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Determination of toxic PAH concentrations for fermentation and evaluation of supernatants and sewage sludge toxicity using TEF indicators

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ABSTRACT

This paper presents the evaluation results of the toxicity of supernatants and sewage sludges using toxicity equivalency factor indicators for polycyclic aromatic hydrocarbons (PAHs) and determines the toxic concentrations of these compounds for the fermentation process. The study was conducted using municipal sewage sludge and sludge from an on-site coking wastewater treatment plant. Municipal sewage sludge (5 samples) constituted the control sample, whereas mixtures of municipal sewage sludge and coke oven sludge were the test samples: S1 municipal sludge with coke oven sludge in the ratio of 20:1 (5 samples) and S2 in the ratio of 6:1 (4 samples). The sludges were incubated at 37°C without light. Quantitative analysis of 16 PAHs was carried out using a gas chromatograph coupled to a mass spectrometer. The determination of PAHs was carried out in the solid and supernatants phase separated from the sludge. The control of fermentation process consisted in the determination of dry matter, mineral and organic compounds, measurement of the amount and composition of biogas. Threshold toxic concentration of PAHs for microorganisms at which the fermentation process breaks down is 2,275 μg/kg dry mass for the solid phase and 4 μg/L – for the sludge liquids. The average toxic equivalency factor value for 16 PAHs for the post-fermentation sludge was 79% lower than the initial value and 570% higher for the sludge liquids. Therefore, recirculation of liquids may adversely affect the efficiency of biological wastewater treatment.

Keywords: Polycyclic aromatic hydrocarbons; Fermentation process; Sewage sludge; Supernatants; Toxicity equivalency factor; Toxic equivalency factor

1. Introduction

Mesophilic fermentation is a biochemical process during which organic polymers, mainly carbohydrates, proteins and fats, are decomposed into methane and carbon dioxide, hydrogen sulfide, nitrogen. It is a multiphase process involving a diverse population of microorganisms. During methane fermentation, the physical and chemical properties of sewage sludge are changed and dry organic matter is reduced, which consequently reduces the harmful effects on the environment [1–3]. Supernatants from the dewatering of post-fermentation sludge are characterized by a high content of biogenic compounds and toxic micropollutants and are returned to the technological sequence of wastewater treatment [4,5]. The correct course of methane fermentation depends on the qualitative composition of the organic substrate as well as the presence and availability of micro- and macroelements for microorganisms.

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It is also important that in the environment, substances toxic to the fermentation biocenosis are not present at all or only in concentrations not exceeding threshold values. Process inhibition under anaerobic conditions can be caused by the presence of substances toxic to microorganisms, both organic and inorganic. During anaerobic stabilization both the compounds introduced with the sludge (external) and the products formed by the transformation of organic polymers during the process (internal) are toxic to microorganisms. Among the compounds present in the substrate the following have an inhibitory effect: oxygen, heavy metals, biologically active organic components of plant protection products or polycyclic aromatic hydrocarbons (PAHs). The compounds formed during fermentation as a result of hydrolysis and other reactions include: organic acids (e.g., propionic acid), ammonia, hydrogen sulfide, and transformation products of various compounds present in the sludge. The toxicity of organic acids, ammonia and hydrogen sulfide depends on the degree of dissociation. It has been shown that undissociated compounds adversely affect the fermentation biocenosis at relatively low concentrations, while compounds in dissociated form have a toxic effect, but only when present in much higher concentrations. The influence of ammonia on the fermentation process is multidirectional and, depending on the concentration, can be stimulating, inhibiting or toxic. Also the impact of sulfur compounds depends on the form of undissociated hydrogen sulfide [6–11]. The effects of selected organic compounds on the methane fermentation process are shown in Table 1.

Disturbance of the correct course of biochemical decomposition of organic compounds during fermentation depends not only on the above-mentioned factors, but can also be caused by changes in pH, temperature fluctuations or accidental oxygen supply, to which methane archeates are particularly sensitive. However, microorganisms involved in the fermentation process have adaptive abilities and show tolerance to constant values of some toxic substances [12]. Pesticides, pharmaceuticals, polychlorinated biphenyls (PCBs), and PAHs have been mentioned among organic pollutants toxic to the consortium of fermentation bacteria [13–16].

PAHs have a planar configuration in which the carbon atoms forming the ring are in the sp2 hybridization state and the p orbitals overlap each other to form a delocalized π orbital. This makes the aromatic system persistent and therefore chemical reactions and biochemical transformations leading to the disruption of aromatic ring only occur under appropriate conditions. Despite their properties classifying them as persistent compounds in the environment, PAHs can undergo physicochemical and biological transformations under changing conditions and in the presence of specific microorganisms. In wastewater treatment plants, PAHs present in wastewater undergo little volatilization or biochemical transformations, while most are adsorbed on solid particles separated from wastewater in the form of sewage sludge [17–19]. Previous studies by the authors have shown that PAHs are mainly accumulated on raw and excess sludge particles [20]. This is due to the physico-chemical properties of these compounds such as hydrophobicity, poor solubility in water [21]. In a municipal wastewater treatment plant, microorganisms are usually not able to degrade PAHs directly. The PAHs in the wastewater cannot be metabolized by aerobic activated sludge microorganisms. Adaptation of the activated sludge microflora to the biodegradation of hydrocarbons is necessary. In order to develop the ability to produce the appropriate enzymes, the microorganisms need some time, which depends on the type of organisms and the properties of hydrocarbon. Activated sludge is an aggregation of various organisms of bacteria, fungi, protozoa, such as: flagellates, rhizopoda, ciliates, polychaetes, oligochaeta, and roundworms. The proportion of each group of organisms depends on: the concentration of organic pollutants, presence of substances inhibiting the metabolism of microorganisms, concentration of oxygen, temperature,

