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Removal of phenanthrene and 4-phenylphenanthrene from wastewater in an integrated technological system

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ABSTRACT

An integrated system, consisting of a bioreactor with a nitrifying immobilized biomass and a membrane reactor, was tested for its ability to remove two polycyclic aromatic hydrocarbons (PAHs)—phenanthrene (PHE) and 4-phenylphenanthrene (4-PPHE)—from wastewater. An almost complete removal of the selected PAHs was observed under the operational conditions applied (0.6 mg PHE/l or a mixture of 0.6 mg PHE/l and 0.6 mg 4-PPHE/l in the influent, retention time in an aerated bioreactor of 1.5 h, transmembrane pressure of the ultrafiltration membrane 1 bar). Permeate concentration was below 2 μg PAH/l. The introduction of the PHE and an increase in organic loading (Q_V) from 4.9 kg chemical oxygen demand (COD)/(m^3 day) to 6.7 kg COD/(m^3 day) resulted in a decline in nitrification efficiency from 87.6 to 53.0%. A further increase in Q_V to 8.4 kg COD/(m^3 day) by supplying an additional 4-PPHE induced neither ammonia oxidation nor PAH removal. The diversity of ammonia-oxidizing bacteria was not affected by operational conditions. The presence of the mixture of two PAHs induced a decline in the diversity of the total bacteria in the immobilized biomass.

Keywords: Phenanthrene; 4-Phenylphenanthrene; Immobilized biomass; Membrane reactor; Biodiversity

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) composed of two or more fused benzene rings, appear in the environment as a result of the incomplete combustion of organic matter. Since they originate from non-point sources, they are ubiquitous in municipal wastewater. PAHs are a recalcitrant group of contaminants. Because of their toxicity, persistence in the environment, low volatility, resistance to microbial degradation, and potential carcinogenic effects, PAHs pose an environmental threat to humans and animals [1]. Phenanthrene (PHE) (3-ring compound) is one of the PAHs found in fossil fuels and it is also used in the production of dyes, drugs, and explosives. The PAHs of up to 3 rings belong to low molecular weight compounds. They are of particular environmental concern because they are water soluble in the range of 1–200 μg/l and are transported with the groundwater [2]. One of the most widespread phenyl derivatives of PAHs from anthropogenic sources are phenylphenanthrenes, which were detected in sedimentary rocks by Rospondek et al. [3]. However, there is lack of data concerning these compounds so their effect on organisms is unrecognized.

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Most PAHs have low water solubility and, as a result, are hydrophobic and are adsorbed onto solid particles during wastewater treatment, making them less available to microorganisms and thus persistent in the ecosystem [4]. There are a lot of data concerning the method of PHE degradation from wastewater in advanced individual oxidation processes (cavitation, photocatalytic oxidation, Fenton's reaction, ozonation, and the use of hydrogen peroxide [5] in the hybrids of the above-mentioned processes [6]), through adsorption on activated carbon [7], or by the application of membrane technology [8].

Systems of activated sludge may also be applied for PAH removal since many bacterial species are able to degrade these compounds in aerobic [9] and anaerobic conditions [10]. Volatilization, biodegradation, and adsorption can be considered as possible mechanisms for PAHs removal in wastewater treatment [11]. However, in a conventional wastewater treatment plant (WWTP), volatilization may contribute to only 1 or 2% of PAH removal [12]. It has been shown that PAH sorption to the flocs of activated sludge is the key removal mechanism due to the high affinity for particulate matter. However, industry surveys claim that sorbed PAHs do not accumulate in the biomass, which indicates their biodegradation [13]. Manoli and Samara [12] reported PAH removal in a conventional activated sludge facility at a level of between 28 and 67% in the primary tank, up to 61% in the secondary stage, and between 37 and 89% in the whole process, which indicates the significance of sorption. In Norwegian domestic WWTPs, combined chemical and biological treatments resulted in the highest removal of the sum of 16 PAHs of between 94 and 100% [14].

The improvement of PAH removal from wastewater can be achieved by the application of membrane bioreactors [15]. These are a fusion of an activated sludge system and membrane technology in which the wastewater is treated at very long sludge age. In research on WWTPs in Italy, the authors proved that PAH removal was enhanced by a long solids retention time (SRT). Clara et al. [16] clearly indicated that a sludge age higher than its critical value determined for the micropollutant is crucial in biodegradation. The importance of SRT in PAH elimination from wastewater explains why systems with immobilized biomass can be successfully used, for example, in the treatment of wastewater containing some refractories from the crude oil processing industry [17].

