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Evaluation of the possibility of PAH degradation by a consortium of fermentation bacteria

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ABSTRACT

The paper presents the results of investigations determining the changes in the concentration of six PAHs (polycyclic aromatic hydrocarbons) in sludge and supernatant liquids during incubation under conditions corresponding to fermentation. Three types of sewage sludge were prepared for technological studies, differing in the initial content of carcinogenic six PAHs. The sludge was inoculated with active fermentation microflora collected from the fermentation chamber from the sewage treatment plant. The SS_1 sample consisted of sludge with six PAHs content of 142.6 μg kg^{-1} dm. In SS_2 sample, the initial content of six PAHs was 257.0 μg kg^{-1} dm and in the SS_3 was 1,003.1 μg kg^{-1} dm. Sludge was incubated for 16 d under anaerobic conditions suitable for the development of particular groups of fermentation bacteria. Quantitative analysis of six PAHs was conducted using a gas chromatograph coupled with a mass spectrometer. PAH determinations were carried out simultaneously in the solid phase and in the supernatant liquid separated from the sludge during their centrifugation. The total content of analyzed six PAHs after the fermentation process was lower than the initial one by 77.8%, 74.4%, and 68.4%, respectively, in SS_1, SS_2, and SS_3 sludge. In supernatant liquids, SS_1 the total concentration of six PAHs before the process was 0.07 μg L^{-1}, and after 16 d of sludge incubation under fermentation conditions, it was higher than the initial one in each case. The balance of PAHs in solid phase and liquids indicates that during biochemical changes of organic compounds carried out by a consortium of fermentation bacteria, degradation of the tested PAHs is possible, with the exception of dibenzo(a,h)anthracente.

Keywords: PAHs; Sewage sludge; Supernatants; Anaerobic digestion

1. Introduction

Methane fermentation is a biochemical process during which complex organic compounds, mainly carbohydrates, proteins, and fats are decomposed into methane and carbon dioxide. Anaerobic stabilization is a multiphase process involving a diverse population of microorganisms [1,2]. During hydrolysis and acid phase, the most important role is played by obligate anaerobes bacteria (*Bacillus*, *Pseudomonas*, *Clostridium*) as well as facultative anaerobes (*Bacillus*, *Pseudomonas*, *Clostridium*, *Streptococcus*, *Enterobacterium*) and aerotolerant organisms (*Aerobacter*, *Alcaligenes*, *Escherichia*). During acetogenesis, acetate bacteria (*Syntrophomonas* sp. and *Syntrophobacter* sp.) take part in the transformation of fatty acids into acetates, formates, methanol, hydrogen, and carbon dioxide, that is, substrates that can be used by methanogenic bacteria (archeons). The final stage of

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fermentation is methanogenesis. Methanogens are obligate anaerobes belonging to Archaeobacteriales, which use hydrogen as a source of energy, and carbon dioxide as a source of carbon [3–6]. One of the most effective methods to reduce polycyclic aromatic hydrocarbons (PAHs) content in the environment is microbiological degradation, which can occur under aerobic or anaerobic conditions. The results of comparative studies indicate that the rate of this process is higher with aerobic bacteria [7,8]. The effectiveness of methane fermentation process with respect to compounds that are persistent, such as PAHs, depends on the presence of microorganisms capable of their decomposition and the content of toxic, for these microorganisms, other chemical compounds. The degradation rate depends on the reaction, temperature, and availability of nutrients [9,10]. PAHs are identified in municipal sewage and accumulated in sewage sludge during their treatment. The balance of PAHs in sewage treatment plants indicates that the load of these compounds discharged with the sludge accounts for approximately 20% of the load discharged with the sewage into the sewage treatment plant [11]. The accumulation of these compounds in the sludge results from physical and chemical properties. PAHs are hydrophobic compounds. They dissolve poorly in water and much better in fats, oils, and organic solvents. Solubility of carcinogenic PAHs ranges from 0.5 to 62 μg L^{-1}. Solubility may increase in the presence of organic compounds such as surfactants or purines. This is due to hydrotrophy. PAHs are compounds with a strong affinity to particulate matter and are therefore primarily adsorbed. The *n*-octanol/water partition coefficients range from 5.74 to 7.66. With the increase in this coefficient, sorption is stronger [12–16]. The content of carcinogenic PAHs in sewage sludge has been presented in Table 1. The presence of PAHs in sludge separated from sewage has been confirmed many times in the literature, but the presented test results are differentiated in terms of the type of sludge (preliminary, excessive, fermented, stabilized, and dehydrated) and the characteristics of treated sewage, including the share and type of industrial sewage.