Table 1
Effect of selected organic compounds on the methane fermentation process [12]

Compound	Concentrations causing 50% decrease in biogas production, mg/L	Compound	Concentrations causing 50% decrease in biogas production, mg/L
Chloroform	15	Formic aldehyde	100
Hydrazine	50	Benzene	1,970
Creolin (mixture of creosol, phenols and resins)	1	Ethylbenzene	340
Dinitrophenol	40	Phenol	1,300
Ethylbenzene	340	Aniline	900
Long-chain fatty acids	500	Benzoic acid	4,250
Fluorinated hydrocarbons	1	Fatty acids	500–1,250
Formaldehyde	70	Tannin	700
Nitrobenzene	10	Volatile terpenes/Pinene	180
Virginiamycin	10	Cationic and anionic detergents	20–50
Akrolina	10	Acetylcholine chloride	300
Acetaldehyde	440	Lauryl choline chloride	50

pH, the time the wastewater is retained in the system, and hydraulic conditions in the bioreactor. Laboratory studies indicate that many bacteria and fungi have the ability to degrade PAHs. The greatest ability to biotransform PAHs are bacteria. Among bacteria, gram-negative bacteria, for example, Pseudomonas, Alcaligenes and Agrobacterium strains, play a major role in the oxidation of aromatic hydrocarbons. However, it should be emphasized that no single species shows the ability to produce such enzymes, with the participation of which it would be possible to degrade all compounds belonging to the PAH group.

According to the classification of IARC (International Agency for Research on Cancer) and US EPA (United States Environmental Protection Agency), most of the compounds from the group of PAHs show toxic effects against microorganisms. It is characteristic that PAHs usually occur in mixtures and under practical (real) conditions during technological processes of wastewater treatment or sludge processing it is not possible to determine which compounds show the strongest toxic properties [22–24]. Toxicity with respect to PAHs can be determined by toxicity equivalency factors (TEF), and the normative PAH content is given as toxic equivalency factor (TEQ). The TEQ value is determined as the sum of products of the concentration of each compound and the TEF values (Section 2.4 – Statistical test) [25]. Table 2 presents IARC and US EPA classification of PAH toxicity and TEF values of individual compounds.

Despite the fact that PAHs are toxic to microorganisms, so far the toxic concentrations of these compounds present in fermented sludge have not been determined for the consortium of microorganisms involved in the process. Therefore, a study was undertaken to determine the threshold concentrations of PAHs in the solids and supernatants for the fermentation process and to evaluate the toxicity of sludge and sludge liquids using TEF toxicity ratios.

2. Materials and methods

2.1. Materials

The study was conducted using sludge collected from a municipal treatment plant and an on-site treatment plant. In the municipal treatment plant, biological treatment of sewage is carried out in bioreactors, where oxidation of organic compounds, phosphorus removal, denitrification and nitrification take place. Sewage sludge is stabilized in two stages: in separate closed chambers and open digesters. A mixture of preliminary and surplus thickened sludge is directed to the digesters. Stabilized sludge is directed to mechanical presses, where it is dewatered with cationic polyelectrolyte. Supernatants from the presses are returned to the main line of the treatment plant. For technological studies five samples were taken from the municipal wastewater treatment plant: preliminary sludge discharged from the gravity thickener (1 sample), excessive sludge discharged from the mechanical thickener (1 sample), mixture of preliminary and excessive sludge from the sludge tank before the digester (3 samples), and fermented sludge from the outflow of separate digesters (5 samples). All samples of municipal sludge and municipal sludge with coke sludge were inoculated with fermented sludge in the proportion 1:1.5.

Coke sludge was taken from the on-site treatment plant. They were collected from the on-site treatment plant. It is a mechanical-biological treatment plant in which prefiltration, tar separation and ammonium nitrogen desorption are carried out. Biological wastewater treatment includes nitrification, denitrification and oxidation of organic

Table 2
US EPA list of PAHs, toxic equivalency factor (TEF) values and IARC classification [22–24]

Name	Abbreviation	Classification		TEF
		IARC	US EPA	
Naphthalene	Naf	2B	*	0.001
Acenaphthylene	Acyl	3	*	0.001
Acenaphthene	Ac	3	*	0.001
Fluorene	Flu	3	D	0.001
Phenanthrene	Fen	3	D	0.001
Anthracene	Antr	3	*	0.01
Fluoranthene	Fl	3	D	0.001
Pyrene	P	3	D	0.001
Benzo[a]anthracene	BaA	2B	B2	0.1
Chrysene	Ch	3	B2	0.01
Benzo[b]fluoranthene	BbF	2B	B2	0.1
Benzo[k]fluoranthene	BkF	2B	B2	0.1
Benzo[a]pyrene	BaP	1	B2	1
Dibenzo[a,h]anthracene	DahA	2A	B2	1
Indeno[1,2,3-cd]pyrene	IP	2B	*	0.1
Benzo[ghi]perylene	BghiP	3	D	0.01

1 – carcinogenic; 2A – probably carcinogenic; 2B – possibly carcinogenic, 3 – not classifiable;
B – probable carcinogens (B2: based on sufficient evidence of carcinogenicity in animals); Group D – not classifiable as to carcinogenicity;
* – not available.

compounds using activated sludge. The final stage of treatment is sedimentation in secondary settling tanks, from where sludge was 4 times collected for technological tests.

