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Control of PAHs degradation process under reducing conditions

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ABSTRACT

The paper presents the results of the study that determined the changes in the concentration of selected polycyclic aromatic hydrocarbons (PAHs) in the sewage sludge during the incubation of sewage sludge under reduction conditions (anaerobic conditions). The sewage sludge was incubated for 16 d at 37°C ± 1°C in the dark. The value of oxidative and reduction potential ranged from –300 to –400 mV. The studies were conducted using sludge differing in their initial PAH content. PAHs were determined in the beginning of process and after 4, 6, 12, and 16 incubation days. The quantification of the three-ring PAHs (acenaphthylene, acenaphthene, fluorene, phenanthrene, and anthracene) was carried out simultaneously in the sewage sludge (solid phase [SP]) and supernatants (liquid phase [LP]). Control of the course of changes in PAH concentrations indicates fluctuations in concentration of these compounds in sludge and supernatant liquids under reduction conditions. The efficiency of three-ring PAH decomposition in sewage sludge was in the range from 33% to 75% and was not correlated with the initial concentration of these compounds. The mass balance of PAHs in SP and supernatant liquids confirms biodegradability of these compounds: their mass loss was within the limits of 13 and 21 μg for 1 L of hydrated sludge incubated for 16 d under reducing conditions. The half-life of PAH decomposition in sewage sludge ranged from 3 to 65 d and in supernatant liquids from 3 to 26 d.

Keywords: Polycyclic aromatic hydrocarbons; Sewage sludge; Supernatants; Degradation

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hydrophobic compounds and dissolve poorly in water. These compounds have a strong affinity to particles and therefore usually occur in the adsorbed form on sewage sludge particles [1–3]. Sewage sludge allocated in municipal and industrial wastewater treatment plants are diversified in terms of quantity and quality. Their quantity and physicochemical properties depend on the quantitative and qualitative characteristics of wastewater and the methods of its treatment [4]. The PAH content in sewage sludge and supernatant liquids is shown in Table 1.

PAHs are considered as difficult to degrade, but under certain conditions, their biodegradation is possible. This process can take place under both aerobic and anaerobic conditions with the use of bacteria, fungi, actinobacteria, and algae. Microorganisms living in the environment are usually not adapted to decomposition of PAH in case of sudden environmental contamination. Therefore, an appropriate adaptation time is needed for inclusion of PAH in the metabolic pathways of microorganisms. This involves developing the ability to produce appropriate enzymes directly or initiating genetic changes that can produce the appropriate enzymes. Biodegradation of PAH may also occur as a result of cometabolic transformations, that is, in the presence of another easily assailable source of carbon. Microorganisms capable of PAH decomposition, among others, include bacteria: *Aeromonas* sp., *Acinetobacter* sp., *Agmenellum*

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Table 1
Concentration PAHs in sewage sludge and supernatants [5–10]

Compounds	Sewage sludge (μg·kg^{-1} dm)				Supernatants (μg·L^{-1})	
	Excess	Digested	Excess dewatered	Digested dewatered	Excess	Digested
Acenaphthylene	4–6,570	2.9	20–6,570	466–569	0.03	0.31
Acenaphthene	3–3,890	39.0	550–3,890	11–13	0.09	0.86
Fluorene	17–11,940	29.9	490–11,940	57–71	0.07	1.34
Phenanthrene	39–28,800	137.9	467–35,100	33–45	0.28	2.33
Anthracene	1–6,140	7.8	40–6,100	25–33	0.02	0.29

PAHs, polycyclic aromatic hydrocarbons.

quadruplicatum, *Alcaligenes denitrificans*, *Arthrobacter* sp., *Bacillus* sp., *Beijerinkia* sp., *Brevibacterium* sp., *Flavobacterium* sp., *Haemophilus* sp., *Mycobacterium* sp., *Oscillatoria* sp., *Pseudomonas* sp., *Rhodococcus* sp., *Sphingomonas paucimobilis*, *S. yanoikuyae*, *Staphylococcus auricularis*, *Strptomyces flavovirens*, sulfate-reducing bacteria, nitrate-reducing bacteria, manganese-reducing bacteria, methaneogenic bacteria; fungi: *Aspergillus niger*, *Cunninghamella elegans*, *Phanerochaete chrysosporium*, *Pleurotus ostratus*; and yeast: *Candida utilis* and *Saccharomyces cerevisiae* [11,12]. Biological transformations of PAHs focus on bioaccumulation and biodegradation [13,14]. These transformations can be carried out both by individual strains of bacteria and by microbial syndromes living in metabiosis. Studies described in the literature indicate that these transformations take place faster in the aquatic environment than the solid matrix of, for example, soil or sludge [12,15]. Previous studies of authors showed increased amounts of PAH in supernatant liquids during anaerobic digestion of sludge, which creates more conditions for biodegradation [10]. Intensification of PAH decomposition was also observed after introduction of microflora adapted to decomposition of these compounds [16]. The effectiveness and rate of biological degradation process depend not only on the ability of microorganisms to PAHs change, but also on reaction, presence of oxygen, temperature, as well as on the availability of nutrients and other carbon sources. Biodegradation occurs not only as metabolic changes, but also as cometabolic transformations. Under anaerobic conditions, the aromatic ring oxidizes at low potential values of redox, leading to the formation of cis-dihydrodiols. The catalyst of these reactions is oxygenase enzyme. Subsequently, the resulting products are transformed to dihydroxylated derivatives and oxidized to catechol. In the presence of abovementioned enzyme, the aromatic ring decomposes and resulting metabolites are transformed on ortho- or meta-pathway. The meta-pathway occurs when the ring is torn between the carbon atom with OH group and the neighboring carbon atom. As a result of subsequent transformations, acetaldehyde and pyruvic acid are formed. While ortho-pathway occurs when the ring is torn between carbon atoms with OH groups, and cis-moucanic acid is formed, and then acetyl-CoA is included into the Krebs cycle. As a result of these changes, acetaldehyde and pyruvic acid are formed [17,18]. Examples of degradation pathways for selected PAHs are shown in Fig. 1.

