

## Removal of polycyclic aromatic hydrocarbons from leachates after autothermal thermophilic aerobic digestion using effective microorganisms

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### ABSTRACT

The autothermal thermophilic aerobic digestion (ATAD) method is increasingly used in Poland and around the world because it allows simultaneous stabilization and hygienization of sewage sludge. After stabilization, sludge is dewatered and then transported to biological systems. It has been proven that leachates after ATAD contain toxic polycyclic aromatic hydrocarbons (PAHs). In addition, their contact with other matrix components often results in the formation of toxic derivatives or forms with more rings. This situation results in a potential threat to the environment, as inferior quality wastewater may enter receiving waters. The scope of the study was to carry out treatment of leachate after ATAD using the activated sludge method in a model system, to which effective microorganisms (EM) was also added. The determination of PAHs in activated sludge, raw and treated leachates was carried out. The aim of the study was to determine the biosorption and removal efficiency of the different groups of PAHs. The greatest biosorption, as well as reduction in total PAHs, occurred in the research stages where EM were used. The addition of EM increased biosorption of 5-ring compounds by 61% and provided removal efficiencies of 81%–91% for this group of PAHs. The average removal efficiency of total PAHs from leachates after ATAD throughout the process ranged from 50% to 96%. The best result was obtained in the stage where EM were present for two weeks.

**Keywords:** Biodegradation; Polycyclic aromatic hydrocarbons; Autothermal thermophilic aerobic digestion; Sewage sludge; Effective microorganisms

### 1. Introduction

Wastewater treatment involves the generation of sewage sludge which, according to the law, must be properly prepared for further use or, if this is not possible, disposed of. For many years, methane fermentation, composting and aerobic stabilization processes have been used to stabilize sewage sludge [1]. Aerobic methods, as well as fermentation carried out under mesophilic conditions, cause only partial hygienization of the sludge. Thus, in order to get rid of pathogenic organisms, separate hygienization methods must be implemented, including liming or drying

[2,3]. Autothermal thermophilic aerobic digestion (ATAD) is a process that provides simultaneous stabilization and hygienization of sewage sludge. The installation occupies a small area, and the tanks in which the stabilization process takes place are completely encapsulated. Designers have also provided for the provision of suitable equipment for reducing gaseous pollutants [4]. The method is increasingly being used in Europe. Installations are also present in Asia (China) and South America. In Poland, as of now, there are 11 ATAD facilities located at municipal wastewater treatment plants and more than half are in the northeastern part of the country [5,6]. Iskra et al. [7] report that more

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ATAD facilities are currently under construction or design. Autothermal thermophilic sludge stabilization is recommended for wastewater treatment plants with a capacity of 20,000 m<sup>3</sup>/d or less.

After the ATAD process, the sludge is either directly used in liquid form for nature or it goes to presses or centrifuges for dewatering. Most technologies used in wastewater treatment plants provide for returning leachates from dewatering to biological systems. Boruszko and Wojciula [8] proved that ATAD leachates can contain significant amounts of polycyclic aromatic hydrocarbons. The concentration of these compounds in some samples was similar to that in petrochemical oven wastewater. As a result, the discharge of leachates to the beginning of the process system can place a burden on municipal treatment plants [9]. In many manufacturing plants, industrial wastewater is subjected to special treatment processes, using membrane techniques or chemical methods such as oxidation or photolysis, among others. For example, ring disruption of polycyclic aromatic hydrocarbons (PAHs) is possible by excitation of the molecules of these compounds with UV-light. Nanofiltration or ultrafiltration, on the other hand, can shift PAHs to another environment, resulting in the disappearance of hydrocarbons in a given portion of the wastewater [10–13]. In the case of wastewater treatment plants with ATAD facilities, the leachates after the dewatering process are not treated. A characteristic feature of PAHs is their susceptibility to transformation under the presence of other environmental components. Compounds with a lower molecular weight can undergo transformation to compounds with a higher number of rings or to dangerous derivatives [14]. Thus, there is a risk that the combination of ATAD leachates with municipal wastewater will cause unfavorable transformations in the biological system.

Polycyclic aromatic hydrocarbons are formed in incomplete combustion processes of organic substances. They are present in industrial wastewater, or a mixture of industrial and municipal wastewater. They enter wastewater treatment plants along with wastewater from manufacturing, such as petroleum, from petrochemical plants, as well as with rainwater runoff from traffic roads [15]. PAHs exist as a light fraction (LMW) and a heavy fraction (HMW). The first type refers to lower molecular weight hydrocarbons containing 2 or 3 aromatic rings. The second group consists of compounds consisting of more rings and characterized by a higher mass. They are called xenobiotics due to their high resistance to decomposition, however, the presence of suitable microorganisms, as well as the provision of certain environmental conditions, can affect their biodegradation [16,17]. The Scientific Committee on Food (SCF) has designated the 15 PAHs most toxic to living organisms. Among them, benzo(a)pyrene was singled out, having a high rate of carcinogenicity and mutagenic activity [18,19]. It has been proven that contact of a PAH mixture with a living organism results in damage to the urinary or immune system [20].

At the moment, there are gaps in the literature on the study of leachates after the ATAD process or the transformation of PAHs in leachates from the dewatering of sewage sludge subjected to stabilization by classical methods. A novelty in terms of the research topic analyzed is the use of effective microorganisms for the degradation of PAH compounds. In the study, it was assumed that the addition of

the effective microorganisms (EM) preparation during biological treatment of leachates would contribute to a better degree of biosorption and biodegradation of PAHs than in the case of treatment with activated sludge only. Thus, the purpose of the study was to determine the degree of PAHs degradation in leachates after ATAD in both cases. During the research experiment, attention was also paid to the exposure time of PAHs to the microorganisms present in the activated sludge chambers. Laboratory analysis included the determination of concentrations of the 16 most common PAHs in samples of activated sludge and raw and treated leachates. Then, the biosorption and removal efficiencies of these compounds were determined for each group of PAHs. The article presents the results from the study, along with their detailed statistical analysis.