The literature data indicate that microorganisms may use hydrocarbons from two to four aromatic rings as the main source of carbon. However, those hydrocarbons that are composed of more rings in a molecule are more resistant to biological decomposition. Anoxic conditions and strong acidification of the substrate significantly limit the decomposition of PAHs, whereas in the presence of oxygen a gradual degradation of PAHs composed of more rings can be obtained [19]. Microorganisms are usually not able to directly decompose micro-contaminants such as PAHs. It is necessary to adapt the microflora to the biodegradation of hydrocarbons present in anaerobic conditions. In order to develop the ability to produce suitable enzymes, microorganisms need a certain amount of time, which depends on the type of organisms and the properties of hydrocarbons. Many bacteria have the ability of PAHs decomposition, but no single species has the ability to produce enzymes that could degrade all impurities in this group. Therefore, the biodegradation of PAHs is a multistage process involving many microorganisms that often show synergistic effects with each other. Microorganisms isolated from sewage sludge and involved in the degradation of PAHs include *Pseudomonas aeruginosa*, *Pseudomons fluoresens*, *Mycobacterium* spp., *Haemophilus* spp., *Rhodococcus* spp., and *Paenibacillus* spp. [20,21].

The aim of this study was to determine the degradation potential of carcinogenic six PAHs [Benzo(b)fluoranthene, B(b)F; Benzo(k)fluoranthene, B(k)F; Benzo(a)pyrene, B(a)P; Dibenzo(a,h)anthracene, D(a,h)A; Indeno(1,2,3,c,d) pyrene I(1,2,3,c,d)P; Benzo(g,h,i)perylene, B(g,h,i)P] in sewage sludge with different amounts of PAHs during incubation under conditions corresponding to the development of fermentation bacteria.

2. Materials and methods

2.1. Fermentation process

Three types of sewage sludge were prepared for technological studies, differing in the initial content of carcinogenic six PAHs. A mixture of raw and excessive sludge was collected from the municipal sewage treatment plant. Raw sludge was collected from the primary tank thickener, while excess sludge was collected from the secondary tank thickener. Under real conditions, sludge processing is carried out in two stages. The first stage consists of mesophilic fermentation at 36°C in closed fermentation chambers, while the second stage consists of aerobic fermentation in open chambers. The SS_1 sample consisted of sludge with PAH content of 142.6 μg kg^{-1} dm (control sample). The next two samples were supplemented with a standard mixture of PAHs. The concentration of PAHs in the prepared samples was then determined as initial. In the SS_2 sample the initial content of six PAHs was 257.0 μg kg^{-1} dm, and in the SS_3 was 1,003.1 μg kg^{-1} dm. Sludge incubation was carried out in glass reactors with a single supply without light access and with the possibility of biogas pressure measurement. The process was conducted in a thermostat at a

Table 1
Concentration PAHs in sewage sludge [17,18] and supernatants [15]

Compounds	Number of rings	Sewage sludge (μg kg^{-1} dm)	Supernatants (μg L^{-1})
Benzo(b)fluoranthene	5	100–1,080	0.06
Benzo(k)fluoranthene	5	110–2,170	0.05
Benzo(a)pyrene	5	133–7,850	0.05
Dibenzo(a,h)anthracene	5	160–14,050	0.03
Indeno(1,2,3,c,d)pyrene	6	120–9,370	0.10
Benzo(g,h,i)perylene	6	350–6,520	0.08

constant temperature of 37°C ± 2°C for 16 d. The samples were inoculated with fermented sludge in order to introduce the appropriate amount of acetate and methanogenic microflora adapted to biochemical transformations of complex organic compounds. The prepared mixtures of SS_1, SS_2, and SS_3 were initially characterized and determined: dry residue and roasted residue. The alkalinity (A), chemical oxygen demand (COD), and volatile fatty acids (VFA) were determined in supernatant liquids from sewage sludge centrifugation. During the fermentation process, atmospheric pressure and biogas pressure were controlled. These measurements were conducted using a manometer at 24 h intervals. Using the Boyle-Mariott equation, the daily volume of biogas produced was calculated from Eq. (1):

$$p_A \times V_A = p_B \times V_B \Rightarrow V_B = \frac{p_A \times V_A}{p_B} \quad (1)$$

where p_A, pressure in the bioreactor (hPa); V_A, volume of free space in the bioreactor (L); p_B, atmospheric pressure (hPa); V_B, calculated volume of biogas (L).