The highest efficiency of biological PAH elimination from wastewater was obtained during the simultaneous removal of phosphorus, nitrogen, and methanol [10]. This could be caused by cometabolism—a widespread process in nature, described as the degradation of a compound by microorganisms which do not obtain energy or carbon for cell growth from the transformation and, therefore, require an alternative source of carbon and energy [18]. Cometabolism can induce a rapid biodegradation of pollutants that would otherwise be broken down very slowly, if at all, in the environment [19]. For example, nitrifying bacteria are capable of cometabolic degradation.

In the current study, a reactor with an immobilized nitrifying biomass as part of the technological system was applied. Such reactors can withstand high hydraulic loading and biomass concentration and their proper exploitation results in low sludge production [20]. Additionally, reactors with immobilized biomass are very effective in maintaining the slow-growing microorganisms in the systems, for example, nitrifying bacteria [21]. In our study, the reactor with the immobilized biomass was integrated with a membrane reactor in the technological system. The ultrafiltration membrane was used to improve the efficiency of PAH removal and acted as a second clarifier. The PHE was selected as a model PAH, because of its occurrence at higher concentrations than other PAHs in municipal wastewater. Wastewater was also supplied with a mixture of PHE and its derivative—4-phenylphenanthrene (4-PPHE). We investigated if there is any relationship between micropollutant removal and nitrification efficiency. The diversity of the microbial community of the immobilized biomass exposed to the selected PAHs was evaluated as well.

2. Materials and methods

2.1. Reactors and operating conditions

The experiment was conducted in an integrated technological system consisting of two stages. In the first stage (stage I°) there was a column reactor with an immobilized biomass and in the second stage (stage II°)—a membrane reactor.

The column reactor (stage I°) (Fig. 1) was filled with a cylinder-shaped porous ceramic support (TAMI Industries, Germany) in which activated sludge was immobilized. The support (external diameter 10 mm, hydraulic diameter 3.6 mm, height 1,178 mm) had three holes through its whole length and a number of macropores inside the ceramics. Pore diameters ranged from 4 to 6 μm and the material porosity was between 35 and 40%. The internal surface of the support was 0.04 m^2 and the total volume 0.1 l. The total volume of the reactor was 0.7 l so the free space outside the support was 0.6 l. After immobilization, the support loading was 10.2 g TSS/l.

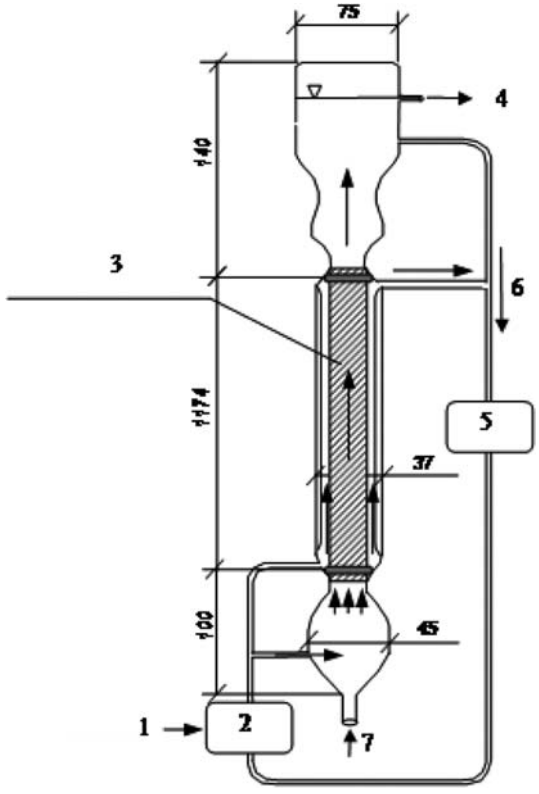

Fig. 1. Scheme of column reactor: 1—influent, 2, 5—pumps, 3—support with immobilized biomass, 4—effluent, 6—internal circulation, 7—air supply.

Activated sludge collected from a municipal WWTP was immobilized by circulating it for 24 h through a ceramic bed.

The bioreactor worked with a continuous flow of 1.6 l/day. Hydraulic retention time (HRT) equaled 1.5 h and the capacity of the internal circulation was 60 l/h. The bioreactor was continuously aerated at the rate of 12 l/h. The whole system was shielded to prevent photolysis of PAH.