## 2. Material and methods

### 2.1. Materials

The leachates after the ATAD process were tested. During the conducted experiment, Greenland's effective microorganisms with the following composition were also dosed: lactic acid bacteria, photosynthetic bacteria, yeast, Azotobacter, cane molasses, total nitrogen at least 0.3%, and potassium converted to K<sub>2</sub>O at least 0.2%. The activated sludge came from one of the wastewater treatment plants of the Podlaskie Province with a p.e. of about 7,000. Microscopic inspection of the composition and condition of the activated sludge was carried out throughout the research experiment, and sample photographs taken of the structure of the activated sludge are shown in Fig. 1.

### 2.2. Methodology of technological research

The research was carried out under laboratory conditions. A model system from the German company GUNT-CE705 was used for biological wastewater treatment using activated sludge, the schematic diagram of which is presented in Fig. 2. The device demonstrates the principles of the activated sludge process, where the nitrification and denitrification process takes place with the help of appropriate microorganisms. Three agitators are present in the system, mixing wastewater with activated sludge, as well as a secondary clarifier for separating treated water from sludge. Table 1 shows the parameters and technical conditions prevailing during operation of the GUNT-CE705 model system.

The research for the present study was conducted for four weeks, during November–December 2020. Preliminary analysis of the research results determined that each week would constitute a separate research phase. During the first and second weeks, only the leachates from dewatering after the ATAD process were present in the model system. In the second and third stages, effective microorganisms were added to the system.

Considering the full cycle of the reactor, which was 24 h, raw and treated leachates were sampled at set intervals. The sampling took place four times during the week. Concentrations of 16 polycyclic aromatic hydrocarbons were determined in the leachates, such as: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene,

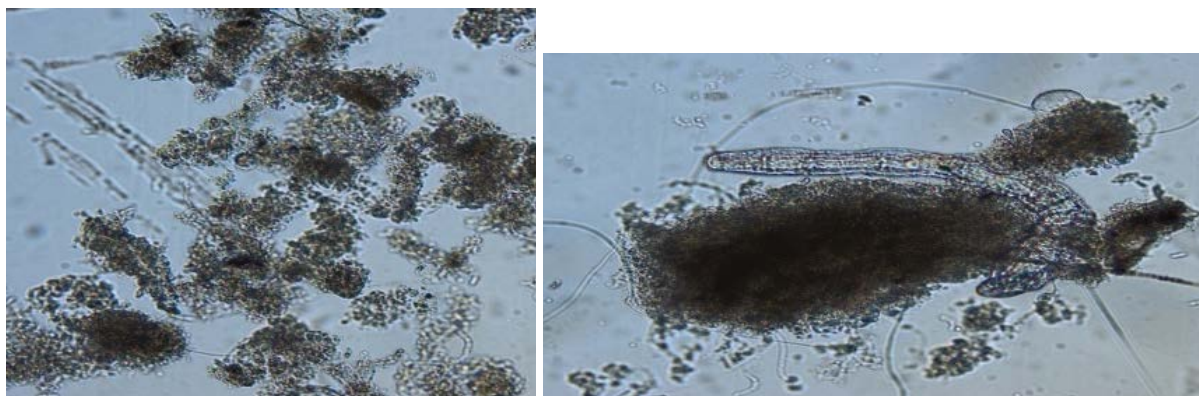


Fig. 1. Flocs of activated sludge used in the study.

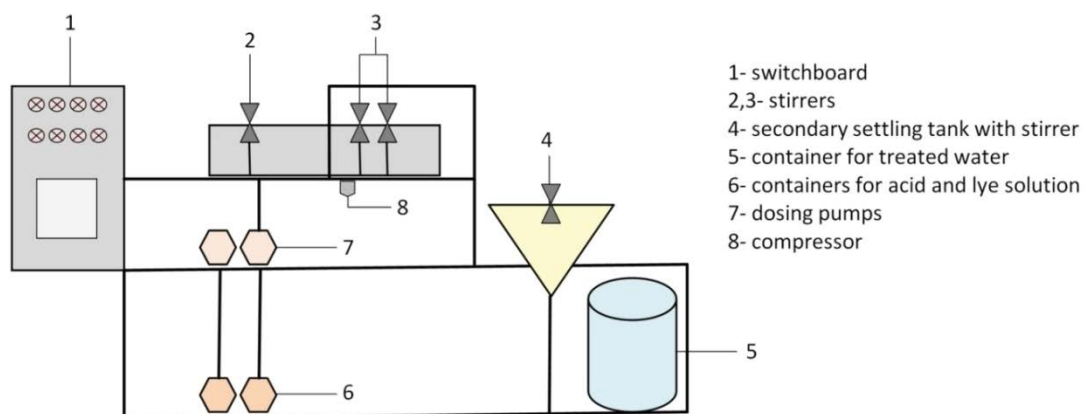


Fig. 2. Demonstration unit of the CE705 model system. Source: own development.

Table 1

Parameters and technical conditions prevailing during the operation of the GUNT-CE705 model system

Parameters/technical conditions	Determined value
Capacity of the raw sewage tank	200 L
Total capacity of the biological reactor	51 L
Capacity of anoxic part (denitrification)	17 L
Volume of aeration zone (nitrification)	34 L
Volume of material in the secondary clarifier	30 L
Volume of material in the container of treated water	80 L
Reaction of the mixture	6.5–7.2 throughout the test period
Temperature prevailing in the system	20°C
Degree of oxygenation in the system	2–5 mg/L, depending on the aeration area
Wastewater pumping capacity	2–2.5 L/h
Average daily wastewater flow through the model system	48–60 L/d
External recirculation	120%
Internal recirculation	250%–300%

benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene, indeno(1,2,3-cd)pyrene and benzo(g,h,i)perylene. Each result presented in the paper is an averaging of three values obtained during laboratory measurements.