2.2. Fermentation process

Fermentation studies were conducted in 1L glass reactors with a single feed without light with biogas pressure measurement capability. The process was carried out in a thermostat at a constant temperature of 37°C ± 2°C. The fermentation process was carried out for 16–20 d. Nine mixed sewage sludge samples differing in coke sludge content were prepared for technological tests. The following mixtures were prepared for fermentation studies: municipal sludge – control sample (5 samples) and municipal sludge (preliminary sludge (1 sample) or excessive sludge (1 samples) or mixture of preliminary and excessive sludge (3 samples)) with coke sludge in proportions 20:1 (S1 – 5 samples), 6:1 (S2 – 4 samples). All samples of municipal sludge and municipal with coke sludge were inoculated with active fermentation microflora taken from a closed chamber of municipal sludge fermentation in a volume ratio 1:1.5. Thus, an appropriate amount of qualitatively diverse microflora (hydrolysing, acetogenic, homoacetogenic, acidogenic sulfate-reducing, nitrate-reducing and methanogenic bacteria) necessary for the course of fermentation was introduced to the prepared mixtures of municipal and coke sludge. In order to define the course of fermentation process, selected physicochemical properties of sludge were determined. In the sewage sludge determined: dry residue, residue after ignition and hydration. In the supernatants from sludge centrifugation, the following were determined: pH, alkalinity, chemical oxygen demand (COD) and volatile fatty acids (VFAs). PAHs were determined in both supernatants and sludges. During the fermentation process the atmospheric and biogas pressure were controlled. These measurements were conducted using a manometer at 24 h intervals. Biogas composition analysis was performed on selected samples using a thermal-conductivity chromatograph (GC-TCD), model Agilent GC 6890, from Agilent Technologies. The following analysis program was selected: oven 50°C, dispenser 100°C, detector temperature 250°C, carrier gas was nitrogen, gas flow rate was 20 mL/min. The determination consisted of calibrating the chromatograph by injecting 100 μL of a standard composition: CO – 0.5%, CO_2 – 28.0%, O_2 – 1.0%, H_2 – 0.5%, CH_4 – 70% and purity: CO – 99.9%, CO_2 – 99.99%, O_2 – 99.997%, H_2 – 99.95%, CH_4 – 99.9%. After calibrating the chromatograph, 100 μL of the test gas was injected.

2.3. Identification of PAHs

Sewage sludge was centrifuged to obtain supernatants. For the study 10 g of centrifuged sewage sludge and 500 mL of separated supernatant liquids were collected. The separation of 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 mL × 3 mL) and then distilled water (2 mL × 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 (produced by AccuStandard, Inc., USA) was used for the analysis. 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 280°C. 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 [11,20,25].

2.4. Statistical test

Student's *t*-test was used to determine the statistical significance of changes in selected physico-chemical ratios. The confidence level was assumed at 0.95. The number determining the degree of freedom was 2 and for this parameter the theoretical value of Student's td distribution was 4.303.

2.5. Toxic equivalency

Determination of the toxicity level of sample, expressed as TEQ was conducted by means of the so-called TEF (Table 1). TEQ was calculated from the results of chemical analyses of concentrations of 16 PAHs (Eq. 1) and TEF coefficients. The total numerical value ∑TEQ of the 16 PAHs is the total TEQ determined for the individual compounds obtained as the product of concentration of a single compound by the corresponding TEF partial factor (Table 1), according to the equation [Eq. (2)]:

$$\mathrm{TEQ} = m_1 \cdot \mathrm{TEF}_i \tag{1}$$

$$\mathrm{TEQ} = \sum \left(m_1 \cdot \mathrm{TEF}_i \right) \tag{2}$$

where m_i – single compound concentration, μg/L, μg/kg dm; TEF_i – toxicity equivalency factors for the *i*-th compound.

3. Results and discussion

3.1. Average PAHs concentrations in sewage sludge and supernatants

The average contents of individual PAHs before and after the fermentation process in municipal sludge and coke sludge and in the supernatants separated from them are presented in Table 3.

The total average content of 16 PAHs in the municipal sludge before the process was 1,643.9 μg/kg dry mass. During the fermentation a decrease in the total content of analysed PAHs by 77% was observed. In the supernatants

Table 3
Average PAHs contents in the mixture of municipal sludge and coke sludge and in the supernatants separated from them before and after the methane fermentation process

PAHs	Control sample (5 samples)				S1 – 20:1; v/v (5 samples)				S2 – 6:1; v/v (4 samples)			
	Sewage sludge, μg/kg dm		Supernatants, μg/L		Sewage sludge, μg/kg dm		Supernatants, μg/L		Sewage sludge, μg/kg dm		Supernatants, μg/L	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Naf	174.7	27.9	0.32	0.41	249.7	34.5	0.53	0.73	348.4	99.1	0.81	2.30
Acyl	30.5	1.5	0.04	0.13	45.6	8.5	0.05	0.16	119.9	30.4	0.17	0.60
Ac	140.2	23.9	0.13	0.42	321.7	39.0	0.25	0.79	568.4	206.4	0.31	2.46
Flu	191.0	33.4	0.12	0.55	268.1	53.3	0.25	1.08	614.5	272.1	0.32	3.41
Fen	307.5	109.4	0.39	0.96	367.8	105.9	0.50	2.49	752.2	302.6	0.53	5.38
Antr	38.7	11.7	0.06	0.26	93.7	30.8	0.09	0.41	144.0	65.4	0.12	0.93
Fl	182.2	23.4	0.16	2.66	273.6	98.0	0.37	4.18	997.8	317.5	1.09	10.47
P	129.8	19.2	0.12	1.83	195.7	74.0	0.30	2.94	726.8	236.8	0.90	7.36
BaA	57.9	8.1	0.05	0.88	81.1	38.2	0.18	1.42	458.8	107.1	0.55	2.84
Ch	67.6	10.8	0.11	0.88	89.4	44.5	0.28	1.51	428.2	141.2	0.87	3.77
BbF	80.6	13.8	0.10	0.99	81.9	43.6	0.30	1.27	337.2	119.9	1.10	2.92
BkF	70.8	11.2	0.09	0.99	62.6	39.7	0.27	1.12	296.8	110.3	0.98	2.85
BaP	55.3	9.9	0.07	0.74	53.9	31.4	0.20	0.81	279.9	102.4	0.74	1.95
DahA	40.7	3.4	0.14	0.33	17.4	16.0	0.14	0.29	86.0	37.0	0.42	0.63
IP	44.5	8.5	0.15	0.50	37.5	23.7	0.21	0.70	138.8	62.3	0.60	0.99
BghiP	46.3	6.5	0.11	0.47	34.6	21.4	0.22	0.55	150.8	57.4	0.72	1.34
∑PAHs	1,643.9	372.8	2.03	12.84	2,274.2	696.0	4.01	20.12	6,448.3	2,267.6	8.85	49.85