Degradation routes of selected compounds with Pseudomonas bacteria are described below. Acenaphtylene degradation is related to the presence of dioxygenation enzyme in the double bond leading to formation of 1,2-dihydroxyacenaphthalene and tautomer of 1-hydroxy-2-ketoacenaphthene. Then 1-hydroxy-2-ketoacenaphthene is oxidized to acenaphthenequinone, which is oxidatively split to produce naphthalene-1,8-dicarboxylate, and then decarboxylated to form 1-naphthoic acid.

Biochemical route of fluorene degradation is initiated in the presence of naphthalene enzyme 1,2-dioxygenase and leads to formation of 9-fluorenol. Then dihydrodiols are formed, which undergo dehydrogenation and then meta-cleavage in the presence of catalytic enzymes. The final product may be phthalates.

The route of phenanthrene degradation is different. Phenanthrene is oxidized under anaerobic conditions in the presence of enzymes to cis-dihydrodiols, then to pyruvate and finally to phthalate.

Bacteria initiate the degradation of anthracene by hydroxylation of the aromatic ring with formation of cis-1,2-dihydroanthracene-1,2-diol. This compound is then transformed to anthracene-1,2-diol. On the meta route 4-(2-hydroxynaph-3-yl)-2-oxobut-3-enoate is formed, which can spontaneously transform into 3-hydroxy-2-naphthoate, which as a result of degradation is split to 2,3-dihydroxynaphthalene and then to phthalate.

The aim of this study was to control changes in concentrations of three-ring PAHs (acenaphthylene, acenaphthene, fluorene, phenyanthrene, and anthracene) in sewage sludge and supernatant liquids during incubation of sludge varying amounts of PAHs under reduction conditions (anaerobic conditions).

2. Materials and methods

2.1. Incubation of sewage sludge under reducing conditions

Qualitative and quantitative research of PAH was conducted using sludge from urban and industrial wastewater treatment plants. Samples of industrial sludge (coke sewage sludge) have been taken from the reservoir of excessive sludge. Wastewater in this treatment plant is destined for biological treatment (denitrification, carbon oxidation, and nitrification). In the municipal treatment plant, wastewater is treated in the process of deformation, denitrification, and nitrification. A mixture of precipitated and superfluous sludge, as well as fermented sludge was taken for the study. Digested sewage sludge, as inoculum, was mixed (mixture of primary and excess sludge inoculated with fermenting sludge at the volumetric ratio of 1:1.5). The studies were conducted