The membrane installation (stage II°) consisted of one filtration module containing one ceramic membrane and was equipped with a tubular heat exchanger. An ultrafiltration membrane (cut-off 150 kDa) (TAMI Industries, Germany) with a filtration area of 0.35 m^2 was used. The maximum working pressure was 10 bar. The installation was operated in batch mode with partial recirculation of the retentate into the process tank, at a transmembrane pressure of 1 bar. In each experimental series, the effluent from the stage I° was introduced into the stage II°.

2.2. Wastewater

Synthetic wastewater was prepared based on Coelho et al.'s [22] formula and modified by adding sodium acetate to imitate municipal sewage. The average pollutant concentrations were: 304 ± 26 mg chemical oxygen demand (COD)/l and 63.6 ± 4.2 mg NH_4-N/l. Wastewater was enriched with two PAHs, namely, a 3-ring PHE ($C_{14}H_{10}$) and a 4-ring 4-PPHE ($C_{20}H_{10}$). The PHE was purchased from Sigma-Aldrich, while the 4-PPHE was synthesized in the Faculty of Chemistry, Jagiellonian University, Krakow. Stock solutions were prepared by dissolution of 60 mg of PHE and 60 mg of 4-PPHE in dimethyl sulfoxide and supplementation with distilled water to 100 ml. One millilitre of these stock solutions were used for preparing 1,000 ml of wastewater.

In series 1, the synthetic wastewater was supplemented with 0.6 mg/l of PHE. In series 2, the synthetic wastewater was enriched with 0.6 mg/l of PHE and 0.6 mg/l of 4-PPHE. The solutions were introduced into the stage I° of the technological system. The control series was conducted with wastewater containing neither PHE nor 4-PPHE.

The experimental series were carried out consecutively in the same reactor. Before the start, activated sludge was immobilized in the support and used throughout the whole experiment. The adaptation period before each experimental series was considered complete when, within seven days, the range of changes of particular parameters of the effluent did not exceed 10%. After adaptation, each research series lasted 2 months. It was assumed that the culturing of the nitrifying biomass would be advantageous for the effective removal of the PHE and 4-PPHE, hence the ammonia concentration was selected as a control parameter.

2.3. Analytical methods

The concentrations of the basic indicators of wastewater (COD, ammonia, nitrite and nitrate nitrogen, total and volatile suspended solids) were assayed according to APHA [23].

2.3.1. PHE and 4-PPHE determination

The 25 ml of sample was shaken with 25 ml of methanol in a 50 ml vial. After shaking, the sample was filtered using a medium quantitative filter. The PHE and 4-PPHE determination was processed by solid phase extraction (SPE) using Discovery DSC-18 columns (Supelco) with 500 mg of sorbent. The SPE stationary phase was conditioned by acetonitrile (3 ml), water (3 ml), and the sample was then passed through the column. The cartridges were cleaned with water (3 ml) and then dried under a vacuum. The analytes were eluted using dichloromethane (5 × 1 ml).

After dichloromethane evaporation in N_2 steam, 1 ml of acetonitrile was added and the PAH concentration was determined by a high performance liquid chromatograph (HPLC) (Varian, Australia) equipped with an autosampler (Model 410), solvent delivery modules, a UV–vis (ProStar 325) and fluorescence detector (ProStar 363). The analyzed PAHs were determined on a Supelcosil LC-PAH column (15 cm × 4.6 mm, 5 μm) with the precolumn (LC-18, 2 cm) (Supelco) at 35˚C under isocratic conditions (flow rate: 0.8 ml/min). A mixture of 78% acetonitrile and 22% water was used as the mobile phase. The HPLC device allowed for simultaneous collection of the data on-line from the UV (254 nm) and fluorescence detectors (250/363 nm). The quantitative determination of PHE and 4-PPHE was performed using the external calibration curve method.

In order to determine the biodegradation susceptibility of wastewater, a respirometric test in the close respirometric unit OxiTop Control OC 110 (WTW, Germany) was used to measure the changes in dissolved oxygen (DO) concentrations in the permeate and retentate. The DO changes in the samples were determined based on the variations of partial pressure inside the measuring vessels. Carbon dioxide, formed during the microbial metabolism, was absorbed by NaOH placed in the small tube above the liquid level in the vessels. The amount of O_2 used by bacteria is proportional to the amount of formed CO_2. The removal of CO_2 from the gas phase induces the pressure drop in the measuring vessel that is automatically converted to the changes of DO in the samples. Based on the changes in DO over time, the reaction rate constants (k) were calculated as first-order kinetics using Statistica 9.0 software.