from the control sludge, the initial total average PAH concentration was 2.03 μg/L. Phenanthrene was present in the highest concentrations in the supernatants before the process as in the solid phase. In the supernatants after the fermentation process, the total average concentration of 16 PAHs was more than 6-fold higher (12.84 μg/L) compared to the initial one. The initial total average of 16 PAH content in the S1 and S2 sludge was higher than that in the control sludge and was 2,274.2 and 6,448.3 μg/kg dry mass, respectively. As in the control sludge, there was a gradual decrease in the total average PAH content with the duration of fermentation by 69% in the S1 mixtures and by 65% in the S2 mixtures. However, this loss was smaller than in the control sludge by a few or several percentage points. A different trend of changes in PAH concentration was observed in the sludge liquids. In both control and coke sludge liquids, PAH concentrations were higher after the fermentation process than the initial ones. The total average concentration of 16 PAHs increased from 4.01 to 20.12 μg/L (more than 5-fold). In S2 liquids, the recorded concentration increase was more than 5-fold (from 8.85 to 49.85 μg/L).

3.2. Physicochemical properties

Determined values of parameters of the fermentation process of municipal sludge with coke sludge which were obtained by analyzing the course of the process in 14 samples are presented in Table 4.

The degree of organic matter decomposition in the control sludge ranged from 15% to 33%, while in the mixtures of municipal sludge with coke sludge it ranged from 14% to 27%. The total biogas production in the control sample reached up to 11.7 L/L. The biogas production during fermentation of sludge with the smallest amount of introduced coke sludge – S1 (20:1; v/v) was on a similar level and did not exceed 10.6 L/L. In contrast, the total amount of biogas in S2 sludge (6:1; v/v) was much lower and did not exceed 4.3 L/L. Therefore, it can be concluded that as the proportion of coke sludge in the fermented mixture increased, the total biogas production was more than 50% lower. The amount of biogas produced per 1 g of organic dry matter in the control sludge ranged from 0.31 to 1.00 L/g dry mass. Lower amounts of biogas by about 65% were obtained during the fermentation of mixtures with the addition of coke sludge, especially when the proportion of the latter was at the level of 15%. The above data on biogas quantities indicate a significant decrease in the activity of microorganisms involved in the fermentation process for mixtures with coke sludge containing significant amounts of PAHs (samples S2). The inhibitory effect of PAHs on the microphase is also confirmed by the amount of methane and carbon dioxide in the biogas. Analysis of the biogas composition showed that in the control sludge the percentage of methane in the biogas was in the range of 52%–74% and the carbon dioxide content was in the range of 28%–39%. The contents of CH_4 and CO_2 in biogas in S1 sludge were in a similar range. However, for mixtures with 15% of industrial sludge (S2 samples), the maximum amount of methane in biogas was 25% lower than the control. The results of statistical calculations determining the significance of changes

Table 4
Summary of the values of the analyzed physico-chemical properties during the fermentation process

Parameters	Control sample (5 sample)	Mix of municipal sludge and coke sewage sludge	
		S1 – 20:1; v/v (5 samples)	S2 – 6:1; v/v (4 samples)
Loss of dry matter (mineral and organic compounds), %	15–33	14–27	16–22
Production of biogas, L/L	3.5–11.7	3.1–10.6	2.6–4.3
CH_4 content in biogas, %	52–74	49–70	51–56
CO_2 content in biogas, %	28–39	28–38	27–31
Amount of biogas from 1 g of organic dry matter introduced into the reactor, L/g	0.31–1.00	0.27–1.00	0.20–0.34

Table 5
Statistical analysis of fermentation process results

Parameters	Mix of municipal sludge and coke sewage sludge	
	S1 – 20:1; v/v (5 samples)	S2 – 6:1; v/v (4 samples)
Loss of dry matter	–	+
Production of biogas	–	+
CH_4 content in biogas	–	–
CO_2 content in biogas	–	–
Amount of biogas from 1 g of organic dry matter introduced into the reactor	–	–

– not statistically significant results; + statistically significant results.

in the values of basic parameters of the fermentation process of municipal sludge with coke sludge are presented in Table 5.

During the fermentation of S1 sludge the addition of industrial sludge in the amount of 5% did not have a significant impact on the obtained values of studied indicators. The addition of coke sludge during S2 sludge fermentation, where its percentage reached 15%, had a significant (unfavourable) impact on dry matter loss and total biogas production.