2.2. Identification of PAHs

For the study, 10 g of centrifuged sewage sludge and 500 mL of separated supernatant liquids were collected. The separation of an organic matrix from sludge was carried out by sonification lasting 25 min with the use of a mixture of cyclohexane and dichloromethane solvents (5:1 v/v). The extracts obtained were centrifuged for 10 min at 9,000 rpm. The separation of organic compounds from liquids was conducted mechanically in a liquid-liquid system with the addition of methanol, cyclohexane, and dichloromethane (20:5:1 v/v/v). In this case, the separation of extracts from the liquid was carried out in a glass separator. Silica gel was used to isolate the analytes from the extracts from other organic substances extracted simultaneously. Before the extracts were introduced, the silica gel column was filled with methanol (2 × 3 mL) and then distilled water (2 × 3 mL) for conditioning. Purified extracts were concentrated in a stream of nitrogen to 2 mL. Determinations were conducted using a gas chromatograph coupled with a mass spectrometer (model GC800/MS800). The standard mixture of PAHs [benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3,3,c,d) pyrene, benzo(g,h,i)perylene] in the mixture of benzene and dichloromethane (produced by AcuStandard Inc., USA) was used for the analysis [15,22]. The determination was based on the injection of 2 μl of extract per DB-5 column (length 30 m, diameter 0.25 mm, thickness 0.25 μm) using helium as a carrier gas. The quantitative determination of PAHs was carried out at 280ºC. The following furnace program was selected: from 40°C to 120°C (heating 40°C min^{-1}), from 120°C to 280°C (heating 5°C min^{-1}), 280°C for 20 min.

2.3. Removal efficiency of polycyclic aromatic hydrocarbons 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 Eq. (2):

$$R = \frac{\left(\mathrm{PAHs}_I - \mathrm{PAHs}_F\right)}{\mathrm{PAHs}_1} \times 100\% \quad (2)$$

where R, reduction percentage (%); $PAHs_I$, initial concentration of an i-th PAH (μg kg^{-1} dm); $PAHs_F$, concentration of an i-th PAH at a given day of the process: the 4th, 8th, 12th, 16th (μg kg^{-1}dm).

2.4. Mass balance of polycyclic aromatic hydrocarbons

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 PAHs in supernatant liquids (S) in relation to the unit volume of dissolved substances and in sewage sludge (SS) was calculated from the Eqs. (3) and (4):

$$S = C_s \times V \quad (3)$$

$$\mathrm{SS} = C_{ss} \times 10^{-3} \times dm \quad (4)$$

where C_s, PAHs concentration in the supernatants (μg L^{-1}); V, volume of supernatants in the hydrated sludge (L L^{-1}); dm, content of dry matter in the hydrated sludge (g L^{-1}); C_{ss}, PAHs concentration in the solid phase (μg kg^{-1} dm).

2.5. Statistical test

For the sludge, evaluation of the results for the addition of coke sewage sludge on the degradation of PAHs t-Student $t_{0.05}$ test was used. The level of confidence was accepted at the 0.95 level. The number specifying the degree of freedom was 4, for this parameter, and the theoretical value of decomposition of the t-Student $t_{0.05}$ was 2.776.

3. Results and discussion

3.1. Physicochemical properties

The dry matter content in SS_1 sludge before fermentation was 14.18 g L^{-1}, while after the process was 12.06 g L^{-1}, and the share of organic matter in the fermented sludge was 60%. COD value in supernatant liquids decreased by 47%. The degree of organic matter decomposition was at the level of 24.7%. In SS_2 sludge, there was a 14% loss of dry organic matter and a 22.5% degree of organic matter decomposition. The share of organic matter in the fermented sludge, similarly as in the SS_1 sludge was 60%. The content of organic compounds determined as COD in supernatant liquids decreased by 44%.

In SS_3 sludge, the content of organic compounds determined as COD after the stabilization process was 710 mg O_2 L^{-1}. Dry mass of sewage sludge after 16 d of fermentation decreased by 16%, the share of organic substances in fermented sewage sludge was 64%.

After the anaerobic stabilization process, alkalinity determined in supernatant liquids in all samples ranged from

2,805 to 2,900 mg $CaCO_3$ L^{-1}. VFA/A quotient in all sludges after the fermentation process did not exceed 0.3.

The largest amount of biogas (4,557 mL L^{-1}) was obtained in the sludge SS_1. At a similar level (4,508 mL L^{-1}), biogas production during the fermentation of SS_2 sludge was maintained. However, the total amount of biogas in SS_3 sludge was much smaller and amounted to 3,667 mL L^{-1}. Therefore, it can be stated that with the increase in PAH concentration, the total biogas production decreased.