### 2.3. Analytical methods

For the study of PAHs, 250 cm<sup>3</sup> each of wastewater material was collected, which was strained with a quality strainer and then extracted. The extraction was carried out

using 100 cm<sup>3</sup> of dichloromethane and 50 cm<sup>3</sup> of hexane on a magnetic stirrer at a speed of 600 rpm for 45 min. After separation of the extract from the leachates, the samples were concentrated to a volume of 1.5 cm<sup>3</sup> at a vacuum of 280 mbar, at 35°C. Samples prepared in this way were subjected to quantitative analysis by gas chromatography coupled with high identification capability GC/MS Agilent 7890B Mass Spectrometry on a DB-5MS column [21].

#### 2.4. Statistical methods

Based on inlet ( $C_{in}$ ) and outlet ( $C_{out}$ ) concentration, treatment efficiency  $\eta$  was calculated as:

$$\eta = \frac{C_{in} - C_{out}}{C_{in}} \quad (1)$$

The dataset was graphically presented by using boxplots [22]. Each boxplot contains two notches and a box with two hinges and a middle bar. The lower and upper hinge represent the first and third quartiles. The middle bar represents the median. To describe a notches range it is helpful to introduce value  $d$  given by the equation

$$d = 1.58 \frac{IRQ}{\sqrt{n}} \quad (2)$$

where: IRQ – interquartile range and  $n$  – number of observations.

Notches extend by a maximum of value  $d$  from the hinge but not further than minimum or maximum value in the data. If there are observations outside the notches, they are plotted using circles and signal possible outliers. To describe each boxplot numerically, the following reporting scheme was used:

$$\frac{\text{mean} \pm \text{sd}(\text{median} \pm \text{mad})}{\text{min}, q_1, q_3, \text{max}} \quad (3)$$

where mean – calculated mean; sd – standard deviation; median – calculated median; mad – scaled median absolute deviation; min – minimum value;  $q_1, q_3$  – quartiles first and third; max – maximum value.

Reported values were round to the first two significant decimal positions of either standard or median absolute deviation – the more accurate of the two was used. The scale for median absolute deviation was equal to:

$$\text{scale} = F_{N(0,1)}^{-1}(0.75) \approx 1.4826 \quad (4)$$

where scale – the scale;  $F_{N(0,1)}^{-1}(\cdot)$  – reversed distribuant of standard normal distribution and is adjusted in such way that the whole calculated deviation is asymptotically equal to standard deviation for normally distributed data.

To assess differences between groups of data, the Kruskal–Wallis test was used [23]. When the test result was significant, post-hoc pairwise Mann–Whitney tests [24] were performed with Benjamini–Hochberg [25]  $p$ -value adjustment.

### 3. Results and discussion

Plots, computations and statistical testing were performed in R statistical environment version 4.2.2 (“Innocent and Trusting”) [26]. The whole dataset was grouped by 4 separate stages of treatment plant work. The following data were plotted and used in further calculations (tables are included as attachments):

- total PAH inlet concentration (Fig. 3, Table 2).
- treatment efficiency for total PAH along with 2, 3, 4, 5 and 6-rings PAH fractions (Fig. 5, Table 4).
- sludge concentration for total PAH along with 2, 3, 4, 5 and 6-rings PAH fractions (Fig. 4, Table 3)

For all statistical tests their  $p$ -value were interpreted as significant, if below  $\alpha = 0.05$  level threshold.

Fig. 3 shows the distribution of PAHs data in the raw leachates after the ATAD process at each test stage. In the first and second stages, the maximum PAHs concentration

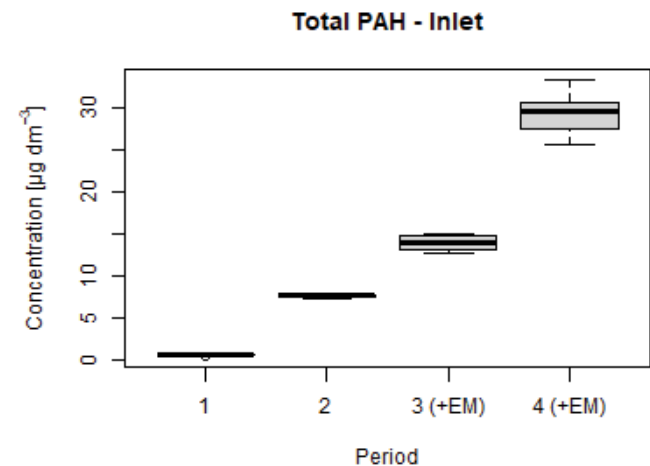


Fig. 3. Concentration of total polycyclic aromatic hydrocarbons in raw leachates after autothermal thermophilic aerobic digestion at each test stage.

Table 2

Values of total polycyclic aromatic hydrocarbons in raw leachates after autothermal thermophilic aerobic digestion at each test stage

Fraction	Stage	Values
Total PAH	1	$0.536 \pm 0.060$ ( $0.547 \pm 0.031$ ) 0.361; 0.531; 0.566; 0.585
	2	$7.61 \pm 0.21$ ( $7.62 \pm 0.23$ ) 7.29; 7.46; 7.72; 8.02
	3	$13.96 \pm 0.88$ ( $14.08 \pm 1.19$ ) 12.69; 13.17; 14.71; 15.03
	4	$29.3 \pm 2.2$ ( $29.6 \pm 2.5$ ) 25.7; 27.6; 30.5; 33.4