3.3. Changes in TEQ value of PAHs in sewage sludge

Based on the determined concentrations of PAHs in sewage sludge and toxicity equivalent factors, the toxicity level of PAHs was calculated. TEQ ratios for individual PAHs in control sludge before and after the process are shown in Fig. 1. TEQ values for individual PAHs before fermentation ranged from 0.0104 (Ac) to 101.0 (BaP). The average TEQ value for BaP in the sludge before the process was 55.3 and was the highest among all the analyzed compounds. After the fermentation process, there was a decrease in all TEQ values for individual PAHs. After the process, the lowest value was recorded for Acyl at 0.0015 and the highest value was recorded for BaP at 37.8. The average TEQ values for individual PAHs after the process were lower than the initial values by 55% for phenanthrene to 93% for acenaphthylene. The average decrease in TEQ values for BaP was 77%.

TEQ ratios for individual PAHs in S1 sludge before and after the process are shown in Fig. 2. During the fermentation of S1 sludge (mixture of municipal and coke sludge in the ratio 20:1; v/v), TEQ values for individual PAHs before the process ranged from 0.0219 (Acyl) to 76.3 (BaP). After the fermentation process, index values ranged from 0.0012 (as before the process for acenaphthylene) to 68.8 for benzo[a]pyrene. The average values for BaP before and after the process were 53.88 and 31.38, respectively. The average decrease in toxicity expressed by TEQ value for individual PAHs ranged from 8 (DahA) to 88% (Ac). For benzo[a]pyrene, the most potent in terms of carcinogenicity, the decrease in toxicity was 77%. As in the control sludge, lower average TEQ values of the analyzed PAHs were recorded compared to the initial ones.

TEQ ratios for individual PAHs in S2 sludge (a mixture of municipal and coke sludge at a ratio of 20:1; v/v) before and after the process are shown in Fig. 3. The range of TEQ values for individual PAHs before the fermentation process was wide from 0.0795 (Acyl) to 345.2 (BaP). The average TEQ values ranged from 0.120 to 279.85. Similar to the case of S1 mixtures also for mixtures with higher amounts of S2 industrial sludge after the fermentation process there was a decrease in all TEQ values for individual PAHs. Average TEQ values ranged from 0.0304 to 102.35. Similarly as in the case of S1 sludge fermentation after the process the lowest value was recorded for Acyl – 0.0124 and the highest for BaP – 119.9. Average decrease of toxicity expressed by TEQ value for individual PAHs ranged from 55% for indeno[1,2,3-c,d]pyrene to 77% for benzo[a]anthracene. The average decrease in toxicity expressed by TEQ value for BaP was 63%.

On the basis of obtained results it should be emphasized that during the fermentation of S1 sludge (20:1; v/v) the introduction of the additional load of PAHs (by 630.3 μg/

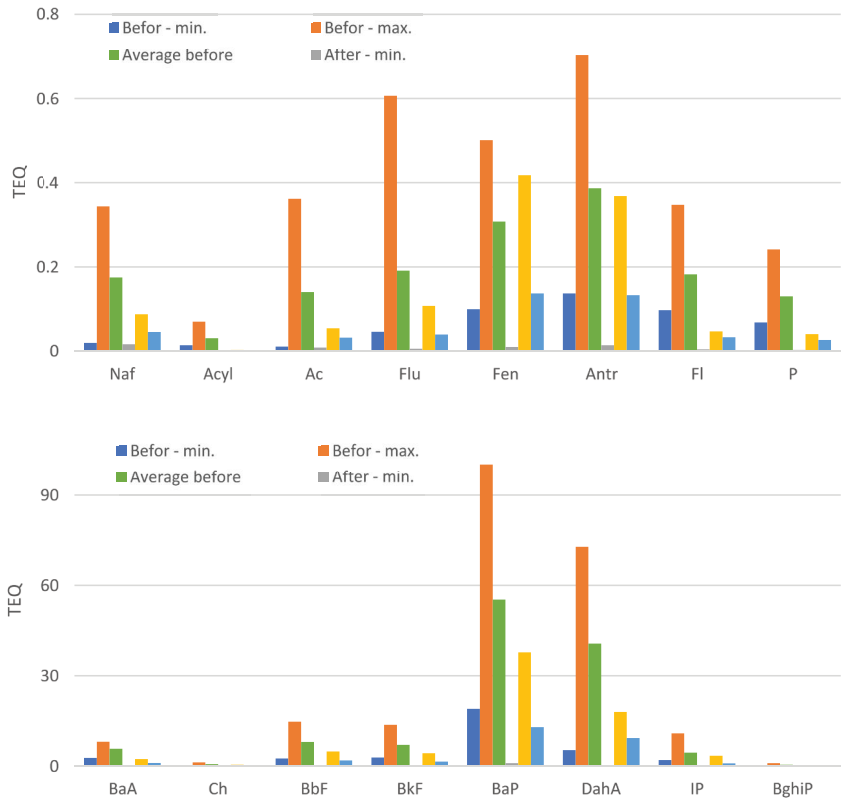

Fig. 1. TEQ value of PAHs in sewage sludge – control sample.

