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Behaviour of PAHs during sewage sludge fermentation in the presence of sulphate and nitrate

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ABSTRACT

In this study, the changes of polycyclic aromatic hydrocarbons (PAHs) in sewage sludge and supernatants during anaerobic digestion under nitrate- and sulphate-reducing conditions were investigated. $NaNO_3$ or Na_2SO_4 were added to the mixed sludge in order to obtain denitrification and sulphate reduction conditions, respectively. Abiotic loses of PAHs simultaneously were observed. Abiotic samples (with sodium azide) were also prepared. All sludge samples (biotic and abiotic) were incubated at 35°C in the dark. PAHs concentration was determined before incubation, after 13 d and after 20 d of incubation. Quantification of 16 PAHs (EPA) was carried out by GC-MS. PAHs were determined both in sewage sludge and in supernatants. During hydrolysis of organic compounds (first phase of fermentation) in the control samples (in sewage sludge and in supernatants) the increase of PAHs concentration occurred (especially naphthalene and 3-ring PAHs). The concentration of 16 PAHs in sewage sludge was reduced by 30% on average under nitrate-reducing conditions after 20 d and by 32% on average under sulphate-reducing conditions, respectively. The effectiveness of reducing of individual hydrocarbons was different. The concentration of carcinogenic PAHs in sewage sludge was reduced by 73% on average under nitrate-reducing conditions and by 79% on average under sulphate-reducing conditions, respectively.

Keywords: Sewage sludge; Supernatants; 16 PAHs; Redox condition; Fermentations

1. Introduction

It is confirmed in the literature the presence of polycyclic aromatic hydrocarbons (PAHs) in soils, waters, sediments, wastewater and sewage sludges [1–3]. It is also proved that the afore mentioned compounds have carcinogenic, mutagenic and teratogenic properties. Although they are regarded as persistent organic pollutants (POPs) they may undergo process of degradation under certain conditions. Eeffectiveness of the degradation depends on the presence of proper microorganisms, temperature, pH, and the presence of oxidants. Decomposition rate of PAHs maybe be 100 times higher under aerobic conditions than under anaerobic conditions. In the water environment PAHs mainly absorb onto solid particles (sediments, sewage sludge in wastewater treatment plant) [4,5]. The presence of PAHs is also confirmed in the raw sewage sludges as well as in the digested sludges [6–8]. Usually the higher concentration of PAHs is found in the digested sludges (after fermentation process) [9]. The former studies proved that during digestion the release of PAHs into supernatants may take place mainly in

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the phase of hydrolysis of organic substrate [10,11]. The available investigations into removal of PAHs from sewage sludges in the process of digestion under different redox conditions are not completed [5,12–14]. The results of investigations described in the literature are not unambiguous and Heterogeneity of sewage sludge as well as the analysis of various hydrocarbons makes difficulties in comparison of the results. Therefore, not only the results in which the increase of efficiency degradation of PAHs in the presence of sulphates and nitrates was obtained are available [6] but the studies with diverse results as well. In the studies on PAHs removal during co-fermentation of sewage sludge and organic fraction of municipal solid waste was proved that the efficiency of PAHs removal for most hydrocarbons was not statistically significant [14].

The studies into changes of PAHs carried out simultaneously in supernatants and solids under denitrification and sulphate reduction conditions and with inhibited activity of microorganisms are limited and further investigations are required. Concentrations in sewage sludge are given in relation to dry weight that decreases during the process of digestion. The content of PAHs in supernatants is skipped in the literary sources. It was proved in the former investigations that supernatants may contain the significant load of PAHs. Therefore, it is necessary to determine simultaneously concentration of PAHs in solid and in liquid phase [10,11]. The aim of the investigations was to compare the changes in the concentration of PAHs in sewage sludges and supernatants during methane digestion conducted under various redox conditions. At the same time, the experiment was carried out with keeping sewage sludge samples without biological activity of the microorganisms.