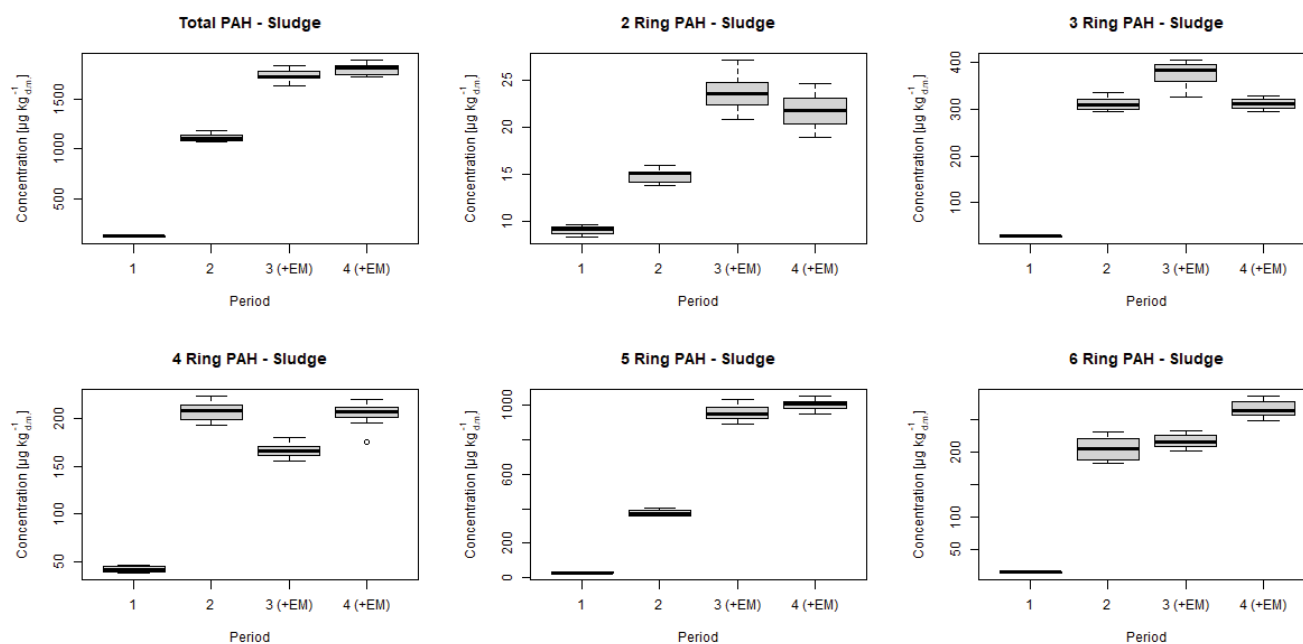


Fig. 4. Polycyclic aromatic hydrocarbons content of sewage sludge at each test stage.

was less than  $10 \mu\text{g}/\text{dm}^3$ . In the weeks where EM was applied to the raw leachates (stage 3 and 4), PAHs concentrations were one and a half to three times higher than in stage 2. The changes seen in Fig. 3 may be due to the specifics of the formulation dosed during the research experiment. The formulation with EM is characterized by containing, among other things, cane molasses, which is a substance produced during the production of cane sugar. Before sugarcane is harvested, it is burnt. In turn, the process of incomplete combustion of organic materials is one of the main sources of the formation of toxic PAHs [27,28]. In view of the above information, it was assumed that the presence of cane molasses in the added formulation could have contributed to these results.

Fig. 4 shows the distribution of data on PAH content in activated sludge, in all research stages. PAHs are separated into groups by the number of rings they contain, and, consequently, their molecular weight. The average biosorption of total PAHs throughout the process ranged from 122 to  $1,807 \mu\text{g}/\text{kg d.m.}$  The lowest content of the tested compounds in the sludge occurred for stage 1, and the highest for stage 4, where EM were set.

It has been proven that strong biosorption of PAHs can reduce the intensity of degradation of these compounds [29]. Such a relationship was noted in the studies conducted for 3- and 6-ring compounds. The 3-ring hydrocarbons underwent significant sorption by microorganisms in stage 3, resulting in a decrease in their average removal efficiency. In the case of 6-ring compounds, this situation occurred in the last week of the study. The average biosorption of total PAHs increased in direct proportion to the length of the tests conducted, as can be read from Table 3. The addition of the EM preparation increased the average sorption of hydrocarbons by microorganisms (except for 4-ring compounds). The most intensive increase was for 5-ring compounds, with a 61% increase.

Statistical testing revealed that in all period comparisons, concentrations were significantly different (Kruskal–Wallis test  $p$  value was less than 0.0001). Post-hoc tests revealed following pairs of insignificantly different periods in case of sludge concentration:

- 3-ring PAH fraction: period 2 and 4;
- 4-ring PAH fraction: period 2 and 4;
- 6-ring PAH fraction: period 2 and 3.

The removal efficiency of PAHs from ATAD leachates, presented later in this paper, can take place through volatilization, biodegradation preceded by biosorption as discussed above, as well as through oxidation of PAHs by enzymes present in microorganisms. Oxidation, in turn, leads to the transformation of PAHs to lower molecular weight compounds, and further transformations can lead to complete degradation [30]. Determination of PAHs in activated sludge was therefore an important factor that demonstrated the relevance of biological processes in the transformation of PAH compounds.

Fig. 5 shows the distribution of data on the removal efficiencies of PAH compounds with different numbers of rings, in all research stages. Detailed values for the mean, median, minimum or maximum can be read in Table 4. The first graph shows the removal efficiency of the sum of all PAHs analyzed. The lowest average value occurred in stage 1, and the highest in stage 4, where EM were present in the system for a period of 2 weeks. Therefore, the presence of specially selected microorganisms was beneficial in removing the sum of PAHs from the leachates after ATAD. The topic of the effect of EM on the decomposition of PAHs in leachates after ATAD has most likely not been addressed by other researchers to date. However, the effectiveness of using EM to treat starch, dairy or individual farm wastewater has been analyzed, with changes in chemical oxygen demand mainly