3.2. Changes of polycyclic aromatic hydrocarbons in sewage sludge

The efficiency of PAH removal during fermentation and critical values of the *t*-Student's test have been presented in Table 2. In SS_1 sewage sludge, the percentage removal of carcinogenic PAHs was 77.8%. The efficiency of PAH removal during fermentation was 74.4% and 68.4% for SS_2 and SS_3 sludge, respectively.

The results of qualitative and quantitative determinations of carcinogenic PAHs during anaerobic incubation in sewage sludge have been presented in Fig. 1. The total initial content of PAHs in SS_1 sludge was 142.6 μg kg^{-1} dm, and at the highest concentration was benzo(b)fluoranthene (35%). With the duration of incubation, the total content of analyzed PAHs decreased and after 16 d, the total content decreased by 79%. After the process, no dibenzo(a,h)anthracene was determined in the digested sludge. In SS_1 sewage sludge, lower contents of analyzed PAHs were observed in comparison with the initial ones.

In SS_2 sewage sludge, the concentration of PAHs before incubation was 257 μg kg^{-1} dm of which benzo(b)fluoranthene constituted 26%. Similarly, as in SS_1 sewage sludge, the process duration gradually decreased with the total content of PAH. The content of PAH after 4, 8, and 16 d was 204.3, 172.3, and 108.4 μg kg^{-1} dm, respectively. After 16 d of sludge incubation, the total content decreased to 65.7 μg kg^{-1} dm, that is, by 74% compared to the initial one.

The total initial content of six PAHs in SS_3 sewage sludge was 1,003.1 μg kg^{-1} dm, while after the anaerobic stabilization process it was 32% lower (317.3 μg kg^{-1} dm). During hydrolysis (4 d) by hydrolyzing bacteria, which results in liquefaction of organic polymers in the tested sewage sludge, a loss of all analyzed PAHs with the participation of enzymes was observed. The exception was benzo(k)fluoranthene and dibenzo(g,h,i)fluoranthene in SS_3 sewage sludge. In subsequent stages, the main role in the decomposition of organic compounds such as amino acids, monosaccharides, organic acids (C1-C6) (formic, acetic, propionic, butyric, valerian, and capronic), alcohols (methanol, ethanol), aldehydes

Table 2
Removal efficiency (%) of PAHs in sewage sludge (*t*-Student $t_{0.05}$ = 2.776)

Compound	Time, day				*t*-Student
	4th	8th	12th	16th	0–16th
		Sewage sludge (SS_1)			
Benzo(b)fluoranthene	6.5	–	–	74.0	52.429
Benzo(k)fluoranthene	34.3	20.1	5.7	76.7	16.692
Benzo(a)pyrene	30.7	–	–	80.5	24.789
Indeno(1,2,3,c,d)pyrene	23.9	3.9	–	82.9	30.818
Benzo(g,h,i)perylene	29.5	1.3	–	80.5	6.316
∑ PAHs	18.5	–	–	77.8	92.417
		Sewage sludge (SS_2)			
Benzo(b)fluoranthene	20.7	38.5	61.8	75.6	26.895
Benzo(k)fluoranthene	14.3	14.1	45.9	63.4	7.111
Benzo(a)pyrene	28.6	35.3	55.6	78.1	19.727
Dibenzo(a,h)anthracene	0.0	23.2	52.7	75.9	16.822
Indeno(1,2,3,c,d)pyrene	27.1	41.1	64.8	80.7	19.412
Benzo(g,h,i)perylene	14.4	40.4	65.1	74.7	7.281
∑ PAHs	20.5	33.0	57.8	74.4	427.444
		Sewage sludge (SS_3)			
Benzo(b)fluoranthene	18.3	15.7	31.2	67.3	18.247
Benzo(k)fluoranthene	–	–	–	53.2	24.158
Benzo(a)pyrene	46.7	35.8	57.0	78.1	19.056
Dibenzo(a,h)anthracene	–	–	6.8	52.3	2.359
Indeno(1,2,3,c,d)pyrene	11.0	–	25.4	66.5	20.033
Benzo(g,h,i)perylene	18.7	5.7	24.7	72.0	8.143
∑ PAHs	10.9	–	29.4	68.4	279.776