Biomass samples for the molecular analyses were taken from the column reactor for the control, series 1, and series 2 in two replicates. Genomic DNA was isolated from the biomass using a FastDNA® SPIN Kit for Soil (Q-Biogene). The quality and quantity of isolated DNA was measured using a Biophotometer (Eppendorf) and by agarose electrophoresis. The partial bacterial 16S rDNA and *amoA* gene sequences were amplified. Primer sequences for each amplification are given in Table 1.

The PCR amplification was carried out using the following program: 95˚C for 5 min, 35 cycles of denaturation at 94˚C for 30 s, annealing at 63˚C (reaction regarding 16S rDNA) or at 60˚C (reaction regarding *amoA*) for 45 s, extension at 72˚C for 1 min, and a final elongation at 72˚C for 5 min. The amplification products were analyzed by electrophoresis in 1.0% agarose gels with ethidium bromide. The size of the PCR products was estimated using a 100 bp O'GeneRuler ladder (Fermentas). The PCR products from both reactions were used for denaturing gradient gel electrophoresis (DGGE).

Table 1
Primers used in the PCR reactions

Primer	Sequence (5´-3´)	Literature
amoA-2R	ccc ctc tgc aaa gcc ttc ttc	[24,25]
amoA-1F	[a]ttt cta ctg gtg gt	
357-F	[b]cct acg gga ggc agc ag	[26]
517-R	att acc gcg gct gct gg	

[a]cgc cgc gcg gcg ggc ggg gcg ggg gcg ggg.
[b]cgc ccg ccg cgc gcg gcg ggc ggg gcg ggg gca cgg ggg g.

The DGGE was performed in the Dcode Universal Mutation Detection System (BioRad). A denaturing environment was created by a constant temperature (60˚C) and a linear gradient of formamide and urea in the gel (30–60%). Electrophoresis was performed in 1x TAE (pH 8.0) at 120 V for 6 h. After electrophoresis, the gels were stained for 30 min in 1x TAE buffer containing a SybrGold solution, viewed under UV light, and analyzed for the PCR-DGGE banding pattern using Image Kodak.

The biodiversity, expressed as the Shannon–Wiener index (H′) [27], was calculated using the following equation:

$$H' = -\sum (n_i/N)\ln(n_i/N)$$

where n_i is the intensity of a band in the lane, and N is the total intensity of all bands in the lane.

A matrix was constructed for all DGGE profiles taking into account the presence or absence of individual bands and the relative contribution of the band (in %) to the total intensity of the lane. This matrix was used to calculate a distance matrix and to create a dendrogram using Tree View32.

3. Results

A nitrifying biomass was cultured in the biological reactor which formed the first stage of the technological system. At a HRT of 1.5 h and an organic loading of the carrier (Q_V) of 4.9 kg COD/(m^3 day), nitrification efficiency was 87.6% (Fig. 2(a)—control series). Ammonium concentration in the effluent equaled 3.8 mg NH_4-N/l, nitrates were the main oxidation product. The immobilized biomass cultivated in this way was exposed to PHE. This was removed with an efficiency of 99.7% (Fig. 2(b)—series 1). The effluent

