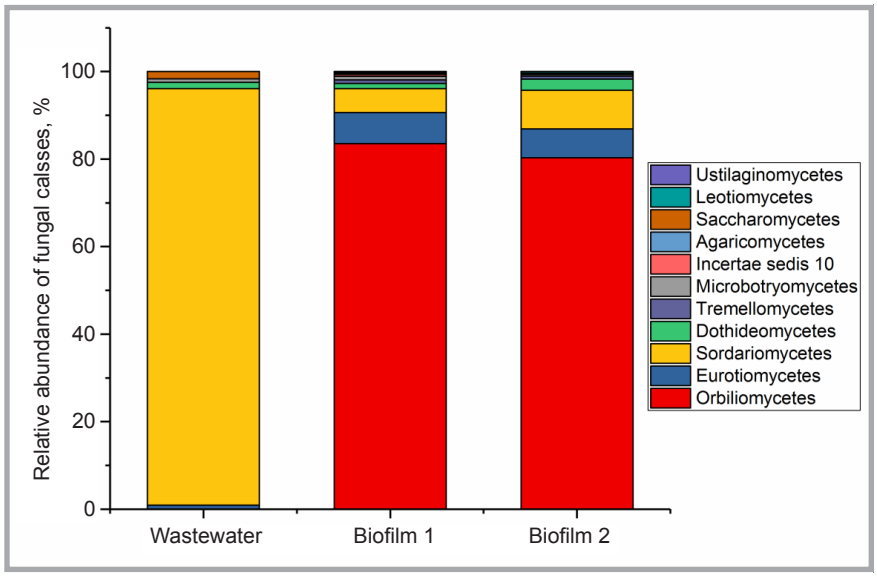

Figure 4. *Fungal classes found in the wastewater and biofilm samples: biofilm 1 – middle of BAF, biofilm 2 – top of BAF.*

el for the raw wastewater, and 31% for the biofilm (***Figure 1***). ***Figure 2*** illustrates the differences in the biodiversity of the raw wastewater and biofilm samples. In the microflora of the wastewater, two phyla dominated: *Proteobacteria* (64%) and *Firmicutes* (35%, ***Figure 2***). Typically, halo- and alkaliphilic bacteria were found in very significant numbers – *Nitrincola ssp*. (38% of all reads) and *Marinilactibacillus ssp*. (7% of all reads). There were no significant differences between the biofilm samples (below 5%, ***Figure 2***). In the wastewater sample three phyla (*Proteobacteria, Firmicutes* and *Bacteroidetes*) made up 99.89% of all reads, while in the biofilm sample eight phyla comprised 90% of the reads (***Figure 2***). However, in the biofilm *Proteobacteria* phylum also dominated (48%). What is more, bacteria dominating in the wastewater were in the insignificant amounts in the biofilm and vice versa, e.g. *Nitrincola ssp*. was assigned in 3 of the 45659 reads for the biofilm sample (0.007%), which means that the ceramsite support enabled the formation of a very specific ecosystem. The Greengenes (13.8) 16S rRNA gene collection did not allow a significant designation of genus and species (***Figure 1***), thus it was not possible to conclude what metabolic processes occurred in the bioreactor. There was only one important finding – the *Nitrospira* family, known for being capable of nitrification, constituted 4% of the biofilm's bacterial microflora.

The presence of fungi in bacterial communities treating complex wastewater may lead to a higher stability of the biodegradation process. Manai et al. [41] showed that the addition of fungal enzymes improved the activated sludge process's resistance to the shock loadings of real textile wastewater. That is why, apart from the bacterial community structure, the fungal biodiversity was also analysed. Unfortunately, only 37% of the reads were assigned to taxonomic categories for the fungi in the biofilm samples (***Figure 3***), which causes that the H index calculated (1.22 ± 0.10) is unrepresentative. Nevertheless, it is in the range of the literature data – 0.78 to 1.76 [18]. It also confirmed the trend observed in the literature – that the bacterial diversity is higher than the eukaryotic diversity [13,18]. In the wastewater almost 100% of the reads were assigned to taxonomic categories – at the phylum and class levels (***Figure 3***). As a result the H index calculated for fungi in the waste-