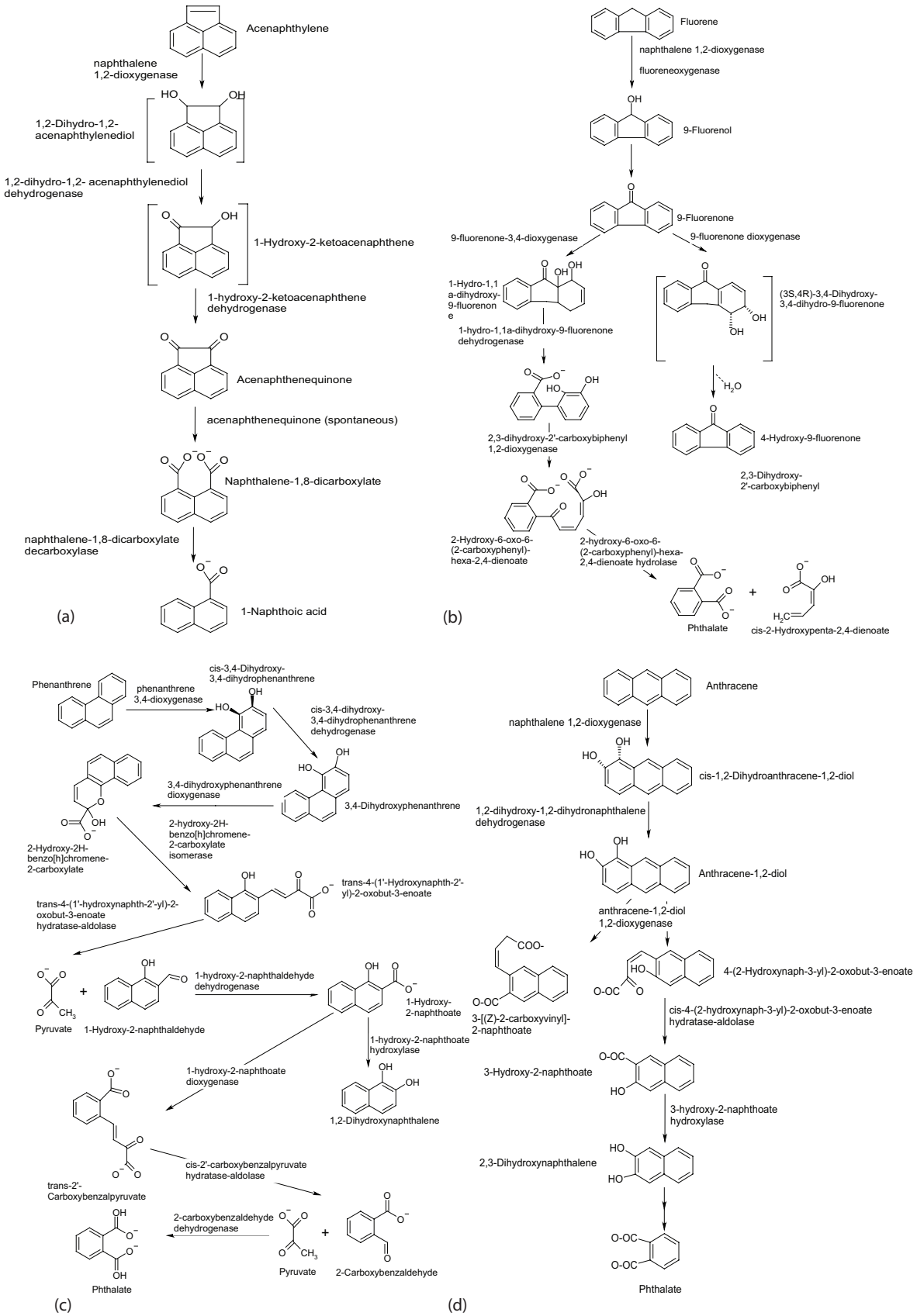

Fig. 1. Pathway of degradation selected PAHs: (a) acenaphthylene [19], (b) fluorene [20], (c) phenanthrene [21,22], and (d) anthracene [23].

using sludge (coke sewage sludge as a main source of PAHs) differing in their initial PAH content. Sample S_1 constituted of sludge with PAH content of 1,595 μg·kg^{-1} dm. In sample S_2, the initial three-ring PAH content was 1,991 μg·kg^{-1} dm, and in S_3 it was 3,491 μg·kg^{-1} dm. The research was carried out in a laboratory system, in glass bioreactors. A total of 15 reactors were prepared of five sludge samples of each type with a volume of 1 L. The sewage sludge was incubated under the same conditions. After 4, 8, 12, and 16 d, sewage sludge from one bioreactor was used for analysis. Incubation of sewage sludge was carried on for 16 d at a temperature of 37 ± 1°C with no access of light, in strongly reducing conditions (anaerobic conditions) with oxidizing and reducing potential of –300 to –400 mV. In order to provide the right contact between biomass and the substrate, the mixture of sewage sludge was mixed once a day, and the pressure of biogas was measured simultaneously.

2.2. Control of physicochemical properties of sludge and supernatant

In order to specify the course of the process, the marking of selected physical and chemical properties of sewage sludge before the process was performed, after 4, 8, 12, or 16 d of incubation from the entire volume of one reactor (scarifying method). For sewage sludge, the following was determined: total suspended solids and volatile suspended solids (VSS). The following was determined in the supernatants obtained from sewage sludge centrifugation: pH, oxidation–reduction potential, alkalinity, and volatile fatty acids (VFAs). During the anaerobic digestion process, pressure and composition of the biogas were monitored. Pressure measurement was conducted at 24 h intervals. The composition of produced biogas was determined by means of gas chromatography (GC) with thermal conductivity detector. Standard mixture was used, with the following composition and concentration (% vol.): CH_4, 70.0%; CO_2, 28.2%; CO, 0.36%; O_2, 0.89%; and H_2, 0.54%. In the biogas, the percentage content of two elementary components was analyzed, that is, CH_4 and CO_2. In the centrifuged sewage sludge, PAHs were marked before the process, after 4, 8, 12, and 16 d of incubation.

2.3. Identification of PAHs

Separation of supernatant liquids was carried out by centrifugation of collected sludge. The determination of PAH concentrations in liquid samples (supernatant liquid) and solid samples (centrifuged sludge) was carried out in three parallel repetitions. The organic matrix was separated from the samples by solvent extraction. The centrifuged sludge was covered with a mixture of cyclohexane solvents: acetone (5:1 v/v, 30:6 mL) and then sonification (25 min) was applied. The extract obtained after this process was poured into centrifuge tubes and centrifuged for 10 min at 9,000 rpm. A mixture of cyclohexane and dichloromethane (5:1 v/v, 20:4 mL) was also used for the extraction of organic matrix from liquid samples (supernatant liquid). In this case, the extraction was carried out mechanically by shaking (60 min). After shaking process, the extracts were separated from samples in the laboratory separator. The extracts obtained from both sludge and liquid were purified under vacuum conditions on columns filled with silica gel. The column filling was conditioned with a mixture of dichloromethane: cyclohexane (1:5 v/v, 3 × 3 mL). The eluates obtained were concentrated to the volume of 2 mL in a stream of nitrogen [24,25].

Qualitative–quantitative analysis of the determined three-ring PAHs was conducted by means of gas chromatography–mass spectrometry method, using GC 8000 gas chromatograph, produced by Fisons, model MS 800. The chromatographic analysis was performed with the use of PAHs standard mixture—16 PAHs (Table 2) were mixed with benzene and dichloromethane (1:1) made by AccuStandard Inc., USA.