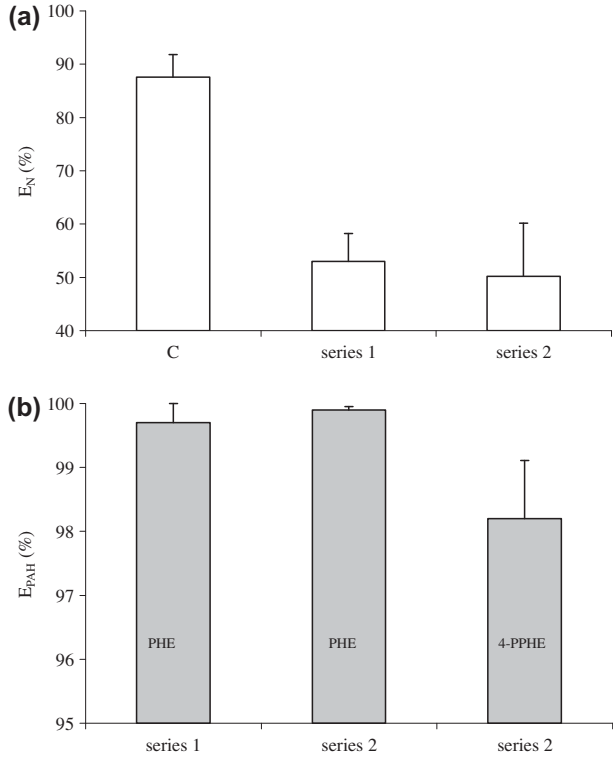

Fig. 2. Performance of the column reactor with the immobilized biomass (stage I°): (a) nitrification efficiency (E_N), and (b) the efficiency of PHE and 4-PPHE removal (E_{PAH}); C—control series.

contained 2 μg PHE/l. Under these conditions, Q_V increased to 6.7 kg COD/(m^3 day)—adding the PHE to the wastewater induced a decrease in nitrification efficiency to 53%. This efficiency was mainly determined by the oxidation of ammonia to nitrite, since the effluent consisted of 28.8 mg NH_4-N/l, 26.1 mg NO_2-N/l, and 0.7 mg NO_3-N/l.

In the environment, PAHs are present in mixtures. For this reason, series 2 was conducted with the influent containing both PHE and 4-PPHE. In the biological reactor (stage I°), PHE was eliminated almost completely (99.9%), whereas the efficiency of removal of 4-PPHE was 98.2% (Fig. 2(b)). The effluent contained 0.6 μg PHE/l and 10.9 μg 4-PPHE/l. In series 2, the presence of PHE and 4-PPHE in the influent resulted in an increase in Q_V to 8.4 kg COD/(m^3 day). However, there were no significant changes in either nitrification efficiency (50.2%) or in the effluent composition. Similar effectiveness of ammonia oxidation to that obtained in series 1 could have been caused by the bacteria's ability to adapt, as the following research series was conducted consecutively in the same reactor.

The DGGE profiles of the 16S rDNA fragment are presented in Fig. 3. Samples 1–3 were taken from the

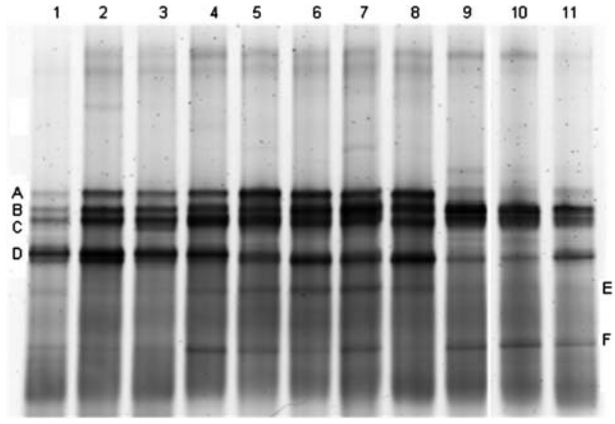

Fig. 3. The DGGE profiles of 16S rRNA gene in bacteria of the nitrifying immobilized biomass: 1–3—samples from control series, 4–5—samples from series 1, and 6–11—samples from series 2.

control series, samples 4 and 5 were taken during the supply of 0.6 mg PHE/l to the reactor, and samples 6–11 were taken when PHE and 4-PPHE, in concentrations of 0.6 mg/l, were added. Analysis of the 16S rDNA gene revealed 11 bands (operational taxonomic units), but in Fig. 3 six bands, the most characteristic for the analysis, were depicted. In all the samples, bands A, B, C, D, and F were common, but bands A and D in samples 9–11 from series 2 had lower intensities than the other samples. B and F was present in all samples, but its occurrence was characterized by a differentiated intensity. B and E was typical for series 1 and for samples 6–8 from series 2.

The biodiversity of the microbial biomass was determined on the 16S rDNA and *amoA* gene patterns from the DGGE technique. The average values of the Shannon–Wiener index (H´), calculated on the basis of the 16S rDNA patterns, varied from 1.90 to 2.13 (Fig. 4). The highest value of H′ was observed during the control series: it remained at an approximately constant level in series 1 and decreased to 1.90 in series 2 when the immobilized biomass was exposed to the mixture of the two PAHs.