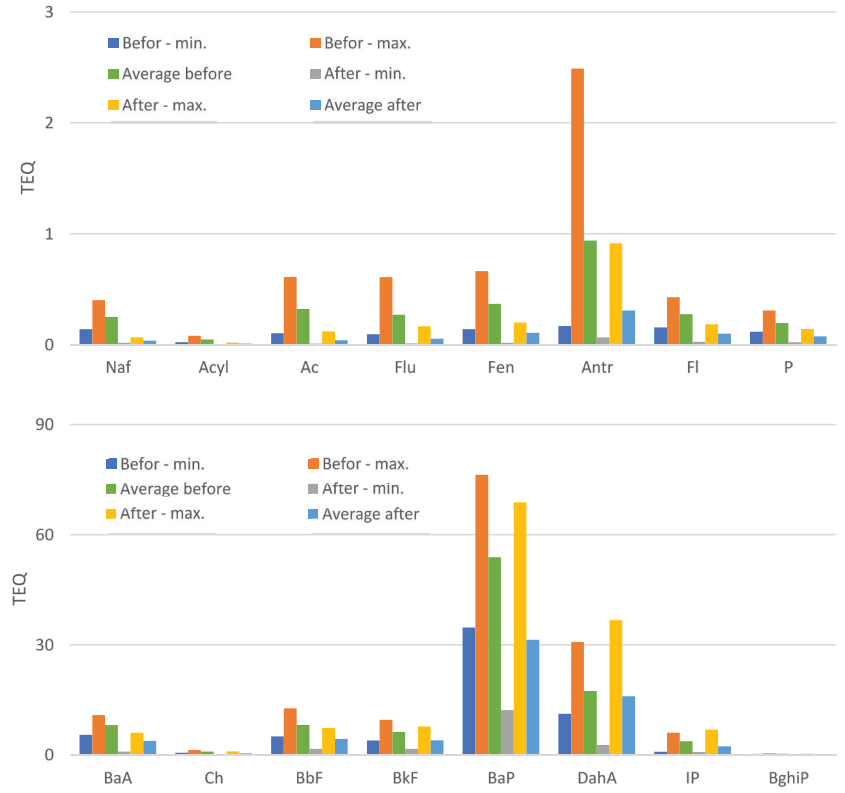

Fig. 2. TEQ value of PAHs in mixed municipal and coke sewage sludges – S1 samples.

Fig. 3. TEQ value of PAHs in mixed municipal and coke sewage sludges – S2 samples.

kg dry mass) to the municipal sludge did not significantly affect the biogas production and the decomposition of organic substances. The methane fermentation process proceeded undisturbed with an average 16WWA concentration of 2,274.2 μg/kg dry mass. During S2 sludge fermentation, the additional average 16WWA concentration introduced into the municipal sludge was 4,804.4 μg/kg dry mass higher for the municipal sludge, which statistically significantly affected the results of this study compared to the municipal sludge. Significantly less amount of biogas was obtained during S2 sludge fermentation. It can be concluded that coke sludge contained components that were toxic to microorganisms.

3.4. Changes in TEQ PAHs in supernatants

As in the sludge, PAHs toxicity levels were calculated from the determined concentrations of PAHs in the supernatants and toxicity equivalency ratios. TEQ ratios for individual PAHs in the supernatants of control, S1, and S2 sludge before and after the process are shown in Figs. 4–6.

In the supernatants obtained from the control sludge, the values of individual TEQs increased compared to the initial values. TEQ values for individual PAHs before the fermentation process were at low levels ranging from 0.000004 (Ac) to 0.26 (DahA). Before the process, the average TEQ values ranged from 0.000035 to 0.14. After the fermentation process, there was an increase in all TEQ values for individual PAHs. The average TEQ values ranged from 0.00013 (Acyl) to 0.736 (BaP). The increase in TEQ for individual compounds before and after the process ranged from 1.3 (Naf) to 16.8 (BaA) (Fig. 4).

In supernatants S1 before the process, the lowest TEQ value was obtained for acenaphthylene (0.00002) and the highest for benzo[b]fluoranthene (0.055). The average values ranged from 0.000046 (Acyl) to 0.198 (BaP). After the process, the highest average TEQ values were obtained for benzo[a]pyrene. The value TEQ for this compound increased more than 4-fold. The highest increase was observed for fluoranthene (more than 11-fold) (Fig. 5).

Pre-process average TEQ values in S2 supernatants ranged from 0.000165 for acenaphthylene to 0.74 for benzo[a]pyrene. After the process, the average TEQ values were in the range of 0.000595 (Acyl) to 1.953 (BaP). After the process, there was an increase in all ratios for each compound (Fig. 6).

Total ratios for solids and supernatants for the control and test sludges are presented in Figs. 7 and 8. In the control sludge before the fermentation process, the total average TEQ value of 16 PAHs obtained from five samples was 115.9 and after the process it decreased to 24.6. The decrease in toxicity expressed by TEQ ratio was 79%.

In S1 sludge, the total average TEQ values for 16WWA after fermentation were 44% lower than the initial values (from 101.5 to 56.9), and in S2 sludge they were 63% lower (from 500.3 to 183.4). A different trend in toxicity changes was observed for supernatants regardless of the fermentation mixture composition. In the control sludge supernatants, an almost 7-fold increase in the total average TEQ value was observed (from 0.19 to 1.29). In the sludge