Table 3  
Sludge concentrations in all periods

Fraction	Stage	Values	Fraction	Stage	Values
Total PAH	1	$\frac{122.4 \pm 7.8 (121.4 \pm 9.9)}{111.3; 116.6; 128.8; 134.9}$	2-ring PAH	1	$\frac{9.01 \pm 0.43 (9.13 \pm 0.42)}{8.25; 8.65; 9.30; 9.56}$
	2	$\frac{1,114 \pm 35 (1,110 \pm 43)}{1,069; 1,083; 1,139; 1,187}$		2	$\frac{14.86 \pm 0.73 (15.11 \pm 0.58)}{13.72; 14.30; 15.25; 15.89}$
	3	$\frac{1,741 \pm 60 (1,732 \pm 51)}{1,637; 1,716; 1,777; 1,839}$		3	$\frac{23.6 \pm 2.0 (23.6 \pm 1.8)}{20.8; 22.5; 24.6; 27.1}$
	4	$\frac{1,807 \pm 55 (1,822 \pm 43)}{1,721; 1,751; 1,842; 1,891}$		4	$\frac{21.8 \pm 1.8 (21.8 \pm 2.1)}{18.9; 20.5; 22.9; 24.6}$
3-ring PAH	1	$\frac{28.6 \pm 1.4 (28.6 \pm 1.7)}{26.3; 27.6; 29.5; 30.9}$	4-ring PAH	1	$\frac{41.5 \pm 2.9 (41.0 \pm 3.8)}{37.5; 39.3; 44.1; 46.1}$
	2	$\frac{311 \pm 12 (308 \pm 15)}{295; 301; 319; 335}$		2	$\frac{207.9 \pm 9.3 (208.3 \pm 9.6)}{193.8; 201.9; 214.8; 223.7}$
	3	$\frac{375 \pm 25 (383 \pm 26)}{325; 360; 393; 403}$		3	$\frac{167.4 \pm 7.3 (166.7 \pm 6.9)}{155.9; 162.1; 169.7; 180.8}$
	4	$\frac{311 \pm 11 (312 \pm 14)}{294; 302; 319; 327}$		4	$\frac{205.2 \pm 11.2 (207.3 \pm 7.6)}{176.3; 202.7; 212.3; 220.8}$
5-ring PAH	1	$\frac{28.9 \pm 2.3 (28.9 \pm 3.2)}{25.6; 26.8; 30.8; 32.2}$	6-ring PAH	1	$\frac{14.5 \pm 1.1 (14.4 \pm 1.5)}{12.9; 13.5; 15.4; 16.1}$
	2	$\frac{375 \pm 16 (371 \pm 16)}{357; 362; 387; 407}$		2	$\frac{205 \pm 16 (204 \pm 23)}{182; 190; 219; 230}$
	3	$\frac{958 \pm 49 (953 \pm 40)}{895; 928; 982; 1038}$		3	$\frac{217.0 \pm 9.8 (215.1 \pm 10.9)}{201.9; 209.4; 225.6; 232.3}$
	4	$\frac{1,002 \pm 30 (1,011 \pm 20)}{949; 984; 1,021; 1,052}$		4	$\frac{267 \pm 13 (264 \pm 15)}{247; 258; 277; 285}$

being evaluated [31–33]. Studies on PAH decomposition with EM have usually been conducted for sewage sludge.

Among hydrocarbons, polycyclic aromatic compounds are the slowest to biodegrade. Microorganisms are able to break down low-molecular-weight aromatic compounds, branched alkanes and n-alkanes faster [34]. This is confirmed by the results of the study, as in the case of 2, 3, 4 and 5-rings hydrocarbons, their removal efficiency was highest when EM had the longest time to adapt to the prevailing conditions in the system. The average removal efficiency of naphthalene, the 2-ring compound, was similar in each test stage, ranging from 81%–87%, with the highest value recorded during stage 1. This situation occurred only for this compound. It is worth noting that the hydrocarbon in question has the lowest molecular weight, high vapor pressure and high volatile losses. Its reduction in the process of biological wastewater treatment may be largely due to the

volatilization phenomenon during mixing or aeration of tank contents [18,35,36]. In the case of 3- and 4-rings hydrocarbons, the maximum efficiency of PAH reduction occurred at weeks 2 and 4. These were stages in which the microorganisms were given time to adapt to the prevailing conditions, since both the technological parameters prevailing in the model system and the composition of the biocenosis were not modified at that time. Microorganisms are capable of inducing changes in genetic properties and producing enzymes that contribute to the decomposition of hydrocarbons, but the condition is to give them adequate time, so it is important to extend the period of exposure of PAHs to microorganisms in activated sludge chambers. Presently, there are gaps in the literature regarding studies of leachates after ATAD or the transformation of PAHs in leachates from dewatering sewage sludge stabilized by classical methods. Włodarczyk-Makuła and Wiśniowska [37] conducted