In Fig. 3, the DGGE profiles of *amoA* are not shown because there were no differences in the number and intensities of the bands in every lane. The calculated H´ index value was about 1.75 during the control series and the PHE and 4-PPHE degradation.

A statistical comparison of the bacterial assemblages of the biomass samples is shown as a dendrogram (Fig. 5). Samples from the control (1–3) and one sample from series 1 were grouped together. Another group was isolated from samples 5–8. The profiles derived from the last stage of the study (samples 9–11) are in the third cluster. This group exhibited a

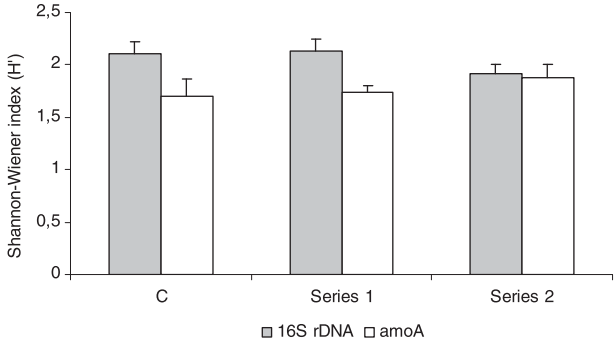

Fig. 4. The values of Shannon–Wiener index (H′) calculated on the DGGE patterns; C—control series.

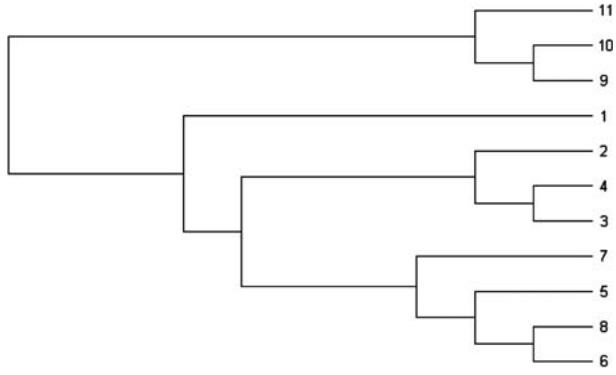

Fig. 5. Distances dendrogram of 16S rDNA fragment generated from the DGGE profiles: 1–3—samples from control series, 4–5—samples from series 1, and 6–11—samples from series 2.

distinct change in the DGGE profiles generated in comparison to the other groups. The samples were clustered mostly according to PAH addition and time of sampling, indicating that the type and concentration of the analyzed compounds were the main factors determining the bacterial composition.

The effluent from the bioreactor was introduced into the membrane filtration (stage II°) to remove suspended solids with potentially adsorbed PAHs. In series 1, PHE was completely removed (Fig. 6). In series 2, the application of membrane filtration resulted in 65.7% of PHE removal and 100% of 4-PPHE removal. The permeate contained 0.2 μg PHE/l. The variation in the efficiency of removal of the selected PAHs could be induced by the different sorption properties of PHE and 4-PPHE.

The permeate was characterized by about 352 mg COD/l. Taking into account the volumes of permeate and retentate obtained, the loading of the organic compounds in the effluent from the second stage of the technological system was included, for most part,

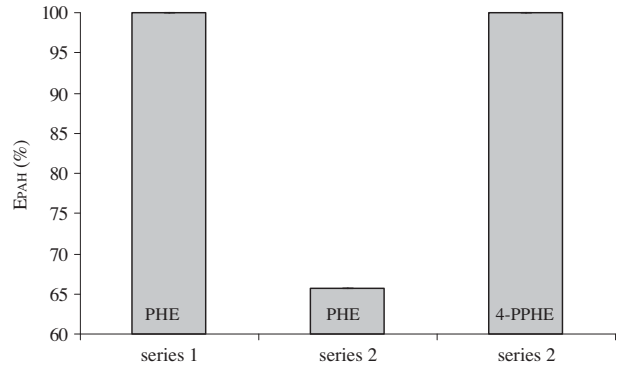

Fig. 6. The efficiency of PHE and 4-PPHE removal from wastewater in the stage II° of the technological system.

in the permeate. The permeate was completely free of suspended solids and contained, at most, a trace amount of PHE. Given the concentration of organics in the effluent in the control series (wastewater with neither PHE nor 4-PPHE)—30 mg COD/l—the level of 320 mg COD/l in the permeate was probably generated by the presence of the intermediate products of PHE or 4-PPHE degradation. For the permeate, the constant of the oxygen uptake rate (k), calculated on the basis of the respirometric test, was 0.08 day^{-1} in series 1 and 2. During membrane filtration, ammonia was removed from wastewater with an efficiency of 24.3% in series 1 and 50.2% in series 2. This generated a total efficiency (for the complete 2-stage system) of 63.4% for series 1 and 84.1% for series 2. Ammonia removal by ultrafiltration was connected with the elimination of biosolids, containing 5–6% of nitrogen, from the bioreactor effluent.