–, no deletion PAHs

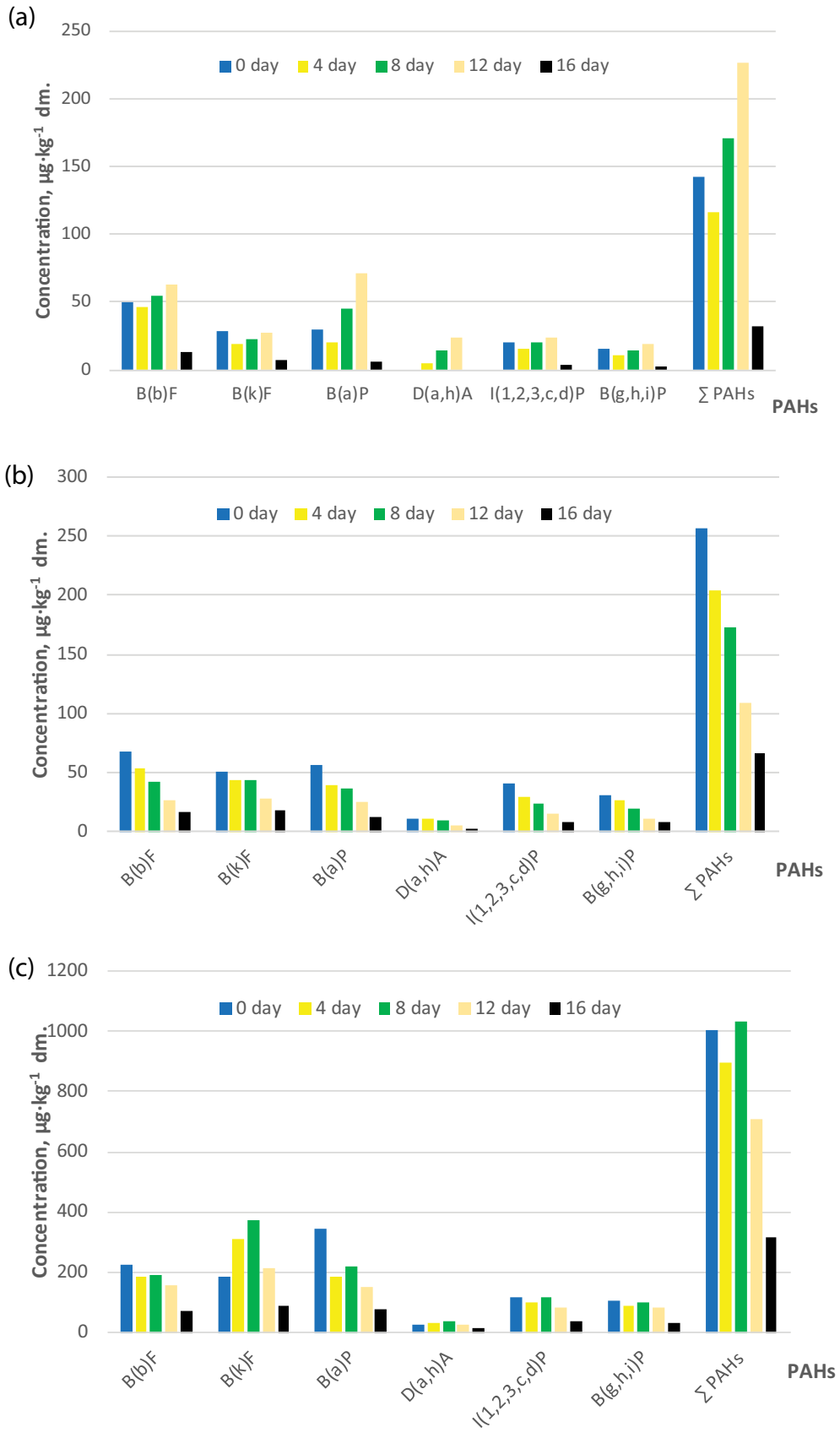

Fig. 1. Changes of PAHs concentration in sewage sludge (a) SS_1, (b) SS_2, and (c) SS_3.