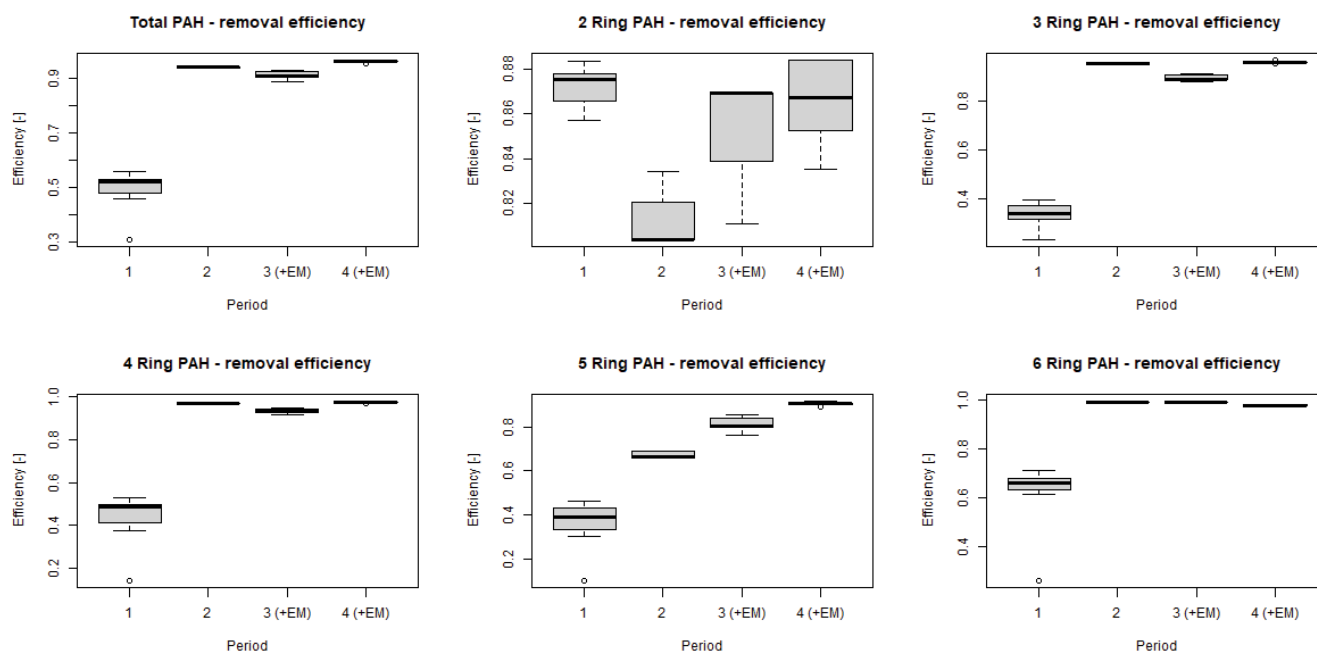


Fig. 5. Efficiency of polycyclic aromatic hydrocarbons removal in each test stage.

a study for filtrate liquids treated from PAHs by aerobic microorganisms. The incubation time was 72 h, and PAH degradation varied between 20%–30%. Manoli and Samara [38] noted a similar relationship. The removal of PAHs from wastewater using activated sludge was a maximum of 89%, however, sorption was by far the predominant process, and little biodegradation occurred. At the same time, it is worth noting that the retention time of wastewater in municipal wastewater treatment plants (into which leachates after ATAD are often discharged) is a maximum of 8 h. In addition, these facilities are not technologically adapted to carry out processes that treat wastewater from PAHs.

The average removal efficiency of 4- and 6-rings HMWs in stage 2 (second week of activated sludge operation) oscillated in the range of 97%–99%. The addition of EM had a negligible effect on changes in the reduction of the hydrocarbons in question. The average removal efficiency of 5-ring PAHs increased with each successive week of the research experiment. The lowest efficiency, at 37%, was recorded in stage 1. In the 2nd week of activated sludge operation, the value increased to 67%, while the last stage provided a reduction efficiency of 91%. The EM used in the conducted studies contributed to significant biosorption and biodegradation of the group of compounds in question. This is particularly important information, since one of the 5-ring aromatic hydrocarbons is benzo(a)pyrene, singled out by the World Health Organization and the International Agency for Research on Cancer (IARC) as the most potent carcinogen among all PAHs. This compound is found most widely in the environment and poses a threat to living organisms [39].

Statistical testing revealed that in all period comparisons, removal efficiencies were significantly different (Kruskal–Wallis test  $p$ -value was less than 0.0001). Post-hoc tests revealed following pairs of insignificantly different periods in case of treatment efficiency:

- 2-ring PAH fraction: treatment efficiency: period 1 and 4, period 3 and 4;
- 6-ring PAH fraction: treatment efficiency: period 2 and 3.

The conducted research experiment made it possible to analyze the transformation of PAH compounds during biological treatment of leachates after ATAD using microorganisms contained in activated sludge, as well as effective microorganisms. The results obtained can be used to control the reduction of PAH compounds in wastewater treatment or pretreatment processes.

#### 4. Conclusions

- The average biosorption of total PAHs throughout the process ranged from 122 to 1,807  $\mu\text{g}/\text{kg}$  d.m. The lowest content occurred for stage 1, and the highest for stage 4, where effective microorganisms were set out.
- The addition of the EM formulation increased the average sorption of hydrocarbons by microorganisms (except for 4-ring compounds). The most intensive increase, 61%, was in 5-ring compounds.
- The average efficiency of removal of total PAHs from leachates after ATAD throughout the process ranged from 50% to 96%. The best result was obtained in stage 4, where effective microorganisms were present for two weeks.
- The effective microorganisms used in the study contributed to significant biosorption and biodegradation of 5-ring aromatic hydrocarbons. Without their participation, the average efficiency ranged from 37% to 67%, and with their participation it was 81%–91%.
- The addition of the EM formulation had a negligible effect on changes in the reduction of 4- and 6-rings aromatic hydrocarbons.