4. Discussion

In the experiment presented, a greater than 98% removal of selected PAHs by a nitrifying immobilized biomass was obtained. Farhadian et al. [28] confirmed that in biofilm reactors the effectiveness of PAH removal can be very high and reach 99%. Probably, the factors positively affecting the rate of the biotransformation of the organic microconstituents include high biomass density [10] and long sludge age (SRT) [16], which are typical for an immobilized biomass. The required SRT should exceed 10 days to ensure the satisfactory removal of biodegradable organic micropollutants. Hence, it can be concluded that WWTPs with biological nutrient removal should be able to eliminate these compounds efficiently. In our research, the bioreactor was operated for a very short HRT of 1.5 h. However, the long SRT probably screened the potential negative effect of a short HRT. The research

can confirm that biomass of nitrification ability can eliminate organic micropollutants effectively. Wood [29] and Verstraete and Philips [30] gave the concept of using nitrifiers for indirect bio-catalysis, known as the phenomenon of cometabolism. According to these authors, during nitrification the generation of OH· radicals by ammonia mono-oxygenase takes place and soluble microbial products are generated that serve as co-substrates for heterotrophic microorganisms. Thus, the nitrification activity can stimulate initiation of the indirect biodegradation of recalcitrant organics [19]. For example, De Heyder et al. [31] obtained a 1.7-fold higher volumetric removal rate of ethene in a packed granular activated carbon reactor by stimulating nitrification. However, the mechanisms of cometabolism dominate at low concentrations of micropollutants [32]. The authors observed the dominance of the cometabolism of ammonia-oxidizing bacteria (AOB) during the biodegradation of the different endocrine disruptors (EDC) in nitrifying activated sludge. The increase in organic loading induced the rate of organic matter degradation, while ammonia oxidation and EDC removal were less effective. Similarly, the research of Giger [33] on the removal of chemical detergents through nitrification-simulated cometabolism proved that organic loading is supposed to be the key parameter responsible for the removal of microconstituents from wastewater. In the research presented, the enhancement of organic loading from 6.7 kg COD/(m^3 day) in series 1, to 8.4 kg COD/(m^3 day) in series 2, did not reduce the effectiveness of PAH removal. However, under these conditions nitrification efficiency was only about 50% and was decreased by 40% as compared to the control series. For this reason, it can be supposed that PAH removal by an immobilized biomass cannot be connected with cometabolism. According to Speitel and Segar [34], the kinetics of cometabolism often are at least 10 times slower than the kinetics of metabolism and during simultaneous metabolism of the growth substrate and cometabolism of the recalcitrant compound, competition for the enzyme may occur. Additionally, the presence of large amounts of carbonaceous substrates often prevents oxygen from being used for nitrification [35]. However, an earlier experiment on nitrogen removal from municipal wastewater by biomass immobilized in a similar support, resulted in a nitrification efficiency greater than 85% at an organic loading of 48.8 kg COD/(m^3 day) [36]. Hence, in this present experiment it could not be the organic loading that limited ammonia oxidation, but the addition of the PAHs which could have induced a decrease in nitrifying ability. Ely et al. [37] proved that a trichloroethylene (TCE) supply caused a decline in ammonia-oxidizing activity due to the TCE-related inactivation of ammonia mono-oxygenase.