The identification was based on injecting 2 μL of extract on the column DB-5 (length 30 m, diameter 0.25 mm, and thickness 0.25 μm) with usage of helium as the carrier gas. For the detection and identification step, the MS 800 spectrometer was used, containing an electron ionization type ion source with the ionization energy of 70 eV. Selective ion monitoring m/z was applied. For each PAH, three representative ions were chosen. Identification ions of PAH are shown in Table 3. Quantitative identification of PAHs was carried out at temperature 280°C. Program of the oven was as follows: from 40°C to 120°C (an increase of 40°C/min), from 120°C to 280°C (an increase of 5°C/min), 280°C for 20 min.

2.4. Removal efficiency of PAHs in sewage sludge

During the interpretation of the results, the decrease in the concentration of selected PAHs in sewage sludge during the anaerobic digestion process was evaluated based on the following formula:

Table 2
Composition of standard mixtures of PAHs

Compound	CAS registry number	Purity % (GC–MS)	Prepared concentration (μg·mL^{-1})	Certified analyte concentration (μg·mL^{-1})
Acenaphthylene	208-96-8	99.2	2,004	1,988
Acenaphthene	83-32-9	100	2,002	2,002
Fluorene	86-73-7	98.1	2,001	1,963
Phenanthrene	85-01-8	99.5	2,004	1,994
Anthracene	120-12-7	97.7	2,048	2,001

CAS, Chemical Abstracts Service; GC–MS, gas chromatography–mass spectrometry; PAHs, polycyclic aromatic hydrocarbons.

$$E = \frac{(c_p - c_i)}{c_p} \times 100, \% \quad (1)$$

where E is percentage of PAHs degradation (%), C_p is initial concentration of an ith PAH (μg·kg^{-1} dm), and C_i is concentration of an ith PAH at a given day of the process: the 4th, 8th, 12th, and 16th (μg·kg^{-1} dm).

2.5. Kinetics of PAHs degradation in sewage sludge

To calculate the half-life decay time, Eq. (2), of PAH, a mathematical description of decomposition rate according to Nerst equation was used for the first-order reaction, Eq. (3):

$$T_{1/2} = \frac{\ln 2}{k} \quad (2)$$

$$\ln \frac{c_0}{c_t} = k \cdot t \quad (3)$$

where k is reaction rate constant (d^{-1}), C_o is initial concentration of PAHs (μg·kg^{-1} dm), C_t is concentration of PAHs after time t (μg·kg^{-1} dm), and t is time of incubation of sewage sludge (d).

Correlation coefficients were calculated using Statistica program to determine the relation between duration of fermentation and the content of PAH in sewage sludge or supernatant liquids.

2.6. Mass balance of PAHs

On the basis of PAH concentrations in supernatant liquids and solids content in relation to dry matter, a mass balance of PAHs was determined. The amount of PAH in supernatant liquids (LP) in relation to the unit volume of dissolved substances and in sewage sludge (SP) was calculated from the formula:

$$LP = C_1 \cdot V \quad (4)$$

$$SP = C_s \times 10^{-3} \text{ s} \quad (5)$$

where C_1 is PAHs concentration in the supernatants (μg·L^{-1}), V is volume of supernatants in the hydrated sludge (L·L^{-1}), s is content of dry matter in the hydrated sludge (g·L^{-1}), and C_s is PAHs concentration in the SP (μg·kg^{-1} dm).

Table 3
Identification ions m/z for chosen PAHs

Ions m/z for PAHs				
Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Anthracene
153	154	167	179	179
152	153	166	178	178
151	152	165	176	176

PAHs, polycyclic aromatic hydrocarbons.

2.7. Statistical test

The significance of changes in PAH in sludge and supernatant liquids was calculated using Excel program and *t*-student test. The confidence level was adopted on the level of 0.95. The number determining degree of freedom was 3, for this parameter the theoretical value of *t*-student td distribution was 2.776.

3. Results and discussion

3.1. Physicochemical properties

The physicochemical properties of the sewage sludge used are shown Table 4.

The total solids in the analyzed sewage sludge S_1, S_2, and S_3 after 16 d of incubation decreased to 12.1 g·L^{-1} (loss of 15%), 13.1 g·L^{-1} (loss of 14%), and 13.6 g·L^{-1} (loss of 16%), respectively. During the process, the decomposition of organic substances in percentage was of 25% in the sewage sludge S_1, and 23% and 22% in the sewage sludge S_2 and S_3, respectively. The oxidation–reduction potential ranged from –300 to –400 mV (average –380 mV). During the incubation, the ratio of VFAs and the alkalinity was not higher than 0.3, and the pH ranged from 7.1 to 8.0. The CH_4 contents in biogas were in the range from 49% to 57% and CO_2 from 27% to 31%. The total volume of biogas produced per 1 g organic dry matter in S_1 sewage sludge was at the level of 0.47 L·g^{-1} of VSS. During the fermentation of S_2 and S_3, sewage sludge biogas production was 0.31 and 0.44 L·g^{-1} of VSS, respectively. In the control reactor, the volume of biogas was at a similar level. Changes in the value of selected indicators were within the range specified in the literature. The incubation process was carried out correctly reproducing the actual conditions prevailing in the sewage treatment plants. Chemical indicators indicate that the sludge after 16 d stabilization was well fermented [26,27].