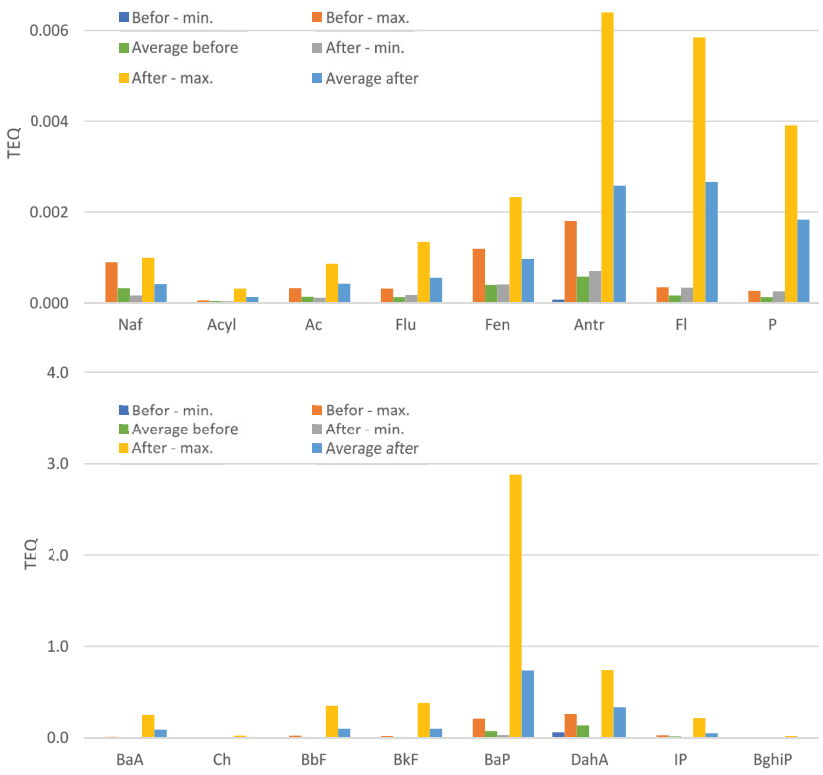

Fig. 4. TEQ value of PAHs in supernatants – control sample.

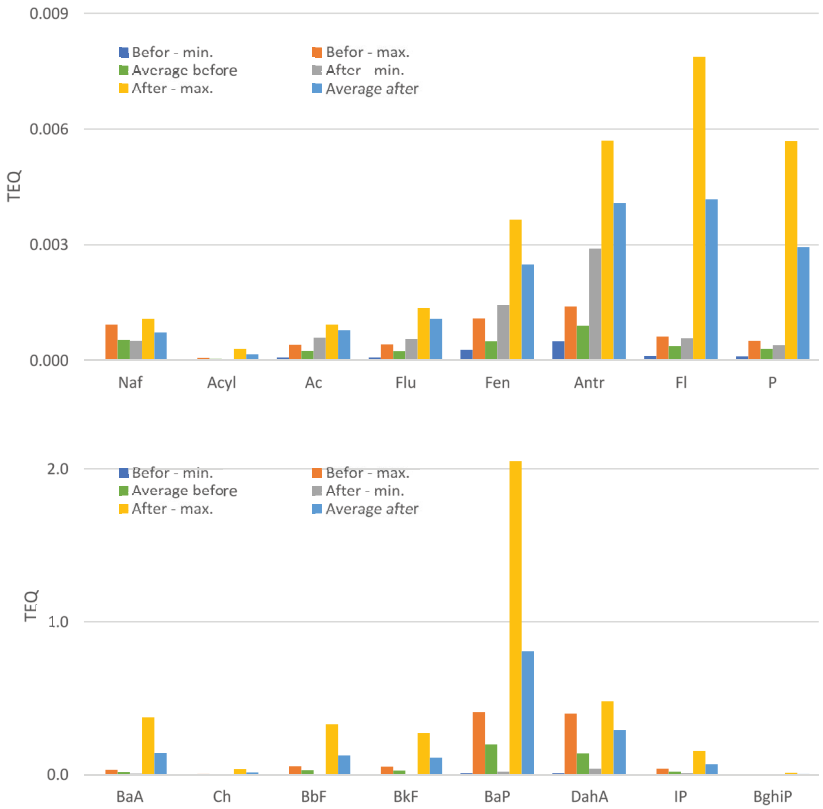

Fig. 5. TEQ value of PAHs in mix of supernatants – S1 samples.

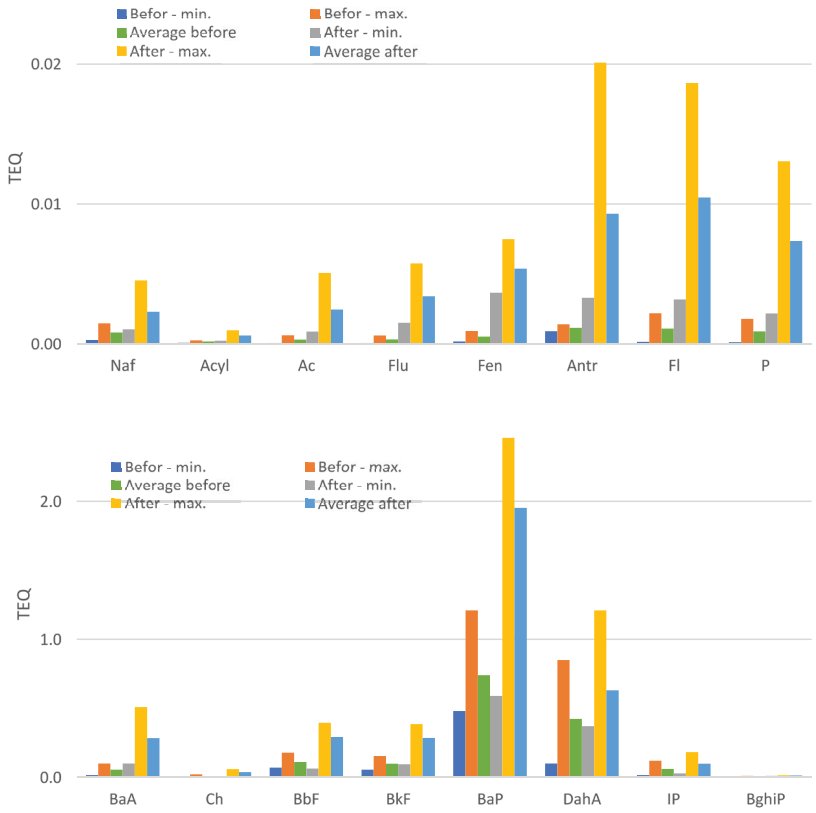

Fig. 6. TEQ value of PAHs in mix of supernatants – S2 samples.