2. Materials and methods

2.1. Materials

The investigations were carried out using sludges originating from a municipal treatment plant. The digestion process takes place in a two-stage closed and opened digestion chamber. Two portions of sewage sludge were used in the studies: digested sludge coming from an effluent from the closed digestion chamber as inoculum and raw sludge originating from a primary settling tank. The samples of raw sludge and digested sludge were prepared (4:1 v/v). The mixture of raw sewage sludge and inoculums samples was primary analyzed by determining physical-chemical properties and used for further studies. Level of mineralization of organic compounds was followed by determining: solid residue and hydration in the sewage sludge and volatile fatty acids content, alkalinity, acidity, pH in the supernatants. Sludge samples were centrifuged 3000 rpm over 10 min in order to separate solid phase from liquid phase (supernatants). The analyses were performed according to applied procedure [15]. Measurements were carried out at the beginning of sewage sludge incubation and after 20 d of digestion. Before fermentation process in raw and digested mixture of sewage sludge concentration of total suspended solids TSS were 40 mg L^{-1}, water contents — 96%. After fermentation process stabilized sludge had high water contents of 98% (concentration of total suspended solids was 20 mg L^{-1}) and low contents of organic substrate matter (40%). The pH was in the range of 7.3–7.5. The decrease in the content of volatile fatty acids 150 mg L^{-1} was observed.

2.2. Experimental procedure

The following samples were prepared for the investigations into degradation of PAHs in sewage sludges:

- the control sewage sludge (biotic samples)
- sludge sample with added sodium sulphate (Na_2SO_4 in the amount of 4 g/L (required to reduction of sulphates),
- sludge sample with added sodium nitrate $NaNO_3$ in the amount of 10 g/L (in order to obtain nitrate–reducing conditions),
- sludge with sodium azide (NaN_3) [12,16] in order to deactivate the microorganisms (abiotic samples),
- sludge sample with added sodium sulphate Na_2SO_4 in the amount of 4 g/L and sodium azide (NaN_3) (abiotic samples with sodium sulphate),
- sludge sample with added sodium nitrate $NaNO_3$ in the amount of 10 g/L and sodium azide (NaN_3) (abiotic samples with sodium nitrate).

The process of digestion was carried out in bioreactors to which the above-mentioned prepared sludge samples were added. Bioreactors were kept in thermostat at 35°C for 20 d. The pressure of biogas produced during digestion of biotic sewage sludges was measured every day. The total volume of obtained gas was 4.7–5.2 L. Everyday control of biogas production is typical measurement of profile of fermentation process.

The influence of added sodium azide on the microorganisms activity was measured with the use of TTC test (dehydrogenase activity). In all samples with the added sodium azide the dehydrogenase activity was equal to zero. The amount of produced biogas during keeping the above mentioned samples was also equal to zero. The PAHs determination were carried out 3 times: at the beginning of experiment, after 13 d of incubation and after 20 d of incubation. PAHs were determined in sludge samples as well as supernatants parallel. The investigations were carried out in duplicates. A *Student t*-test was used in order to assess the statistical significance of results. The critical value was read from tables for specified degree of freedom (n-2) and at a confidence level of 95%. Theoretical value of decomposition t_d ranged 4,303 [17].

2.3. PAHs analysis

PAHs were determined using gas chromatography–mass spectrometry. Preparation of samples consisted of the extraction by the mixture of solvents [18,19]. Extraction process for sewage sludge samples (solid phase) with cyclohexane and chloromethane mixture (in the ratio 5:1 (v/v)) as a solvents was carried out in ultrasonic bath for 20 min. The solvents with organic compounds were separated in centrifuge. The extraction process for supernatants (liquid samples) with methanol, cyclohexane and dichloromethane mixture (in the ratio 25:5:1 (v/v)) as a solvents was carried out in horizontal shaker. The solvents with organic compounds were separated in laboratory glass separator. Prepared extracts (from solid and liqiud samples) were concentrated to a volume of 3 mL under nitrogen steam. Afterwards extracts were purified using SPE columns packed with silica gel under vacuum conditions. Subsequently extracts were concentrated again to a volume 1 mL and then analyzed using gas chromatography method. Qualitative and quantitative analysis was done using Fisons gas chromatograph (model CGC8000/MS800). 16 PAHs were determined among which eight were proved to have carcinogenic properties. The following PAHs according to US EPA list were analyzed [20]:

- 2-ring hydrocarbon: naphthalene (Naph),
- 3-ring hydrocarbons: acenaphthylene (Acyl), acenaphthene (Ac), fluorene (Flu), phenanthrene (Phen), anthracene (Ant),
- 4-ring hydrocarbons: fluoranthene (Fl), pyrene (Pyr), benzo(a)anthracene (BaA), chrysene (Ch),
- 5-ring hydrocarbons: benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DahA) and
- 6-ring hydrocarbons: indeno(1,2,3,c,d)pyrene (IP), benzo(ghi)perylene (Bper).