Table 4  
Removal efficiencies in all periods

Fraction	Stage	Values	Fraction	Stage	Values
Total PAH	1	$\frac{0.497 \pm 0.066 (0.521 \pm 0.023)}{0.309; 0.488; 0.531; 0.561}$	2-ring PAH	1	$\frac{0.8723 \pm 0.0094 (0.8753 \pm 0.0039)}{0.8570; 0.8695; 0.8776; 0.8835}$
	2	$\frac{0.9412 \pm 0.0018 (0.9415 \pm 0.0024)}{0.9388; 0.9391; 0.9431; 0.9431}$		2	$\frac{0.812 \pm 0.012 (0.804 \pm 0.000)}{0.804; 0.804; 0.819; 0.834}$
	3	$\frac{0.9100 \pm 0.014 (0.908 \pm 0.016)}{0.887; 0.906; 0.922; 0.930}$		3	$\frac{0.855 \pm 0.024 (0.870 \pm 0.000)}{0.811; 0.842; 0.870; 0.870}$
	4	$\frac{0.9616 \pm 0.0023 (0.9623 \pm 0.0011)}{0.9555; 0.9606; 0.9629; 0.9647}$		4	$\frac{0.867 \pm 0.016 (0.867 \pm 0.023)}{0.835; 0.853; 0.884; 0.884}$
3-ring PAH	1	$\frac{0.337 \pm 0.045 (0.339 \pm 0.043)}{0.231; 0.318; 0.366; 0.396}$	4-ring PAH	1	$\frac{0.442 \pm 0.106 (0.485 \pm 0.054)}{0.142; 0.430; 0.495; 0.528}$
	2	$\frac{0.9542 \pm 0.0029 (0.9540 \pm 0.0046)}{0.9509; 0.9509; 0.9576; 0.9576}$		2	$\frac{0.96909 \pm 0.00014 (0.96906 \pm 0.00016)}{0.96895; 0.96895; 0.96927; 0.96927}$
	3	$\frac{0.895 \pm 0.013 (0.890 \pm 0.017)}{0.878; 0.888; 0.907; 0.914}$		3	$\frac{0.933 \pm 0.011 (0.931 \pm 0.013)}{0.917; 0.930; 0.943; 0.948}$
	4	$\frac{0.95932 \pm 0.00334 (0.95975 \pm 0.00069)}{0.95187; 0.95861; 0.95985; 0.96601}$		4	$\frac{0.97543 \pm 0.00193 (0.97627 \pm 0.00037)}{0.97171; 0.97479; 0.97648; 0.97798}$
5-ring PAH	1	$\frac{0.366 \pm 0.099 (0.388 \pm 0.063)}{0.099; 0.342; 0.430; 0.464}$	6-ring PAH	1	$\frac{0.633 \pm 0.121 (0.663 \pm 0.028)}{0.260; 0.641; 0.681; 0.715}$
	2	$\frac{0.6727 \pm 0.0146 (0.6648 \pm 0.0057)}{0.6609; 0.6609; 0.6924; 0.6924}$		2	$\frac{0.99311 \pm 0.00057 (0.99321 \pm 0.00074)}{0.99240; 0.99240; 0.99371; 0.99371}$
	3	$\frac{0.811 \pm 0.031 (0.807 \pm 0.035)}{0.763; 0.802; 0.838; 0.854}$		3	$\frac{0.9932 \pm 0.0016 (0.9936 \pm 0.0018)}{0.9906; 0.9921; 0.9940; 0.9952}$
	4	$\frac{0.9076 \pm 0.0060 (0.9102 \pm 0.0013)}{0.8926; 0.9045; 0.9105; 0.9163}$		4	$\frac{0.9796 \pm 0.0021 (0.9797 \pm 0.0020)}{0.9769; 0.9774; 0.9811; 0.9837}$

- The highest average efficiency of naphthalene removal occurred in stage 1. The observed regularity is most likely due to the physico-chemical properties of the compound, which ensure its high volatility, among other things, during the process of aeration of wastewater.
- The analysis of the results of the study showed that a longer opportunity for microorganisms to adapt to new conditions (stage 2 and 4) resulted in higher removal efficiencies of 3- and 4-rings hydrocarbons than when the time was shorter (stages 1 and 3).

## References

- [1] Ł. Górski, B. Buszewski, Methods of sludge stabilization and sanitary, *Ecol. Chem. Eng. S*, 9 (2002) 1501–1519.
- [2] S. Borowski, Aerobic thermophilic stabilization on municipal sludges, *Environ. Pollut. Control*, 4 (2000) 21–25.
- [3] M. Sobiepański, R. Kostrzewa, Odwadnianie i higienizacja osadu ściekowego w procesie zautomatyzowanym przy pomocy sterownika cyfrowego, *Technika*, 12 (2015) 2880–2884.
- [4] O. Augustin, I. Bartkowska, L. Dzienis, Efficiency of Wastewater Sludge Disinfection by Autoheated Thermophilic Aerobic Digestion (ATAD), W: IWA Specialist Conference: Moving Forward: Wasterwater Biosolids Sustainability: Technical, Managerial and Public Synergy, Conference Proceedings, Canada, June 24–27, 2007, pp. 1037–1043.
- [5] S. Liu, N. Zhu, L.Y. Li, The one-stage autothermal thermophilic aerobic digestion for sewage sludge treatment: stabilization process and mechanism, *Bioresour. Technol.*, 104 (2011) 266–273.
- [6] I. Bartkowska, *Autothermal Thermophilic Aerobic Digestion*, Seidel-Przywecki Publishing, 2017.
- [7] K. Iskra, J.M. Miodoński, Ł. Krawczyk, K. Citko, Analysis of efficiency of autothermal thermophilic aerobic digestion of sewage sludge in full scale, *Gas Water Sanit. Eng.*, 10 (2020) 8–12.