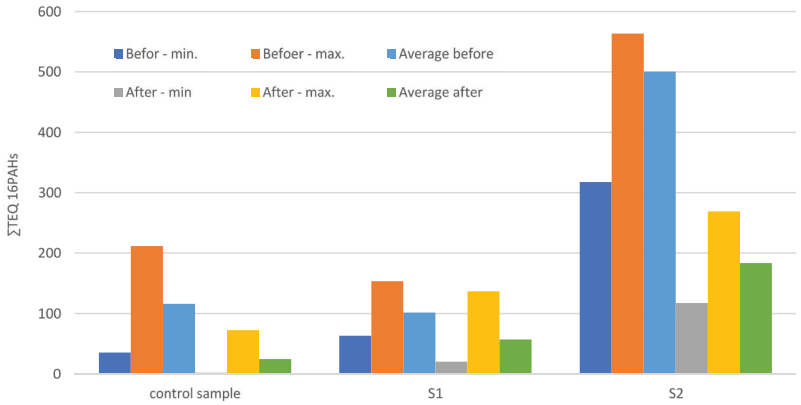

Fig. 7. ∑TEQ value of 16 PAHs in sewage sludge.

supernatants of the pre-treatment S1 mixtures, the total average TEQ value of 16WWA increased almost 4-fold (from 0.41 to 1.51). In S2 sludge, supernatant liquids with coke sludge at 15%, the TEQ values increased more than 3-fold (from 1.15 to 3.63).

The total average concentration of 16 PAHs in the supernatants separated from control sludge was 2.03 μg/L, while in the supernatants from S1 and S2 sludge it was 4.01 and 8.85 μg/L, respectively. The average concentrations of 16 PAHs increased during each fermentation. The final average concentration for the supernatants from the control sludge was 12.84 μg/L, and in the supernatants from the S1 and S2 sludges were 20.12 and 49.85 μg/L, respectively. The fermentation process proceeded smoothly when the average concentration of 16WWA before the process was 4.01 μg/L.

4. Summary

Analysis of changes in TEQ values for individual PAHs in sludge and supernatants shows that during fermentation there is a decrease in sludge toxicity (TEQ values) in

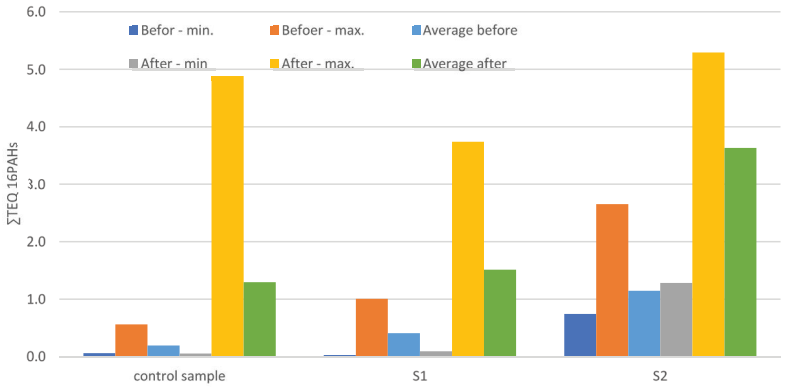

Fig. 8. ∑TEQ value of 16 PAHs in supernatants.

sewage sludge with a simultaneous increase in the toxicity of supernatants. As the proportion of coke sludge increased, the toxicity of PAHs to the fermentation bioflora increased. This resulted in gradual inhibition of metabolic processes of microorganisms and breakdown of methane fermentation process, which translated into lower biogas production, lower methane content in biogas and lower loss of organic dry matter. It should be added that an increase in the toxicity of supernatants was found during the study. This is important because these liquids either directly or after the sludge dewatering process in municipal treatment plants are returned to the wastewater treatment line. Thus, the raw wastewater stream is enriched with additional pollutants that are toxic to microorganisms. It is important during wastewater treatment carried out with the participation of microorganisms living in the activated sludge. In real conditions at continuous operation of the wastewater treatment system, the efficiency of PAHs biodegradation is low. The increase in toxicity of supernatants returned to the beginning of the technological system may lead to a decrease in the effectiveness of wastewater treatment process in terms of the removal of other easily biodegradable organic pollutants as well as biological transformation of biogenic compounds. Research results indicate that during fermentation, the TEQ value of sludge decreases several times, but the post-fermentation sludge is still toxic. It is of importance for the natural management of sludge.

Based on the experiments conducted in 9 samples of municipal sludge and coke sludge mixtures and the results obtained, the following can be concluded:

- PAHs at a total concentration of 2,275 μg/kg dry mass for solids and 4 μg/L – for liquids the fermentation process proceeded without interference.
- PAHs present in the average concentration of 6,448.3 μg/kg dry mass and in the supernatants – 8.85 μg/L have a toxic effect on the biocenosis, causing a significant inhibition of biogas production by 65%, reduction in the amount of methane by 24% and reduction in the degree of organic matter decomposition by 33%.
- during fermentation, a decrease in toxicity expressed as TEQ ratio for the solid phase and an increase in TEQ ratio for the sludge liquids were observed.
- recirculation of toxic supernatants into the treated wastewater stream poses a threat to activated sludge microorganisms.
- natural use of sludge may pose a threat of toxic PAH contamination of soils.

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