In order to verify the applied procedure of preparation of sludge samples and supernatants the recovery of standard mixture (Accu Standard) 16 PAHs in benzene and dichloromethane in the concentration of 2000 μg per 1 L was also made. Standard mixture in the concentration of 8000 μg kg^{-1} dry sludge was added to sewage sludge and the amount of 32 μg L^{-1} was added to supernatants, respectively. Then, the samples were carefully shaken, extracted and analyzed for PAHs according to procedure described above. Average PAHs recoveries from solid phase (sewage sludge) ranged from 44 to 92%. The recoveries of analyzed organic compounds from liquid phase (supernatants) were in the range of 46–108%. The recoveries obtained in the study correspond to data found in literature (0–128%), [8,18] for complex organic matrixes (industrial wastewater, sewage sludge).

3. Results

3.1. Changes in PAHs concentration in sewage sludge

The initial concentration of total 16 PAHs in sludge was equal to 1260 μg/kg of dry weight (d.w.). Concentration of 8 carcinogenic PAHs was 582 μg/kg of d.w., 4-ring hydrocarbons amounted to 30% of the total contents of PAHs. The concentration of 3-ring and 6-ring hydrocarbons varied from 170 to 369 μg/kg. d.w. Changes in the total concentration of hydrocarbons in sludges in biotic control samples before digestion, after 13 d and after 20 d are given in Fig. 1. The concentration of complex organic compounds in the control sludge samples increased in the primary phase of digestion (hydrolysis). It was observed that concentration of 16 hydrocarbons exceeded 2749 μg/kg.d.w. (content of 8 hydrocarbons was at the level 1050 μg/kg.d.w.) after 13 d. In the former studies the higher concentrations of PAHs in the primary phase of digestion were already found [10,11]. In this study the significant increase occurred in naphthalene, 3-ring and 4-ring hydrocarbons. In the final step of the investigations the total concentrations of carcinogenic decreased to the amount of 333 μg/kg.d.w. Changes in the total concentra-

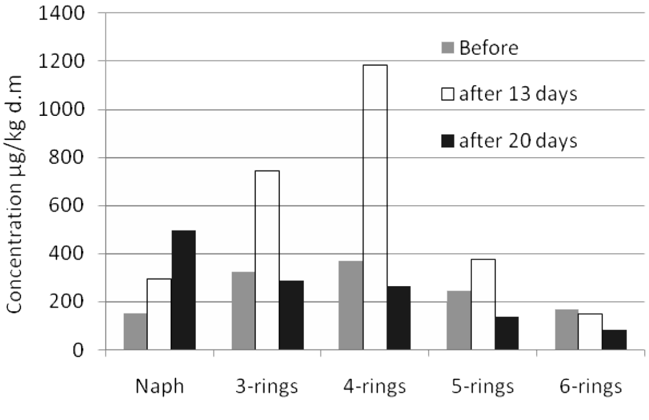

Fig. 1. Changes of PAHs in sewage sludge — control (biotic) samples.

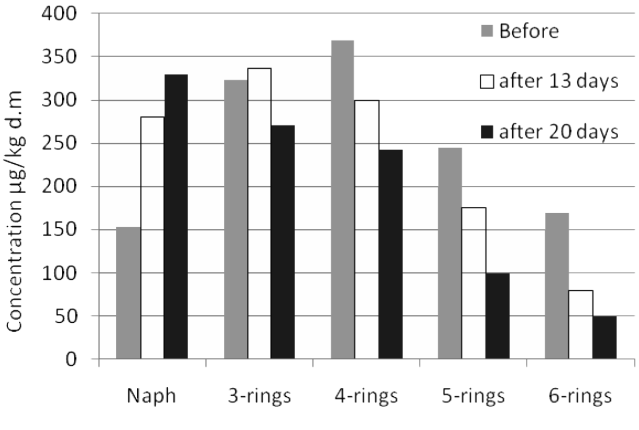

Fig. 2. Changes of PAHs in sewage sludge — abiotic samples.

tion of hydrocarbons in sludges in abiotic samples before digestion, after 13 d and after 20 d are presented in Fig. 2. In the sludge samples with the inhibited activity of microorganisms (abiotic samples with added sodium azide) the gradual decrease of studied compounds was found. The final total concentration of 16 PAHs was equal to 990 µg/kg.d.w. (concentration of carcinogenic compounds was 220 µg/kg.d.w. that constituted 37% of the initial content).