water (0.94) is not much lower than for the biofilm. In the contrary to bacteria, above 90% of microorganisms assigned were identified at the species level (***Figure 3***). *Ascomycota* phylum dominated in the biofilm as well as in the wastewater – 98.3 ± 0.8% and 99% of all reads assigned, respectively. *Basidiomycota* phylum constituted 1.5 ± 0.6% in the biofilm and 0.8% in the wastewater samples. *Zygomycota* was present in the biofilm at a very low level (0.2 ± 0.1%) and not observed in the wastewater sample. There were significant differences at the class level between the biofilm and wastewater samples (***Figure 4***). In the wastewater *Sordariomycetes* constituted 95% of species assigned (***Figure 4***). What is more, one species dominated – *Petriella sordida* made up 74% of all species. In the biofilm the *Orbiliomycetes* class outdid the others – 81.9 ± 2.3% of the whole fungi population (***Figure 4***),with the dominating species being *Arthrobotrys oligospora* (82 ± 2%). It is probable that *Arthrobotrys oligospora* dominated the biofilm's microflora as this fungi is capable of using a wide range of carbohydrates [42] and belongs to facultative nematophagous fungi, which are able to capture bacteria, amoeba and other soil organisms as well as to digest them [43]. Thus, it may both degrade organic pollutants and refresh the bacterial community as a predator.

Biodegradation of pretreated wastewater

An HRT equal to 48 h was enough, even for the biodegradation of the raw wastewater, resulting in 96% removal of biodegradable organic compounds (measured as BOD_5) and over 60% of organic carbon compounds, measured as COD (61%) and TOC (65%, ***Table 3***). Although the coagulation/flocculation process removed a certain amount of pollutants, the efficiency of sole biodegradation of the pretreated wastewater was only slightly lower when measured for BOD_5 and COD (***Table 3***). TOC removal was even a little bit higher for the pretreated wastewater (***Table 3***). Generally, the physico-chemical pretreatment by means of coagulation with PAX 18 influenced the textile wastewater biodegradability insignificantly. This is in agreement with the fact that values of BOD_5/COD ratios for both raw and pre-treated wastewater were similar (***Table 1***).

Table 3. *Biodegradation efficacy of raw and chemically pre-treated wastewater.*

Parameter		Raw wastewater		Wastewater after coagulation	
		Effluent	Removal, %	Effluent	Removal, %
pH, –		8.21±0.16	–	8.09±0.20	–
Conductivity, mS·cm^{-1}		13.50±3.24	–	10.02±4.11	–
BOD_5, mgO_2·dm^{-3}		11.63±6.00	95.91±2.31	11.41±4.78	94.82±1.58
COD, mgO_2·dm^{-3}		322±96	61±13	251±71	52±18
TOC, mgC·dm^{-3}		80.6±33.4	65±21.8	46.4±17.8	66.6±11.5
TN, mgN·dm^{-3}		85.0±43.3	16.0±23.9	59.4±47.4	0±19
TP, mgP·dm^{-3}		2.5±1.5	6.1±20.7	0.2±0.1	32.1±9.7
SAC, m^{-1}	436 nm	36.0±24.6	33.5±19.7	31.8±22.4	12.1±29.3
	525 nm	33.1±26.3	35.9±10.9	36.8±28.2	10.7±29.5
	620 nm	21.9±13.2	24.8±15.8	30.5±27.5	23.9±33.3

Table 4. *Main results of coagulation process for different doses of PAX 18.*

Parameter		Raw wastewater				Wastewater after biodegradation		
Coagulant dose, cm^3·dm^{-3}		0.8	1.2	1.6	2.0	0.8	1.2	1.6
Sludge volume, cm^3·dm^{-3}		70	160	140	Flotation	80	90	150
Mohlmann index, cm^3·mg^{-1}		110	240	n.a.	n.a.	229	158	234
Parameter		**Removal, %**						
BOD_5		20	22	32	n.a.	n.a.	n.a.	n.a.
COD		21	37	41	42	32	41	46
TP		88	92	93	94	96	97	98
SAC	436 nm	30	48	58	47	45	59	66
	525 nm	28	45	56	42	42	57	64
	620 nm	23	38	49	38	44	60	69

Coagulation/flocculation process

PAX 18 enabled the removal of organic carbon compounds up to 42% for the raw wastewater (measured as COD, ***Table 4***) and up to 46% for the biotreated wastewater (***Table 4***). GilPavas and co-workers [24] obtained similar results using aluminium sulfate – 48% COD removal. PAX 18 was very efficient in phosphorus removal – above 88% (***Table 4***) for both types of wastewater. This coagulant was medium effective in decolouration – between 23 and 58% for the raw wastewater and between 42 and 69% for the biotreated wastewater (***Table 4***). PAX 18 lowered the toxicity of about 77% in the case of the raw wastewater and by over 30% in the case of the biotreated effluent.