Table 4
The physicochemical properties of the sewage sludge

Parameters	Unit	Sewage sludge		
		Coke	Preliminary and excessive	Digested
pH	–	7.2	6.6	7.1
Alkalinity	mg CH_3COOH·L^{-1}	1,100	295	3,360
VFAs	mg $CaCO_3$·L^{-1}	369	729	137
TSS	g·L^{-1}	17.6	10.6	17.4
VSS	g·L^{-1}	12.2	7.8	11.5

TSS, total suspended solids; VFAs, volatile fatty acids; VSS, volatile suspended solids.

3.2. Control of PAHs concentration in the sewage sludge and in the supernatants

Table 5 shows the results of quality and quantitative three-ring PAH determinations marked during the test.

Values of *t*-student td test were also given, indicating the significance of PAH content differences before and after the sludge SP. The total initial content of three-ring PAH in S_1 sludge was 1,595 μg·kg^{-1} dm. During sludge incubation, the concentration of tested compounds gradually decreased: after 4 d, it was 1,025 μg·kg^{-1} dm; after 8 d, it was 804 μg·kg^{-1} dm; after 12 d, it was 582 μg·kg^{-1} dm; and after 16 d, it remained at this level and was 594 μg·kg^{-1} dm. Fluorene was the dominant factor before and after the phenanthrene process. During the incubation of S_2 sludge, the concentration of three-ring PAH prior to the process was 1,991 μg·kg^{-1} dm and decreased to 488 μg·kg–^{1} dm after 16 d. As in S_1 sludge, fluorene had the largest part in total concentration of three-rings PAH before the process, whereas phenanthrene and fluorene after the process. The initial total three-ring PAH content in S_3 sludge was 3,491 μg·kg^{-1} dm of which 34% was acenaphthene. After 4 d of incubation, the total content of three-ring PAH in sludge was at a similar level, and after 8 d it was 2,857 μg·kg^{-1} dm. After the incubation process, the total content decreased to 2,338 μg·kg^{-1} dm, and fluorene dominated in the sludge. Critical values of *t*-student test $t_{0.05}$, determining the significance of differences of PAH content in sludge before and after the process, indicate that observed changes in sludge content for all hydrocarbons were statistically significant.

Efficiency of PAH removal during sludge incubation is shown in Fig. 2. In S_1 sludge, the percentage removal of PAH was 62%. While PAH removal efficiency was 75% and 33%, respectively, for S_2 and S_3 sludge. In S_1 sludge, the removal efficiency for acenaphthylene, acenaphthene, and fluorene increased with the duration of process in relation to the initial content. In the case of phenanthrene, the percentage removal increased to 12 d, while in 16 d the lowest percentage of removal was achieved. In case of anthracene up to 4 d its content in sludge increased, while from 8 d the analyzed hydrocarbon loss was observed and thus the efficiency of its removal increased. During the storage of S_2 sludge under specified conditions for all determined compounds, the efficiency of their removal increased. Greater PAH loss during the incubation of S_2 than S_1 sludge was observed for phenanthrene and anthracene. Therefore, industrial sludge could be a carrier of microorganisms capable of biodegradable of these PAHs. Such microorganisms are methanogenic arecheans, which show the ability to degrade hydrocarbons [28]. The presence of these microorganisms in sewage and sludge is confirmed in literature [29]. Therefore, their presence contributed to an increase in biodegradability of these hydrocarbons. This phenomenon was also observed in previous studies during codigestion of a mixture of coke sludge rich in microflora from municipal sludge [10,16]. In case of S_3 sludge, the highest efficiency of removal of all analyzed PAHs was achieved in the period from 12 to 16 incubation days.

Table 5
Changes of PAHs concentration in sewage sludge (μg·kg^{-1} dm) under reduction conditions (*t*-student $t_{0.05}$ = 2.776)

Compound	Time (d)					*t*-Student
	Before	4th	8th	12th	16th	0–16th
Sewage sludge, S_1						
Acenaphthylene	70	30	23	17	1	12.960
Acenaphthene	362	298	229	160	54	51.267
Fluorene	607	276	216	156	107	47.205
Phenanthrene	501	339	272	204	418	7.342
Anthracene	56	82	64	46	14	10.286
∑ three-ring PAHs	1,595	1,025	804	582	594	74.937
Sewage sludge, S_2						
Acenaphthylene	79	43	42	36	11	13.545
Acenaphthene	609	343	373	251	120	81.617
Fluorene	608	374	417	265	165	31.636
Phenanthrene	559	382	384	243	165	56.271
Anthracene	137	86	88	46	27	137.375
∑ three-ring PAHs	1,991	1,227	1,304	842	488	159.356
Sewage sludge, S_3						
Acenaphthylene	162	139	91	81	62	49.850
Acenaphthene	1,190	1,069	821	695	600	7.373
Fluorene	1,057	1,099	901	817	776	9.370
Phenanthrene	906	948	858	816	764	38.486
Anthracene	176	200	186	136	136	3.653
∑ three-ring PAHs	3,491	3,456	2,857	2,545	2,338	27.242

PAHs, polycyclic aromatic hydrocarbons;

In studies described in literature, the problem of PAH in supernatant liquids is usually omitted and concentrations of PAH in sludge are expressed in relation to dry matter [30]. However, the author's research carried out earlier confirms the possibility of releasing PAH from sludge to supernatant liquid under anaerobic conditions [10]. PAH concentrations in supernatant liquids are shown in Table 6.