Changes in the total concentration of hydrocarbons in sludges amended with sodium sulphate and sodium nitrate in biotic samples before digestion, after 13 d and after 20 d are given in Figs. 3 and 4, respectively. Changes in the total concentration of hydrocarbons in sludges with added sodium sulphate and sodium nitrate in abiotic samples before digestion, after 13 d and after 20 d are given in Figs. 5 and 6, respectively. In sludge samples both with added Na_2SO_4 or $NaSO_4$ as an acceptor of electrons the degradation of PAHs was also observed. After 22 d of incubation the total concentration was lower than the initial contents of 32% and 30%, respectively. A *Student t*-test was used in order to assess the statistically significance addition of reagents in the sludge samples. On the basis of statistical calculations it can be concluded that the addition of these reagents impacted significantly the process of degradation of carcinogenic PAHs. Changes in the concentration of these compounds in sludge undergo the digestion process under denitrification conditions

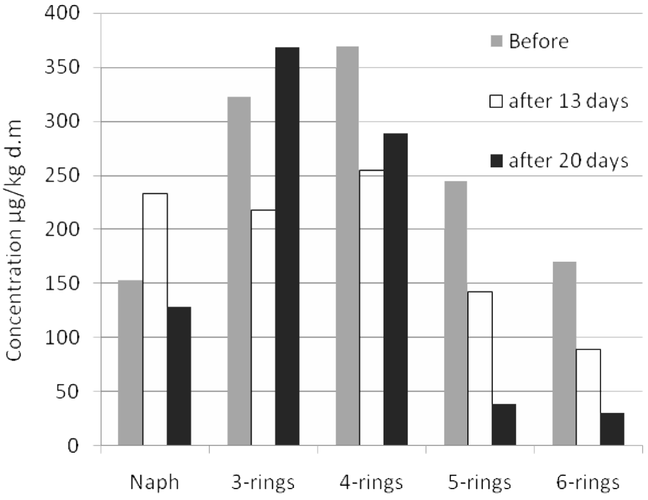

Fig. 3. Changes of PAHs in sewage sludge —samples under sulphate reducing conditions (biotic samples).

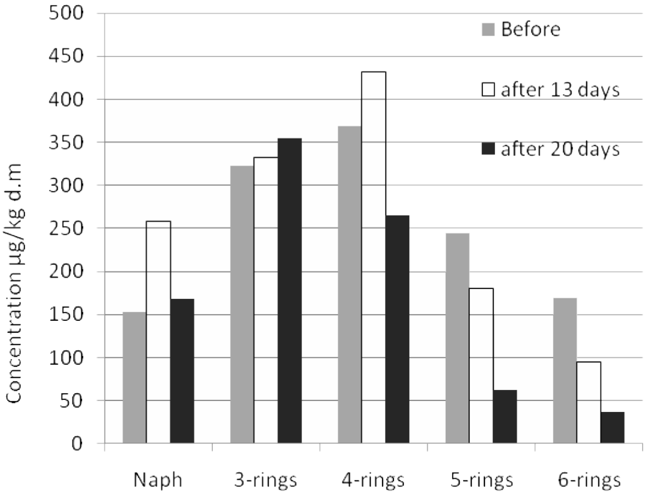

Fig. 4. Changes of PAHs in sewage sludge — samples under nitrate reducing conditions (biotic samples).

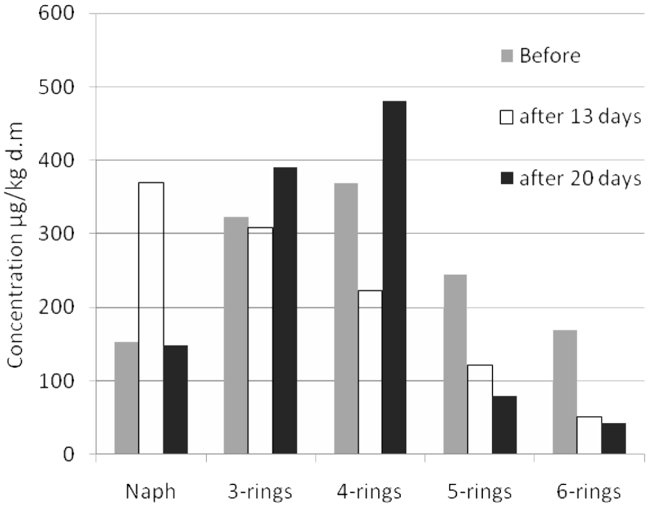

Fig. 5. Changes of PAHs in sewage sludge — abiotic samples with sodium sulphate.