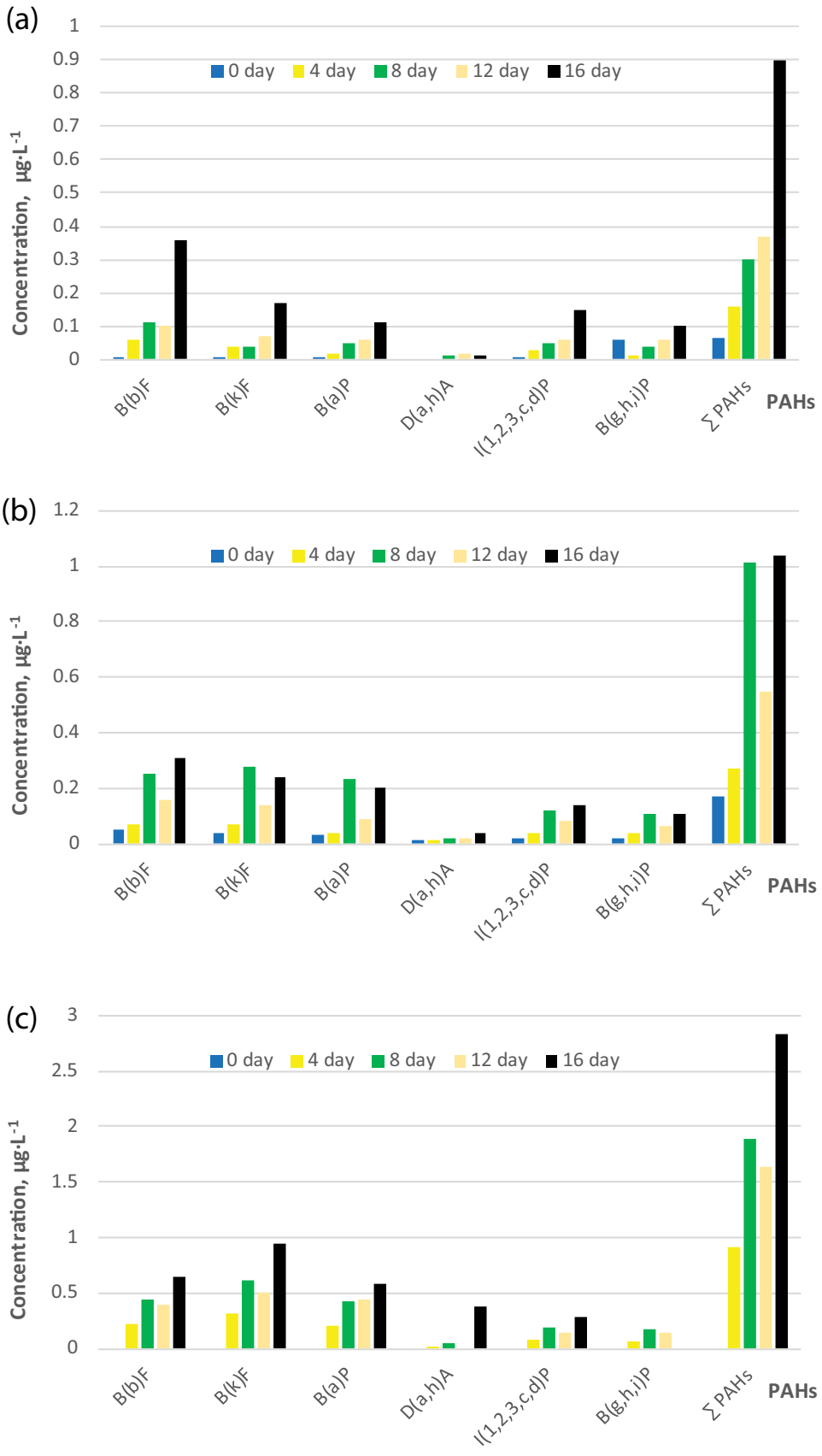

Fig. 2. Changes of PAHs concentration in supernatants (a) SS_1, (b) SS_2, and (c) SS_3.

were played by acidogenic and acetogenic bacteria (8–12 d). The concentration of PAHs in SS_1 sewage sludge increased. At the same time, an increase in PAH levels was observed in supernatant fluids. The analyzed compounds could also be periodically determined at higher concentrations due to the decomposition of complex organic polymers. During this time, simple organic compounds are formed, which are more readily available to bacteria and thus inhibit hydrocarbon metabolism. In the case of SS_2 sewage sludge during acidogenesis and acetogenesis, the concentration of analyzed PAHs decreased further. In SS_3 sewage sludge, the increase of all PAHs occurred on the 8th day of the process. The increase of PAHs in the previous phase caused a disturbance in the activity of methanogens, which resulted in a decrease in biogas. At the same time, after exhaustion of readily available substrate in the last stage of fermentation, degradation of PAHs was possible, which confirms the loss of all analyzed PAHs as compared to the concentration prior to the process.

3.3. Changes of polycyclic aromatic hydrocarbons in supernatants

In supernatant liquids from SS_1 sewage sludge, the initial total concentration of PAHs was 0.066 μg L^{-1}. In supernatant liquids after incubation the total concentration of six PAHs was higher than the initial more than 13 times (0.9 μg L^{-1}). The total concentration of six PAHs in supernatant liquids from SS_2 sewage sludge increased from 0.17 to 1.04 μg L^{-1}. Higher concentrations of all analyzed PAHs were observed in supernatant liquids compared to the initial concentration.