Epoly CRD removed up to 31% of organic carbon compounds from the raw wastewater (***Table 5***). The best results were obtained for a coagulant dose of 0.8 g·dm^{-3}. This coagulant caused an increase in BOD_5 (up to 24%), as a result of which the BOD_5/COD ratio increased

Table 5. *Main results of coagulation process for different doses of Epoly CRD.*

Parameter		Raw wastewater					Wastewater after biodegradation				
Coagulant dose, g·dm^{-3}		0.4	0.6	0.8	1.0	1.2	0.4	0.6	0.8	1.0	1.2
Sludge volume, cm^3·dm^{-3}		130	180	190	195	200	74	100	130	125	140
Mohlmann index, cm^3·mg^{-1}		225	230	235	240	227	200	217	251	208	233
Parameter		**Removal, %**									
BOD_5		n.a.	n.a.	-17	-24	-13	n.a.	n.a.	n.a.	n.a.	n.a.
COD		21	28	32	31	30	22	36	16	5	-7
TP		6	12	13	16	15	11	19	7	8	10
SAC	436 nm	59	85	93	94	95	53	88	91	91	92
	525 nm	66	90	96	97	97	59	91	92	94	95
	620 nm	64	92	99	99	99	59	92	96	96	97

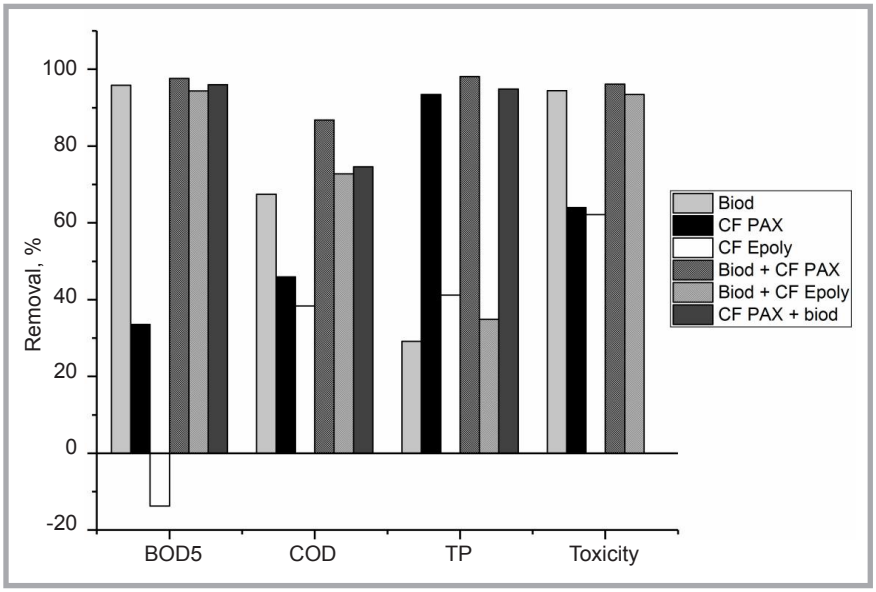

Figure 5. *Removal of organic carbon compounds and total phosphorus after different treatments and their combinations.*