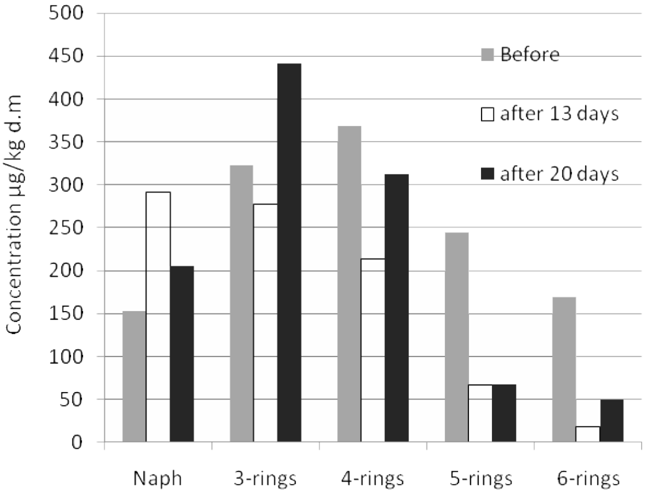

Fig. 6. Changes of PAHs in sewage sludge — abiotic samples with sodium nitrate.

were similar to those carried out under sulphate reducing conditions. The final total concentration of 8 PAHs was lower than the initial content of 74% and 79% in the presence sulphate and nitrate, respectively. The presence of both reagents $NaNO_3$ and Na_2SO_4 in abiotic samples was not significant. At the end of the experiment the total concentration of 16 PAHs in these samples decreased of 10–15% as compared to initial concentration.

3.2. Changes in PAHs concentration in supernatants

The initial concentration of 16 PAHs in supernatants was 5.2 µg/L on average. Changes in the concentration of hydrocarbons in supernatants during the process of sludge digestion are given in Fig. 7. In the control sample (biotic sample) almost 2.5 times higher concentration of hydrocarbons was found after 13 d of incubation (13.3 µg/L). As it was proved in the former studies hydrocarbons may have released to the water phase during phase of hydrolysis. At the end of the experiment the total concentration of 16 PAHs was higher than initial concentration of 80%. In the control samples of supernatants the total concentration of carcinogenic hydrocarbons was higher than the initial contents of 50%. In Fig. 8 changes of PAHs in abiotic samples of supernatants are given. PAHs concentration in abiotic samples gradually lowered from 5.2 µg/L to 4.3 µg/L.

Changes in the concentration of hydrocarbons in supernatants in the presence of sodium sulphate and sodium nitrate during the process of sludge fermentation are given in Figs. 9 and 10, respectively. Concentrations of PAHs in supernatants in the presence of sulphates and nitrates varied from 6.4 to 7.5µg/L after 13 d of digestion. The decrease in the concentration of PAHs in supernatants occurred in the final phase of incubation. The level of degradation of 5-ring hydrocarbons in supernatants dur-

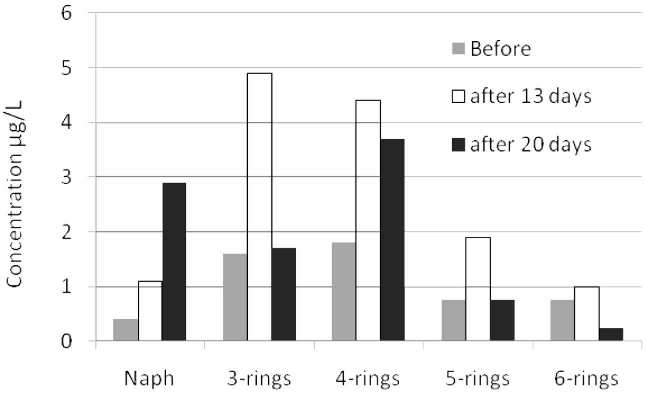

Fig. 7. Changes of PAHs in supernatants — control (biotic) samples.