In supernatant liquids from *S*1 sludge, the initial total three-ring concentration of PAH was 0.024 μg·L^{-1}. Both before and after the incubation process in supernatant liquids, acenaphthylene appeared below the limit of determination. During anaerobic incubation, gradually higher concentrations of PAH were observed up to 12 d. However in the final period, the concentration of these compounds

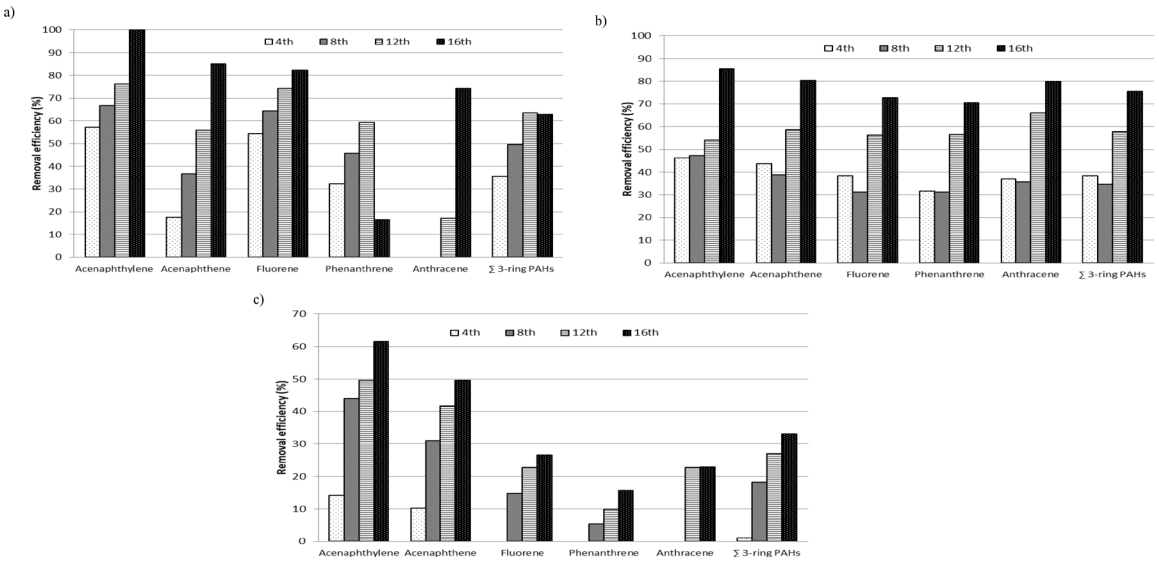

Fig. 2. Removal efficiency (%) of PAHs in sewage sludge: (a) S_1, (b) S_2, and (c) S_3.

Table 6
Changes of PAHs concentration in supernatants (μg·L^{-1}) under reduction conditions (*t*-student $t_{0.05}$ = 2.776)

Compound	Time (d)					*t*-Student	
	Before	4th	8th	12th	16th	0–16th	12–16th
Sewage sludge, S_1							
Acenaphthylene	nd	nd	nd	nd	nd	–	–
Acenaphthene	0.004	0.06	0.10	0.75	0.36	34.902	24.742
Fluorene	0.006	0.08	0.18	0.59	0.52	25.572	2.951
Phenanthrene	0.007	0.12	0.19	0.68	0.68	33.483	0.053
Anthracene	0.007	0.06	0.19	0.21	0.64	64.592	14.333
∑ three-ring PAHs	0.024	0.32	0.66	2.23	2.20	69.835	0.967
Sewage sludge, S_2							
Acenaphthylene	0.03	0.04	0.06	0.26	0.11	1.905	4.143
Acenaphthene	0.22	0.26	0.53	1.45	0.87	21.667	11.889
Fluorene	0.28	0.26	0.57	1.36	1.20	12.333	3.458
Phenanthrene	0.38	0.31	0.81	1.35	1.66	12.869	5.333
Anthracene	0.08	0.07	0.16	0.43	0.31	5.476	4.586
∑ three-ring PAHs	0.99	0.94	2.13	4.85	4.15	25.426	4.086
Sewage sludge, S_3							
Acenaphthylene	0.04	0.10	0.13	0.23	0.13	4.091	8.751
Acenaphthene	0.17	0.63	0.84	1.38	1.01	33.549	6.151
Fluorene	0.15	0.63	0.82	1.48	1.33	33.571	3.364
Phenanthrene	0.19	0.61	0.99	1.87	1.87	30.636	0.325
Anthracene	0.07	0.13	0.18	0.40	0.25	3.529	9.063
∑ three-ring PAHs	0.62	2.10	2.96	5.36	4.59	16.167	11.624

nd, not detected; PAHs, polycyclic aromatic hydrocarbons.