In the study presented in this report, the diversity of the microbial community as expressed by the Shannon–Wiener index (H´) was calculated as the response of biomass exposition to selected PAHs. A high H´ value indicates high biodiversity in a microbial community. The addition of PHE into the influent did not affect the diversity of the total bacteria (H´ = 2.13). Only exposing the biomass to a mixture of the two analyzed PAHs induced a decline in diversity. This was confirmed by H´ decreasing to 1.90. Furthermore, an analysis of the genetic distance confirmed these changes, indicating that the DGGE profiles for the immobilized biomass not exposed to PAHs, but exposed to PHE, were similar, whereas the profiles obtained for the biomass with PHE and 4-PPHE were grouped in a separate cluster. The exposure of the immobilized biomass to micropollutants and to increasing organic loadings up to 8.4 kg COD/(m^3 day) did not affect the biodiversity of the AOB, but the activity of the nitrifiers declined. Similarly, Kurola et al. [38], when analyzing the structure of AOB populations in oil-contaminated soil by the DGGE analysis of partial 16S rDNA sequences, observed the presence of stable AOB populations in the soil during the period of the study. However, the diversity tended to decrease after the aromatic compound degradation, compared to the diversity in the control [39]. According to Xia et al. [40], an increase in organic loading limits the diversity because a high organic carbon concentration in wastewater favors fast-growing bacteria which tend to be dominant over slow-growing populations. Still, the issues of microbial diversity related to micropollutant removal from wastewater have so far not been well recognized.

There is a great deal of research concerning the treatment of mixtures of pollutants. Some PAHs can inhibit degradation of others due to toxicity [41] or degradation of the mixture is inhibited because of the total PAH concentration [10]. In our study, the relatively lower efficiency of removal of 4-PPHE (98.2%) as compared to the efficiency of removal of PHE (99.9%), could result from the higher total PAH concentration in the wastewater as compared to the series with a single PAH. The toxicity should not be considered, since the presence of the PAH mixture did not induce a decrease in nitrification efficiency, as compared to the experiment with PHE as the only PAH in the influent. Besides, an immobilized biomass is considered to be more resistant to unfavorable conditions than a suspended one [42] due to diffusion limitations. Kovarova-Kovar and Egli [43] explained that biodegradation may not occur because of a lack of

enzyme induction if the concentration of a pollutant decreases below a certain level. However, in our research 98.2% of 4-PPHE was eliminated from wastewater, so the concentration of selected PAHs did not cause the enzyme repression. In contrast, Yuan et al. [44] reported that the degradation of five selected PAHs was more effective when they were in a mixture because the presence of all of them provides more carbon sources, or cross-acclimation can enhance the biodegradation rate.

The application of the membrane filtration turned out to be an advantageous solution for the removal of the residuals of PAHs or PAHs sorbed to biosolids. The concentrations of the examined compounds in the retentate were lower by about 50% than would have resulted from a material balance. This may indicate that some parts of the compounds could be adsorbed by the membrane material. However, biodegradation of PAHs earlier sorbed on solids could have followed, so that we did not observe the accumulation of the tested PAHs in the concentrate. According to Stringfellow and Alvarez-Cohen [13], biosorption of PAHs may be advantageous in PAH elimination because sorbed PAHs are retained in the treatment system for a longer period than the HRT, so that the bacteria have more time for biodegradation. In our research, the permeate was characterized by 0.2 μg PHE/l only when a mixture of the two PAHs was in the influent. However, on the basis of the concentration of organic compounds, we concluded that the permeate contained intermediate products of PHE or 4-PPHE degradation of a molecular weight lower than 150 kDa. The results of the respirometric test confirmed this thesis. The small values obtained for the constant of oxygen uptake rate (k) in the permeate ($0.08\,day^{-1}$) indicated the low biodegradation susceptibility of the permeate in spite of high COD. It is commonly known that k depends mainly on the rate of biological oxidation of the organic substances. Therefore, for example, in raw municipal wastewater k is significantly higher ($0.3–0.5\,day^{-1}$) than in the same wastewater after biological treatment ($0.2\,day^{-1}$). Pitter, Chudoba [45] stated that values of k lower than $0.2\,day^{-1}$ indicated the presence of compounds of low biodegradability.

5. Summary

The research on the efficiency of removal of selected PAHs from wastewater revealed that the application of a biological reactor with a nitrifying immobilized biomass followed by membrane filtration constituted a promising technological solution. Almost total PAH removal was achieved both when PHE was present as a single micropollutant and for a mixture of PHE and 4-PPHE. The supply of PHE resulted in the nitrification efficiency decrease of 40%, whereas the addition of the mixture of PHE and 4-PPHE did not affect further inhibition of ammonia removal. However, the introduction of PAHs did not induce a decline in the biodiversity of the AOB and only slightly decreased the index of diversity for the total bacteria, but only in the case of the addition of a PAH mixture. This may be proof of the lessened sensitivity of the immobilized biomass to the toxic loadings than that of the suspended biomass.

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