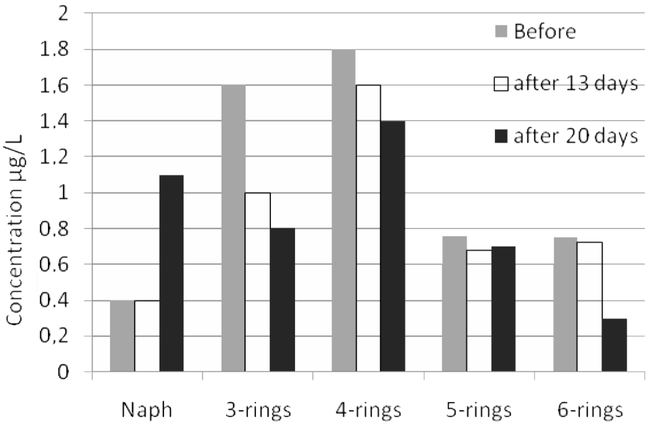

Fig. 8. Changes of PAHs in supernatants —abiotic samples.

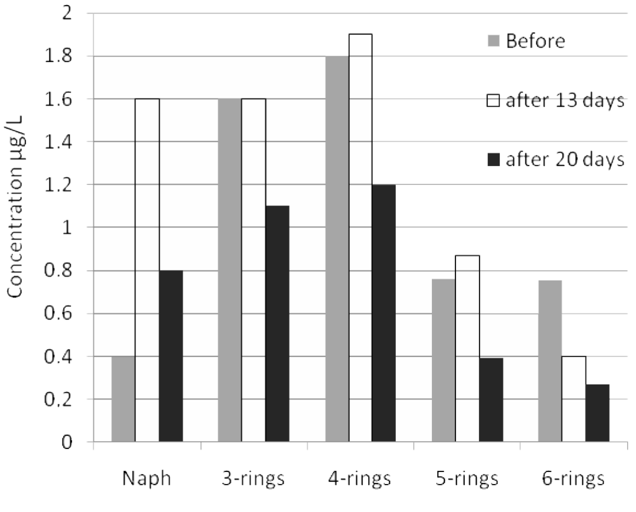

Fig. 9. Changes of PAHs in supernatants — samples under sulphate reducing conditions (biotic samples).

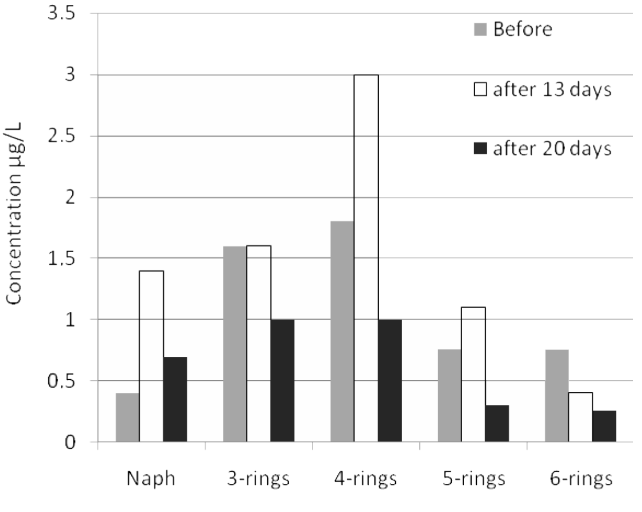

Fig. 10. Changes of PAHs in supernatants —samples under nitrate reducing conditions (biotic samples).

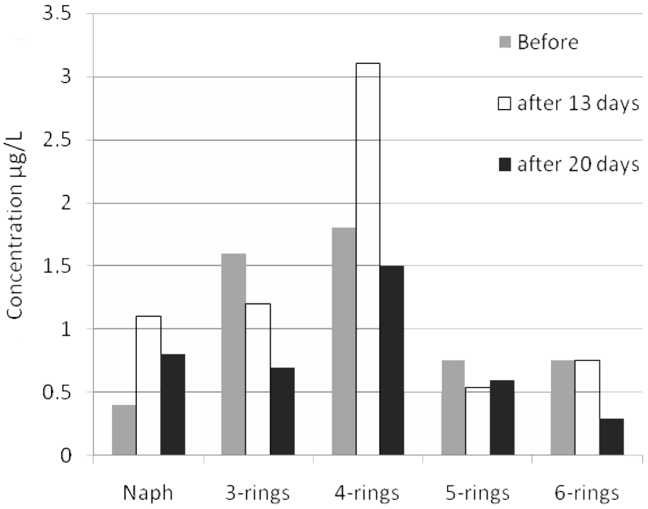

Fig. 11. Changes of PAHs in supernatants — abiotic samples with sodium sulphate.