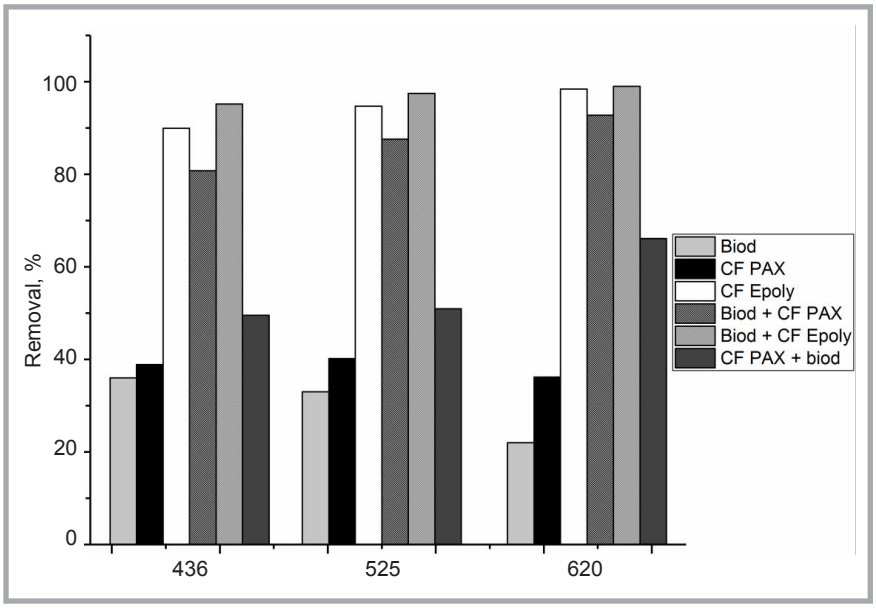

Figure 6. *Removal of colour after different treatments and their combinations.*

from 0.31 up to 0.55. Epoly CRD was insufficient in phosphorus removal – up to 16% (***Table 5***). Irrespective of the wastewater type, Epoly CRD was very effective in colour removal – up to 99% (***Table 5***). The coagulant led to a decrease in the raw wastewater toxicity (about 62%) but did not have a significant influence on the biotreated wastewater toxicity.

Comparison of different treatment effectiveness

In this section all removal effectivities were calculated on the basis of the mean parameter values of the raw wastewater (collected in ***Table 1***).

Figures 5 and ***6*** show that the best results were obtained after the combined treatment in which the biodegradation was followed by coagulation with PAX18. Such a combination enabled the removal of 98% BOD_5, 87% COD, 88% TOC, 48% TN, 98% TP, 98% toxicity and colour between 81% at 436 nm and 93% at 620 nm. The biodegradation prior to the coagulation led to similar results to those in the opposite sequence (biodegradation after coagulation). Chhabra and co-workers [44] achieved comparable decolourisation results – 80% using laccase enzyme with ABTS as the biodegradation step before coagulation with alum. However, they reported better effects in the opposite sequence – 85% colour removal.

Analysis of the results obtained also revealed that the biodegradation was a very efficient process in organic carbon (above 95% measured as BOD_5 and over 60% as COD and TOC) and toxicity removal (94%). However, the biological treatment was insufficient in terms of the total phosphorus (29%) and colour depletion (up to 36%).

The coagulation with PAX18 had a lower efficiency in organic carbon removal than the biodegradation (***Figure 5***) – up to 32% BOD_5, 42% COD and 32% TOC. However, it was very effective in the total phosphorus depletion – up to 94%.

Although coagulation with Epoly CRD was the best solution for colour removal (***Figure 6***), this process was ineffective in organic carbon compound and total phosphorus removal (***Figure 5***). The biodegradation followed by coagulation with Epoly CRD resulted in significantly lower total phosphorus removal in comparison to the best combination, leading to the much better decolourisation than in the combined enzymatic and alum treatment, as described by Chhabra et al. [44].

Conclusions

The results presented in this paper confirmed the suitability of biodegradation in BAFs and coagulation with PAX18 for the treatment of highly contaminated wastewater from the textile industry – a waste stream of the water cycle proposed in a dyeing plant.

Among the fillings tested, ceramsite showed the best properties – the material did not clog during five months of operation. Additionally, the biofilm formed on this support was characterised by high bacterial and fungal diversity, which resulted in good treatment efficiency (above 95% measured as BOD_5 and over 60% as COD and TOC) of the wastewater, which had high acute toxicity towards *Vibrio fisheri* and a BOD_5/COD ratio typical for mixtures biodegradable by selected microorganisms.

The combination of the biological and physicochemical processes revealed the advantages of both of them – the high removal of organic carbon compounds via biodegradation and the total phosphorus via coagulation. As a result the effective-

ness of the combined treatment achieved a removal of over 98% of BOD_5, 87% of COD, 88% of TOC, 98% of the total phosphorus and 98% of toxicity. What is more, the decolourisation was also high – between 81 and 93% (depending on the wavelength). The biodegradation prior to coagulation led to slightly better results than in the opposite sequence (biodegradation after coagulation). However, it has to be stressed that other textile wastewater may be more toxic towards microorganisms than that used in this study, in which case coagulation might be used before biodegradation. For each dye plant investigations should be performed in order to check which sequence is better in the particular case.

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