Prior to incubation in supernatant liquids (SS_3) none of the analyzed PAHs was determined. After the process, only benzo(g,h,i)perylene was not determined in supernatant liquids. The total concentration of PAHs in SS_3 liquids after the process was 2.83 μg L^{-1} (Fig. 2).

3.4. Mass balance of polycyclic aromatic hydrocarbons in solid phase of sewage sludge and in supernatants

On the basis of PAH concentrations and dry matter content in sewage sludge, the amounts of these compounds in solid and liquid phase in a unit volume were calculated. The results of calculated PAH balance in both phases (solid and liquid) for SS_1 sewage sludge have been presented in Table 3. The total amount of PAH in both phases before the process was 2.02 μg, whereas after the fermentation process it decreased to 1.27 μg. The mass balance of six PAHs in individual phases shows that in the solid phase before fermentation the PAH content was at the level of 2.02 μg, whereas after the fermentation process it amounted to 0.39 μg. Thus, the loss of hydrocarbons in the solid phase was 1.63 μg. However, in supernatant liquids the amount of PAH increased by 0.88 μg after fermentation in comparison with the initial one. Thus, total hydrocarbon losses for solid and liquid phases were 0.75 μg per unit volume of sludge.

In supernatant liquids, SS_2 the calculated increase of six PAHs was at the level of 0.86 μg, and in the solid phase the loss was 3.05 μg. The total amount of PAHs in sewage sludge and supernatant liquids before the process was 4.06 μg, and after 16 d decreased to 1.87 μg. Therefore, the loss of PAH in a unit volume was in the range of 2.19 μg (Table 4).

Before the process, the amount of PAHs in SS_3 sewage sludge and separated supernatant liquids was 16.42 μg in total, whereas after the process was 7.1 μg. Also in this mixture, a decrease in the amount of PAHs in the solid phase and an increase in the liquid phase were observed. In the sludge after the process the total content of six PAHs decreased from 16.42 to 4.35 μg, and in the liquid phase was from 0 to 2.75 μg. The total loss of PAHs during the 6 d anaerobic stabilization process was 9.32 μg (Table 5).

After sludge thickening and dewatering, supernatant liquids are in most cases recycled to raw wastewater, thus the amount of PAHs in the liquids may have a significant role in the balance of these compounds in the wastewater treatment plant. The analysis of PAH changes in both phases shows that during the fermentation process it is possible to release PAHs from sewage sludge and accumulate them in supernatant liquids. Taking into account the fact that supernatant liquids from sludge fermentation are usually recycled into the sewage treatment plant, the burden of large amounts of PAHs can significantly increase the concentration of these pollutants in the sewage.

The studies carried out by other authors did not always provide unambiguous results of PAHs during the incubation of sewage sludge under anaerobic conditions. Before

Table 3
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture SS_1, μg

PAHs	Before incubation		After incubation		Degradation
	Solid phase of sewage sludge	Supernatants	Solid phase of sewage sludge	Supernatants	
Benzo(b)fluoranthene	0.70	0	0.16	0.35	0.20
Benzo(k)fluoranthene	0.40	0	0.08	0.17	0.16
Benzo(a)pyrene	0.42	0	0.07	0.11	0.24
Dibenzo(a,h)anthracene	0	0	0	0.01	*
Indeno(1,2,3,c,d)pyrene	0.29	0	0.04	0.14	0.11
Benzo(g,h,i)perylene	0.21	0	0.04	0.10	0.08
∑ PAHs	2.02	0	0.39	0.88	0.75

*, increase of PAHs concentration

Table 4
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture SS_{2}, μg

PAHs	Before incubation		After incubation		Degradation
	Solid phase of sewage sludge	Supernatants	Solid phase of sewage sludge	Supernatants	
Benzo(b)fluoranthene	1.03	0.05	0.22	0.30	0.56
Benzo(k)fluoranthene	0.77	0.03	0.24	0.23	0.33
Benzo(a)pyrene	0.85	0.02	0.16	0.20	0.51
Dibenzo(a,h)anthracene	0.17	0.01	0.04	0.04	0.10
Indeno(1,2,3,c,d)pyrene	0.62	0.02	0.10	0.13	0.40
Benzo(g,h,i)perylene	0.47	0.02	0.10	0.11	0.28
∑ PAHs	3.91	0.15	0.86	1.01	2.19