- [8] D. Boruszko, A. Wojciula, Heavy metals and polycyclic aromatic hydrocarbons in leachates from autothermal thermophilic aerobic digestion as a potential threat to the environment in north-eastern Poland, *Stud. Quat.*, 39 (2022) 15–22.
- [9] M. Gajewska, Treatment of reject water from digested sludge dewatering in multistage constructed wetland, *Ecol. Eng. Environ. Technol.*, 25 (2011) 86–98.
- [10] D. Dąbrowska, A. Kot-Wasik, J. Namieśnik, Degradation of organic compounds in the environment, *Ecol. Chem. Eng. S*, 9 (2002) 1077–1096.
- [11] M. Litter, N. Quici, Photochemical advanced oxidation processes for water and wastewater treatment, *Recent Patent Eng.*, 4 (2010) 217–241.
- [12] M. Smol, M. Włodarczyk-Makula, Preliminary removal of PAHs from coke oven wastewater, *LAB*, 17 (2012) 28–31.
- [13] A. Rubio-Clemente, R.A. Torres-Palma, G.A. Peñuela, Removal of polycyclic aromatic hydrocarbons in aqueous environment by chemical treatment: a review, *Sci. Total Environ.*, 478 (2014) 201–225.
- [14] G. Kupryszewski, *Introduction to Organic Chemistry*, Polish Scientific Publishers, Warsaw, 1997.
- [15] P. Ofman, I. Skoczko, PAH removal effectiveness comparison from hydraulic fracturing model wastewater in SBR reactors with granular and flocced activated sludge, *Desal. Water Treat.*, 134 (2018) 41–51.
- [16] D. Gateuille, O. Evrard, I. Lefevre, E. Moreau-Guigon, F. Alliot, M. Chevreuil, J.M. Mouchel, Mass balance and decontamination times of polycyclic aromatic hydrocarbons in rural nested catchments of an early industrialized region (Seine River Basin, France), *Sci. Total Environ.*, 470–471 (2014) 608–617.
- [17] B. Macherzyński, M. Włodarczyk-Makula, Effect of composition of the sewage sludge on degradation of low molecular weight PAHs in the fermentation process, *Eng. Prot. Environ.*, 17 (2014) 533–545.
- [18] K. Hussain, R.R. Hoque, S. Balachandran, S. Medhi, M.G. Idris, M. Rahman, F.L. Hussain, Monitoring and Risk Analysis of PAHs in the Environment, C. Hussain, Ed., *Handbook of Environmental Materials Management*, Springer, Cham, 2018, pp. 1–35. Available at: [https://doi.org/10.1007/978-3-319-58538-3\\_29-2](https://doi.org/10.1007/978-3-319-58538-3_29-2)
- [19] A. Sapota, Polycyclic aromatic hydrocarbons, *Principles and Methods of Assessing the Working Environment*, 2 (2002) 179–208.
- [20] A. Zasadowski, A. Wysocki, Some toxicological aspects of polycyclic aromatic hydrocarbons (PAHs) effects, *Ann. National Inst. Hyg.*, 53 (2002) 26–35.
- [21] E.W. Rice, R.B. Baird, A.D. Eaton, *Standard Methods for the Examination of Water and Wastewater*, 23rd ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C., 2017.
- [22] R. McGill, J.W. Tukey, W.A. Larsen, Variations of Box Plots, *The American Statistician*, 32 (1978) 12–16.
- [23] M. Hollander, A.D. Wolfe, *Nonparametric Statistical Methods*, John Wiley & Sons, New York, 1973, pp. 115–120.
- [24] M. Hollander, A.D. Wolfe, *Nonparametric Statistical Methods*, John Wiley & Sons, New York, 1973, pp. 68–75.
- [25] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, *J. R. Stat. Soc. B*, 57 (1995) 289–300.
- [26] R Core Team, *A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2022.
- [27] S.A.V. Tfouni, M.C.F. Toledo, Determination of polycyclic aromatic hydrocarbons in cane sugar, *Food Control*, 18 (2007) 948–952.
- [28] L. Jamir, V. Kumar, J. Kaur, S. Kumar, H. Singh, Composition, valorization and therapeutical potential of molasses: a critical review, *Environ. Technol. Rev.*, 10 (2021) 131–142.
- [29] W. Stringfellow, L. Alvarez-Cohen, Evaluating the relationship between the sorption of PAHs to bacterial biomass and biodegradation, *Water Res.*, 11 (1999) 2535–2544.
- [30] K. Klimaszewska, Biodegradation and occurrence of four polycyclic aromatic hydrocarbons in aquatic sediments, *Biotechnology*, 1 (1998) 140–148.
- [31] G. Zhou, J. Li, H. Fan, J. Sun, X. Zhao, *Starch Wastewater Treatment with Effective Microorganisms Bacteria*, 2010 4th International Conference on Bioinformatics and Biomedical Engineering, Chengdu, China, 2010, pp. 1–4.
- [32] M.T. Rashid, J. West, *Dairy Wastewater Treatment with Effective Microorganisms and Duckweed for Pollutants and Pathogen Control*, M.K. Zaidi, Ed., *Wastewater Reuse–Risk Assessment, Decision-Making and Environmental Security*, NATO Science for Peace and Security Series, Springer, Dordrecht, 2007.
- [33] K. Józwiakowski, *The Evaluation of Usability of EM-Farming™ Preparation for Work Optimization of Preliminary Settling Tanks*, Polish Academy of Sciences, Cracow Branch, 2008, pp. 159–167.
- [34] H. Kołoczek, P. Kaszycki, *Bioremediation of Refinery Pollutants in the Groundwater Environment: Methods for Removing Hydrocarbon Pollutants from the Groundwater Environment*, AGH University Publishing House, Cracow, 2006.
- [35] B. Nas, M.E. Argun, T. Dolu, H. Ateş, E. Yel, S. Koyuncu, S. Dinç, M. Kara, Occurrence, loadings and removal of EU-priority polycyclic aromatic hydrocarbons (PAHs) in wastewater and sludge by advanced biological treatment, stabilization pond and constructed wetland, *J. Environ. Manage.*, 268 (2020) 110580, doi: 10.1016/j.jenvman.2020.110580.
- [36] K. Shimada, M. Nohchi, X. Yang, T. Sugiyama, K. Miura, A. Takami, K. Sato, X. Chen, S. Kato, Y. Kajii, F. Meng, S. Hatakeyama, Degradation of PAHs during long range transport based on simultaneous measurements at Tuoji Island, China, and at Fukue Island and Cape Hedo, Japan, *Environ. Pollut.*, 260 (2020) 113906, doi: 10.1016/j.envpol.2019.113906.
- [37] M. Włodarczyk-Makula, E. Wiśniowska, Evaluation of degradation possibility of PAHs by microorganisms obtained from reject waters, *Proc. ECOpole*, 12 (2018) 599–610.
- [38] E. Manoli, C. Samara, The removal of polycyclic aromatic hydrocarbons in the wastewater treatment process: experimental calculations and model predictions, *Environ. Pollut.*, 51 (2008) 477–485.
- [39] M. Rusin, E. Marchwińska-Wyrwał, Health hazards involved with an environmental exposure to polycyclic aromatic hydrocarbons (PAHs), *Environ. Med.*, 3 (2014) 7–13.