decreased. The total concentration of three-ring PAH was higher in comparison with the initial in 12 d of almost 100 times (2.23 μg·L^{-1}). The total concentration of three-ring PAH in S_2 supernatant liquids increased from 0.99 to 4.85 μg·L^{-1} (almost five times) in 12 d, while in S_3 it was 0.62 μg·L^{-1}, and after 12 d it increased to 5.36 μg·L^{-1}. In supernatant liquids, the concentration of all analyzed PAHs was higher than in the initial concentration. In all supernatant liquids, the total concentration of three-ring PAH decreased from 12 d. Analyzing individual hydrocarbons for all sludge, it can be observed that in case of phenanthrene, the concentration in S_1 and S_3 sludge liquids remained at the same level, while for S_2 sludge liquids an increase was observed. Anthracene increase was also observed, but only in S_1 sludge liquids. Higher concentrations of PAHs up to 12 d can be explained by the release of these compounds from microbial cells as a result of their decomposition, desorption from solid particles, and decomposition of compounds with more rings. After adaptation of microorganisms settling in sewage sludge to PAH under reduction conditions, these compounds were subject to biological transformations. As a result, PAH concentrations in both sludge and supernatant liquids decreased rapidly. Phenanthrene dominated in supernatant liquids before and after the incubation process. For most hydrocarbons, $t_{0.05}$ values of *t*-student distribution were higher than critical ($t_{0.05}$ = 2.776). The acenaphthylene concentration difference in the LP for S_2 sludge was statistically insignificant. The critical values of *t*-student test $t_{0.05}$ were also calculated, determining the significance of PAHs concentrations differences from 12 to 16 d. For all hydrocarbons, changes in liquid content recorded during this period of time were also statistically significant. Statistically insignificant differences were observed for phenanthrene, for supernatant liquid from S_1 and S_3 sludge, which concentrations remained at the same level, and for the total concentration of three-ring PAH in supernatant liquids from S_1 sludge. Based on determined concentrations of PAHs and the content of dry matter in sludge, the amounts of these compounds were calculated in SP and LP in unit volume. Results of calculated PAH balance in both phases for S_1 sludge are presented in Table 7.

The total amount of PAH in sludge was 22.64 μg, while after the incubation process it decreased to 9.29 μg. The mass balance of five PAH in individual phase shows that in the SP before to incubation, PAH content was 22.61 μg, while after the process it was 7.15 μg. Therefore, hydrocarbon loss in the SP was 15.46 μg. However, after incubation an increase in PAH by 2.09 μg compared with the initial level was observed in supernatant liquids. Therefore, total hydrocarbon losses for the solid and LPs were 13.35 μg in the unit volume of sludge.

In supernatant liquids separated from S_2 sludge, the calculated increase of three-ring PAH was at the level of 3.07 μg and in the SP the loss was 23.92 μg. The total amount of PAH in sludge and supernatant liquids before the process was 31.26 μg, and after 16 d it decreased to 10.41 μg. Therefore, PAH loss in unit volume was in the order of 20.85 μg. Changes in individual hydrocarbon content of S_2 sludge are presented in Table 8.

Before incubation, the total amount of PAH in 1 L of S_3 sludge was 57.36 μg, and after the process it was 36.26 μg. In supernatant liquids, PAH content increased almost eight times compared with the initial value, while in sewage sludge the amount of these compounds decreased by 25.00 μg. The difference in PAH content in sludge and supernatant liquids before the process, and content of these compounds after the process was 21.10 μg (Table 9).

3.3. Half-life and correlation coefficient of PAHs in sewage sludge

The half-life of these compounds (Table 10) was calculated on the basis of determined PAH contents in sewage sludge and supernatant liquids. For individual hydrocarbons, the half-life of degradation in sludge was between 3 and 65 d. Phenanthrene was the most solid compound, which value in the half-year of disintegration was up to 65 d. In case of supernatant liquids, the half-life was between 3 and 26 d. Fluorene was the most solid compound in all supernatant liquids.

The results obtained from conducted research allowed for the following ranking of analyzed PAHs in sewage sludge and supernatant liquids in descending order due to the half-life of decomposition.

Sewage sludge:

S_1: Phenanthrene > Anthracene > Acenaphthene = Fluorene > Acenaphthylene
S_2: Phenanthrene > Fluorene > Acenaphthene = Anthracene > Acenaphthylene
S_3: Phenanthrene > Anthracene > Fluorene > Acenaphthene > Acenaphthylene

Table 7
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture S_1

PAHs	Before incubation		After incubation		Degradation (μg)
	Sewage sludge (μg)	Supernatants (μg)	Sewage sludge (μg)	Supernatants (μg)	
Acenaphthylene	0.99	0.00	0.00	0.00	0.99
Acenaphthene	5.13	0.00	0.65	0.35	4.13
Fluorene	8.60	0.01	1.29	0.51	6.81
Phenanthrene	7.10	0.01	5.04	0.66	1.41
Anthracene	0.79	0.01	0.17	0.62	0.01
∑ three-ring PAHs	22.61	0.03	7.15	2.14	13.35

PAHs, polycyclic aromatic hydrocarbons.

Table 8
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture S_2

PAHs	Before incubation		After incubation		Degradation (μg)
	Sewage sludge (μg)	Supernatants (μg)	Sewage sludge (μg)	Supernatants (μg)	
Acenaphthylene	1.20	0.03	0.15	0.10	0.98
Acenaphthene	9.27	0.22	1.56	0.85	7.07
Fluorene	9.24	0.27	2.15	1.17	6.20
Phenanthrene	8.50	0.37	2.15	1.61	5.11
Anthracene	2.08	0.08	0.36	0.31	1.49
∑ three-ring PAHs	30.29	0.97	6.37	4.04	20.85

PAHs, polycyclic aromatic hydrocarbons.