Table 5
Mass balance of PAHs in the solid and liquid phases before and after the incubation process in the mixture SS_{3}, μg

PAHs	Before incubation		After incubation		Degradation
	Solid phase of sewage sludge	Supernatants	Solid phase of sewage sludge	Supernatants	
Benzo(b)fluoranthene	3.66	0	1.00	0.63	2.03
Benzo(k)fluoranthene	3.06	0	1.20	0.92	0.93
Benzo(a)pyrene	5.65	0	1.04	0.57	4.04
Dibenzo(a,h)anthracene	0.46	0	0.18	0.36	*
Indeno(1,2,3,c,d)pyrene	1.86	0	0.52	0.27	1.07
Benzo(g,h,i)perylene	1.73	0	0.41	0	1.33
∑ PAHs	16.42	0	4.35	2.75	9.32

*, increase of PAHs concentration

the process of municipal sludge fermentation in the studies conducted by Bernal–Martinez, the concentration of 12 PAHs was 30.7 μg kg^{-1} dm, and after the stabilization process, there was a loss of 28%. In supernatant liquids, the concentration of PAH decreased by nearly 52% [23]. However, in the studies conducted by Wiśniowska and Janosz–Rajczyk, the concentration of six PAHs in sewage sludge before the process was 788 μg kg^{-1} dm, and increased after the process by 1,013 μg kg^{-1} dm [24]. In other studies, the total content of PAHs in sewage sludge and supernatant liquids was higher than before the fermentation process by 131%–167% and 180%–220%, respectively [25]. In previous studies by co-authors, an increase in PAH concentration in supernatant liquids was observed [15]. In the studies conducted by Boruszko, the initial content of six PAHs was 125.7 μg kg^{-1}, after 7 d an increase to 143.0 μg kg^{-1} was recorded, which may be a result of decomposition of other complex compounds. In subsequent fermentation stages, the content of six PAHs in the sludge gradually decreased [26]. A similar relation was noted in earlier studies by the authors [15]. Results of these studies indicated the possibility to determine increased concentrations of PAHs at the initial fermentation stage in relation to the initial one. During methanogenesis, the PAH content gradually decreased, which confirmed the possibility of PAH degradation under these conditions.

The balance of PAHs in both phases indicates that PAHs released into the liquid are more readily available for the microflora composed of a mixed bacterial population. During anaerobic incubation, the concentrations of PAHs change both in the sludge and supernatant liquids separated from them. Under anaerobic conditions, the loss of PAHs in sewage sludge may be caused by the biodegradation of these compounds and by strong sorption on solid particles. The increase of PAHs in supernatant fluids is possible due to the release of PAHs from the sewage sludge on which they were previously absorbed and after the decomposition of microbial cells and their desorption to the liquid.

Microbiological degradation of PAHs is related to the presence of bacterial consortiums capable of decomposing these compounds. Higher PAH content in the fermented sewage sludge than in the fermented sludge confirms the biotransformation of organic matter contained in the sludge. As a result of these transformations, the analyzed hydrocarbons may be formed periodically. Moreover, as the studies indicate, PAHs may penetrate the bacterial cells and release after their decomposition [27]. The loss of PAHs is possible during the fermentation of sewage sludge thanks to appropriate microorganisms, which use hydrocarbons as a source of carbon and energy [26]. Hydrocarbons may be used as the basic or additional source of carbon, depending on the type of bacteria and environmental conditions.

The main purpose of PAHs biodegradation during the sewage sludge fermentation process is their conversion to CO_2. The conducted research confirms only partial conversion of PAHs to carbon dioxide. Incomplete biodegradation leads to the formation of intermediates that are still toxic to the environment of methane bacteria. These results indicate that the loss of PAHs under anaerobic conditions is influenced by many factors. These discrepancies also result from the variable chemical composition of the sludge and varied activity of microorganisms capable of PAHs biodegradation and process conditions [2,8,28].

4. Conclusions

The following conclusions can be drawn from the research results:

- The total concentration of PAHs in incubated sewage sludge in the presence of fermentation microflora was different in solid and liquid phase: the total concentration of six PAHs in sewage sludge after incubation was lower than the initial one by 68%–78%, whereas in liquids after 16 d of incubation under anaerobic conditions it was 3–14 times higher than the initial one.
- The solid-liquid mass balance determined for hydrated sewage sludge indicates that the mixed population of fermentation microflora has the ability to biodegrade PAHs. The loss of these compounds ranged from 0.75 to 9.32 μg.
- With the increase in PAH concentration in sewage sludge, the total biogas production decreased, which indicates the adverse effect of PAHs on methanogens.

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