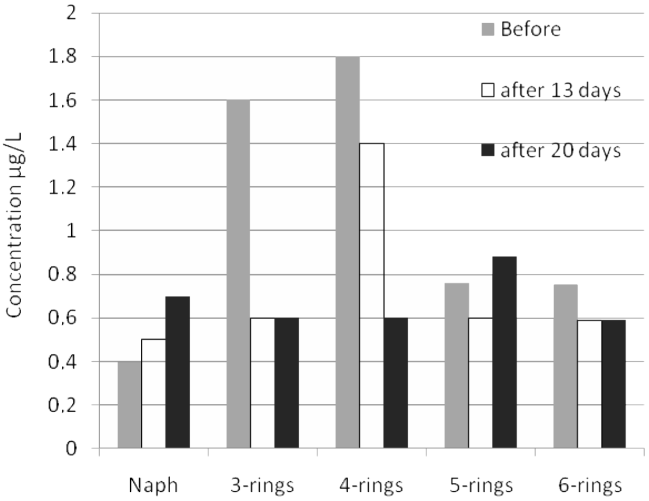

Fig. 12. Changes of PAHs in supernatants — abiotic samples with sodium nitrate.

ing the process of digestion amounted to 30%, whereas 6-ring hydrocarbons in supernatants during the process of digestion amounted to 40%. The concentrations of the remaining hydrocarbons (naphtalene, 3-ring and 4-ring hydrocarbons) were high. Carcinogenic compounds were lower than initial content in the presence of sodium sulphate and sodium nitrate of 37% and 31%, respectively. In Figs. 11–12 changes of PAHs concentration in abiotic samples with sodium sulphate and sodium nitrate are given, respectively. Abiotic losses of PAHs were in the range of 26–36%. At the end of the experiment (after 20 d) the total concentrations of carcinogenic PAH were lower than the initial concentrations of 17–19%.

4. Conclusions

The total concentration of 16 PAHs in sewage sludge was reduced by 30% on average under nitrate-reducing conditions after 20 d and was reduced by 32% on average under sulphate-reducing conditions, respectively. Due to the fact that in control sewage sludges the final concentration was at he same level as the initial content it can be concluded that assuring conditions reducing sulphates or nitrates allowed to increase the efficiency of degradation of PAHs. However, not all hydrocarbons were identically subjected to process of decomposition. The total concentration of 8 carcinogenic compounds in sewage sludge was reduced by 73% on average under nitrate-reducing conditions after 20 d and was reduced by 79% on average under sulphate-reducing conditions, respectively, whereas in control samples — 43%. It is worth to mention that the significant increase of naphthalene and 3- and 4-ring hydrocarbons took place in the initial period of incubation. It was due to the fact of decomposition of complex polymers. The increase of concentrations of these compounds had significant impact on the content of sum of 16 PAHs. It indicates that interpretation of the results on the basis of changes of total content of 16 PAHs in sewage sludges is simplified as the removal of individual hydrocarbons is diverse. Comparing the behaviour of hydrocarbons in biotic to abiotic sludges it can be concluded that the efficiency of PAHs removal was higher in the presence of microorganisms. In abiotic sludges losses of PAHs did not excess 20% and they were statistically insignificant. The abiotic losses may be related to the evaporation, sorption as well as reactions with other compounds of sewage sludges.

The significant increase of concentration of low-ring hydrocarbons in the initial phase of digestion process both in solid and in supernatants was found. During hydrolysis of multiple organic compounds the studied hydrocarbons are released to the liquid phase. 5-rings and 6-rings of hydrocarbons in supernatants reduced 40% under nitrate-reducing conditions and 30% under sulphate-reducing conditions. It indicates of the possibility of biodegradation of examined PAHs also in supernatant under denitrification process and under sulphate-reducing conditions except for naphthalene and 3-rings of hydrocarbons. The degradation of carcinogenic PAHs in supernatants ranged 31% under nitrate-reducing conditions and 37% under sulphate-reducing conditions, whereas, in the control samples the increase in the concentration of PAHs of 53% occurred. The contribution of abiotic processes in the removal of carcinogenic PAHs in supernatants was significant. In the samples with the inhibited activity of microorganisms the decreases of hydrocarbons ranged to 36%.

Acknowledgement

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