Table 9
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture S_3

PAHs	Before incubation		After incubation		Degradation (μg)
	Sewage sludge (μg)	Supernatants (μg)	Sewage sludge (μg)	Supernatants (μg)	
Acenaphthylene	2.63	0.03	0.84	0.13	1.69
Acenaphthene	19.36	0.16	8.16	0.98	10.38
Fluorene	17.20	0.14	10.55	1.30	5.49
Phenanthrene	14.74	0.18	10.39	1.82	2.71
Anthracene	2.86	0.06	1.85	0.24	0.83
∑ three-ring PAHs	56.79	0.57	31.79	4.47	21.10

PAHs, polycyclic aromatic hydrocarbons.

Table 10
The half-life period PAHs in sewage sludge and supernatants

Compound	Half time (d)					
	Sewage sludge			Supernatants		
	S_1	S_2	S_3	S_1	S_2	S_3
Acenaphthylene	3	6	12	NA	3	5
Acenaphthene	6	7	16	4	5	9
Fluorene	6	8	36	22	22	26
Phenanthrene	61	9	65	NA	NA	NA
Anthracene	8	7	43	NA	8	6

NA, not applicable.

Supernatants:

S_1: Fluorene > Acenaphthylene
S_2: Fluorene > Anthracene > Acenaphthene > Acenaphthylene
S_3: Fluorene > Acenaphthene > Anthracene > Acenaphthylene

During the process, the longest decomposition compound was phenanthrene, while the shortest half-life in all sludge was acenaphthylene. It can be assumed that under anaerobic conditions relatively fast decomposition of this compound is possible.

The correlation coefficients are shown in Table 11. For most of analyzed compounds, the absolute value is close to unity (>0.9), which indicates an almost complete correlation between the duration of process and concentration of a given compound. The lowest correlation coefficient was obtained for acenaphthene and phenanthrene (0.3–0.4), which can be interpreted as an average correlation.

Table 11
Correlation coefficient PAHs in sewage sludge and supernatants

Compound	Correlation coefficient					
	Sewage sludge			Supernatants		
	S_1	S_2	S_3	S_1	S_2	S_3
Acenaphthylene	–0.9135	–0.9295	–0.9747	NA	0.6351	0.7127
Acenaphthene	–0.3074	–0.9393	–0.9872	0.7182	0.7727	0.8550
Fluorene	–0.8966	–0.9409	–0.9320	0.9232	0.9002	0.8556
Phenanthrene	–0.4063	–0.9692	–0.9026	0.9380	0.9599	0.9706
Anthracene	–0.7531	–0.9648	–0.7750	0.8985	0.8309	0.7843

NA, not applicable.

In sewage sludge, the content of individual PAHs decreased after 16-d incubation. On this basis, it can be concluded that PAH decomposition may occur in sludge during incubation under reducing conditions. Similar results were obtained in previous studies by the authors. During 20-d fermentation, when the initial three-ring PAH concentration was 304 μg·kg^{-1} dm, the percentage removal of three-ring PAH of 28% (218 μg·kg^{-1} dm) was achieved. When the initial total three-ring PAH content was 1,055 μg·kg^{-1} dm, the loss was 63% and after 20 d the total concentration was 389 μg·kg^{-1} dm [10]. According to studies described in literature, under anaerobic conditions may reach to decrease and increase in the content of analyzed compounds [31–33]. Research results indicate that loss of PAHs during anaerobic processes is affected by many factors related to the conditions of study. These discrepancies are also due to the variable chemical composition of sludge and varying activity of microorganisms capable of PAH

biodegradation. In research conducted by Wiśniowska and Janosz-Rajczyk [31], the total content of five PAHs (three-ring) in sewage sludge before fermentation was 152 μg·kg⁻1 dm, and after the process it decreased to 84 μg·kg⁻1 dm. In studies conducted by Bernal-Martinez et al. [32], an increase of 13 PAHs in sewage sludge by 12.5% was achieved. However, other publications of these authors reported a decrease in PAHs [33]. There are few literature reports on the half-life of PAH in sewage sludge under anaerobic conditions. For example, in studies on the storage of sewage sludge under aerobic conditions for 12 weeks and under the same conditions as inhibition of microbial activity by the addition of sodium azide, the half-life of three-ring PAH in biologically active sewage sludge after 4 weeks ranged from 39 to 271 d and in inactive sewage sludge from 11 to 1,785 d [34]. In studies on soil reclamation with sewage sludge, the half-life of fluorine, phenanthrene and anthracene degradation was 387, 208, and 300 d, respectively [35]. Therefore, it can be concluded that the half-life for individual compounds under reduction conditions, which was calculated in own studies not exceeding 65 d, was significantly shorter than the half-life for PAH under aerobic conditions described in the literature.

4. Conclusions

Based on the study results, the following conclusions can be drawn:

- Control of the course of changes in PAH concentrations indicates fluctuations in concentration of these compounds in sludge and supernatant liquids under reduction conditions (anaerobic conditions).
- The efficiency of three-rings PAH decomposition in sewage sludge was in the range from 33% to 75% and was not correlated with the initial concentration of these compounds.
- In supernatant liquids, the concentration of three-ring PAH increased in the initial period of sludge incubation, while in the final period—the concentration of most analyzed compounds decreased. This indicates biodegradation of these compounds after initial adaptation period.
- The mass balance of PAHs in SP and supernatant liquids confirms biodegradability of these compounds: their mass loss was in the range of 13–21 μg for 1 L of hydrated sludge incubated for 16 d under reducing conditions.
- The half-life of decomposition of PAH in sewage sludge ranged from 3 to 65 d and in supernatant liquids from 3 to 26 d.

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